ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON THE PREVALENCE OF THERMOPHILIC *CAMPYLOBACTER* SPECIES IN RAW MEAT IN ADDIS ABABA AND DEBRE ZEIT, ETHIOPIA

BY

LEMMA DADI

JUNE 2007

DEBRE ZEIT, ETHIOPIA

TABELE OF CONTENTS

Page

LIST OF TABLES

LISTS OF FIGURE

ABBRIVATIONS

AI	Artificial Insemination
AID	Acquired Immunodeficiency Syndrome
µg	Micro gram
µm	Micrometer
cAMP	Cyclic Adenosine Monophosphate
CLSI	Clinical Laboratory Standard Institute
CT	Cholerae Toxin
ELISA	Enzyme Linked Immuno Sorbent Assay
GBS	Guillain-Barre`syndrome
GM	Ganglioside Membrane
H_2S	Hydrogen sulfide
HIV	Human Immuno deficiency Virus
HL	Heat Labile
HS	Heat-Stable
ISO	International Organization for Standardization
IU`	International Unit
LT	Heat-Labile Toxin
mg	milligram
MH	Mueller-Hinton
MIC	Minimum Inhibitory Concentrations
ml	milliliter
mm	millimeter
NaCl	Sodium chloride
NMSA	National Meteorology Service Agency
PCR	Polymerase Chain Reaction
ppm	parts per million
VMAT	Vaginal mucus agglutination test
rpm	revolution per minute

ABSTRACT

A study on the prevalence of thermophilic *Campylobacter* species was under taken in selected abattoirs, ,butchers and supermarkets in two cities (Debre Zeit and Addis Ababa)of Ethiopia from November 2006 to April 2007.The study was conducted on raw meat (beef, chicken meat, goat meat, sheep meat and pork). A total of 540 raw meat samples consisting of 227 beef, 92 goat meat, 114 sheep meat, 60 chicken meat and 47 pork were collected and analysed during the study period. Thermophilic *Campylobacter* species were isolated and identified according to the techniques recommended by the International Organization for Standardization (ISO/CD 10272-1 and 10272-2, 2002).

Out of 540 raw meat samples analyzed 50 (9.3%) samples were positive for thermophilic *Campylobacter* species. The most prevalent species recovered from the samples was *Campylobacter jejuni* (78.0%), followed by *C. coli* (18.0%) and the remaining 4.0% of the isolates were identified as *C. lari.* Out of the samples examined chicken meat was the most frequently contaminated food item with thermophilic *Campylobacter* species with an over all prevalence of 21.7%.Of the examined sample 10.5% sheep meat, 8.5% pork 7.6% goat meat, and 6.2% beef samples were also positive for thermophilic *Campylobacter* species. The 21.7% prevalence of thermophilic *Campylobacter* species in row chicken meat was statistically significant. *Campylobacter jejuni* was the predominant species 78.0% isolated from raw meat samples examined in the current study. Its prevalence was higher in beef with the isolation rate of 85.7% followed by 84.6% in chicken meat, 83.3% in sheep meat, 71.4% in goat meat and 25% in pork. The difference in the distribution of the *C. jejuni* among different raw meat types was statistically significant. *Campylobacter coli* was the second predominant thermophilic *Campylobacter* species isolated (18.0%) from raw meat analyzed. *C. coli* was the predominant isolate from pork accounting for (50%) of the pork isolates. *Campylobacter lari* the least prevalent species accounting for 4.0% of the total isolates recovered from raw meat examined. The result of the present study antimicrobial sensitivity test indicates that the overall resistance of 20.0% was developed to streptomycin. Similarly, 14.0% and 12.0% resistances were developed against gentamicin and kanamycin, respectively. Of the total isolates 10.0% were

resistant to tetracycline and ampicillin. Multi drug resistance to two or more drugs was detected in 20% of the isolates.

The microbiological examination in the current study suggested that thermophilic *Campylobacter* species particularly *C. jejuni* was found in the raw meat analysed. The development of antimicrobial resistance was observed in a considerable number of isolates. This indicates the possible risk of food borne campylobacteriosis particularly for elderly and immunocompromised individuals in Ethiopia.

Keywords: Antibiogram, prevalence, raw meat, Thermophilic *Campylobacter* species, Addis Ababa, Debre Ziet, Ethiopia.

1. INTRODUCTION

Campylobacter is one of the most common bacterial genera, containing several species of both public and animal health importance (Acha and Szyfres, 2001). They are distributed worldwide and many species are commensal on the mucus membrane of the oral cavity and intestinal tract while a few species occurs in the prepuce of bulls and the genital tract of cows in the herd, where bovine genital campylobacteriosis is or, has been present (Ellen *et al.*, 1994). The organisms can be found in poultry, pigs, sheep, cattle and other food animal species and in bulk milk samples, tissue specimens from beef cattle and raw ground beef. Several species of birds and rodents may act as reservoirs and are the ultimate sources for most human infections (Radostits, 2001). Most of outbreaks in humans are associated with consumption of raw milk or surface water where as most sporadic cases are often associated with improper handling and consumption of undercooked poultry or cross contamination of food by raw poultry. Infection results from consumption of contaminated food, food products, water and/or contact with feces of animals (especially young animals with diarrhea). Traveling in high prevalence areas is also considered as risks factors in human disease (Friedman, *et al*., 2000). Similarly, occupational exposure may also cause infection and disease in workers in animal health facilities, animal shelters poultry processing plants, animal agriculture and rendering-plants (Prescott and Munroe, 1982).

The most common causes of campylobacteriosis in food producing animals include *C. fetus* subsp. *venerealis, C. fetus* subsp. *fetus, C. jejuni, C. mucosalis, C. hyointestinalis, C. coli, C. lari and C. upsaliensis* (Quinn *et al.*, 2002).The other species *Campylobacter pylori, C. intestinalis and C. fetus subsp.fetus* have been isolated from blood, spinal fluid synovial fluid, heart, brain, liver and meninges and aborted fetus (Davis *et al.*, 1990). Among these species of *Campylobacter*, except the genital form of infection, all infections in animals are acquired through oral-fecal route (Gyles and Thoen, 1993). The *Campylobacter* species cause abortion, stillbirth, infertility, enteritis and diarrhea in animals (Acha and Szyfres, 2001; Quinn *et al.*, 2002).

Campylobacter enteritis in man has been diagnosed with increasing frequency over recent years, mainly due to *C. jejuni*, *C. coli* and *C. lari* (Davis *et al.*, 1990; Radostits, 2001). Thermophilic *Campylobacter* species, particularly *C. jejuni* and C. *coli*, are recognized as one of the most frequent causes of acute diarrheal disease in humans throughout the world (Nachamkin, 1995). They are among the major microorganisms causing diarrhea in Europe, the United States and other industrialized countries. Thermotolerant Campylobacter, particularly *C. jejuni* and *C. coli*, are presently the most common causes of human food borne infections in several developed countries where the number of reported cases of campylobacteriosis by far has exceeded the number of cases of salmonellosis (Altekruse *et al*., 1999; Friedman *et al*., 2000). Besides, campylobacter infections are hyperendemic among the infant populations of underdeveloped countries (Jiménez *et al*., 1994). *Campylobacter jejuni* is considered to be one of the principal bacterial agents causing enteritis and diarrhea in man, particularly in developed countries, where the incidence is higher than enteritis caused by *Salmonella* species. Over 80% of cases are caused by *C. jejuni* and about 10% of cases are caused by *C. coli*. Other Campylobacter species, such as *C. upsaliensis* and *C. lari*, may also be associated with human diarrhea (Lastovica & Skirrow, 2000). In susceptible humans, *C. jejuni* and *C.coli* infection is associated with acute enteritis and abdominal pain lasting for up to 7 days or more. Although such infections are generally self-limiting, complications can arise and may include bacteraemia, reactive arthritis, and abortion (Skirrow & Blaser, 2000). The infection is a very wide spread diarrheal disease in developing world commonly affecting babies and young children and in individuals with debilitating diseases such as HIV/ AIDS (Tee *et al.,* 1995). Campylobacter infections usually occur following ingestion of improperly handled or under cooked food and campylobacteriosis is considered as zoonotic disease, and animals such as poultry, pigs, cattle, sheep and goat may act as reservoirs for *Campylobacter* (Jimenez, *et al.*, 1994**).**

Campylobacter enteritis is a self-limiting disease, and antimicrobial therapy is not generally recommended. However, antimicrobial agents are recommended for extraintestinal infections and for treating immunocompromised persons. Erythromycin and ciprofloxacin are the drugs of choice (Engberg *et al*., 2001). The rate of resistance to these drugs is increasing in both developed and developing countries, although the incidence is higher in developing countries (Steinbrückner, *et al*. 2000). Use of these drugs for infections other than gastroenteritis and self-

medication are often the causes of increase in resistance in developing countries; where as in developed countries, resistance is due to their use in food animals (Feierl, *et al*., 2001).

There have been limited studies conducted on human campylobacteriosis in Ethiopia. A study conducted at Tikur-Anbessa and Ethio-Swedish children's hospital indicated that thermophilic *Campylobacter* species are important causes of diarrhea both in adults and children in which *C. jejuni* accounted for 82.4% of the isolates (Asrat *et al.*, 1999). A cross sectional study conducted on urban and rural farm animals indicated that thermophilic *Campylobacter* species are significantly identified with prevalence of 39.6% in the fecal sample (Kassa, *et al*., 2005). The antibiogram conducted on these isolates indicates that some of the bacteria showed a range of resistance to certain antimicrobial agents (Asrat *et al.*, 1999; Kassa *et al*., 2007). However no citable work has been done so far regarding the prevalence of thermophilic *Campylobacter* species in foods of animal origin, and the role foods of animal origin as the epidemiologic factor for human campylobacteriosis.

The objectives of this study are to: -

- To study the prevalence and distribution of thermophilic *Campylobacter* species in raw meat.
- Identify the principal food category which represents the most significant vehicle of campylobacter infection.
- To determine the antibiogram status of each isolates

2 LITERATURE REVIEW

2.1 The genus Campylobacter

The genus Campylobacter comprises a number of species (Wallace *et al.*, 1998). Members of the genus are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. These bacteria are microaerophilic. They neither ferment nor oxidize carbohydrates. Some species, particularly *C. jejuni, C. coli* and *C. lari*, are thermophilic, growing optimally at 42°C (Hirsh and Zee, 1999).

They can colonize mucosal surfaces usually the intestinal tract of most mammalian and avian species. The species of *C. jejuni* comprises two subspecies (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*) that can be distinguished on the basis of nitrate reduction (subsp. *doylei* does not reduce nitrate). Subspecies *jejuni* is much more frequently isolated than subspecies *doylei.* The *Campylobacter jejuni* and *C. coli* are the major pathogenic species of zoonotic importance (Skirrow and Blaser, 2000).

Several species of genus *Campylobacter* are known to cause different types of disease in farm animals, some of which are potentially zoonotic (Radostits *et al.*, 1994). *Campylobacter fetus* subsp. *venerealis* is the cause of infertility and abortion in cattle, while *C. fetus* subsp. *fetus* causes sporadic abortion in cattle and enzootic abortion in sheep and has been associated with bacteraemia in man. Campylobacters implicated in enteric disease of animals and humans are *C. jejuni, C. coli, C. concisus, C. hyointestinalis, C. mucosalis, C. lari and C. upsaliensis* (Hirsh and Zee, 1999). The *C. sputorum* subsp. *mucosalis* is frequently found in association with a group of enteric diseases in pigs called “porcine intestinal adenomatosis” complex and recently *C. hyointestinalis* has also been isolated from the same lesion (Radostitis *et al*, 1994).

2.1.1. Taxonomy and Classification

The family Campylobacteriaceae is classified under Phylum Proteobacteria, Class Epsilon proteobacteria and Order Campylocacteriales (Vandamme, and ON, 2001). Family Campylobacteriaceae contains the genera Campylobacter and Arcobacter (previously with in the genus Campylobacter). During the last 30 years differentiation of the Campylobacter genus from Vibrio, groups of Campylobacter-like organisms were detected, described and identified as new species, or as different biotypes of existing species. Phylogenetic studies in the late 1980's revealed genotypic heterogeneity among the species with three major clusters, rRNA homology groups, were formed (Vandamme *et al*., 1991). Campylobacter was thus divided into three genera with revised genus descriptions, and new names were proposed for the remaining two genera: Arcobacter and Helicobacter (Schumacher, *et al.*, 1992).

The genera Campylobacter and Arcobacter formed a family of gram-negative, nonsaccharolytic bacteria with microaerobic growth requirements and a low G+C content. Members of this family are encountered mainly as commensals or parasites in humans or domestic animals. The family also contains misclassified species (*Bacteroides ureolyticus*) and strains originally described as 'free-living Campylobacters' (*Sulfurospirillum* species.) (Schumacher, *et al.*, 1992).

Campylobacter contains DNA with a Guanine and Cytosine (G+C) content between 29 and 36% (Veron and Chatelain, 1973). Most species are microaerophilic, although some show a range of oxygen tolerance, some are almost anaerobic, and others grow best in the presence of 5-10% oxygen (Doyle, 1986). The aerobic species of Campylobacter were reclassified as genus Arcobacter in (Vandamme *et al*., 1991).

In 1963, when Campylobacter was first described, the genus comprised of two species. At present, the genus contains 16 species, and six subspecies. The 16s rRNA gene, used to determine phylogenetic relationships among all living organisms, played a major role in a previous extensive rearrangement of Campylobacter taxonomy (Vandamme *et al*., 1991). *Campylobacter* species implicated in enteric disease of animals and humans include *C. jejuni*,

C. coli, C. lari, C. upsaliensis, C. concisus, C. helviticus, C. hyointestinalis and *C. mucosalis. Campylobacter jejuni* and *C. coli* are the major causes of gastroenteritis in people and non-human primates, and have been found in faecal samples from dogs and cats with diarrhea, with *C. jejuni* being more common of the two (Hirsh and Zee, 1999).

2.1.2. Morphology and Staining reaction

The thermophilic microorganisms belonging to the genus Campylobacter are spiral or curved Gram-negative nonsporeforming rods, 0.2–0.5μm wide and 0.5–0.8μm long. Their characteristic corkscrew-like motion is due to the presence of a single flagellum (occasionally multiple) at one or both cell poles. Nutrient limitation, aeration of the medium and the presence of free radicals all affect the transition from spiral to coccoid morphology. This morphologic transition may be caused by certain changes in the structure of the peptidoglycans, mainly due to its enzymatic degradation (Wallace *et al.*, 1998). They are microaerophilic in nature and survive for a longer time outside the host. The organisms are susceptible to drying, high oxygen conditions and low pH. (Pattison, 2001)

2.1.3. Cultural Characteristics

Thermophilic *Campylobacter* species are microaerophilic, requiring an atmosphere containing 5% oxygen and 10% carbon dioxide concentration for growth producing grayish, mucoid and spreading colonies. Some such as *C. jejuni*, *C. coli* and *C. lari* will grow at 42^0C, a characteristic that is useful for their selectivity in isolation from intestinal source (Hirsh and Zee, 1999). The *Campylobacter jejuni* and *C. coli* are best isolated from faecal samples on selective media containing antimicrobial agents (e.g. Campy CVA containing Cefoperazone, Vancomycin and Amphotericin B). Other species could also be isolated under the same condition (Quinn *et al.*, 2002).

2.1.4. Biochemical Characteristics

Members of the genus Campylobacter are chemoorganotrophs, unable to ferment or oxidize carbohydrates. They obtain energy through the respiratory chain, from the metabolism of amino acids, and from the metabolism of intermediates of the tricarboxylic acid cycle (Amano and Shibata 1992).The *Campylobacter* species are oxidase positive. Though they possess catalase and superoxidedismutase, these enzymes are overwhelmed by the excess of hydrogen peroxide and superoxide anions formed when they are grown in the presence of atmospheric concentration of oxygen (Hirsh and Zee, 1999). Susceptibility or resistance to nalidixic acid or cephalothin, hydrogen sulfide production, nitrate reduction growth at 25^{O}C or 45^{o}C and the catalase reaction are some of the criteria on which a definitive identification of the *Campylobacter* species is based (Table 1) (Quinn *et al.*, 2002).

Table 1: Basic phenotypic characteristics of thermophilic *Campylobacter* species

Characteristics	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Growth on Preston selective agar at 42^{o}C	+	+	+	+
Growth in 3.5% NaCl	–	–	–	–
Catalase	+	+	+	- or slight
Indoxyl acetate hydrolysis	+	+	-	+
Nalidixic acid	S	S	R	S
Cephalothin	R	R	R	S
Nitrate reduction	+	+	+	+
H_2S	+	+	+	+

Source: - Ellen *et al*., 1994

Key: + = positive; - = negative; S = sensitive; R = resistant

2.1.5. Typing of *Campylobacter* Species

Biotyping

The research in thermophilic *Campylobacters* species necessarily involves examination of the colonies appearing in selective media after an incubation period of 48h at 42°C under microaerophilic conditions. Non-hemolytic gray or colorless colonies, either plain aqueous with irregular edges, or round-convex with regular round edges, should be investigated. Suspicious colonies are picked and subjected to at least three tests such as direct microscopic examination of motility and cell morphology, Gram-staining, which should be negative and oxidase production, which should be positive(Quinn *et al.*, 2002). Most thermotolerant *Campylobacter* species involved in food-borne bacterial enteritis, except for *C. upsaliensis*, also produce catalase. *C. jejuni, C. coli* and *C. lari* can be distinguished based on the growth at 25°C and 43°C, susceptibility to nalidixic acid, hippurate-hydrolysis and hydrogen sulfide production in an iron-containing medium.

The classification of campylobacters based only on biochemical tests is complex. However, the determination of biochemical features is the most widely employed identification strategy applied to Campylobacter, which justifies the optimization of phenotypic analysis for the differentiation of *Campylobacter* species. The application of numerical analysis of phenotypic features to campylobacters was considered by Neill *et al.* (1985). More recently, On and Holmes (1995) designed a scheme comprising the investigation of 67 phenotypic characters in the genera *Campylobacter*, *Helicobacter* and other related taxa, obtaining a final scheme that proved to be a valuable tool for identification at species and subspecies level in most strains studied and whose results coincide with previous results obtained by RNA and DNA sequencing . According to the study by On and Holmes(1995), *C. coli, C. jejuni* subsp. *jejuni* and *C. lari* are closely related, a similar result having been obtained from phylogenetic studies based on the analysis of 16S rRNA sequences (vandamme *et al*.,1991).

Serotyping

Serotyping of Campylobacter is done as recommended by International Committee for Microbiology, and is based on isolation and typing of the organism (Korolik *et al*., 1988).

Serotyping of Campylobacter involve haemagglutination of heat-stable (HS or O) antigens, which were later confirmed to be the O (somatic) antigens. *Campylobacter jejuni* and *C. coli* have their own types of O antigen, although some cases (< 4.5%) of cross reaction have been reported. Serotyping was also reported to be done based on the heat stable (HS) and heat labile (HL) antigens (Patton *et al*., 1985). Detection of O antigens by direct agglutination of *C. jejuni* and *C. coli* cells could able replace passive haemagglutination (Frost *et al*., 1998).

In 1980, Penner and Hennessy described a passive haemagglutination procedure for serotyping *Campylobacter jejuni* subsp. *jejuni* on the basis of soluble heat-stable antigens (Penner and Hennessy, 1980). Penner method uses passive haemagglutination, whereby the supernatant from a boiled cell suspension is used to sensitize erythrocytes that are later mixed with antisera to demonstrate agglutination and this system recognizes 65 serotypes in total, and comprises 47 antisera for *C. jejuni* and 18 antisera for *C. coli* (Penner *et al*., 1983). A comparative analysis of O and HL serogrouping of human *C. jejuni* isolates has showed conserved associations between specific O and HL antigens (Jackson *et al*., 1998).Lior *et al*, in 1982, published amd proposed a new scheme (a slide agglutination test) for detection of heat stable antigens which recognizes 130 serotypes of *C. jejuni, C. coli* and *C. lari*. The proportion of isolates that give a result from serotyping can be low, but, in general, non-type ability in human and veterinary strains is less than 20% (Nielson, *et al*., 1997).

Resistotyping and phage typing

The susceptibility profiles of *Campylobacter* species with respect to several antimicrobial agents has been considered for taxonomic purposes (Ribeiro *et al.*, 1996). The methods of choice to evaluate susceptibilities to antimicrobial agents are determination of minimum inhibitory concentrations (MIC) in Mueller-Hinton agar and standardized disk diffusion in agar on selected plate media. The increasing isolation rate of *Campylobacter* strains resistant to an increasing number of antimicrobial agents especially the quinolones together with the conjugational exchange of resistance against others such as tetracycline or kanamycin has reduced the taxonomic value of resistotyping studies. The interest for susceptibility studies, however, has increased from the therapeutic point of view.

2.2. Epidemiology

Campylobacter species have been known as the cause of diseases in animals since 1913 (Blaser and Reller, 1981). Prior to 1970s, *Campylobacter* species were known primarily to veterinary microbiolo*g*ists as the organisms that caused spontaneous abortions in cattle and sheep and as a cause of other animal pathologene (Jay, 2000); but they have been recently recognized as a cause of human disease (Fox, 1998). Both Domestic and wild birds as well companion animals are the known reservoirs for *Campylobacter* species. The *Campylobacter* species are microaerophilic inhabitants of the gastrointestinal tract of various animals, including, cattle, sheep, goat, poultry and pig as well as the reproductive tract of various animal species, which acts as source of infection (Ellen *et al*., 1994; Jay, 2000).

2.2.1. In cattle

Bovine genital campylobacteriosis is a true venereal disease that is characterized primarily by early embryonic death, infertility a protracted calving season, and abortion (Lofstedt, 1998). The disease is common in beef breeds worldwide. The causative agent of bovine abortion, *Campylobacter fetus* subsp. *venerealis* survives on the penis and prepuce of bulls especially within mucosal crypts and within the female reproductive tract (Radostits, 2001). The *Campylobacter fetus* subsp. *fetus* causes sporadic abortion in cattle and is isolated from the reproductive tract of bull and cows, and there are some evidences that it may cause infertility as in the classical "bovine venereal campylobacteriosis" syndrome. The *C. jejuni* is isolated sporadically from aborted bovine fetus (Acha and Szyfres, 2001). Abortion occurs in infected cattle to the extent of 3% to 5% usually after 5 months of gestation but occasionally at 2 to 3 months (Ellen et al., 1994). *Campylobacter jejuni* is recognized to be a potential cause of mastitis (Quinn et al., 2002)

Both C. *jejuni* and *C. fetus* subsp. *fetus* are incriminated as a cause of diarrhea in cattle (Quinn *et al.*, 2002). Experimentally the organism causes a mucoid diarrhea often with dysentery and fever in calves. The disease is mild and unapparent, without fever, and may be manifested by mild depression and soft feces with the occasional mucous.

Most *Campylobacter* species are found in the alimentary system of healthy, carrier or diarrheic animal (Hirsh and Zee, 1999) the exception being *C. fetus* subsp. *venerealis*, which is found only in the reproductive tract of cattle and *C. sputorum* subsp. *bobulus* which is found only in the reproductive tract of cattle and sheep (Ellen *et al.,* 1994) and also transmitted by contaminated instruments, bedding or by artificial insemination (AI) using contaminated semen. Bulls can also transmit the infection mechanically for several hours after mating with an infected cow (Lofstedt, 1998). The organism remains at the cervicovaginal junction until the end of estrus, then multiply at this site and when conditions are suitable move into the uterus. Further multiplication perhaps active invasion result in inflammation of the uterus with resultant endometritis and cessation of pregnancy and the animal will turn to estrus (Hirsh and Zee, 1999). In female animals, infected bull deposit *C. fetus* subsp. *venerealis* in the vagina at coitus (Radostits, 2001)

2.2.2. In Sheep and Goats

Abortion, stillbirths and weak lambs characterize campylobacteriosis in sheep during late pregnancy. *Campylobacter jejuni* and *C. fetus* subsp. *fetus* are the causative agents of this disease. The infection is highly contagious and may cause 70% of the ewes to abort when the organism is newly introduced into a flock (Delong *et al*., 1996). In Britain, it is the third most common cause of ewes' abortion (Quinn *et al*., 2002). *Campylobacter fetus* subsp. *fetus* is one of the agents of ovine campylobacteriosis that cause abortion in sheep. It is also incriminated as an enteric pathogen that causes enteritis and diarrhea in many species (Smith, 1996). Outbreaks of severe gastroenteritis in fattening lambs have been attributed to *C. jejuni* (Radostits *et al*., 1994).

Isolation of *Campylobacter* species from rectal swabs or feces samples of healthy goats is being reported. The *Campylobacter* species were isolated from goats in different part of the world. In Canada 2.7% of the animals studied found positive for *Campylobacter jejuni* (Prescott and Bruin-Mosch 1981). Turkson *et al.* (1988), in Kenya, found *Campylobacter* species in 6.3% of the goats sampled, and Abrahams *et al.* (1990) in Ghana detected *C. jejuni,* but not *C. coli*, in a high proportion (33.3%) of the goats tested. These different carriage rates could be attributable

to contact of goats with other animal species. Thus, Jiwa *et al.* (1994) explored the prevalence of *Campylobacter* species in healthy goats kept under various management systems in Tanzania and observed that goats kept away from other farm animals, irrespective of whether the management system was good or poor, were negative for *Campylobacter* species.

Campylobacteriosis due to *C. fetus* subsp. *fetus* and *C. jejuni* have been reported to cause sporadic abortion in goats. In one out break in the United States, 5 of 21 late-pregnant goats aborted, and 2 of the does became systemically ill; *C. jejuni* was later isolated from diarrheic feces in the same herd. In South Africa where campylobacter abortion appear more common, as many as 30% of aborted kids have grossly visible liver necrosis. The placenta is often edematous with necrosis of cotyledons (Smith and Sherman, 1994).

Susceptible ewes and does may acquire infection through ingestion of forage contaminated with fetal materials or uterine discharges; other sources of infection may include feces of carrier sheep and other mammals and various birds such as magpies, sparrows, starlings, pigeons and other birds commonly found around livestock (Delong *et al.,* 1996). The placenta, fluids and fetus have been found to contain large number of the organism and act as a source of infection to susceptible animals (Hirsh and Zee, 1999).

2.2.3. In Pigs

Pigs seem to be a natural reservoir for *Campylobacter* species with prevalence between 50% and 100% and excretion levels ranging from 10^2 to 10^7 CFU/g, but opposite to most other animals, pigs show a dominance of *C. coli* (Alter *et al*., 2005; Boes *et al*., 2005). Nevertheless, American studies showed that *C. jejuni* may constitute a majority (up to 87%) of the *Campylobacter* species. detected in hog farms (Young *et al*., 2000). A high prevalence of *C. jejuni* has been reported from porcine livers (Kramer *et al*., 2000). The *Campylobacter jejuni* may co-exist with *C. coli* in pigs, but are typically present in 10–100-fold lower numbers than *C. coli* (Madden *et al*., 2000; Jensen *et al*., 2006).

The organism may cause diarrhea in nursing piglets and in weaned pigs with high rate of isolation from small intestine (Radostits *et al.*, 1994). The *C. sputorum* subsp. *mucosalis* is frequently found in association with a group of enteric diseases in pigs called "porcine intestinal adenomatosis" complex. Recently, *C. hyointestinalis* has also been isolated from the same lesion. Young adults show cutaneous pallor, weakness and black tarry feces (Radostits *et al.*, 1994). *Campylobacter coli* have been isolated from small intestine of diarrheic piglets and described to cause dysentery and necrotizing ileitis with mortality rate of more than 50%. Older animals suffer from diarrhea, rapid weight loss and mortality rate of 2-20% (Buxton and Fraser, 1977). However, free-range pigs from a single organic farm seemed to be colonized with Campylobacter earlier in life than conventional pigs (Alter *et al*., 2005). Outdoor organic pigs may differ from conventional pigs with respect to the occurrence of Campylobacter. For example, the lower animal density probably reduces the infection pressure and roughage stimulates the intestinal flora, which is likely to reduce the susceptibility to infections (Mikkelsen *et al.*, 2004).

Pigs acquire infection through ingestion of feed that has been contaminated with feces of reservoir host (Buxton and Fraser, 1977). The pigs appear to be the likely source of the Campylobacter contamination in the paddock environment (Jensen, *et al.*, 2006).

2.2.4. In birds

The thermophilic members of the genus Campylobacter attribute campylobacteriosis in birds to infections. The three species of significance for bird campylobacteriosis are *C. jejuni, C. lari and C. coli* (Calnek *et al.*, 1991). The *C. jejuni* is the cause of "vibrionic hepatitis" in poultry and has been isolated from a wide range of animals and birds with or without diarrhea (Smith, 1996). The *C. coli* isolated occasionally from the intestinal tract of poultry and its derived meat products, while *C. lari* isolated mainly from free living marine birds (Calnek *et al.*, 1991).

Poultry primarily serve as reservoir host for thermophilic *Campylobacter* species and up to 90% of broilers may be infected, while 100% of turkeys and 88% of domestic ducks may harbor the organisms. Various species of *Campylobacter* have been isolated from free-ranging pigeons in

the USA and Japan. Infection has been recorded among game birds, including partridges, pheasants and quails which act as reservoir of infection (Shani, 1998). Despite the fact that *C. jejuni* is the most common intestinal commensal in floor housed turkeys, broilers and layer birds, there is no evidence to show that campylobacters can be transmitted vertically by either transovarially or penetrating the egg shell after oviposition (Calnek *et al.*, 1991).

2.3. Reservoirs

The Campylobacter jejuni and *C. coli* are generally considered commensals of livestock, domestic pet animals and birds. However, they have been associated with disease in a wide range of hosts. In cats and dogs, especially young animals or animals under stress, *C. jejuni* is associated with diarrhea and this is a well-recognized source of human infection (Weijtens, 1999). Dogs and cats are also frequently colonized by *C. upsaliensis* (dogs) and *C. helveticus* (cats). Outbreaks of Campylobacter-associated enteritis have been reported in some animals including breeding groups of non-human primates and even small laboratory-reared mammals. Large numbers of campylobacters have been isolated from young livestock, including piglets, lambs and calves, with enteritis, but the organisms are also found in healthy animals. In birds, especially poultry, disease is rare, if it occurs at all, despite high levels of colonization with *C. jejuni* or *C. coli*. Outbreaks of avian hepatitis have been reported, but the pathogenic role of *Campylobacter* species in this is unclear. One possible exception is ostriches where campylobacter-associated death and enteritis occurs in young birds. *Campylobacter* species are frequently found in wild birds (Broman *et al*., 2002). *Campylobacter jejuni, C. coli, C. hyointestinalis* and *C. sputorum,* as well as *C. fetus* may also be associated with infections of the reproductive tract (Newell *et al.*, 2001). In cattle, all these strains can be associated with abortion. In sheep up to 20% of campylobacter-associated abortions are due to *C. jejuni* or *C. coli.* Such infections are presumably a consequence of translocation from the gastrointestinal tract or via an ascending route.

Animal and animal by products are the sources of infection for human beings and susceptible animal species (Hirsh and Zee, 1999). The *C. jejuni* is not an environmental organism, but is associated with warm-blooded animals. A high percentage of all major meat producing animals

have been shown to contain these organisms in their feces, with poultry being prominent. Its prevalence in fecal sample often ranges from around 30% to 100% (Jay, 2000). The *C. jejuni* and *C. coli* have been isolated from the intestine of healthy farm animals, poultry, pets, zoo animals and wild birds. *Campylobacter jejuni* and *C. hyointestinalis* are found in the rumen and intestine of normal adult cattle and calves. *Campylobacter coli* and *C. jejuni* were isolated from rectal swabs taken from dairy cows. *Campylobacter jejuni* has been isolated from samples collected from various species of animals as shown in Table-2.

Table 2: *Campylobacter jejuni* isolation rates from various samples taken from different food animal

Specimen	Rate of isolation
Eviscerated chicken	72% - 80%
Chicken intestinal content	39% - 83%
Chicken liver	4.5%
Raw milk	30%
Swine intestinal content	61%
Swine feces	66%- 87%
Swine carcasses	22%
Sheep feces	73%
Sheep carcasses	24%
Eviscerated turkey	94%

Source: Jay, (2000)

2.4. Virulence factors

An important mechanism by which bacterial enteropathogenes induce diarrhea is through the production of potent toxins. They produce cytotoxin, enterotoxin or both. Cytolethal distending toxin activity causes certain cell types to become slowly distended, progressing to death (Lindblom *et al.,* 1995). Bacterial toxins in general have been conveniently classified as either membrane damaging, such as haemolysins and phospholipases, or intracellular acting, such as the toxins produced by *Corynebacterium diphtheriae*, *Vibrio cholerae,* and *Shigella dysenteriae*

(Radostitis *et al.*, 1994). The latter group is probably directly associated with the mechanisms for inducing diarrhea. These toxins are proenzymes that share several modes of action. They bind to specific receptors on the plasma membrane. *Campylobacter jejuni* secretes toxin similar in activity to the enterotoxin of *Vibrio cholerae* (CT) and the heat-labile toxin (LT) of *Escherichia coli* by increasing intracellular cyclic adenosine monophosphate (cAMP) level and cytoskeletal rearrangement followed by cell death (cytolethal distending toxin) a protein that was shown to induce hepatitis in mice (hepatotoxins) and protein that has hemolytic activity (haemolysins) (Hirsh and Zee, 1999). Both toxins are immunologically similar and bind to the same ganglioside on the target cells. *Campylobacter coli* and *C. lari* produce uncharacterized substances with cytotonic and cytotoxic activity (Jay, 2000).*Campylobacter jejuni* secretes a toxin similar to a cholera toxin and heat labile toxin (HL) of Escherichia coli by increasing intracellular cAMP and cytoskeletal rearrangements. Both toxins are immunologically related and bind to the same ganglioside (GM) on the surface of the target cell. It also produces a mannose-resistant adhesion that binds to a fructose-containing receptor on the target cell. In addition, it survives inside mononuclear phagocytes, implying the existence of other important as yet unidentified surface structure (Hirsh and Zee, 1999).

2.5. Diagnosis

Diagnosis of animal campylobacteriosis is based on isolation and identification of the organism from suspected samples as well as serological tests (Shani, 1998).

2.5.1. Direct Examination

The Grams stained smear from fecal material or aborted fetal stomach contents will reveal numerous slender, curved rods in cases of diarrhea produced by *C. jejuni.* A modified acid-fast stain is also used to demonstrate organisms best in smear (Hirsh and Zee, 1999). Impression smears of the intestine of swine with proliferative enteritis contain similar rods. A characteristic darting motility of *C. jejuni* can be examined by dark-field or phase contrast microcopy of fecal smear of acute stage diarrhea (Lofstedt, 1998).

2.5.2. Culture and Identification

The *Campylobacter* species can be isolated from fetal abomasal content in sheep (Smith and Sharman, 1994) it can also be isolated from feces, cecal and jejunal contents and from liver tissue, bile and blood in systemic infection of poultry (Calnek *et al.*, 1991). In pigs, isolation of the organism from feces is difficult due to intracellular localization of the bacteria (Quinn *et al.*, 2002). In cow bulk tank milk samples, several studies have reported a frequency of isolation that ranged between 0.4% and 12.3% for *C. jejuni* (Morgan *et al*., 2003).

The specimen should be transported kept either in Lary-Blair transport medium or in Campy thio, a thioglycollate broth base with 0.16% agar and vancomycin (10 mg/L), trimethoprim (5mg/L), cephalothin (10mg/L), Polymyxin B (2,500U/L) and amphotericin B (2mg/l). The same antimicrobial agents are incorporated into Brucella agar base with 10% sheep blood to produce Campy-BAP, one of the selective agars that are useful for cultivation of *Campylobacter* species, another selective media such as Campy-CVA containing antimicrobial agents like cefoperazone, vancomycin and amphotericin-B can also be used for the isolation of the organism (Quinn *et al.*, 2002).Preston campylobacter selective agar supplemented with 10% lysed horse blood and Preston antibiotic agents(Polymyxin B (2,500IU), Rifampicin (5.0 mg), trimethoprim(5.0mg) and cyclohexamide (50.0mg)) is preferable in the isolation of thermophilic Campylobacter species. Incubation is done at 37^0C in an atmosphere containing 5% Oxygen and 10% carbon dioxide. Plates are examined within 48 hours (Hirsh and Zee, 1999). Thermophilic *Campylobacter* species, *C. jejuni, C. coli* and *C. lari* should be cultured and incubated at 42^0 C under microaerophilic conditions for 48 hours (Quinn *et al.*, 2002). The most common agents of gastroenteritis, *C. jejuni* and *C. coli* are able to grow at 42^oC and are resistant to cephalothin, characteristics useful for their initial isolation. The number of colonies doesn't increase at this temperature, but the colonies appear sooner and are larger and the growth most faecal flora is inhibited (Ellen *et al*., 1994). The evaluation of susceptibility to nalidixic acid has also been considered as an important test to distinguish between traditionally sensitive species, such as *C. jejuni* and *C. coli*, and the resistant thermotolerant species *C. lari* (Salama *et al.*, 1990). The *C. jejuni* is the only species that hydrolyses sodium hippurate (Quinn *et al.*, 2002).

Almost all pathogenic *Campylobacter* species are Oxidase and catalase positive and unable to grow in 3.5% NaCl. Additionally, susceptibility to nalidixic acid and cephalothin, are important differential characteristics among species (Table 1). Susceptibility is determined by inoculating a 5% sheep blood agar plates or Mueller-Hinton agar plate supplemented with 5% horse blood, with a 0.5 McFarland turbidity suspension of the organism as for agar disk diffusion susceptibility testing placing 30μg of Nalidixic acid and Cephalothin antibiotic disks on the agar surface and incubating microaerobically at 37^{o}C

2.5.3. Immunodiagnosis

Even though imunodiagnosis is not used for the diagnosis of intestinal disease produced by campylobacters, antibody responses measured by enzyme linked immunosorbent assay (ELISA), have been applied for epidemiological purpose (Hirsh and Zee 1999). The ELISA has been used to test vaginal mucus and reported to be more sensitive and able to detect a wider range of antibody responses. The vaginal mucus agglutination test (VMAT) for *C. fetus* subsp. *venerealis* is accurate if carried out 2-7 months post infection (Quinn *et al.*, 2002), and also valuable, but because of variability in individual responses, at least 10% of the herd or at least 10 cows should be sampled (Lofstedt, 1998). As *C. jejuni* is the normal inhabitant of the intestine of normal animals, its isolation from feces may not necessarily be significant. A four-fold increase in agglutinating antibody titer to the bacterium would suggest involvement of the organism in the diarrhea. Assays using polymerase chain reaction (PCR) have been developed to amplify DNA from feces (Hirsh and Zee, 1999).

2.2.4. Public Health Significance of *Campylobacter* species

Campylobacteriosis is a collective description for infectious diseases caused by members of the bacterial genus Campylobacter (Nachamkin and Blaser, 2000). The *Campylobacter* species is recognized worldwide as an important food borne pathogen. Thermophilic *Campylobacter* species have received considerable attention in recent years as a major cause of bacterial enteritis in man (Healing *et al.*, 1992). Campylobacter is recognized as one of the principal

causes of human acute gastro-enteritis worldwide (Allos, 2001). The only form of campylobacteriosis of major public health importance is campylobacter enteritis due to *C. jejuni* and *C. coli*. *Campylobacter lari* has also been implicated in two fatal cases of bacteraemia (Nachamkin, *et al*., 1984) and diarrhea (Tauxe *et al.,* 1985); in sporadic cases with gastrointestinal symptoms and in a water-borne outbreak (Broczyk *et al.,* 1987).Human gastroenteritis caused by these organisms is frequently associated with consumption of red meat and poultry meat (Beuchat, 1985). The rate of *Campylobacter* infections worldwide has been increasing, with the number of cases often exceeding those of salmonellosis and shigellosis (Altekruse *et al*., 1999). *Campylobacter* is one of the most frequently isolated bacteria from stools of infants with diarrhea in developing countries a result of contaminated food or water.

There is a significant increase in incidence of acquired campylobacter infection both in industrialized and developing countries (Acha and Szyfres, 2001). In countries where records are kept, campylobacters are now reported to be the leading cause of bacterial diarrhea in man and isolation and incidence rates in some developing countries have increased since their initial reports (Coker and Adefeso, 1994), with most being isolated from <2-year-old children with diarrhea (Albert *et al*., 1999). In some reports the isolation rates for symptomatic and asymptomatic children were not statistically significant. *Campylobacter* isolation rates in developing countries range from 5 to 20% (Oberhelman and Taylor 2000). Value as high as 14.9% in controls have been observed (Megraud *et al.*, 1990). However, campylobacter is not frequently recovered from asymptomatic persons in developed countries, as observed in the Netherlands, where a 0.5% isolation rate has been reported (de Wit *et al.*, 2001).The infective dose of *campylobacter* ranges from $5x10^2$ -10^4 cells, depending on the strain, environmental stress and susceptibility of the host (Wallace *et al*., 1998).

Campylobacteriosis affect all age groups, however, infections are recognized with increasing frequency in infants, children and in aged individuals suffering from deliberating disorder such as HIV/AIDS and in homosexual men, where, the infection is associated with prostitis (Davis *et al.*, 1990). Campylobacter associated diarrhea and bacteraemia occur in HIV/AIDS patients' worldwide (Tee *et al.,* 1995). For instance, *C fetus* subsp. *fetus* most often causes serious systemic infection in immunocompromised hosts (Davis *et al.*, 1990). The disease does not

appear to be important in adults in developing countries. Acquisition of the pathogen because of poor sanitation and contact with animals early in life may explain the isolation from healthy children. In contrast, infection occurs in adults and children in developed countries. Poor hygiene and sanitation and the close proximity to animals in developing countries all contribute to easy and frequent acquisition of any enteric pathogen, including campylobacter. Although infections in infants appear to decline with age, a comprehensive community-based cohort study in Egypt has shown that infection could be pathogenic regardless of the age of the child, underscoring the need for strengthening prevention and control strategies for campylobacteriosis (Reo *et al.*, 2001).Human campylobacteriosis is often acquired early in life in developing countries and thus disease is commonest in infants, and there are a relatively high proportion of asymptomatic carriers. In developed countries, infection generally occurs rather in the later age. The highest incidence of disease occurs in children and young adults and there are few asymptomatic carriers (Quinn *et al.,* 2002). The disease is also an important cause of "travelers' diarrhea". Generally it is recognized when accompanied by predisposing debilitating factors such as pregnancy, premature birth chronic alcoholism, and neoplasia and cardio vascular disease (Ellen *et al.*, 1994). Fever malaise, headache and sometimes aching of limbs and colic abdominal pain with nausea but rarely vomiting precedes the onset of diarrhea. The organisms are regularly found in small intestine. There is good evidence that humans contract infection from food, particularly chickens and milk borne outbreaks have been recorded (Acha and Szyfres, 2001).

Sources of infection and mode of transmission of disease in man

The presence of campylobacters in the migratory birds is the indication of the large distances that campylobacters can be transferred (Humphrey and Muscat, 1995) and they are known reservoirs and responsible for shedding of the bacteria to cause contamination of the environment. *Campylobacter* species is widespread in the environment, where they are a sign of recent contamination with animal and avian feces, agricultural run off and sewage effluent. Intestinal carriage of *Campylobacter* species is ubiquitous in livestock, domestic animals, wild birds and poultry and depends on the environmental factors (Humphrey and Muscat, 1995). C*ampylobacter jejuni* commonly is found as a commensal in the gastrointestinal tract of wild

and domestic ruminants, swine, dogs, cats, fowl, and rodents, and these reservoirs are the ultimate sources for most human infections.

Campylobacter jejuni and *C. coli* have been isolated from chicken, goat, sheep and pigs in developing countries. Strains isolated from chicken and human were phenotypically and genotypically correlated, confirming that chickens are important source of human campylobacteriosis in developing countries. Disease caused by *C. jejuni*, now the leading cause of bacterial food poisoning, most often spread by contact with raw or undercooked poultry and a single drop of juice from a contaminated chicken is enough to make someone sick with campylobacteriosis. The major source of humans being infected by *Campylobacter* species has been reported to be poultry meat contaminated with the intestinal contents of chickens (Nadeau *et al.,* 2002). In recent years, researches have also been focused on farm animals in order to develop control strategies against campylobacter infection (Fitzgerald *et al.* 2001). However, most of the previous studies have investigated *C. jejuni* and *C. coli* in diarrheic animals such as cattle and sheep (Adesiyun *et al.,* 2001; Acha *et al.*, 2004). *Campylobacter coli* have been isolated from the intestinal content of 99% of pigs at slaughter (Radostits *et al*, 1994). Six percent of fresh pork and liver has been found contained campylobacter with *C. coli* being more abundant than *C. jejuni* (Jay, 2000).

A number of transmission vehicles, including food products have been implicated in the transmission of *Campylobacter* species to humans (Jorgensen *et al*., 2002). The most significant risk factors identified include the consumption and/or handling of raw or undercooked poultry or other meats, raw milk and surface waters. Cross contamination of ready to eat foods during food preparation as well as direct contact with animals has been identified (Adak *et al*., 1995). Food animals may act as asymptomatic intestinal carriers of campylobacter and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (Whyte *et al*., 2003). Previous studies recorded infection rates in live broilers ranging from 0 to 100% (Moore *et al*., 2003) with prevalence up to 60% in cattle (Neilson *et al*., 1997). Campylobacter prevalence of up to 100% has also been reported on dressed poultry carcasses (Dominguez *et al*., 2002) with significantly lower prevalence of the organism generally reported on beef carcasses (Madden *et al.,* 2001). Other foods from which Campylobacter has been

recovered include raw milk and milk products (Lacerc *et al*., 2002).It is now accepted that campylobacteriosis is predominantly acquired through the consumption of contaminated foods (Anonymous, 1995). *Campylobacter jejuni* is a frequent cause of diarrhea/dysentery in children, which is often related to pet keeping and chicken meat consumption as well as untreated drinking water (Ali *et al*., 2003).

Human beings in developed societies acquire *C. jejuni* from asymptomatic or symptomatic companion animals (dogs and cats) and from foods such as raw milk, water, poultry products and unhygienic food preparation (Reo *et al.*, 2001; Alterknus *et al*., 1998). Contaminated, under cooked poultry has been reported to be responsible for more than 50% of cases investigated in humans (Shani, 1998).

Occupational exposure may cause infection and disease in workers in animal health facilities, animal shelters, and poultry processing plants, animal agriculture, and rendering plants. Family members of the aforementioned also are at increased risk of infection (Prescott and Munroe 1982). It has also been reported that there is a strong association between campylobacter infection and residence on a farm. Risks of acquiring campylobacteriosis in developing countries include the presence of an animal in cooking area, uncovered garbage in cooking areas and lack of piped waters (Reo *et al.*, 2001). Poor hygienic and sanitation and close proximity to animals in developing countries contributes to the easy and frequent acquisition of any enteric pathogens including campylobacter (Oberhelman and Taylor, 2000).

The source of human campylobacteriosis is almost always food (Acha and Szyfres, 2001) and most sporadic cases probably arise from consumption of improperly handled poultry or contact with infected pets (Hirsh and Zee, 1999). Several research works so far conducted overseas, on various foods of animal origin indicated that different foods of animal origin particularly raw red meat are contaminated with enteropathopgenic *Campylobacter* species as shown in Table 3. These authors have reported thermophilic *Campylobacter* species at varying rate of recovery from different raw red meat samples collected from various sources.

Table 3: Prevalence of *Campylobacter* species in raw red meat overseas

Country	Product	No. Samples tested	No. (%)*Campylobacter*	Reference
Australia	Beef carcasses:	124	1 (0.8)	Vanderlinde *et al.*, 1998
Australia	Sheep carcasses, domestic	140	231 (2.1)	Vanderlinde *et al.*, 1999
	Sheep carcasses, export	330	137(0.9)	
Belgium	Pork carcasses	49	1 (2.0)	Korsak *et al.*, 1998
	Beef carcasses	62	6 (10.0)	
Canada	Pork carcass	463	78 (12.1)	Lammerding *et al.*, 1988
	Beef carcass	598	135 (14.7)	
	Veal carcass	267	115 (34.5)	
Canada	Pork carcass diaphragms	200	47 (23.5)	Mafu *et al.*, 1989
England	Minced meats	135	3 (2.2)	Bolton *et al.*, 1985
England	Beef	127	30 (23.6)	Fricker and Park, 1989
	Pork	158	29 (18.4)	
	Lamb	103	16 (15.5)	
England	Raw sausages	1197	4 (0.3)	Little and de Louvais, 1998
	Raw burgers	1015	10 (1.0)	
England and Wales	Minced beef	2015	21 (1.0)	Turnbull and Rose, 1982
	Minced pork	342	1 (0.3)	
Ireland	Minced beef	20	4 (20.0)	Cloak *et al.*, 2001
	Pork	20	0	
Ireland	Raw beef	221	7 (3.2)	Whyte *et al.*, 2004
	Raw pork	197	10 (5.1)	
	Raw lamb	262	31 (11.8)	
Italy	Pork	27	1 (3.7)	Zanetti *et al.*, 1996
Italy	Beef	151	2 (1.3)	Pezzotti *et al.*, 2003
	Pork	175	18 (10.3)	
Japan	Beef	94	2 (2.1)	Tokumaru *et al.*, 1990
	Pork	52	0	
Japan	Deer meat	30	0	Kanai *et al.*, 1997
Netherlands	Pig carcasses after evisceration	210	19 (9.1)	Oosterom *et al.*, 1985

2.2.5. Status of Campylobacteriosis in Ethiopia

There are very few studies conducted on human campylobacteriosis in Ethiopia. A study conducted at Tikur-Anbessa and Ethio-Swedish children's hospital indicated that *Campylobacter* species are an important cause of diarrhea both in adults and children in which *C. jejuni* accounted for 82.4% of the isolates (Asrat *et al.*, 1999). A cross sectional study conducted on urban and rural farm animals indicated that thermophilic *Campylobacter* species are significantly identified with prevalence of 39.6% in the fecal sample (Kassa *et al*., 2005). The antimicrobial test conducted on these isolates indicates that some of the bacteria showed a range of resistance to certain antimicrobial agents (Asrat *et al.*, 1999; Kassa *et al*., 2007). However no citable work has been done so far regarding the prevalence of thermophilic *Campylobacter* species in foods of animal origin, particularly in raw meat which is most commonly discriminated as the major source of human infection and the role of animal products in the epidemiology of human campylobacteriosis. Therefore this study was undertaken to determine the rate of isolation these species from raw meat of different animal species and their antimicrobial sensitivity pattern.

2.3. Treatment and Prevention.

Campylobacter enteritis is a self-limiting disease, and antimicrobial therapy is not generally recommended. However, antimicrobial agents are recommended for extraintestinal infections such as Guillain-Barre`syndrome (GBS) that is serious consequence of diarrhoeal disease characterized by polyneuritis of the peripheral nerves, which may lead to either a transient or long-term paralysis (Blaser *et al.*, 1983), for treating immunocompromised persons. Antimicrobial therapy is not required except in severe cases long-lasting campylobacter enteritis and systemic infections (Aarestrup and Engberg, 2001). Erythromycin and ciprofloxacin are normally considered the drug of choice for campylobacter enteritis (Engberg *et al*., 2001). Intravenous aminoglycosides are recommended for the treatment of serious campylobacter bacteraemia and other systemic infections (Tajada *et al.*, 1996). The rate of resistance to these drugs is increasing in both developed and developing countries, although the incidence is higher in developing countries. Use of these drugs for infections other than gastroenteritis and self-

medication are often the causes of resistance in developing countries; in developed countries, resistance is due to their use in food animals and travel to developing countries (Steinbrückner *et al*., 2000; Feierl *et al*., 2001). There is growing scientific evidence that the use of antibiotics in food animals particularly in developed countries leads to the development of resistant pathogenic bacteria that can reach humans though food chain (Van Looveren *et al*., 2001; Avrain *et al.,* 2003). The available information on antimicrobial susceptibilities of thermophilic campylobacters has been found to differ in different countries (Van Looveren *et al*., 2001; Avrain *et al.,* 2003; Ishihara *et al.,* 2004), but resistance has been reported to be increasing particularly to macrolides and fluoroquinolones (Aarestrup and Engberg, 2001).The increase in erythromycin resistance in developed countries is often low and stable at approximately 1% to 2%; this is not true for developing countries (Steinbrückner *et al*., 2000; Feierl *et al*., 2001). For example, in 1984, 82% of campylobacter strains from Lagos, Nigeria, were sensitive to erythromycin; 10 years later, only 20.8% were sensitive (Coker and Adefeso, 1994). In addition, resistance to another macrolide, azithromycin, was found in 7% to 15% of campylobacter isolates in 1994 and 1995 in Thailand (Steinbrückner, *et al*., 2000). The increasing rate of resistance to the fluoroquinolones, ciprofloxacin limits its clinical usefulness. In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from zero before 1991 to 84% in 1995 (Hoge *et al*., 1998). Recent data have shown a marked increase in resistance to quinolones in developed countries (Steinbrückner *et al*., Feierl *et al*., 2001).

In Ethiopia, there are few reports on the antimicrobial susceptibility pattern of campylobacters isolated from humans. Resistance was found against ampicillin in 60% of the isolates and against trimethoprim-sulphamethoxazole in 58.8%.resistance to both ampicillin and trimethoprim-sulphamethoxazole was found in 38% of the strains (Asrat *et al*., 1999). Resistance of 20% to ampicillin and 37.5% to trimethoprim-sulphamethoxazole was reported in strains isolated from food animals (Kassa *et al*., 2007).

3. MATERIALS AND METHODS

3.1. Study Area

The study was undertaken in Addis Ababa, the capital city of Federal Republic of Ethiopia and Debre Zeit city. Addis Ababa lies in the central high lands of country at an altitude of 2500 meter above sea level and with an estimated human population of about 3 million. The average annual temperature and rainfall are 21^{o} C and 1800mm, respectively. Addis Ababa has a relative humidity varying between 70% and 80% during the rainy season and 40% to 50% during the dry season (NMSA, 2003).

Debre Zeit is located 45 km south east of Addis Ababa. The area is located at 9^{O} N latitude and 40^{o}E longitude at an altitude of 1850 meter above sea level. It has an annual rainfall of 866 mm which occurs from June to September and the dry season extends from October to February. The average temperature is 14^{o}C to26^{o}C with a relative humidity of 61.3 % (CSA, 2001).

3.2. Study Design

A study was conducted on selected abattoirs (n=3), butchers (n=35) and supermarkets (12) where raw meats of different food animal (beef, goat meat, sheep meat, pork and chicken meat) are processed or sold to customers.

3.3 Sample Collection, Handling and Transportation

All beef, goat meat, sheep meat, pork and chicken meat samples purchased from super markets were either deep frozen or refrigerated and wrapped by polyethylene plastic bags. The samples were procured once a week from abattoirs, retail super markets and butchers. The samples were labeled for sample type (beef, goat meat, sheep meat, chicken meat and pork), sample number, code of sample source, date of sampling and storage state (fresh, refrigerated or frozen). The samples from super markets were collected in plastic materials with which purchased meat is distributed to customers or sterile plastic bags for samples collected from abattoir and butchers. A total of 540 raw meat samples were procured from different sources over a period of 6months

from November 2006 to April 2007 as shown in Table 4. Samples were then transported on cold chain using icebox and ice packs, to the Microbiology laboratory of the FVM, AAU, Debre Zeit, for Microbiological analysis. The samples were kept under refrigeration temperature of $+4^{o}C$ until being processed with in 24hours of collection. Samples were analyzed microbiologically according to the International Organization for Standardization (ISO/CD 10272-1 and 10272-2, 2002).

Table 4. Showing the number and type of raw meats collected from abattoir, butcher and supermarkets.

Types raw meat examined	Source		
	Abattoirs No. (%)	Butchers No. (%)	Supermarkets No. (%)
Beef (n=227)	138(60.7)	69 (30.4)	20 (8.8)
Sheep meat (n=114)	93(81.6)	10 (8.8)	11(9.6)
Goat meat (n=92)	67(72.8)	11(12.0)	14 (15.2)
Pork (n=47)	30(63.8)	-	17 (36.2)
Chicken carcass (n=60)	30(50.0)	-	30 (50.0)
Total (n=540)	**358 (66.3)**	**90(16.7)**	**92 (17.0)**

3.4 Sample Preparation and Enrichment

The samples were thawed at room temperature for 4 to 6 hours before processing and weighed to10g (analytical unit). None chopped food samples were aseptically removed and chopped using sterile pair of forceps and scissors and placed in 90 ml of Preston broth (Oxoid, England) supplemented with Preston Campylobacter Selective Supplement (polymyxin B 2,500IU, rifampicin 5.0 mg, trimethoprim 5.0mg and cyclohexamide 50.0mg) (SR0117E, Oxoid, Hampshire England) and 5% lysed horse blood, in sterile plastic bags and homogenized for 1 minutes in a laboratory stomacher (Seward, England). The homogenized material was then transferred in to a sterile container and additional broth was added to minimize headspace with in the bottle. Following processing all the samples were incubated at $42^{o}C$ for 48h.

3.5 Plating and Identification

All broth cultures were subsequently subcultured on to Preston campylobacter selective agar plates supplemented with Preston Campylobacter Selective Supplement (polymyxin B 2,500IU, rifampicin 5.0 mg, trimethoprim 5.0mg and cyclohexamide 50.0mg) (SR0117E, Oxoid, Hampshire England) and 5% lysed horse blood. The plates were then incubated at 42^{o}C for 48hours in a microaerobic atmosphere which was achieved in by using CampyGen^{TM} gas generating kits (5%O_2 and 10% CO_2) (Oxoid, England). The isolation media and conditions used in this study were adopted as described by Wang *et al.*, (2002). The growth of thermophilic campylobacter was detected by their characteristic appearance on culture media i.e. the presence of mucoid grayish white colonies resembling droplets of water sprayed on the medium. Preliminary identification was performed based on the characteristic Gram-staining reactions (Figure 1), motility and positive tests for oxidase, catalase, H_2S production and Nitrate reduction. The type strains *C. jejuni* (NCTC 11351), *C. coli* (LMG 6440) and *C. lari* (NCTC 11352) were included as positive controls.

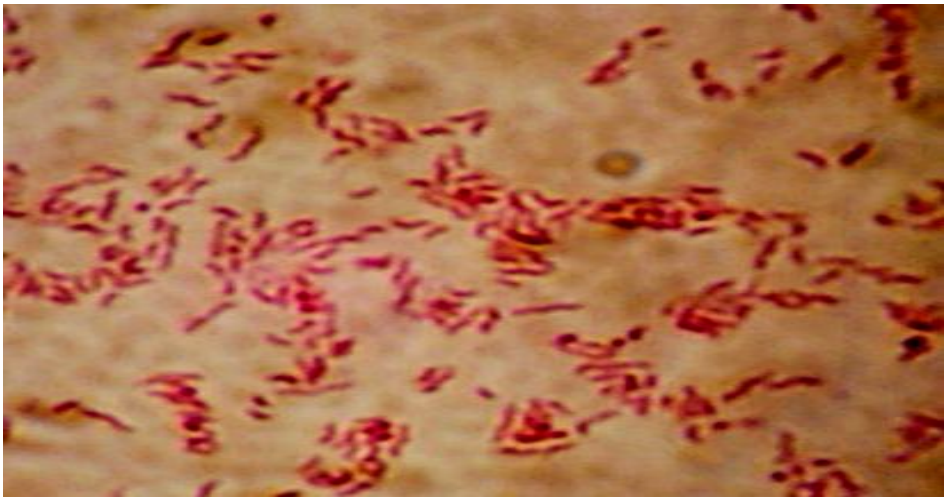

Figure 1: A characteristic spiral, curved and flying sea gull morphology of gram negative *Campylobacter* species (1000X).

3.6. Species differentiation

All isolated strains identified as thermophilic campylobacters were tested for hippurate hydrolysis and susceptibility to nalidixic acid and cephalothin. These parameters formed the basis for the identification of *C. jejuni, C. coli and C. lari.* For hippurate hydrolysis test, two or three colonies were taken from 48hours Colombia blood agar plate culture and emulsified in 1% sodium hippurate broth and incubated with loosen caps at 42^{o}C for 48h in a microaerobic condition. Following incubation the broth culture was centrifuged at 2500rpm for 2 minutes and 0.8ml of the supernatant was transferred to a sterile clean tube. The hydrolysis of hippurate was detected by addition of 0.2ml acidic ferric chloride and gentle shaking. A dense brown persistent precipitate which indicates the presence of benzoic acid that results from hippurate hydrolysis was considered as a positive test

3.7 Antimicrobial susceptibility testing.

All campylobacter isolates were tested by agar disc diffusion method against nine antimicrobial agents. Amoxycillin (20 µg), ampicillin (10µg), chloramphenicol (30µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), norfloxacin (10 µg), streptomycin (10 µg) and tetracycline (30 µg) (Oxoid, Hampshire, England) were test for each isolates recovered from the raw meat samples analyzed. Campylobacter strains were first grown on Colombia blood agar plates were *transferred* to normal saline and its turbidity was adjusted with a 0.5 McFarland turbidity standard then spread onto Mueller-Hinton (MH) agar(Oxoid, England) supplemented with 5% lysed horse blood. The discs containing antimicrobial compounds were laid on to the plates and pressed gently to ensure adherence to the plate surface. The plates were incubated at 37^{o}C for 24hours in a microaerobic conditions. The diameter of the growth inhibition zone was measured according to the (NCCLS, 2000).

3.8 Data management and analysis

Microsoft Excel and Intercooled Stata7 were employed for data entry, computation of descriptive statistics. Difference in the isolation rate of the species between different raw meat types was determined by Chi-square (X^2) test. Descriptive statistics such as percentages and proportions were used to describe/present the nature and the characteristics of the data.

4 RESULTS

4.1: Prevalence of thermophilic Campylobacter species

The overall prevalence of thermophilic *Campylobacter* species in raw meat samples (beef, goat meat, sheep meat, chicken meat and pork) procured from different outlets (abattoirs, butchers and supermarkets) was 9.3% (Table 5). The number and percentage of the strains from each food samples were 13/60(21.7%) chicken meat, 12/114(10.5%) sheep meat, 4/47(8.5%) pork, 7/92 (7.6%) goat meat and 14/227(6.2%) beef. In this study chicken meat was found to be the most frequently contaminated compared to the other raw meat types examined, with the contamination rate of 21.7% ($p<0.05$). There was no significant differences in the prevalence rate ($p>0.05$) were observed among other raw meat types analysed. The distribution of thermophilic *Campylobacter* species was found to be relatively higher in samples collected from abattoirs 38/328 (10.6%) where as no statistical difference was observed between samples obtained from butchers 6/90(6.7%) and supermarkets 6/92(7.6%) ($p>0.05$) (Table 5).

Table 5: The prevalence of thermophilic *Campylobacter* species isolated from raw meat obtained from different sources

Meat type. tested	No. of positive samples / No samples examined (%)			
	Abattoir	Butcher	Supermarket	Total
Beef	9/138 (6.5)	4/69(5.8)	1/20(5.0)	14/227(6.2)
Sheep meat	11/93(11.8)	1/10(10.0)	0/11(0)	12/114 (10.5)
Goat meat	6/67(9.0)	1/11(9.0)	0/14 (0)	7/92(7.6)
Pork	3/30 (10.0)	-	1/17(5.9)	4/47(8.5)
Chicken meat	8/30 (26.7)	-	5/30(16.7)	13/60(21.7)
Total	38/328(10.6)	6/90(6.7)	7/92(7.6)	50/540(9.3)

The number of *C. jejuni, C. coli and C. lari* per raw meat type were beef 12/2/0, sheep meat10/2/0, goat meat 5/2/0, pork 1/2/1 and Chicken meat 11/1/1, respectively (Table 6). The most prevalent species recovered from the samples was *Campylobacter jejuni* (78.0%),

followed by *Campylobacter coli* (18.0%) and the remaining 4.0% of the isolates were identified as *Campylobacter lari.* In the current study the prevalence of *C. jejuni* was found to be highest in beef (85.7%) followed by 84.6% in chicken, 83.3% in sheep meat, 71.4% in goat meat and 25.0% in pork (Table 6). The difference in the distribution of *C. jejuni* among various raw meat types was statistically significant ($p<0.05$).However, the distribution difference between the *C. coli* and *C. lari* was not significant ($p>0.05$). *Campylobacter lari* was recovered less frequently only from chicken meat and pork with the recovery rate of 1.7% and 2.1% from chicken meat and pork, respectively (Table 6).

Table 6: Distribution of thermophilic *Campylobacter* species isolated from different meat sources

Number and type of positive sample	No. (%) *Campylobacter* species		
	C. jejuni	***C. coli***	***C. lari***
Beef(n=14)	12(85.7)	2(14.3)	-
Sheep meat(n=12)	10(83.3)	2(16.7)	-
Goat meat(n=7)	5(71.4)	2(28.6)	-
Pork(n=4)	1(25.0)	2(50.0)	1(25.0)
Chicken carcass(n=13)	11(84.6)	1(7.6)	1(7.6)
Total(50)	39(78.0)	9(18.0)	2(4.0)

4.2. Antimicrobial susceptibility test result.

The result of antimicrobial susceptibility testing for *C. jejuni, C. coli and C. lari* isolated from raw meat sample against nine selected antimicrobial agents is shown in Table 7.The current study on antimicrobial sensitivity test of thermophilic *Campylobacter* species recovered from different raw meat types revealed a varying degree of susceptibility to antimicrobial agents tested. The overall susceptibility of 98% to erythromycin, 92% to chloramphenicol and 88% to tetracycline has been revealed by the isolates. Susceptibility as low as 24% to 32%, was shown to streptomycin and gentamicin, respectively (Table 7). Susceptibility difference to

antimicrobials tested was also observed among the three species. All of the *C. lari* isolates were found to be completely susceptible to ampicillin, gentamicin, chloramphenicol, streptomycin and tetracycline. The difference in susceptibility among the three species, to ampicillin, amoxycillin and streptomycin was statistically different ($p < 0.05$) (Table 7)

Table 7. Antibiotic susceptibility/resistance profiles of *C. jejuni, C. coli* and *C. lari* raw meat isolates against various antibiotic agents

Antimicrobials tested	Susceptibility Pattern of *Campylobacter* species												P value
	C. jejuni			*C. coli*			*C. lari*			Overall susceptibility pattern			
	S*	I*	R*	S*	I*	R*	S*	I*	R*	S*	I*	R*	
AML	33(84.6)	5(12.8)	1(2.6)	4(44.4)	3(33.3)	2(22.2)	1(50.0)	1(50.0)	-	38(76.0)	9(18.0)	3(6.0)	0.016*
AMP	29(74.4)	5(12.8)	5(12.8)	9(100)	-	-	2(100)	-	-	40(80.0)	5(10.0)	5(10.0)	0.047*
C	37(94.9)	2(5.1)	-	7(77.8)	-	2(22.2)	2(100)	-	-	46(92.0)	2(4.0)	2(4.0)	0.696
CN	14(35.9)	23(59.1)	2(5.1)	-	4(44.4)	5(55.6)	2(100)	-	-	16(32.0)	27(54.0)	7(14.0)	0.192
E	38(97.4)	-	1(2.6)	9(100)	-	-	2(100)	-	-	49(98.0)	-	1(2.0)	0.57
K	33(84.6)	2(5.1)	4(10.3)	6(66.7)	2(22.2)	1(11.1)	1(50.0)	-	1(50.0)	40(80.0)	4(8.0)	6(12.0)	0.185
NOR	31(79.5)	8(20.5)	-	9(100)	-	-	1(50.0)	1(50.0)	-	41(82.0)	9(18.0)	-	0.317
S	6(15.4)	25(64.1)	8(20.5)	4(44.4)	3(33.3)	2(22.2)	2(100)	-	-	12(24.0)	28(56.0)	10(20.0)	0.003*
Te	36(92.3)	1(2.6)	2(5.1)	-	6(66.7)	3(33.3)	2(100)	-	-	38(76.0)	7(14.0)	5(10.0)	0.961

Note: - AML=amoxycillin, AMP= ampicillin, C= chloramphenicol, CN= gentamicin, E= erythromycin, K= kanamycin, NOR = norfloxacin, S= streptomycin, Te = tetracycline S* = susceptible, I*= intermediate and R*= resistant

Of the 50 thermophilic *Campylobacter* species isolated in the present study 10(20.0%) were found to be resistant to at least two drugs tested. (Table 8).Out of the isolates developed multiple drug resistant 4(40%) were those recovered from beef sample.

Table 8 Showing multi-drug resistance in certain thermophilic *Campylobacter* species isolates.

Multi-resistant Species	No.(%) of isolate multi-drug resistant	Types of drugs resisted	Source
C. jejuni	1 (2.0)	S, AMP, CN, K	Beef
C. jejuni	2 (4.0)	S, AMP, Te, K	Sheep meat and Beef
C. coli	1 (2.0)	S, CN, K,	Sheep meat
C. jejuni	1 (2.0)	S, Te, AMP	Beef
C. coli	2 (4.0)	AML, CN	Chicken
C. coli	3 (6.0)	K, CN	Sheep meat and Beef
Total	10(20.0)		

Note: - AML=amoxycillin, AMP= ampicillin, CN= gentamicin, K= kanamycin, S= streptomycin and Te = tetracycline

The antibiotic susceptibility profile showed a significant difference ($p<0.05$) in the susceptibility pattern to various antimicrobial agents among isolates recovered from different raw meat types. Isolates recovered from goat meat were found to be 100% susceptible to all antimicrobial agents tested except to streptomycin and gentamicin to which they showed 0% and 57.1% susceptibility, respectively (Table 9). Similarly, of the *Campylobacter* species isolated from sheep meat only 8.3% and 16.7% were found to be susceptible to streptomycin and gentamicin, respectively. While the remaining isolate showed a susceptibility ranging from 57.1% to 100%. *Campylobacter* species recovered from beef revealed a susceptibility ranging from 71.4% to 100% to various antimicrobial agents tested. Thermophilic *Campylobacter* species recovered from chicken meat were found to be 100% susceptible ampicillin and tetracycline. Only 25% of the isolates recovered from pork were found susceptible to amoxycillin while 100% of the isolates were completely susceptible to chloramphenicol and erythromycin (Table 9)

Table 9 Percentage susceptibility thermophilic *Campylobacter* species isolated from different raw meat types.

Antimicrobial tested	% respective isolates susceptible					Total No.(%) isolate susceptible	P value
	Beef (n=14)	Sheep meat (n=12)	Goat meat (n=7)	Pork (n=4)	Chicken meat (n=13)		
AML	10(71.4)	8(66.7)	7(100)	1(25.0)	12(92.3)	38(76.0)	0.009*
AMP	8(57.1)	9(75.0)	7(100)	3(75.0)	13(100)	40(80.0)	0.02*
C	13(92.8)	12(100)	7(100)	4(100)	10(76.9)	46(92.0)	0.77
E	4(28.6)	2(16.7)	0(0)	1(25.0)	9(69.2)	16(32.0)	0.56
CN	14(100)	12(100)	7(100)	4(100)	12(92.3)	49(98.0)	0.01*
K	11(78.6)	9(75.0)	7(100)	1(75.0)	12(92.3)	40(80.0)	0.008*
NOR	13(92.8)	8(57.1)	7(100)	3 (75.0)	10 (76.9)	41(82.0)	0.34
STR	1(7.1)	1(8.3)	4(57.1)	2(50.0)	4(30.8)	12(24.0)	0.03*
Te	9(64.3)	10(83.3)	7(100)	2(50.0)	10(76.9)	38(76.0)	0.31

Note: - AML=amoxycillin, AMP= ampicillin, C= chloramphenicol, CN= gentamicin, E= erythromycin, K= kanamycin, NOR = norfloxacin, S= streptomycin, Te = tetracycline and n= number of isolate tested

5 DISCUSSIONS

During the last decade, numerous studies have indicated Campylobacter infections as the primary bacterial infections of humans, and foods of animal origin were incriminated as the main sources (Friedman *et al*., 2000; Oberhelman and Taylor, 2000). Raw meats and its products are commonly consumed in traditional Ethiopian diets, but campylobacteriosis is rarely studied compared to other countries. In the present study thermophilic *Campylobacter* species were isolated from chicken meat, beef, sheep meat, goat meat and pork at different recovery rates. The result of the present study indicated that chicken meat was the most frequently contaminated compared to the other raw meat types examined, with the contamination rate of 21.7% ($p<0.05$), which is relatively lower than the 68.1% prevalence reported by Kassa *et al.,* (2007). Wide variation (0–90%) in the prevalence of campylobacter in fresh poultry meat had been reported in different countries (Jacobs-Reitsma, 2000; Cloak *et al*., 2001; Jorgensen *et al.*, 2002; Whyte *et al*., 2003). Slaughterhouse studies have shown that the main source of contamination of *C. jejuni* in chicken meat is their intestinal contents (Newell *et al.*, 2001; Berrang *et al.,* 2004). In the present study speciation of the isolates confirmed that *C. jejuni* was the most prevalent species identified from raw chicken meat samples, with an isolation rate of 84.6%. Other studies (Neilson *et al*., 1997; Jorgensen *et al*., 2002; Whyte *et al*., 2003) reported prevalence of campylobacter in raw chicken meat with a recovery rate of *C. jejuni* ranging from 8 to 98%.

The prevalence of thermophilic *Campylobacter* species in raw sheep meat in the present study was 10.5% indicating that sheep meat is the second most frequently contaminated food of animal origin next to chicken meat. This result is higher when compared with the prevalence rate of(2.1%) in Australia (Vanderlinde *et al.,* 1999), 5.1% in Pakistan (Hussain, *et al.* 2007) and 8.1% in Norway (Rosef *et al.,* 1983) but was comparable with the 11.8% prevalence from raw lamb reported in Ireland (Whyte *et al*., 2004).The result of current study was relatively smaller than 15% prevalence in lamb reported in Portugal(Cabrita *et al.,* 1992),15.5% prevalence in sheep carcass recorded in England (Fricker and Park, 1989) and 20% in Brazil(Aquino *et al.*, 2002).

The isolation rate was found to be higher in samples collected from abattoirs (11.8 %) and purchased from butchers (10.0%) than those from supermarkets (0%). The

predominant species of *Campylobacter* identified from sheep meat in this study was *C. jejuni* (76.9%) followed by *C. coli* (23.1%).This result is higher than the 59.3% *C. jejuni* recovery rate from sheep feces (Kassa *et al.* 2007) and the 40.5% isolation rate from the intestines of sheep at slaughter in United Kingdom (Stanley *et al*., 1998).

The isolation frequency of campylobacter in pork in the present study was 8.5%. The overall prevalence of thermophilic *Campylobacter* species in the present study was higher than a 0% prevalence recorded in Ireland (Cloak *et al.,* 2001) and Netherlands (Oosterom *et al.,* 1985), 2.0% prevalence in Belgium (Korsak *et al.,* 1998) and 5.1% prevalence in Ireland (Whyte *et al*., 2004). The current recovery rate was lower than the 9.1% prevalence recorded in pig meat after evisceration in Netherlands (Oosterom *et al.,* 1985), 18.4% prevalence from pork in England (Fricker and Park, 1989) and 50% prevalence reported in feces of pigs in Jimma zone of Ethiopia (Kassa *et al*., 2007).Prevalence as high as 79.3% was reported earlier for campylobacter infection amongst piglets in Trinidad in a herd-based study (Adesiyun *et al*. 1992b). In the current study, *Campylobacter coli* was the most dominant species isolated from pork at the rate of 50% and the remaining part accounted for *C. jejuni* and *C. lari,* 25% each. The recovery rate recorded in our study is lower when compared with the (60%) and 100% recovery rate reported by Hoorfar *et al*., (1999) and Kassa *et al*. (2007), respectively. Several workers have reported a higher carriage rate of *C. coli* than *C. jejuni* among healthy pigs, providing evidence that *C. coli* is a normal flora of these animals (Rosef *et al.,* 1983; Cabrita *et al*., 1992; Aquino *et al*., 2002). Although there are reports that as compared to other animal species with the exception of the avian species, pigs to be known to have relatively high prevalence of campylobacter infection (Tuckson *et al*. 1988; Adesiyun *et al*. 1992b) the present study indicates that pork to be the third most contaminated food item next to chicken and sheep meat. The relatively high contamination of pork meat may therefore pose a health hazard to consumers of improperly cooked pork or pork products since *Campylobacter* species, particularly *C. jejuni* and *C. coli* are known causes of gastroenteritis in humans (Nielsen *et al* 1997)

The frequency of isolation of thermophilic *Campylobacter* species in goat meat was 7.6% indicating that it is the fourth most contaminated raw meat of the various raw meat of food animal examined in the present study. In Canada, *Campylobacter jejuni* was

identified in 2.7% of the animals studied (Prescott and Bruin-Mosch 1981) which is lower as compared with the present findings. Turkson *et al.* (1988), in Kenya, also recorded *Campylobacter* species at the recovery rate of 6.3% in goat meat sample. *Campylobacter jejuni* were recovered with the isolation rate of 71.4% while *C. coli* accounts for 28.6% of goat isolates. The result was higher than the (33.3%) prevalence of *C. jejuni* recorded in Ghana Abrahams *et al.* (1990). Similar to other raw meat types, the recovery of rate of thermophilic *Campylobacter* species was found to be higher in samples procured from abattoirs (85.7%) as compared to those collected from butchers and supermarkets (14.3%).

The present study indicates that beef was the least contaminated raw meat giving a recovery rate of 6.2% of thermophilic *Campylobacter* species with *C. jejuni* being the dominant isolate (85.7%) ($p<0.05$) followed by *C. coli* (14.3%). The present result is in close agreement with the 6.5% prevalence recorded in bovine liver in Turkey (Acik and Cetinkaya 2005) but lower than the prevalence of 10.0% in beef in Belgium (Korsak *et al.*, 1998), 10.9% prevalence in beef carcass in Pakistan (Hussain, *et al.* 2007), 14.7% in Beef in Canada (Lammerding *et al.*, 1988), 20% in minced beef in Ireland (Cloak *et al.*, 2001), 23.6% in beef in England (Fricker and Park, 1989), 34.5% in veal carcass in Canada (Lammerding *et al.*, 1988). The current figure was also smaller than the 12.7% prevalence recorded by Kassa *et al*. (2007) in feces of cattle. Similarly, 13.6% prevalence was recorded in fecal sample of healthy beef cattle (Inglis *et al.*, 2004).The isolation of the organism from the feces in a relatively higher level may not be surprising as *Campylobacter* species are the normal flora gastrointestinal tract of normal animal. Other researchers also reported recovery rates lower than the present findings. For instance the prevalence of 0.8% in Australia (Vanderlinde *et al.*, 1998), 1% in England and Wales (Turnbull and Rose, 1982), 2.1% in Japan (Tokumaru *et al.*, 1990) and 3.2% in Ireland (Whyte *et al*., 2004) has been recorded. Similarly, other studies have demonstrated low prevalence in beef, documented as 0.9% (Kwiatek *et al.* 1990) and 1.3% (Pezzotti *et al*. 2003). In the past, research in cattle was limited, but has been increasing as outbreaks of human campylobacteriosis have been traced to foods of cattle origin (Padungton and Kaneene, 2003). *Campylobacter* species was reported at different prevalence rate of in different bovine breeds. For instance, prevalence of 15% in beef calves, 37.7% in dairy herds and 89.4% in beef cattle at slaughter house have been reported (Padungton and Kaneene, 2003). The recovery rate of the organism was found

to be similar in sample types collected from abattoirs (5.8%) than those from butchers (5.8%) and supermarkets (5.0%).

Generally the variation in the prevalence of campylobacter isolation rate from raw meat reported in other studies from the present may be a result of different sampling techniques employed and laboratory methodologies employed and may also be due to the reason that the studies were carried out in different countries at different times.

The antimicrobial sensitivity test of the present study indicates that the overall resistance of 20%, 14% and 12% was attributed to streptomycin, gentamicin and kanamycin, respectively. The frequency of tetracycline and ampicillin resistance among the species was 10%. There was an overall resistance of 20.5% to streptomycin, 12.8% to ampicillin, 10.3% kanamycin; and 5.1% to tetracycline and gentamicin in *C. jejuni* (Table 7). Low and high level of resistance to gentamicin (0–11.9%), streptomycin (0–48%) and tetracycline (0–96%) has been reported among *Campylobacter* species isolated from food animals in different parts of the world (Padungton and Kaneene, 2003). Resistance of 44%, 34%, and 14% to tetracycline, ampicillin, and erythromycin described by Kristi *et al*., (2007) in Estonia is higher than the present study. This high rate of resistance may be associated with indiscriminate use of these antimicrobials as chemotherapeutic agents more frequently in Estonia than our country. Varying degree of susceptibility, 98.0%, 94.1% and 92.2% was observed per the whole isolates of thermophilic *Campylobacter* species to erythromycin, chloramphenicol and tetracycline, respectively. The results in the present study indicated that thermophilic *Campylobacter* species showed a relatively low resistance to norfloxacin, chloramphenicol, erythromycin, norfloxacin, amoxycillin and tetracycline (0–5.9%)(Table 7). Low level resistance to chloramphenicol in our series of *Campylobacter* species were found, in accordance with the result presented by Saenz *et al.,* (2000). Similar result has been documented by Kassa *et al.,* (2007). *Campylobacter jejuni* isolates were 97.4%, 94.9% and 92.3% susceptibility to erythromycin, chloramphenicol and tetracycline, respectively. However, increased resistance of *C. jejuni* to tetracycline and ampicillin was also reported by Kiseon *et al.,* (2007). The 74.4% susceptibility of *C. jejuni* to ampicillin detected in the present study is higher than the 45% and 38.8% susceptibility documented by Saenz *et al.*, (2000) in Spain and Kiseon *et al.,* (2007) in Korea, respectively. Resistance ranging from 10 to 100% was showed by *Campylobacter lari* to chloramphenicol, tetracycline, gentamicin, streptomycin, amoxycillin and kanamycin

(Table 7). The *C. coli* isolates were 100% susceptible to norfloxacin, ampicillin, and erythromycin. The frequency of multi drug resistant strains (20%) in this study was higher than the 14.5% resistance in Ethiopia (Kassa *et al*., 2007) but lower than the 60% multi resistance reported in Belgium (Van Looveren *et al*., 2001).The frequency of susceptibility to antimicrobial agents was found to differ among the isolates recovered from different raw meat types with less susceptibility being revealed by those strains isolated from beef sheep meat and pork (Table 9).This result may explain the frequency of antimicrobial use in the respective species of animal species.

6. CONCLUSION AND RECOMENDATIONS

The investigation of campylobacter in raw meat in Addis Ababa and Debre Zeit showed 9.3% prevalence. Higher isolation rate was observed in samples collected from abattoirs (10.9%) compared to those samples procured from butchers (6.7%) and supermarkets (7.6%).Of raw meat types examined chicken meat was found to be the most frequently contaminated with the recovery rate of 21.7%.

A total of 50 Campylobacter isolates consisting of three species were identified from the samples examined. The most dominant isolate was *Campylobacter jejuni* (78.0%) followed by *C. coli* (18.0%) and *C. lari* (4.0%).

The overall resistance of 20.0%and 14.0% and 12.0% was developed against streptomycin gentamicin and kanamycin, respectively. Of the total isolates10.0% were also found to be resistant to tetracycline and ampicillin.

The existence of thermophilic *Campylobacter* species in raw meat and the isolation of resistant isolates highlight the threat to public health. This is higher particularly to those who have direct contact with food animals and raw animal products (farmers, abattoir workers and animal health personnel), immunocompromised individuals, elderly and to consumers who have habit of eating raw or under cooked meat.

To control and prevent campylobacter infection and contamination in live animal and animal products, it is crucial that risk reduction strategies should be used. Throughout the food chain that is from farm to fork. Based on the findings of the present study the followings are recommended:

- The contamination of raw meat in this study indicates the need in improvement of microbiological quality of raw meat. Good manufacturing practice and hygienic standards in raw meat production should be implemented.

- Standard abattoir and personnel hygiene should be employed to control contamination of meat and meat products with campylobacter.

- Awareness should be created among the public about the risks associated with consumption of raw or under cooked meat.

- Antimicrobial agents must be used wisely both in animals and humans.

- Further studies should be under taken in order to establish exactly the contamination points along raw meat production chain so the corrective measures can be taken.

7. REFERENCES

Aarestrup, F.M., and Engberg, J. (2001): Review: Antimicrobial resistance of thermophilic Campylobacter. Vet. Res.**31**:311-321

Abrahams, C. A., Agbodaze, D., Nakano, T., Afari, E. A., Longmatey, H .E. K. **(**1990): Prevalence and antibiogram of *Campylobacter jejuni* in domestic animals in rural Ghana. *Archives of Environ. Health.* **45**: 59-62.

Acha, P. N. and Szyfres, B. (2001): Zoonoses and communicable diseases common to man and animals. 3rd ed. PAHO Washington D C. **1:** 67-78.

Acha, S. J., Kuhn, I., Jonsson, P., Mbazima, G., Katouli, M., Mollby, R. (2004): Studies on calf diarrhea in Mozambique: prevalence of bacterial pathogens. *Acta. Vet. Scand.* **45**:27–36.

Acik, M.N., and Cetinkaya, B. (2005): The heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from healthy cattle. *L. A. Microbiol.*, **41**: 397–403.

Adak, G.K., Cowden, J.M., Nicholas, S., Evans, H.S. (1995): The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of Campylobacter infection. *Epidemiol. Infect***. 115**: 15–22.

Adesiyun, A. A., Kaminjolo, J. S., A., Loregnard, R., Kitson-Piggot, W. (1992b): Campylobacter infections in calves, piglets, lambs and kids in Trinidad. *Br. Vet. J.* **148:** 547-556.

Adesiyun, A. A., Kaminjolo, J. S., Ngeleka, M., Mutani, A., Borde, G., Harewood, W. and Harper, W. (2001): A longitudinal study on enteropathogenic infections of livestock in Trinidad. *Rev Soc Bras Med Trop* **34**:9–35.

Albert, M. J., Faruque, A.S., Faruque, S.M., Sack, R.B., Mahalanabis, D. (1999): Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. *J. Clin. Microbiol.* **37:**3458-3464.

Ali, A. M., Qureshi, A .H., Rafi, S., Roshan, E., Khan, I., Malik, A.M., Shahid, S.A. (2003): Frequency of *Campylobacter jejuni* in diarrhea/dysentery in children in Rawalpindi and Islamabad. *J. Pak. Med. Assoc*. **53**: 517–520.

Altekruse, S. F., Stern, N. J., Fields, P. I., Swerdlow, D. L. (1999): *Campylobacter jejuni* an emerging foodborne pathogen. *Emerg. Infect. Dis.* **5**:28-35

Alter, T., Gaull, F., Kasimir, S., Gurtler, M., Mielke, H., Linnebur, M., Fehlhaber, K. (2005): Prevalence and transmission routes of *Campylobacter* species. Strains within multiple pig farms. *Vet. Microbiol*. **108**: 251–261.

Alterknus, S. F., Swerdlow, D. L., Stern, N. J. (1998): Microbial food-borne pathogen: *Campylobacter jejuni. Vet. Clin. North Am. Food Anim. Pract.* **14**:31-40.

Amano, K., Shibata, Y. (1992): Structural studies of peptidoglycans in *Campylobacter* species. *Microbiol. Immunol.* **36**:961–967.

Anonymous, (1995): Pathogen Reduction; Hazard Analysis, Critical Control Point (HACCP) Systems; proposed rule. *Fed. Register* **60:** 6774–678.

Aquino, M. H., Pacheco, A. P., Ferreira, M. C., Tibana, A. (2002): Frequency of isolation and identification of thermophilic campylobacters from animals in Brazil. *Vet. J.* **164**:159–16

Asrat, D., Hathaway, A., Ekwall, E. (1999): Antimicrobial sensitivity pattern of Campylobacter strains isolated from patients in Tikur Anbassa and Ethio-Swedish Children's Hospitals, Addis Ababa, Ethiopia. *Ethiop. J. Health Dev*. **13:** 41–44.

Avrain, L., Humbert, F., L'Hopsitalier, R., Sanders, P., Vernozy-Rozand, C., Kempf, I. (2003): Antimicrobial resistance in Campylobacter from broilers: Association with production type and antimicrobial use. *Vet. Microbiol*. **96**:267–276

Berrang, M. E., Smith, D. P., Windham, W. R., Feldner, P. W. (2004): Effect of intestinal content contamination on broiler carcass Campylobacter counts. *J. Food Prot*. **67**: 235–238.

Beuchat, L. R. (1985): Efficacy of media and methods for detecting and enumerating *Campylobacter jejuni* in refrigerated chicken meat. *J. Appl. Microbiol.* **50:**934-939.

Blaser, M. J., Reller, L. B. (1981): Campylobacter enteritis. *New Engl. J. Med*. **305**: 1444-1452.

Blaser, M.J., Duncan, D.J., Warren, G.H., Wang, W.L. (1983): Experimental *Campylobacter jejuni* infection of adult mice. *Infect. Immun*. **39:** 908–916

Boes, J., Nersting, L., Nielsen, E.M., Kranker, S., Enoe, C., Wachmann, H. C., Baggesen, D. L. (2005): Prevalence and diversity of *Campylobacter jejuni* in pig herds on farms with and without cattle or poultry. *J. Food Prot*. **68**:722–727.

Bolton, F.J., Hawkins, H.C., Hutchinson, D.N. (1985): Biotypes and serotypes of thermophilic campylobacters isolated from cattle, sheep and pig offal and other red meats. *J. Hyg.*, Cambridge, **95**:1-6.

Broczyk, A., Thompson, S., Smith, D., Lior, H. (1987): Water-borne outbreak of *Campylobacter laridis*-associated gastroenteritis, Lancet **1**: 164–165.

Broman, T., Palmgren, H., Bergström, S., Sellin, M., Waldenström, J., Danielsson-Tham, M. L., Olsen, B. (2002): *Campylobacter jejuni* in black-headed gulls (Larus ridibundus): Prevalence, genotypes, and influence on *C. jejuni* epidemiology. *J. Clin. Microbiol*, **40**: 4594-4602.

Buxton, A. and Fraser, G. (1977): Campylobacter. In: Animal microbiology. Black Well Scientific Publications. London. **1**:247-251.

Cabrita, J., Rodrigues, J., Braganca, F., Morgado, C., Pires, I., Goncalves, A. P. (1992): Prevalence, biotypes, plasmid profile and antimicrobial resistance of Campylobacter isolated from wild and domestic animals from northeast Portugal. *J. Appl. Bacteriol*. **73:** 279–285.

Calnek, B. W., John, B. H., Beard, C. W., Reid, W. M., Yoder, H. W. (1991): Campylobacteriosis. In: Diseases of poultry. 9th ed. Iowa State University Press. Ames. Iowa. Pp 237-241.

Cloak, O. M., Duffy, G., Sheridan, J. J., Blair, I. S., McDowell, D. A. (2001): A survey on the incidence of *Campylobacter* species and the development of surface adhesion polymerase chain reaction assay for the detection of *Campylobacter jejuni* in retail meat products. *Food Microbiol*. **18:** 287–298.

Coker, A. O., Adefeso, A. O., (1994): The changing patterns of *Campylobacter jejuni/ coli* in Lagos, Nigeria after ten years. *East Afr Med J;* **71:**437-340

CSA. (2001): Central Statistical Authority, Federal democratic Republic of Ethiopia, central statistical investigation, Statistical abstract.2001

Davis, B. D., Dulbecco, R., Eisen, H. N., Gonsbery, H. S. (1990): *Campylobacter* species. *In*: Microbiology. 4thed .The Murray printing Company London. Pp. 586-87.

de Wit, M. A. S., Koopmans, M. P. G., Kortbeek, L. M., van Leeuwen, N. J., Bartelds, A.I.M., van Duynhovenm, Y. T. H. P. (2001): Gastroenteritis in sentinel general practices, the Netherlands. *Emerg. Infect. Dis.***7**: 82-91.

Delong, W. J., Jaworski, M. D., Ward, A. C. S. (1996): Antigenic and restriction enzyme analysis of *Campylobacter* species associated with abortion in sheep. *Am. J. Vet. Research.* **57**:163-67.

Dominguez, C., Gomez, I., Zumalacarregui, J. (2002): Prevalence of *Salmonella* and *Campylobacter* species. in retail chicken meat in Spain. *Int. J. Food Microbiol*. **72**:165–168.

Doyle, M. P. (1986): *Campylobacter jejuni*. In: Food-borne Microorganisms and Their Toxins: Developing Methodology. Pierson, M. D. and Stern, N. J., Eds. Marcel Dekker Inc. New York. Pp. 312-318

Ellen, J. B., Lance, R.P., Sydney M. F. (1994): Epidemiology and pathogenesis of Campylobacter infection. In: Diagnostic Microbiology. 9thed MOSBY-Year Book Inc. Toronto, Pp. 437-441.

Engberg, J., Aarestrup, F.M., Taylor, D.E., Gerner-Smidt, P., Nachamkin, I. (2001): Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* resistance mechanisms and trends in human isolates. *Emerg. Inf. Dis*.**7**:24–34.

Feierl, G., Wagner, U., Sixl, B., Grisold, A., Daghofer, E., Marth, E. (2001): Epidemiology of campylobacteriosis and development of resistance in Styria, Austria. *Int J Med Microbiol*; **291**:9.

Fitzgerald, C., Stanley, K., Andrew, S., Jones, K. (2001): Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. *Appl. Environ. Microbiol.* **67**:1429–1436.

Fox, J. C. M. (1998): Campylobacteriosis. In: The Merck Veterinary Manual 8th ed. National Publishing Inc. Philadelphia, Pp 11-18.

Fricker, C.R. and Park, R. W. A.,(1989): A two year study of the distribution of thermophilic Campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. J. Appl. Bacteriol. **66**:477-490

Friedman, C. R., Neimann, J., Wegener, H. C, Tauxe, R. V. (2000): Epidemiology of *Campylobacter jejun*i infections in the United States and other industrialized nations. *In*: Campylobacter, Second Edition, Nachamkin I. & M.J. Blaser, eds. ASM Press, Washington DC, USA, Pp.121-138.

Frost, J. A, Oza, A. N., Thwaites, R. T., Rowe, B. (1998): Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. *J. Clin. Microbiol.* **36:**335–339.

Gyles, C. L. and Thoen C. O. (1993): Campylobacter. In: Pathogenesis of Bacterial Infections in Animals. 2nded. Iowa State University Press/ AMES. IOWA Pp 162-170.

Healing, T. D., Greenwood, M. H., Pearson, A. D. (1992): Campylobacters and enteritis. *Rev. Med. Microbiol*. **3:**159-167

Hirsh, D. C. and Zee, Y. C. (1999): Campylobacter. (Digestive and Reproductive tract). In: Veterinary Microbiology. Black well Science. Inc. Melbourne, Pp. 192-195.

Hoge, C.W., Gambel, J.M., Srijan, A., Pitarangsi, C., Echeverria, P. (1998): Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clin Infect Dis;* **26:**341-345.

Hoorfar, J. Nielsen, E. M., Stryhna, H., Andersenb, S., (1999): Evaluation of two automated enzyme-immunoassays for detection of thermophilic campylobacters in fecal samples from cattle and swine. *J. Microbiol. Meth*. **38**:101–106.

Humphrey T. J. and Muscat, I. (1995): Incubation temperature and isolation of *Campylobacter jejuni* from food, milk and water. Let. *Appl. Microbiol*. **9:**137-139.

Hussain, I., Muhammad, S. M., Akhtarb, M., Khan, A. (2007): Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiology* **24**: 219–222.

Inglis, G. D., Kalischuk, L. D., and Busz H. W. (2004): Chronic shedding of *Campylobacter* species in beef cattle. *J. Appl. Microbiol*. **97:**410–420.

Ishihara, K., Kira, T., Ogikubo, K., Morioka, A., Kojima, A., Kijima- Tanaka, M., Takahashi, T., Tamura, Y. (2004): Antimicrobial susceptibilities of Campylobacter isolated from food-producing animals on farms (1999–2001): results from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Int. J. Antimicrob*. Agents **24:** 261–267.

ISO/CD 10272-1 and 10272-2. (2002): Microbiology of food and animal feeding stuffs - horizontal method for the detection and enumeration of Campylobacter growing at 41.5 degrees Celsius. Part 1: Detection method; Part 2: Colony count technique. International Organization for Standardization (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale **56**:1211. Geneva 20, Switzerland.

Jackson, C.J., Fox, A.J., Jones, D.M., Wareing, D.R.A., Hutchinson, D.N. (1998): Associations between heat-stable (O) and heat-labile (HL) serogroup antigens of *Campylobacter jejuni*: evidence for interstrain relationships within three O/HL serovars. *J. Clin. Microbiol.* **36**:2223–2228.

Jacobs-Reitsma, W. F. (2000): Campylobacter in the food supply. *In*: Nachamkin, I., Blaser, M.J. (Eds.), Campylobacter, second ed. American Society for Microbiology, Washington, DC, and Pp. 467–481.

Jay, J. M. (2000): Campylobacteriosis. *In*: Modern Food Microbiology 6thed. An Aspen publisher Inc. Maryland, Pp. 560-563.

Jensen, A. N., Andersen, M. T., Dalsgaard, A., Baggesen, D. L., Nielsen, E. M. (2006): Development of real-time PCR and hybridization methods for detection and identification of thermophilic *Campylobacter* species. in pig faecal samples. *J. Appl. Microbiol*. **99:** 292–300.

Jiménez, A., Velázquez, J. B., Rodríguez, J., Chomón, B., Villa, T. G. (1994): Biotyping of *Campylobacter jejuni* and *Campylobacter coli* infections in Spain. *J. Infect.* **29**:305–310.

Jiwa, S. F. H., Kazwala, R. R., Namahungu, E. (1994): Prevalence of *Campylobacter* species in clinically normal goats kept under various management systems in urban Tanzania. *Small Rum. Res.* **15**:97-100.

Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R A., Bolton, F. J., Frost, J. A., Ward, L., Humphrey, T. J. (2002): Prevalence and numbers of *Salmonella* and *Campylobacter* species on raw, whole chickens in relation to sampling methods. *Int. J. Food Microbiol.* **76:** 151–164.

Kanai, Y., Hayashidani, H., Kaneko, K.I., Ogawa, M., Takahashi, T., Nakamura, M. (1997): Occurrence of zoonotic bacteria in retail raw game meat in Japan with special reference to *Erysipelothrix*. *J. Food Prot*.; **60**: 328-331.

Kassa, T., Gebre-selassie, S., Asrat, D. (2005): The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, southwest Ethiopia. *Ethiop. J. Health Dev*.**9:**225-229.

Kassa, T., Gebre-Selassie, S., Asrat, D. (2007): Antimicrobial susceptibility patterns of thermotolerant Campylobacter strains isolated from food animals in Ethiopia *Vet. Microbiol*. **119**:82–87.

Kiseon, H., Sung, S. J., Euiyoung, C., Sunggi, H., Sangryeol, R. (2007): Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. *Int. J. Food Microbiol*.**114:** 50–59.

Korolik, V., Moorthy L., Coloe P. J. (1988): Differentiation of *Campylobacter jejuni* and *Campylobacter coli* strains by using endonuclease DNA profiles and DNA fragment polymorphisms. *J. Clin. Microbiol.* **33**:1136–1140.

Korsak, N., Daube, G., Ghafir, Y., Chahed, A., Jolly, S., Vindevogel, H. (1998): An efficient sampling technique used to detect four foodborne pathogens on pork and beef carcasses in nine Belgian abattoirs. *J. Food Prot*.; **61**: 535-541

Kramer, J. M., Frost, J. A., Bolton, F. J., Wareing, D. R. (2000): Campylobacter contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. *J. Food Prot*. **63:** 1654–1659.

Kristi, P., Mati, R., Hannu, K., Marja-Liisa, H. (2007): PFGE genotyping and antimicrobial susceptibility of Campylobacter in retail poultry meat in Estonia. *Int. J. Food Microbiol*.**114:**105–112.

Kwiatek, K., Wajton, B., Stern, N.J., (1990): Prevalence and distribution of *Campylobacter* species on poultry and selected red meat carcasses in Poland. *J. Food Prot*. **53:** 127–130.

Lacerc, V., Dufour, B., Lombard, B., Gauchard, F., Garin-Bastuji, B., Salvat, G., Brisabois, A., Poumeyrol, N., De Buyser, M. L., Gnanou-Besse, N., Lahellec, C. (2002): Pathogens in meat and milk products: surveillance and impact on human health in France. *Livestock Prod. Sci*.**76**: 195–202.

Lammerding, A.M., Garcia, M.M., Mann, E.D., Robinson, Y., Dorward, W.J., Truscott, R.B., Tittiger, F. (1988): Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. *J. Food Prot.;* **51**: 47-52.

Lastovica, A. J. and Skirrow M. B. (2000): Clinical significance of Campylobacter and related species other than *Campylobacter jejuni* and *Campylobacter coli.* In: Campylobacter, Second Edition, Nachamkin I. & M.J. Blaser, eds. ASM Press, Washington DC, USA, 89-120.

Lindblom, G. B., Sjogren, E., Hansson, W. J., Kaijser, B. (1995): *Campylobacter upsaliensis*, *Campylobacter sputorum* biovar *sputorum* and *Campylobacter*

concisus as a common cause of diarrhea in Swedish children. *Scand. J. Infect. Dis.* **27:**187-188.

Lior, H. (1982): New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli* and "*Campylobacter laridis*". *J. Clin. Microbiol.* **20**:636–640.

Little, C.L., de Louvais, J. (1989): The microbiological examination of butchery products and butchers' premises in the United Kingdom. *J. Appl. Microbiol*.; **85**: 177-186.

Lofstedt, R. (1998): Bovine genital campylobacteriosis. In: The Merck Veterinary Manual8thed. National Publishing Inc. Philadelphia, pp. 997-998.

Madden, R.H., Espie, W.E., Moran, L., McBride, J., Scates, P. (2001): Occurrence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* and *Campylobacter* species. on beef carcasses in Northern Ireland. *Meat. Sci*. **58**:343–346.

Madden, R.H., Moran, L., Scates, P. (2000): Optimizing recovery of *Campylobacter* species. from the lower porcine gastrointestinal tract. *J. Microbiol. Meth*. **42**: 115–119.

Mafu, A.A, Higgins, R., Nadeau, M., Cousineau, G., (1989): The incidence of *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* in swine carcasses and the slaughterhouse environment. *J. Food Prot*; **52**: 642-645.

Megraud, F., Boudraa, G., Bessaoud, K., Bensid, S., Dabis, F., Soltana, R., *et al*. (1990): *Microbiol.* **20**: 338-340.

Mikkelsen, L. L., Naughton, P. J., Hedemann, M. S., Jensen, B. B. (2004): Effects of physical properties of feed on microbial ecology and survival of *Salmonella enterica* serovar Typhimurium in the pig gastrointestinal tract. *Appl. Environ. Microbiol*. **70:** 3485–3492.

Moore, J. E., Wilson, T. S., Wareing, D. R. A., Murphy, P. G., (2003): Occurrence and characterization of thermophilic *Campylobacter* species. in a poultry processing plant in Northern Ireland. *Irish Vet. J*. **56**:95–98.

Morgan, F., Massouras, T., Barbosa, M., Roseiro, L., Ravasco, F., Kandarakis, I., Bonnin, V., Fistakoris, M., anifantakis, E., Jaubert, G. and raynal-ljutovac, K. (2003): Characteristics of goat milk collected from small and medium enterprises in Greece, Portugal and France. *Small Rum. Res.* **47**: 39-49.

Nachamkin, I. (1995): *Campylobacter* and *Arcobacter*, p. Manual of clinical microbiology, 6thed. American Society for Microbiology. Washington, D.C. 483-491.

Nachamkin, I. and Blaser M. J. (2000): Campylobacter. 2nded. Washington: American Society for Microbiology.

Nachamkin, I., Stowell, C., Skalina, D., Jones, A. M., Hoop II, R. M. (1984): *Campylobacter laridis* causing bacteremia in an immunosuppressed patient. *Ann. Intern. Med*. **101**: 55–57.

Nadeau, E., Messier, S. and Quessy, S., (2002): Prevalence and comparison of genetic profiles of Campylobacter strains isolated from poultry and sporadic cases of campylobacteriosis in humans. *J Food Prot* **65**:73–78.

National Committee for Clinical Laboratory Standards (2000): Performance Standards for Antimicrobial disc Susceptibility Tests, Approved Standard M2-A7, 7th ed. National Committee for Clinical Laboratory Standards, Wayne, PA.

National Meteorology Service Agency (2003): Rainfall and Temperature data, Addis Ababa, Ethiopia. N.M.S.A.

Neill, S. D., Campbell, J. N., O'Brien, J. J., Weatherup, S. T., Ellis, W. A. (1985): Taxonomic position of *Campylobacter cryaerophilus* species. *Int J Syst. Bacteriol.* **35**:342–356.

Neilson, E. M., Engberg, J., Madsen, M., (1997): Distribution of serotypes of *Campylobacter jejuni* and *Campylobacter coli* from Danish patients, poultry, cattle and swine, FEMS. *Immunol. Med. Microbiol*. **19**:47–56.

Newell, D. G., Shreewe, J. E., Toszeghy, M., Domingue, G., Bull, S., Humphrey, T., Mead, G. (2001): Changes in the carriage of Campylobacter strains by poultry carcasses during processing in abattoirs. *App. Env. Microbiol*. **67**: 2636–2640.

Oberhelman, R. A and Taylor, D. N. (2000): Campylobacter infection in developing countries. Campylobacter. In: Nachakin, I and Blaser, M.J. 2nd ed. Washington DC, *Am. Soci Microbiol*. pp. 139-153.

On, S. L. W, Holmes, B. (1995): Classification and identification of Campylobacters, Helicobacters and allied taxa by numerical analysis of phenotypic characters. *Syst. Appl. Microbiol.* **18**:374–390

Oosterom, J., Dekker, R., De Wilde G.J.A., van Kempen-de T. F, Engels G.B. (1985): Prevalence of *Campylobacter jejuni* and *Salmonella* during pig slaughtering. The Veterinary Quarterly; **7**: 31-32.

Padungton, P., Kaneene, J. B. (2003): Review: *Campylobacter* species in human, chickens, pigs and their antimicrobial resistance. *J. Vet. Med. Sci*. **65**:161–170.

Pattison, M. (2001): Practical intervention strategies for Campylobacter. *J. Appl. Microbiol.* **90**:121-125.

Patton, C. M., Barrett, T. J., Morris, G. K. (1985): Comparison of the Penner and Lior methods for serotyping *Campylobacter* species. *J. Clin. Microbiol*.**22**:558–565.

Penner, J. L. and Hennessy, J. N. (1980): Passive haemagglutination technique for serotyping *Campylobacter foetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J. Clin. Microbiol*. **12**:732.

Penner, J. L., Hennessy, J. N., and Congi, R. V. (1983): Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *Eur. J. Clin. Microbiol.* **2**:378.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M., Perin, R. (2003): Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in Northeastern Italy. *Int. J. Food. Microbiol.* **82**: 281–287.

Prescott, J. F., and Bruin-Mosch, C. W. **(**1981): Carriage of *Campylobacter jejuni* in healthy and diarrheic animals. *Ame. J. Vet. Res.* **42**: 164-165.

Prescott, J. F., and Munroe, D. L. (1982): *Campylobacter jejuni* enteritis in man and domestic animals. *J. Am. Vet. Med. Assoc.* **181**: 1524-1530.

Quinn, P. J., Carter, M. E., Markey, B., Carter, G. R. (2002): Campylobacteriosis. In: Clinical Veterinary Microbiology. Mosby International Committee, London, pp. 268-672.

Radostits, O. M. (2001): Campylobacter infection. *In*: Herd Health: Food Animal Production Medicine 3rd ed. W.B. Saunders Company. Philadelphia pp. 155-156.

Radostits, O. M., Blood, C. D., Gay, C. C. (1994): Diseases caused by *Campylobacter* species. In: Veterinary Medicine. A textbook of the disease of cattle, sheep, pig, goat and horse 8th ed.W.B. Saunders Company Philadelphia pp. 882-883.

Reo, M. R., Naficy, A. B., Savarino, S. J., Abu-Elyazeed., R., Wierzba, T. F., Peruski, L. F, *et al*.(2001): Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *Am. J. Epidemiol.* **154**:166-173.

Ribeiro, C. D., Thomas, M. T., Kembrey, D., Magee, J. T., North, Z. (1996): Resistotyping of campylobacters: fulfilling a need. *Epidemiol Infect* **116**:169–175.

Rosef, O., Gondrosen, B., Kapperud, G., Underdal, B., (1983): Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl. Environ. Microbiol*. **46**:855–859.

Saenz, Y., Zarazaga, M., Martalantero, M., Gastanars, J., Baquero, F., and Torres (2000):Antibiotic resistance in Campylobacter strains isolated from animals, food, and humans in Spain in 1997-1998. Antimicrobial agents and chemotherapy, **44**: 267-271.

Salama, S. M., Bolton, F. J., Hutchinson, D. N. (1990): Application of a new phagetyping scheme to campylobacters isolated during outbreaks. *Epidemiol Infect* **104**:405–411.

Schumacher, W., Kroneck, P. M. H., Pfennig N. (1992): Comparative systematic study on "*Spirillum* 5175*", Campylobacter and Wolinella* species. Description of "Spirillum" 5175 as *Sulfurospirillum deleyanum. Arch. Microbiol.* **158**: 287-194.

Shani, S. M. (1998): Avian campylobacter infection. In: the Merck Veterinary Manual 8th ed. National publishing Inc. Philadelphia Pp. 1902-1903.

Skirrow, M. B. and Blaser, M. J. (2000): Clinical aspects of Campylobacter infection. In: Campylobacter, Second Edition, Nachamkin I. & M.J. Blaser, eds. Pp 231-235

Smith, B. P., (1996): Campylobacter abortion. In: Large Animal Internal Medicine. 2nd ed. W.B. Saunders Company. Philadelphia Pp. 1557-1558.

Smith, M. C. and Sherman, D. M. (1994): Campylobacteriosis. In: Goat medicine. Williams and Wilkins. A Waverly Company Philadelphia, USA. P 424.

Stanley, K. N., Wallace, J. S., Currie, J. E., Diggle, P. J., Jones, K. (1998):. Seasonal variation of thermophilic campylobacters in lambs at slaughter. *J. Appl. Microbiol*. **84:**1111–1116.

Steinbrückner, B., Ruberg, F., Vetter-Knoll, M., Kist, M. (2000): Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated in Freiburg from 1992 to 2000. *Int. J. Med. Microbiol*.; **291**:8.

Tajada, P., Gomez-Garces, J.L., Alos, J.I., Balas, D., Cogollos, R. (1996): Antimicrobial susceptibility of Campylobacter jejuni and campylobacter coli to 12 beta-lactam agents and combinations beta-lactam inhibitors.Antimicrob. Agents Chemother. **40**:1924-1925

Tauxe, R. V., Patton, C. M., Edmonds, P., Barrett, T. J., Brenner, D. J., Blake, P. A. (1985): Illness associated with *Campylobacter laridis*, a newly recognized *Campylobacter* species, *J. Clin. Microbiol***. 21**: 222–225.

Tee, W., Kaldor, J. and Duoyer, B. (1995): Emergence of multi drug resistance in *Campylobacter jejuni* isolated from three patients infected with HIV. *J. Clin. Infect. Dis.* **21**: 634-38.

Tokumaru, M., Konuma, H., Umesako, M., Konno, S., Shinagawa, K. (1990): Rates of detection of *Salmonella* and *Campylobacter* in meats in response to the sample size and the infection level of each species. *Int. J. Food Microbiol;* **13**: 41-46.

Turkson, P. K., Lindquist, K. J. and Apperud, K. (1988): Isolation of *Campylobacter* species and *Yersinia enterocolitica* from domestic animals and humans patients in Kenya. *Acta Patholo. Microbiol. Immunolo. Scand.* **96**: 141-146.

Turnbull, P.C.B, Rose, P. (1982): *Campylobacter jejuni* and salmonella in raw red meats. *J. Hyg*., Cambridge; **88**: 29-37.

Van Looveren, M., Daube, G., De Zutter, L. Dumont, J.M., Lammens, C., Wijdooghe, M., Vandamme, P., Jouret, M., Cornelis, M., Goossens, H. (2001): Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium. *J. Antimicrob. Chemother*. **48**: 235–240.

Vandamme, P. and ON, S. L. W. (2001): Recommendations of the Subcommittee on the taxonomy of *Campylobacter* and related bacteria. *Int. J. Syst. Evol. Microbiol* **51**:719–721.

Vandamme, P., E. Falsen, R., Rossau, B., Hoste, P., Segers, Tytgat, R., and De Ley, J., (1991): Revision of Campylobacter, Helicobacter, and Wolinella taxonomy: emendation of generic description and proposal of Arcobacter. *Int J Syst Bacteriol.* **41**:88. 199.

Vanderlinde, P.B, Shay, B., Murray, J. (1998): Microbiological quality of Australian beef carcass meat and frozen bulk packed meat. J. Food Prot.; **61**: 437-443.

Vanderlinde, P.B., Shay B., Murray J. (1999): Microbiological status of Australian sheep meat. Journal of Food Protection; **62**: 380-385.

Veron, M. and. Chatelain, R. (1973): Taxonomic study of the genus Campylobacter Sebald and Veron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith & Taylor) Sebald and Veron. *Int. J. Syst. Bacteriol.* **23**:122

Wallace, J. S., Stanley K. N. and Jones, K. (1998): The colonization of turkeys by thermophilic Campylobacters. *J. Appl. Microbiol.* **85**:224-230.

Wang, G., C. Clark, G., Taylor, T. M., Pucknell, C. Barton, C., Price, L., Woodward, D. L., and Rodgers, F. G. **(**2002): Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J. *Clin. Microbiol*. **40:**4744–4747.

Weijtens, M.J.B.M., Reinders, R.D., Urlings, H.A.P., van der Plas, J. (1999): Campylobacter infections in fattening pigs; excretion pattern and genetic diversity. *J. Appl. Microbiol*. **86**: 63–70.

Whyte, P., McGill K, Cowley D, Madden RH, Moran L, Scates P, Carroll C, O'Leary A, Fanning S, Collins JD, McNamara E, Moore JE, Cormican M. (2004): Occurrence of *Campylobacter* in retail foods in Ireland. *Int. J. Food Microbiol;* **95**:111-118.

Whyte, P., McGill, K., Collins, J. D. (2003): An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. *Food Microbiol*. **20:** 111–117.

Young, C. R., Harvey, R., Anderson, R., Nisbet, D., Stanker, L. H. (2000): Enteric colonization following natural exposure to Campylobacter in pigs. *Res. Vet. Sci.* **68**: 75–78.

Zanetti, F., Varoli, O., Stampi, S., DeLuca, G. (1996): Prevalence of thermophilic Campylobacter and *Arcobacter butzleri* in food of animal origin. *Int. J. Food Microbiol*. **33**: 315–321.

8 ANNEXES

Annex 1. Sample collection sheet for bacteriological analysis

Date of collection	Sample source	Sample type	Sample code	TNS	Remark

Note: TNS= Total number of sample

Annex 2. Location of sampling super markets, types and number of samples

Source	Name	Sample type	TNS	Location
Supermarket	Abadir	MB, SM,CM	8	Addis Ababa
Supermarket	Beloyans	B, MB, SM	6	Addis Ababa
Supermarket	Bambis	MB, P, SM	7	Addis Ababa
Supermarket	Shisolomon	B,P,GM	8	Addis Ababa
Supermarket	Novis-1	MB, P,CM	8	Addis Ababa
Supermarket	Edget	CM, B	9	Addis Ababa
Supermarket	Alema	CM,P,B	7	Debre Zeit
Supermarket	Meskerem	MB, P, SM	8	Addis Ababa
Supermarket	Negash	B, MB, SM	8	Addis Ababa
Supermarket	Abebe Abshir	MB, P, SM	5	Addis Ababa
Supermarket	Loyal	CM, B,GM	8	Addis Ababa
Supermarket	Central	MB, P, SM	10	Addis Ababa
Total			92	

Note: MB=minced beef, B=beef, SM= sheep meat P= pork, GM= goat meat, CC= chicken meat, TNS= Total number of sample

Annex 3. Microbiological Activities in the isolation and identification of thermophilic *Campylobacter* species

Sample code	Growth at on selective medium at 42^{o}C (P/N)	Morphology	Motility test	Grams reaction	Catalase test	Oxidase test	H_2S production	Nitrate reduction	Hippurate hydrolysis

Note: - P/N = Positive/Negative

Annex 4: Location of sampling abattoirs, types and number of samples used for the study.

Source	Name	Sample type	TNS	Location
Abattoir	ELFORA		**140**	Debre Zeit
Abattoir		Sheep meat	50	Debre Zeit
Abattoir		Goat meat	50	Debre Zeit
Abattoir		Beef	10	Debre Zeit
Abattoir		Chicken carcass	30	Debre Zeit
Abattoir	D/Z Public abattoir		**37**	
Abattoir		Sheep meat	08	Debre Zeit
Abattoir		Goat meat	07	Debre Zeit
Abattoir		Beef	22	Debre Zeit
Abattoir	A.A Public abattoir		**181**	Addis Ababa
Abattoir		Sheep meat	35	Addis Ababa
Abattoir		Goat meat	10	Addis Ababa
Abattoir		Beef	106	Addis Ababa
Abattoir		Pork carcass	30	Addis Ababa

Note: TNS= Total number of sample

Annex 5: Location of sampling butchers, types and number of samples

Source	Name	Sample type	TNS	Location
Butcher	Ienay	B,SM,	3	Addis Ababa
Butcher	Bekele	B	2	Addis Ababa
Butcher	H/Mariam	B,SM,GM	4	Addis Ababa
Butcher	Selam	SM, GM	3	Addis Ababa
Butcher	Fasika	B, SM	4	Addis Ababa
Butcher	Tadesse	B	2	Addis Ababa
Butcher	Betelihem	B,GM	4	Addis Ababa
Butcher	Tinsae	GM,B	2	Addis Ababa
Butcher	Tsegaye	SM,B,GM	3	Addis Ababa
Butcher	Lambu	B	2	Addis Ababa
Butcher	Tenama Ye Harer senga	B,SM	3	Addis Ababa
Butcher	Mirt	B	2	Addis Ababa
Butcher	Bash Woldwe chilot	B,SM,GM	4	Addis Ababa
Butcher	K/Giorgis	B,SM	5	Addis Ababa
Butcher	Tiegist	B	1	Addis Ababa
Butcher	Dessalegn	B,GM	4	Addis Ababa
Butcher	Biherawi	SM	3	Addis Ababa
Butcher	Assela	B,SM	3	Addis Ababa
Butcher	Mugib	B,	1	Addis Ababa
Butcher	Tinsae	B	2	Addis Ababa
Butcher	Chercher	B	2	Addis Ababa
Butcher	Fasika	B,SM	1	Addis Ababa
Butcher	Bere Lelimat	B	2	Addis Ababa
Butcher	Ashu	B,GM	3	Addis Ababa
Butcher	Erikum	B,SM	3	Addis Ababa
Butcher	Kebele 10 public	B	2	Addis Ababa
Butcher	Kidus Mikael	B	4	Addis Ababa
Butcher	Ayalew Kiristian Sigabet	B	3	Addis Ababa
Butcher	Tsehay	B,SM	2	Addis Ababa
Butcher	Adaa	B	1	Debre Zeit
Butcher	Nice	B	2	Debre Zeit
Butcher	Shewa cottage	B	1	Debre Zeit
Butcher	Kana Zegelila	B	3	Debre Zeit
Butcher	Genet	B	2	Debre Zeit
Butcher	Kulubigebriel	B	3	Debre Zeit
Total			90	Debre Zeit

Note: B= beef, SM=sheep meat, GM=goat meat

Annex 6: Bacteriological media and Chemicals, Prepared and utilized in the current study.

Campylobacter Agar Base (CM689, OXOID, ENGLAND)

Formula per Liter: Lab-Lamco' Powder 10.0g, peptone 10.0 g, Sodium chloride 5.0 g, Agar 12.0g.

Direction: Suspend 18.5g of Campylobacter Agar Base (CM 689) in 475ml of distilled water and bring to boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Cool to 50^{o}C. Aseptically add 25ml of Lysed Horse Blood (SR117) reconstituted as Directed.

Tryptic Nitrate Medium (DEFCO, France)

Formula per Liter**:** Bacto Tryptose 20.0g, Bacto Dextrose 1g, Disodium phosphate 2g, potassium nitrate, Bacto Agar 1g.

Direction: To rehydrate the medium suspend 25grams in 100ml, cold distilled water heat to boil, to dissolve completely. Sterilize in the autoclave for 15 minutes at 121^{o}C. Inoculate with test organism and incubate at 37^{o}C for 24hours. Test for nitrate reduction.

Reagent A

Sulfalinic acid 5g

Acetic acid (5M)

Reagent B

N-N-dimethyl-naphthylamine 3ml

Acetic acid (5M) 500ml

Add 3drops of reagent A then 3 drops of reagent B to the suspension of organism in broth. Wait for 30 minute for the production of red color, indicating the presence of nitrate reduction product, nitrite. The presence of unreduced nitrate can be detected by adding a pinch of zinc powder to the broth if the red color didn't develop.

Difco™ Columbia Blood Agar Base
Approximate Formula per Liter
Pancreatic Digest of Casein, 10.0 g ,Proteose Peptone, 5.0 g, Yeast Extract, 5.0 g, Beef Heart Digest, 3.0 g, Corn Starch, 1.0 g, Sodium Chloride, 5.0 g, Agar,12g.

Difco™ Columbia Blood Agar Base (DEFCO, BACTON, DICKINSON France S.A.).

Direction: Suspend 39 g of Columbia Blood Agar Base powder in 1litre of distilled water and heat with frequent agitation and boil for 1 minute to completely dissolve the powder and autoclave at 121°C for 15 minutes.

For preparation of blood agar, cool the base to 45-50°Cand add 5% sterile, defibrinated blood, mix well and dispense an approximate of 12ti15ml in to sterile Petri dishes.

Mueller- Hintons Agar (DEFCO, BACTON, DICKINSON France S.A.)

Formula: Beef Infusion 300.0g, Casamino Acid Technical17.5g, Starch 1.5g, Agar 17.0g

Direction: Suspend 38g of the powder in 1 liter of purified water; Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^{o}C for 15 minutes. Avoid over heating. Cool to 50^{o}C. Aseptically add 25ml of horse Blood.

SIM Medium (BBL, Bacton Dickinson Microbiology System)

Formula per Liter: Pancreatic Digest of Casein 20g, Peptic Digest of Casein 6.1g, Ferrous Ammonium Sulphate 0.2, Sodium Thiosulfate 0.2g, Agar 3.5g

Direction: Suspend 30g of the powder in 1 liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute and autoclave at 121^{o}C for 15 minutes.

Sodium Hippurate Broth, BBLTM (Becton, Dickinson, USA).

Formula per Liter: Heart Muscle, Infusion from (Solids)10.0 g, Peptic Digest of Animal Tissue 10.0 g, Sodium Chloride 5.0 g, Sodium Hippurate, 10.0 g

Direction: Dissolve 10g in 1 liter of distilled water and distribute in to final containers. Sterilize by autoclaving at 121^{o}C

Ferric Chloride Test Reagent

Formula for acidic ferric chloride solution……..... 12%

Ferric chloride . . . . . 12.0 g

Concentrated hydrochloric Acid . . . . . 5.4 ml

Distilled water . . . . . 94.6 ml

Direction

1. Add approximately 75ml of distilled water to a 100ml volumetric flask.
2. With transfer pipette add 5.4ml HCl to flask, running the acid down the sides of the flask.
3. Add 12.0 g of ferric chloride.
4. Dissolve by warming flask gently, swirling contents to mix well.
5. Bring volume up to 100ml with distilled water so the final solution appears orange in color.

Procedure:

- Inoculate tubes with one to two isolated colonies from pure culture plate.
- Include an uninoculated tube as a negative control and a positive control (type cultures).
- Incubate tubes with loosened caps for 48 h at 35 ± 2°C in a microaerobic atmosphere.

- Following incubation, centrifuge all cloudy cultures and use the supernatant in the test.
- Aseptically transfer its 0.8ml supernatant to small test tubes using sterile pipette, add 0.2ml of 12% acidic ferric chloride and gently shake.

A positive test for hippurate hydrolysis is indicated by production of a brown flocculants, insoluble precipitate that persists on shaking.

Tryptone Soya Broth (CM0129, OXOPID, ENGLAND)

Formula per Liter: Pancreatic digest of casein 17.0g; peptic digest of soybean meal 3.0g; sodium chloride 5.0g; Dibasic potassium phosphate 2.5g; Glucose 2.5g

Direction: Dissolve 30g in 1 liter of distilled water and distribute in to final containers. Sterilize by autoclaving at 121^{o}C

Brucella Broth (B2900-02, Oxoid, England)

Formula per Liter: Casein Digest Peptone 10.0g, Yeast Extract 2.0g, Sodium chloride 5.0g, Sodium bisulfate 0.1,Peptic Digest of Animal Tissue 10.0g and Dextrose 1.0g.

Direction: Dissolve 28grams per liter of distilled water and heat with stirring until completely dissolved Dispense into appropriate containers; loosen caps and autoclave for 15 minutes at 121^{o}C.

9. CURRICULUM VITAE

Name	Lemma Dadi
Date of Birth	March 12, 1980
Place of birth	Addis Ababa, Ethiopia
Sex	Male
Nationality	Ethiopian
Marital status	Single
Children	No
Religion	Orthodox
Language Proficiency	Affan Oromo, Amharic and English
E. mail	ayantuulemma2009@yahoo.com
	Tel. 09 11 16 29 58
	01 11 22 91 36(Home)

Educational background

1979-1986 E.C.	Qusquam Taitu Bitul Elementary School
1987-1990 E.C.	Entoto Vocational, Technical and Academic School

1991-1992 E.C.	Addis Ababa University, Faculty of Science, Addis Ababa (Fresh man courses).
1993-1997 E.C.	Addis Ababa University, Faculty of Veterinary Medicine., Debre Zeit (Courses of general Veterinary medicine).
1998-1999 E.C.	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Master of Veterinary Science in Veterinary Microbiology).

Research paper

- A cross sectional study on bovine mastitis in small holder dairy farms in Degem District of North Shoa Zone, Ethiopia. DVM Thesis (2005) FVM, AAU, (Unpublished).

Other papers

- Zoonotic diseases associated with dairy production. Seminar paper (2004).
- Campylobacter and its public health significance. Seminar paper (2006).

Additional trainings and certificates

- Computer literacy: Basic computer application software courses, June/2003-December 15/2004. (Diploma).
- Second grade driving license

Work experience

As externship student from September, 2004-June, 2005 North Shoa, research in dairy farms, abattoir work, laboratory and clinical work activities. Microbiological analysis techniques of different samples originated from dairy farms and veterinary clinics has

been developed in the years 2006 - 2007 at the microbiology laboratory of Aklilu Lemma Institute of pathobiology

10. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university that all sources of material used for the thesis have been duly acknowledged.

Name: Lemma Dadi

Signature_____

Date of submission_____

This thesis has been submitted for examination as an advisor

Prof. L. Muniyappa_____

Dr. Daniel Asrat _____