

ADDIS ABABA UNIVERSITY

SCHOOL OF VETERINARY MEDICINE

**ISOLATION OF THERMOPHILIC *CAMPYLOBACTER* SPECIES IN CHICKEN MEAT
AND IN FECAL SAMPLES TAKEN FROM POULTRY FARMS IN DEBRE ZEIT,
ETHIOPIA**

BY

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Debre Zeit, Ethiopia

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**A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial
fulfillment of the requirements for Degree of Masters of Sciences in Veterinary Public
Health**

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CI	Confidence interval
cAMP	Cyclic adenosine monophosphate
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
GI	Gastrointestinal
FDA	Food and drug administration
HIV	Human immunodeficiency virus
NCLLS	National committee for clinical laboratory standards
OR	Odds ratio
PCR	Polymerase chain reaction
ReA	Reactive arthritis
rRNA	Ribosomal ribonucleic acid
spp.	Species
subsp.	Sub-species
TMAO	Trim ethylamine-N-oxide hydrochloride
UK	United Kingdom
USA	United States of America
WHO	World health organization
χ^2	Chi-square

ABSTRACT

A cross-sectional study was conducted from October, 2010 to May, 2011 on apparently healthy broiler chickens (n=263) to assess the extent of colonization and the subsequent contamination of chicken carcass with thermophilic *Campylobacter* species in Debre Zeit. The study involved six randomly selected broiler farms (2 large and 4 small scale farms) situated in Debre Zeit, where broilers are raised and slaughtered. From the total of 526 fecal and carcass swab samples, thermophilic *Campylobacter* species were isolated in 284 (54%) samples. Thermophilic *Campylobacter* spp. were isolated from 149 (56.65%) of the 263 fecal swab samples investigated from six poultry farms. Among the 149 viable isolates, 141 (94.63%) were identified as *C. jejuni* while only 8 (5.37%) were identified as *C. coli*. On the other hand, 135 (51.33%) of the carcass swab samples were found positive for thermophilic *Campylobacter* spp. 133 (89.26%) of these isolates were identified as *C. jejuni* and only 2 (1.34%) were isolates of *C. coli*. There were statistically significant differences in the isolation rates of *Campylobacter* species among large and small production scale, and amongst the farms studied. However, there were no statistically significant differences in the isolation rate of *Campylobacter* species between the sexes and between the types of sample examined. The results of this study indicate that thermophilic campylobacters are very frequent among poultry in Ethiopia, suggesting possible risks of infection to people through the consumption of contaminated poultry products or through contact with infected poultry.

Key words: *Campylobacter*, thermophilic, broiler, prevalence, carcass, feces, isolation, Debre Zeit.

1 INTRODUCTION

Campylobacter species are bacteria that commonly infect a broad range of livestock species, pets and wild animals. Campylobacters are mainly spiral-shaped, S-shaped or curved, rod-shaped bacteria (Pal, 2007). There are 16 species and six subspecies assigned to the genus *Campylobacter*. These organisms are unable to ferment or oxidize carbohydrates and most species prefer a micro-aerobic (containing 3-10% oxygen) atmosphere for growth (Lawson *et al.*, 2001).

Several species of genus *Campylobacter* are known to cause different types of disease in farm animals, some of which are potentially zoonotic. The most important species of *Campylobacter* in veterinary medicine are *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* (Tesfaye *et al.*, 2005). *Campylobacter fetus* subsp. *veneralis* is 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. *C. sputorum* subsp. *mucosalis* is frequently found in association with a group of enteric disease in pigs called “porcine intestinal adenomatosis” complex. Recently, *C. hyointestinalis* has also been isolated from the same lesion (Radostitis *et al.*, 1994).

Avian species, especially domestic poultry including chickens, turkeys, ducks and geese, are frequently infected with the members of *Campylobacter* species, primarily *Campylobacter jejuni* and *Campylobacter coli* (Stephens *et al.*, 1998; Sabin *et al.*, 2003; Pal, 2007). In poultry, they tend to multiply in large numbers in the hindgut, principally in the cecae. As enteric organisms, *C. jejuni* and *C. coli* are well adapted to the avian host and reside in the intestinal tract of birds. Despite extensive colonization, *Campylobacter* infections produce little or no clinical diseases in poultry (Lee and Newell, 2006). However, infectious hepatitis associated with *C. coli* and *C. jejuni* has been reported in ostriches, causing a high morbidity and mortality (Sabin *et al.*, 2003).

Campylobacteriosis, a human enteric infection caused by thermophilic campylobacters, is a well established foodborne zoonotic disease (Humphrey *et al.*, 2007). Humans are normally exposed to *Campylobacter* spp. through an oral pathway by handling and consuming uncooked meat, especially poultry products, faecal contaminated water and even raw vegetables (Chai *et al.*, 2007). Fecal shedding is presumably an important factor in the dissemination of organisms around large broiler flocks once the first bird becomes colonized. Certainly, once flock colonization is detected, bird-to-bird transmission within flocks is extremely rapid, and the majority (up to 100%) of birds in a positive flock are colonized within only a few days (Shreeve *et al.*, 2000; Milfin *et al.*, 2001).

Many pathogen-specific virulence determinants may contribute to the pathogenesis of *Campylobacter* infection, but none has a proven role. Suspected determinants of pathogenicity include chemotaxis, motility, and flagella, which are required for attachment and colonization of the gut epithelium. Once colonization occurs, other possible virulence determinants are iron acquisition, host cell invasion, toxin production, inflammation and active secretion, and epithelial disruption with leakage of serosal fluid (Altekruse *et al.*, 1999).

Campylobacter jejuni, *C. coli*, *C. lari* and *C. upsaliensis* are often referred to as thermophilic or thermotolerant *Campylobacter*. This refers to the fact that most strains of the named species, together with *C. helveticus*, prefer a slightly higher growth temperature (42°C) than other *Campylobacters* (Doyle and Roman, 1981).

Although thermophilic campylobacters are not significant pathogens of poultry, they are of importance to food safety and public health. Most frequently reported (90%) human campylobacteriosis are due to *C. jejuni* subspecies *jejuni* and *C. coli*. *C. lari* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease (Lawson *et al.*, 2001).

The high prevalence of *Campylobacter* in the intestinal tract of market age poultry results in frequent contamination of poultry carcasses in processing plants. Thus, poultry products at retail are often contaminated by members of *Campylobacter* species (Jorgensen *et al.*, 2002; Lee and Newell, 2006; Son *et al.*, 2007). During slaughtering, the damage of intestinal tract integrity can

lead to direct contamination. Contamination can also occur directly through air, bird to bird, via equipment and water (Jones *et al.*, 1991; Corry and Atabay, 2001).

Campylobacter has emerged as a leading bacterial cause of food borne gastroenteritis in humans around the world (Mead *et al.*, 1999). There is significant increase in the incidence of acquired *Campylobacter* infection both in industrialized and developing countries (Acha and Szyfres, 2001). Most of *Campylobacter* related illnesses in humans are sporadic and characterized by self limiting watery and/or bloody diarrhea, abdominal cramp and fever (Pal, 2007); however, severe conditions may occur in immunocompromised patients, requiring antibiotic treatment (Mead *et al.*, 1999). Handling and eating raw or undercooked poultry meat is considered a major source of human campylobacteriosis (Wesley *et al.*, 2000; Corry and Atabay, 2001; Pal, 2007).

Campylobacter spp. could cause serious complications related to acute bacterial enteric disease in humans throughout the world (Nachamkin, 1995; Mead *et al.*, 1999). The most important pathogenic strains belong to the group of thermotolerant campylobacters. *C. jejuni* and *C. coli* are associated with human illness (Wesley *et al.*, 2000; Pal, 2007). Among children, especially in developing countries, *Campylobacter* infections can cause severe life-threatening diarrheal disease (Friedman *et al.*, 2000). In recent years, reports show that *Campylobacter* species are becoming one of the major causes of childhood diarrhea (Gedlu and Assefa, 1996; Asrat *et al.*, 1997).

Rarely, some long-term consequences can result from a *Campylobacter* infection. Some people may have bacteraemia, reactive arthritis following campylobacteriosis, and some individuals develop Guillain-Barré syndrome, a post-infectious autoimmune disease characterized by acute and progressive neuromuscular paralysis (Friedman *et al.*, 2000).

Housed poultry can be maintained free of *Campylobacter* infection by consistent application of excellent biosecurity. The control of *Campylobacter* spp. in broiler production is principally one of identifying ways by which the three-week *Campylobacter*-free period can be extended until slaughter age. Essentially, this means either preventing the entry of the bacteria into the flock and/or improving the resistance of the flock (Altekruse *et al.*, 1999).

It is generally assumed that campylobacters contaminate poultry meat during processing, surviving throughout the food chain supply to constitute a risk to human health. Human campylobacteriosis is the most common cause of food poisoning in much of the industrialized world (ACMSF, 2004), and the reduction and/or elimination of *C. jejuni* in the food chain, particularly from chicken products, is a major strategy in efforts to control this disease (WHO, 2002).

Cooking all poultry products thoroughly, preventing cross-contamination in the kitchen, avoiding consumption of non-pasteurized milk and untreated surface water, washing hands carefully, use of vaccinations, and education in hygienic handling of foods for abattoir workers, caterers and those involved in the production of raw meat is essential to keep *Campylobacter* contamination to a minimum in humans (ACMSF, 2004; Pal, 2007).

Despite the scientific advances made, *Campylobacter* remains a poorly characterized microorganism, which impacts on its epidemiology and control (ACMSF, 2004). For a variety of reasons, our understanding of the molecular and cellular pathogenesis of *Campylobacter* infection has lagged far behind that of other enteric pathogens. Advances in the control of diseases such as tuberculosis, brucellosis and trichinosis in animals have reduced the hazards posed to workers in the meat industry and to the consumers of meat. There is, therefore, a need for continued fundamental research (Bourke *et al*, 1998).

In addition, little is known about the presence of these bacteria in various food animals as possible sources of infection to humans in Ethiopia (Tefera *et al.*, 2009). Therefore, this study was conducted in Debre Zeit, Ethiopia with the following objectives:

General objective: To study the prevalence of thermophilic *Campylobacter* species in broiler flocks and broiler carcasses in Debre Zeit.

Specific objectives:

To estimate the prevalence of thermophilic *Campylobacter* species in fecal samples of chickens slaughtered in poultry farms located in Debre Zeit.

To know the prevalence of thermophilic *Campylobacter* species in carcass swab samples taken from chickens slaughtered in poultry farms located in Debre Zeit.

2.1.1. *Campylobacter* species

Campylobacter species are gram-negative, spiral-shaped, 3-5 µm long, and motile. They are the second most common cause of bacterial gastroenteritis in humans. *Campylobacter* species are found in the environment, particularly in the gastrointestinal tract of animals. *Campylobacter* species are found in the environment, particularly in the gastrointestinal tract of animals. *Campylobacter* species are found in the environment, particularly in the gastrointestinal tract of animals. *Campylobacter* species are found in the environment, particularly in the gastrointestinal tract of animals.

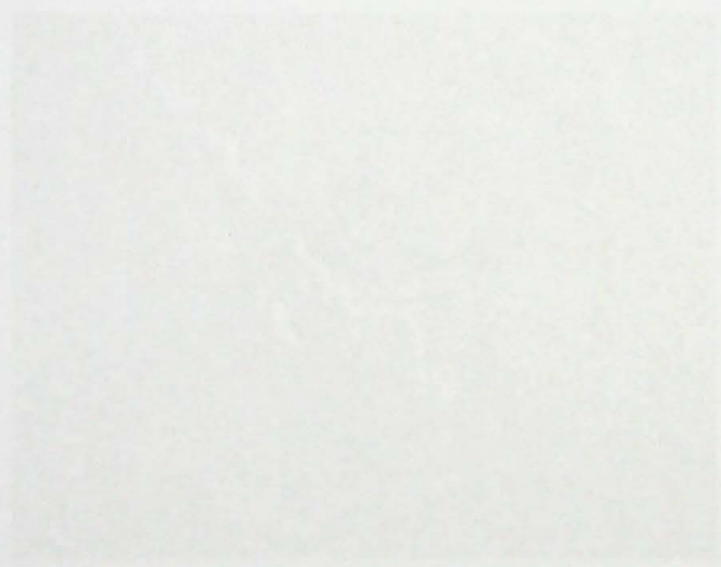


Fig. 2.1.1. *Campylobacter* species

2.1.2. *Campylobacter* and *Chlamydia* spp. in *Campylobacter* species

The family *Campylobacteriaceae* is composed of the genera *Campylobacter*, *Campylobacter*, *Campylobacter*, and *Campylobacter*. The family *Campylobacteriaceae* is composed of the genera *Campylobacter*, *Campylobacter*, *Campylobacter*, and *Campylobacter*. The family *Campylobacteriaceae* is composed of the genera *Campylobacter*, *Campylobacter*, *Campylobacter*, and *Campylobacter*. The family *Campylobacteriaceae* is composed of the genera *Campylobacter*, *Campylobacter*, *Campylobacter*, and *Campylobacter*.

2 LITERATURE REVIEW

2.1 The genus *Campylobacter*

Campylobacters are mainly spiral-shaped, S-shaped or curved, rod-shaped bacteria. There are 16 species and six subspecies assigned to the genus *Campylobacter*, of which the most frequently reported (90%) in human disease are *C. jejuni* subspecies *jejuni* and *C. coli*. *C. lari* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease. Most species prefer a micro-aerobic (containing 3-10% oxygen) atmosphere for growth (Lawson *et al.*, 2001).

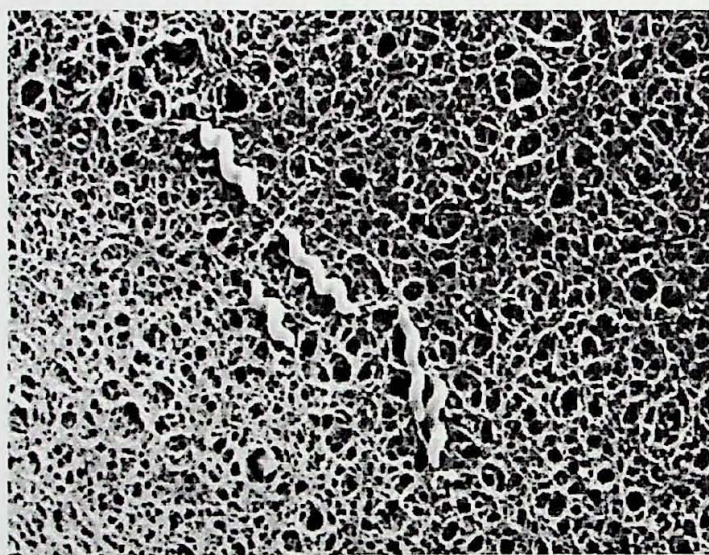


Fig. 1: Scanning electron microscopy of *Campylobacter jejuni*, 7200X (Gregory and Marshall, 2004).

2.1.1 Taxonomy and Classification of *Campylobacter* species

The family *Campylobacteriaceae* is classified under Phylum Proteobacteria, Class Epsilon proteobacteria and Order Campylocacteriales (On and Holmes, 1995; Vandamme, 2000). The family *Campylobacteriaceae* includes the genera *Campylobacter* and *Arcobacter* (previously within the genus *Campylobacter*). During the last thirty years of 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 the 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, 2000). The family 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 guanine and cytosine content. Members of this family are encountered mainly as commensals in humans or domestic animals. The family also contains misclassified species and strains originally described as 'free-living *Campylobacter*' (*Sulfurospirillum* species) (Schumacher *et al.*, 1992). 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* (Vandamme, 2000).

In 1963, when *Campylobacter* was first described, the genus comprised of two species. Presently, the genus *Campylobacter* includes 16 species and six sub-species. The 16 rRNA gene, used to determine phylogenetic relationships among all living organisms, played a major role in all previous extensive rearrangement of *Campylobacter* taxonomy (Vandamme, 2000).

Several species of genus *Campylobacter* are known to cause different types of disease in farm animals, some of which are potentially zoonotic. *Campylobacter fetus* subsp. *veneralis* is 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. *C. sputorum* subsp. *mucosalis* is frequently found in association with a group of enteric disease in pigs called "porcine intestinal adenomatosis" complex. Recently, *C. hyointestinalis* has also been isolated from the same lesion (Radostitis *et al.*, 1994).

DNA: rRNA hybridization has demonstrated that *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* form a separate rRNA sub cluster within RRNA cluster I (consisting the genus *Campylobacter*) in rRNA super family VI. *C. helveticus* is a later described species, which shows

close DNA homology with *C. upsaliensis*. Recently, *C. insulaenigrae*, a new distinct species most closely related to *C. jejuni* and *C. lari*, is described (Vandamme, 2000).

Campylobacter jejuni, *C. coli*, *C. lari* and *C. upsaliensis* are often referred to as thermophilic or thermotolerant *Campylobacter*. This refers to the fact that most strains of the named species, together with *C. helveticus*, prefer a slightly higher growth temperature than other *Campylobacters* (Doyle and Roman, 1981).

However, the terminology is not completely clear-cut, as neither *C. jejuni* subsp. *doylei* nor the recently described *C. insulaenigrae* grows at 42⁰c. Moreover, several other *Campylobacter* species grow at 42⁰c and consequently may be called thermotolerant campylobacters. It is notable that the described use of “themophillic” and “thermotolerant” is appropriate only in strict context of campylobacters, as general terms referring to microorganisms that thrive in or tolerate temperatures above 50⁰c. In this regard, all campylobacters belong to the mesophilic organisms.

As one of the leading bacterial causes of human enteritis worldwide, *C. jejuni* is the species that has been most extensively studied. The organism was first isolated from cattle with infectious diarrhea. Unfortunately, the original strains were lost, and the species was redefined when it was transferred to the new genus *Campylobacter* (Veron and Chatelain, 1973). The species *C. jejuni* includes two subspecies (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*) that can be discriminated on the basis of several phenotypic tests (nitrate reduction, selenite reduction, sodium fluoride, and safranine) and growth at 42°C (subsp. *doylei* does not grow at 42°C). Subspecies *jejuni* is much more frequently isolated than subspecies *doylei* (Garritty *et al.*, 2005).

The second most common *Campylobacter* spp. isolated from humans is *C. coli*. The organism was first isolated from swine with swine dysentery (Doyle, 1944), and is the primary *Campylobacter* spp. found in swine. As in the case of *C. jejuni*, the original strains were lost and the current type strain is from a later date (Veron and Chatelain, 1973). *C. coli* differs phenotypically from *C. jejuni* primarily by its inability to hydrolyze hippurate. *C. lari* differs from other thermophilic *Campylobacter* spp. in its resistance to nalidixic acid and ability to grow anaerobically in the presence of trim ethylamine-N-oxide hydrochloride, TMAO (Benjamin *et al.*, 1983; Waldenstrom *et al.*, 2002).

2.1.2 Morphology and staining reaction

Thermophilic microorganisms that belong to the genus *Campylobacter* are spiral or curved, Gram negative, non spore forming rods, 0.2-0.5 micrometers wide and 0.5-0.8 micrometers long. Their characteristic cork screw-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 peptidoglycans, mainly due to its enzymatic degradation (Wallace *et al.*, 1998).

These thermophilic *Campylobacter* species are microaerophilic in nature and survive a longer time outside the host. The organisms are susceptible to drying, high oxygen conditions and low pH (Lema and Daniel, 2008).

2.1.3 Cultural Characteristics

Thermophilic *Campylobacter* species are microaerophilic requiring an atmosphere containing 10% carbon dioxide and 5% oxygen concentration for their growth producing grayish, mucoid and spreading colonies. *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* grow at 42 °C, a characteristic that is useful for their selectivity in isolation from intestinal source (Hirsh and Zee, 1999). *C. jejuni* and *C. coli* are best isolated from fecal samples on selective media containing antimicrobial agents. 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 metabolism of amino acids, and from metabolism of intermediates of tricarboxylic acid cycle (Amano and Shibata, 1992).

Campylobacter species are positive for oxidase and catalase tests. 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).

Table 1: Basic phenotypic characteristics of thermophilic *Campylobacter* species (Ellen *et al.*, 1994).

Characteristics	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. upsaliensis</i>
Growth on selective media at 42 ⁰ C	+	+	+	+
Catalase	+	+	+	-/slight
Oxidase	+	+	+	+
Hydrogen sulphide production	+	+	+	+
Nitrate reduction	+	+	+	+
Nalixidic acid	S	S	R	S
Cephalothin	R	R	R	S
Hippurate hydrolysis	+	-	-	-

Key: + positive, - negative, R resistant, S sensitive

Susceptibility or resistance to nalidixic acid or cephalothin, nitrate reduction, hydrogen sulphide production and growth at 25 ⁰C or 42 ⁰C are other criteria on which definitive identification of *Campylobacter* species is based. *C. jejuni* and *C. coli* are distinguished by hippurate hydrolysis, since the former is positive for the test (Quinn *et al.*, 2002).

2.2 Reservoirs of *Campylobacter* species

C. jejuni and *C. lari* 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 in stress, *C. jejuni* is associated with diarrhea and this is a well recognized source of human infections (Weijtens, 1999; Pal, 2007). 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 small laboratory reared mammals. Large numbers of *Campylobacter* species have been isolated from young livestock, including piglets, lambs and calves, with enteritis, but these organisms are also harbored in apparently healthy animals (Jay, 2000).

In birds, especially poultry, disease is rare, if it occurs at all, despite high levels of colonization with *C. jejuni* and *C. coli*. Outbreaks of avian hepatitis have been reported, but the pathogenic role of *Campylobacter* species is unclear. One possible exception is *Campylobacter* associated death and enteritis that occurs in young ostriches. *Campylobacter* species are frequently found in wild birds (Borman *et al.*, 2002; Pal, 2007).

C. jejuni, *C. coli*, *C. hyointestinalis* and *C. sputorum*, as well as *C. fetus* may also be associated with infections of the respiratory 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* and *C. coli*. Such infections are presumably a consequence of translocation from gastrointestinal tract or via an ascending route. Animal and animal products are sources of human infection and susceptible animal species (Hirsh and Zee, 1999).

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 the organisms in their feces, with poultry being prominent. Its prevalence in fecal samples often ranges from around 30 to 100% (Jay, 2000).

C. jejuni and *C. coli* have been isolated from intestines of healthy farm animals, poultry, pets, zoo animals and wild birds. *C. jejuni* and *C. hyointestinalis* are found in the rumen and intestine normal adult cattle and calves. *C. coli* and *C. jejuni* were isolated from rectal swabs taken from dairy cows. *C. jejuni* has been isolated from samples collected from various species of animals (Weijtens, 1999).

2.3 Responses of thermophilic *Campylobacter* species to stress

There is debate as to the extent to which *Campylobacter* is sensitive to environmental stresses. A long-held view is that the organism is unusually sensitive, and there is no doubt that *Campylobacter* appears to lack many adaptive responses which can be correlated with resistance in other bacteria. For example, analysis of the genome indicates that the organism does not possess the RpoS global regulator which, in a number of Gram-negative organisms, is the basis for the survival of the bacterial cell in stationary phase and during exposure to many types of environmental stress. Recent work has shown, however, that *C. jejuni* can mount adaptive responses to both acidic and aerobic conditions (Murphy *et al.*, 2003a).

In addition, there is increasing recognition that *Campylobacter* is more resistant to stress than had initially been thought. One theory is that the methods used to assess survival have caused confusion. Because the methods have not been optimized for the recovery of damaged cells, they have provided the opportunity for an overestimation of sensitivity (Murphy *et al.*, 2003b).

2.3.1 Response to high temperature

Although thermophilic in nature, *Campylobacter* spp. are readily inactivated by heat and do not survive pasteurization treatments or typical cooking procedures. The organism does exhibit a heat shock response, with at least 24 proteins being preferentially synthesized by *C. jejuni* immediately after exposure to temperatures above that which is optimal for growth. The response mechanism is still being investigated, but it appears to be different to those seen in other bacteria. It has been suggested that the heat shock response may play a role in both thermotolerance and colonization, as mutants deficient in one of the heat shock proteins have severely reduced growth at 46°C and are unable to colonize chickens (ACMSF, 2004).

2.3.2 Response to low temperature

Campylobacter jejuni does not appear to produce cold shock proteins, which may explain why it cannot grow below 30°C. Unlike other bacteria, which show a gradual reduction in growth rate near their minimal growth temperature, *C. jejuni* shows a rapid decline. However, at temperatures as low as 4°C, the organism can respire, generate ATP, and move towards favorable environments, although it is unable to replicate. At lower temperatures, viability is lost rapidly

but the organism can still be isolated from frozen meats and poultry products (Murphy *et al.*, 2003a).

2.3.3 Response to oxidative stress

Most *Campylobacter* spp. are microaerophilic, although a few species have now been found to grow under strictly aerobic or anaerobic conditions. Those that are microaerophilic, which includes the main species linked to food borne disease, have an inherent sensitivity towards oxygen and its reduction products, and require cellular defenses for survival during exposure to air. Oxidative stress has been shown to contribute to the freeze-thaw induced killing of *Campylobacter* (Murphy *et al.*, 2003a).

2.3.4 Response to osmotic stress

Many bacteria have regulatory mechanisms which enable them to resist osmotic stress, for example through the synthesis or transport of compatible solutes. Such systems appear to be absent in *Campylobacter*, and this may explain why the organism is relatively sensitive to osmotic stress. That said, there is evidence that the organism can be recovered from dry surfaces 24 hours after contamination, albeit in low numbers (ACMSF, 2004).

2.4 Pathogenesis and virulence factors of thermophilic *Campylobacter* species

Whilst the mechanism of *Campylobacter* infection in humans is still not fully understood, some of the factors essential for pathogenesis have now been identified. Infection appears to include at least two stages namely, the organism adhering to the intestine of the animal and producing toxin, then invading and proliferating within the intestinal mucosa. Motility is thought to contribute significantly to both colonization and the development of disease, and understanding of how this works is increasing, genes having been identified for regulatory components of a chemotaxis system and candidate receptors for signal detection (ACMSF, 2004).

The pathogenesis of infection involves both host and pathogen-specific factors. The health and age of the host and *Campylobacter*-specific humoral immunity from previous exposure influence clinical outcome after infection (Altekruse *et al.*, 1999).

Many pathogen-specific virulence determinants may contribute to the pathogenesis of *Campylobacter* infection, but none has a proven role. Suspected determinants of pathogenicity include chemotaxis, motility, and flagella, which are required for attachment and colonization of the gut epithelium. Once colonization occurs, other possible virulence determinants are iron acquisition, host cell invasion, toxin production, inflammation and active secretion, and epithelial disruption with leakage of serosal fluid (Altekruse *et al.*, 1999).

An important mechanism by which bacterial enteropathogens 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 toxins produced by *Corynebacterium diphtheriae*, *Vibrio cholerae* and *Shigella dysenteriae* (Radostitis *et al.*, 1994). The latter group probably directly associated with the mechanisms inducing diarrhea. These toxins are proenzymes that share several modes of action. They bind to specific receptors on the plasma membrane (Hirsh and Zee, 1999).

C. jejuni secretes a toxin similar in activity to the enterotoxins of *Vibrio cholerae* and the heat labile toxin of *Escherchia coli* by increasing the intracellular cyclic adenosine monophosphate (cAMP) level and cytoskeletal rearrangement followed by cell death due to cytolethal distending toxin, a protein that was shown to induce hepatitis in mice (hepatotoxin), and a protein that has haemolytic activity (haemolysin). Both toxins are immunologically similar and bind to the same ganglioside on target cells (Hirsh and Zee, 1999). *C. coli* and *C. lari* produce uncharacterized substances with cytotoxic and cytotoxic activity (Jay, 2000).

Invasion mechanisms also remain poorly understood, although there is recent evidence to suggest that they are strain-specific. Flagella mediated motility is thought to be a major contributing

factor, with invasion likely to involve changes to the host cell membrane and/or the internal cytoskeletal structure. *C. jejuni* secretes proteins which are felt to be essential for internalization of the organism into mammalian cells, these being synthesized during interaction with epithelial cells. Following invasion, *C. jejuni* appears to be largely confined to membrane-bound vacuoles, although some organisms have been detected free in the cytoplasm (ACMSF, 2004).

The mechanism by which *Campylobacter* causes diarrhea is becoming understood, although it is apparent that various hosts react differently to invasion, with fluid secretion being dependent on the extent of the host response and degree of epithelial damage. The process of cell adhesion, internalization and movement of the organism across host cells attracts white blood cells to the site of infection, and granules released by these cells during *Campylobacter* phagocytosis cause tissue damage, inflammation and, ultimately, diarrhea. (Nachamkin *et al.*, 1998).

2.4 Epidemiology of *Campylobacter* species

Campylobacters are widely distributed and occur in most warm-blooded domestic, production and wild animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep, ostriches and shellfish; and in pets, including cats and dogs (Friedman *et al.*, 2000; Pal, 2007).

Prior to 1970s, *Campylobacter* species were known primarily to veterinary microbiologists as the organism that caused spontaneous abortions in cattle and sheep, and as the cause of animal's pathologies (Jay, 2000); but they have been recently recognized as a cause of human disease (Fox, 1998). Both domestic and wild birds as well as companion animals are known reservoirs of *Campylobacter* species. These species are microaerophilic inhabitants of the gastrointestinal (GI) tract of various animal species, which act as source of infection (Ellen *et al.*, 1994; Jay, 2000).

Campylobacter jejuni and *C. coli* are common worldwide causes of human gastroenteritis, in the developed world as well as developing countries. The reported incidences vary between countries, probably due to difference in surveillance systems as well as real differences in incidence (EFSA, 2006). Especially, mild *Campylobacter* infections can be expected to be substantially underreported. Some efforts to estimate the true campylobacteriosis incidence have

been made. Some estimate the true incidence of *Campylobacter* to be 5 to 8 times higher than the reported incidence (Mafu, 2000).

2.4.1 Epidemiology of *Campylobacter* species in Poultry

Chickens are notorious for the fact that they carry bacteria such as *Campylobacter* and *Salmonella*, which can cause zoonoses by contamination of the end product, without hampering growth and development of the chicken itself. This carrier status can only be explained by the inability of the chickens immune system to eliminate the pathogen, this in turn being due to insufficient humoral responses towards the polysaccharides of the bacterial capsule (Jeurissen, 1998).

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

Poultry primarily serves as reservoir host of 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, pheasants and quails which act as reservoirs of the infection (Shani, 1998).

Despite the fact that *C. jejuni* is prevalent as an intestinal commensal in floor housed broiler breeds, turkeys and layer type breeder chicken, there is no evidence to show that *Campylobacter* can be transmitted vertically by either transovarially or penetrating the egg shell after ovipositor (Calnek *et al.*, 1991).

Raw chicken is known to be contaminated with *Campylobacter* spp. at a high frequency, often in excess of 50% (ACMSF, 2004; Wilson, 2002). Poultry is frequently colonized with *C. jejuni* (65–95%) less often with *C. coli* and rarely with other *Campylobacter* species (Newell and Wagenaar, 2000). Higher incidence in poultry is attributed to the body temperature of the chicken which is

approximately 41 to 42 °C (Altekruse *et al.*, 1999). As the temperature optimum for thermophilic campylobacters corresponds to the body temperature of birds rather than mammals, they seem to be well adapted to the avian gut, and birds have been suggested as natural hosts for these organisms (Newell and Wagenaar, 2000).

Colonization rates in broiler chickens are age-related. Most flocks are negative until 2 weeks of age. Once *Campylobacter* colonization occurs in a broiler flock, transmission, via coprophagy, is extremely rapid and up to 100% of birds within a flock can become colonized within 72 hours. Samples from live birds, destined for the food chain, should, therefore, be taken as close to slaughter as possible (Newell and Wagenaar, 2000).

The uneven distribution of campylobacters amongst different species of birds is mainly influenced by the feeding behavior of the birds (Waldeström *et al.*, 2002). *Campylobacter* spp. found in wild birds include the most common human pathogens *C. jejuni* and *C. coli*. Moreover, a substantial proportion of isolates are identified as *C. lari* (Broman *et al.*, 2002).

Sources of Campylobacter spp. in chickens

Over the last 25 years, since the identification of poultry meat as an important source of human infection with *Campylobacter* spp., there have been many studies in many countries into the epidemiology of this zoonotic pathogen in poultry production. As with many areas of science, there is a degree of dispute over the importance of the various routes of infection, which are identified as contaminated water, contaminated feed, carry-over from a previous flock, domestic and/or wild animals, contaminated transport crates, vehicles and personnel at flock, thinning, feed withdrawal, and the external environment around the broiler house (EFSA, 2006).

Wild birds have been suggested to be important reservoirs of campylobacteriosis infection in broilers and humans. However, comparisons of *C. jejuni* subtypes from humans and chickens reveal only a few common subtypes (Broman *et al.*, 2002).

Although the epidemiology of *Campylobacter* infection in chickens has some similarities to that of *Salmonella* spp., there is one important difference. *Salmonella* primarily enters poultry flocks when the chicks are very young. *Campylobacter* is rarely found in broiler flocks until the birds are in the third week of life. There is currently no agreement on the reason(s) for this delay but

the following have been suggested as having a role: maternal antibodies in young chicks, as most broiler-breeder flocks are *Campylobacter*-positive and anti-*Campylobacter* antibodies may be present in egg yolks; and the presence of bacterial floras antagonistic to *Campylobacter* spp. in young birds (EFSA, 2006).

2.4.2 Epidemiology of *Campylobacter* species 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 (Radostitis *et al.*, 1994). *C. fetus* subsp. *fetus* causes sporadic abortion in cattle and is isolated from the reproductive tract of bulls and cows, and there are some evidences that it may cause infertility. *C. jejuni* is isolated sporadically from aborted bovine fetus (Acha and Szyfres, 2001).

C. jejuni has been shown experimentally to be a potential cause of mastitis. Both *C. jejuni* and *C. fetus* subsp. *fetus* are incriminated as cause of diarrhea in cattle (Quinn *et al.*, 2002). Experimentally, these organisms cause a mucoid diarrhea often with dysentery and fever in calves. Most *Campylobacter* species are found in the alimentary system of health, carrier or diarrheic animal (Hirsh and Zee, 1999), the exception being *C. fetus* subsp. *venerealis*, which is found only in reproductive tract of cattle and *C. sputorum* subsp. *bobulus* which is found only in reproductive tract of sheep (Ellen *et al.*, 1994) and also transmitted by contaminated instruments, bedding or during artificial insemination using contaminated semen. Bulls can also transmit the infection mechanically for several hours after matting with an infected cow (Lofstedt, 1998).

2.4.3 Epidemiology of *Campylobacter* species in sheep and goat

Abortion, still births and weak lambs characterize campylobacteriosis in sheep in late pregnancy. *C. jejuni* and *C. fetus* subsp. *fetus* are causative agents of this disease. The infection is highly contagious and may cause up to 70% of the ewes to abort when the organism is newly introduced to the flock (DeLong *et al.*, 1996). In Britain, it is the third most common cause of ewe's abortion (Quinn *et al.*, 2002). Apart from the fact that *C. 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 enteritidis and diarrhea in many animal species (Smith, 1996).

Campylobacter species have been isolated from goats all over the world. In Canada, 2.7% of the animals studied were found positive for *C. jejuni* (Prescott and Bruin-Mosch, 1981). Turkson and others (1988) have found *Campylobacter* species in 6.3% of goats sampled in Kenya. Abreham and others (1990), in Ghana, detected *C. jejuni* but not *C. coli*, in higher proportion (33.3%) of goats tested. These different isolation rates could be attributable to contact of goats with other animal species. Campylobacteriosis due to *C. fetus* subsp. *fetus* and *C. jejuni* have been reported to cause sporadic abortion in goats (Smith and Sherman, 1994).

Susceptible ewes may acquire infection through ingestion of forage contaminated with fetal material or uterine discharges. Other sources include feces of carrier sheep and other mammals and various birds commonly found around livestock (DeLong *et al.*, 1996). The placenta, fluids and fetus have been found to contain large numbers of the organisms and act as source of infection to susceptible animals (Hirsh and Zee, 1999).

2.4.4 Epidemiology of *Campylobacter* species in pigs

Pigs seem to be reservoir of *Campylobacter* species with prevalences between 50-100%. However, opposite to most other animals, pigs show dominance of *C. coli* (Alter *et al.*, 2005; Boes *et al.*, 2005). Nevertheless, American studies showed that *C. jejuni* may constitute a majority of (up to 87%) of *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 *C. jejuni* may co-exist with *C. coli* in pigs, but are typically present in 10 to 100-fold lower than *C. coli* (Madden *et al.*, 2000; Jensen *et al.*, 2006).

The organism may cause disease in nursing piglets and in weaned pigs with high rate of isolation from small intestine. *C. sputorum* subsp. *mucosalis* is frequently found in association with a group of enteric diseases called “porcine intestinal adenomatosis” complex. Recently, *C. hyointestinalis* has also been reported from same lesion. Young adults show cutaneous pallor, weakness and black tarry feces (Radostitis *et al.*, 1994).

C. coli have been isolated from small intestine of diarrheic piglets and described to cause dysentery and necrotizing ileitis with mortality rate 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* species earlier in life than conventional pigs (Alter *et al.*, 2005).

Outdoor organic pigs may differ from conventional pigs with respect to the occurrence of campylobacteriosis. For example, the lower animal density probably reduces the infection pressure and rough stimulates the intestinal flora, which is likely to reduce the susceptibility to infections (Mikkelsen *et al.*, 2004).

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

2.4.5 Epidemiology of *Campylobacter* spp. in Humans.

Infection with a *Campylobacter* species is one of the most common causes of human bacterial gastroenteritis (Saenz *et al.*, 2000). There are 12 species of *Campylobacter* of which four (*C. jejuni*, *C. coli*, *C. laris* and *C. upsaliencies*) are known to cause enteric infections in humans (Mitike *et al.*, 2000). Poultry meat is assumed to be one of the main sources of food borne *Campylobacter* infections in accordance with the greatest proportion of *Campylobacter*-positive food samples (EFSA, 2006; Humphrey *et al.*, 2007).

Campylobacter is the most commonly reported bacterial cause of gastroenteritis in the developed world (EFSA, 2006). An estimated 2 million cases of *Campylobacter* enteritis occur annually in the U.S., accounting for 5-7% of cases of gastroenteritis. Furthermore, in the United Kingdom during 2000, *Campylobacter jejuni* was involved in 77.3% in all cases of food borne illness. About 15 of every 100,000 people are diagnosed with campylobacteriosis every year, and with many cases going unreported, up to 0.5% of the general population may unknowingly harbor *Campylobacter* in their gut (Saenz *et al.*, 2000).

In 2004, an incidence of 51.6 cases per 100,000 inhabitants was reported by 24 EU member states. The incidence varied widely between countries, ranging from <0.1 to 303 cases per 100,000 inhabitants. There has been a marked increase in the number of human *Campylobacter* cases in Norway since 1997, the annual incidence being around 100 cases per 100,000 of the population (EFSA, 2006). In the Netherlands, the estimated number of cases is 80,000 per year in a population of 16 million (Havelaar *et al.*, 2007).

Generally, developing countries do not have national surveillance programs for campylobacteriosis; therefore, incidence values in terms of number of cases for a population do not exist. Availability of national surveillance programs in developed countries has facilitated monitoring of sporadic cases as well as outbreaks of human campylobacteriosis (Tauxe, 1992; Altekruse *et al.*, 1999; Rao *et al.*, 2001). Most estimates of incidence in developing countries are from laboratory-based surveillance of pathogens responsible for diarrhea. *Campylobacter* isolation rates in developing countries range from 5 to 20% (Oberhelman and Taylor, 2000). Table 1 shows isolation rates for some developing countries from studies of diarrhea in children under the age of 5 years.

Despite the lack of incidence data from national surveys, case-control community-based studies have provided estimates of 40,000 to 60,000/100,000 for children <5 years of age (Oberhelman and Taylor, 2000; Frost, 2001). In contrast, the figure for developed countries is 300/100,000. Estimates in the general population in developing and developed countries are similar, approximately 90/100,000 (Oberhelman and Taylor, 2000), confirming the observation that campylobacteriosis is often a pediatric disease in developing countries. The isolation and incidence rates in some developing countries have increased since their initial reports. This increase has often been attributed to improved diagnostic methods (Akitoye *et al.*, 2002).

Seasonal variation

A striking feature of campylobacteriosis in temperate countries is seasonal variation, with one or two incidence peaks occurring in spring, summer or early autumn. The seasonal variations in 9 European countries show a remarkably consistent pattern from year to year (Nylen *et al.*, 2002). The seasonality pattern is still largely unexplained, although it has been shown to be related to climatic factors. Seasonal peaks in *Campylobacter* spp. prevalence in broilers and other potential

sources have been suggested to be related to the seasonal variation in humans. Others propose flies as important vectors for infection transmission during summer (Nichols, 2005).

In Wales, consumption or handling of milk contaminated by bird pickling at milk bottles was associated with *Campylobacter* infections during a spring incidence rise. Another suggests cause of seasonal peaks is human behaviors that may be more common during the warmer season such as swimming in lakes and rivers, and drinking water from streams and lakes (Nylen *et al.*, 2002).

In contrast to developed countries, *Campylobacter* enteritis has no seasonal preference epidemics in developing countries. Isolation peaks vary from one country to another and also within countries. The lack of seasonal preference may be due to lack of extreme temperature variation as well as lack of adequate surveillance for epidemics (Akitoye *et al.*, 2002).

Table 2: Isolation rates of *Campylobacter* from diarrhea specimens from children under the age of 5 in selected developing countries.

Country	Isolation rate (%)	Reference
Algeria	17.7	Megraud <i>et al.</i> , 1990
Bangladesh	17.4	Albert <i>et al.</i> , 1999
Brazil	9.9	Mangia <i>et al.</i> , 1993
Cameroon	7.7	Koulla-Shiro <i>et al.</i> , 1995
Egypt	9.0	Rao <i>et al.</i> , 2001
Ethiopia	13.8	Gedlu and Aseffa, 1996
Guatemala	12.1	Ramiro Cruz <i>et al.</i> , 1994
Jordan	5.5	Na'was and Abo-Shehada, 1991
Nigeria	16.5	Coker and Adefeso, 1994
Tanzania	18.0	Lindblom <i>et al.</i> , 1995
Thailand	13.0	Echeverria <i>et al.</i> , 1989
Zimbabwe	9.3	Simango and Nyahanana, 1997

2.5 Public Health Significance of Campylobacteriosis

Awareness of the public health implications of *Campylobacter* infections has evolved over more than a century. In 1886, Escherich observed organisms resembling campylobacters in stool samples of children with diarrhea. In 1913, McFaydean and Stockman identified campylobacters (called related *Vibrio*) in fetal tissues of aborted sheep. In 1957, King described the isolation of related *Vibrio* from blood samples of children with diarrhea, and in 1972, clinical microbiologists in Belgium first isolated campylobacters from stool samples of patients with diarrhea. The development of selective growth media in the 1970s permitted more laboratories to test stool specimens for *Campylobacter*. Soon *Campylobacter* spp. were established as common human pathogens (Kist, 1985).

Campylobacteriosis is a collective description for infectious diseases caused by members of the genus campylobacter (Nachamkin and Blaser, 2000). Campylobacters are recognized worldwide as important food borne pathogens (Pal, 2007). 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 gastroenteritis worldwide (Allos, 2001; Pal, 2007). The only form of campylobacteriosis of major public health importance is *Campylobacter* enteritis due to *Campylobacter jejuni* and *C. coli*. *C. 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 water borne outbreaks (Broczyk *et al.*, 1987).

Human gastroenteritis caused by these organisms is frequently associated with consumption of red meat and poultry meat (Beuchat, 1985; Pal, 2007). The rate of *Campylobacter* infection worldwide has been increasing, with the number of cases often exceeding those of Salmonellosis and Shigellosis (Altekruse *et al.*, 1999).

Campylobacterial infections are the most common cause of bacterial enterocolitis in humans. Among children, especially in developing countries, *Campylobacter* infections can cause severe

life-threatening diarrheal disease (Friedman *et al.*, 2000). In recent years, reports show that *Campylobacter* species are becoming one of the major causes of childhood diarrhoea (Gedlu and Assefa, 1996; Asrat *et al.*, 1997).

There is significant increase in the incidence of acquired *Campylobacter* infection both in industrialized and developing countries (Acha and Szyfres, 2001). In countries where records are kept, campylobacters are being reported to be the leading cause of bacterial diarrhea in man, and isolation and incidence rates in some developing countries increased since their initial reports (Coker and Adefeso, 1994), with most being isolated from less than 2-year-old children with diarrhea (Albert *et al.*, 1999). In some reports, isolation rates for symptomatic and asymptomatic children were not statistically significant (Oberhelman and Taylor, 2000).

Campylobacteriosis affects all age groups; however, infections are recognized with increasing frequency in infants, children and aged individuals suffering from debilitating disorders such as HIV/AIDS and in homosexual men, where the infection is associated with proctitis (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 systematic 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 infected animal in early 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 in developing countries 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 the disease is most common in infants, and there is relatively high proportion of asymptomatic carriers. In developed countries, infection generally occurs rather in the later stage. The highest

incidence of the 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 factor such as pregnancy, premature birth, chronic alcoholism, and neoplasia and cardiovascular disease (Ellen *et al.*, 1994). Fever, malaise, headache and sometimes aching of limbs and colicky abdominal pain with nausea but rarely vomiting, precede 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 (Acha and Szyfres, 2001; Pal, 2007).

Poultry plays a significant role in the causal chain of events leading to human food borne illness. The human infectious dose is thought to be quite low and a single live chicken could potentially carry millions of human infectious doses of *Campylobacter*. Of course, the microbiological loading may be significantly reduced during slaughter and processing, but poultry meat will still pose a heavy challenge to hygienic measures along the supply chain right into kitchens, both in the home and in catering establishments (ACMSF, 2004).

The consumption of undercooked poultry and cross-contamination of other foods with drippings from raw poultry are leading risk factors for human Campylobacteriosis (Altekruse *et al.*, 1999; Acha and Szyfres). Many chicken flocks are silently infected with *Campylobacter*; that is, the chickens are infected with the organism but show no signs of illness. *Campylobacter* can be easily spread from bird to bird through a common water source or through contact with infected feces. When an infected bird is slaughtered, *Campylobacter* can be transferred from the intestines to the meat (Butzler and Skirrow, 2009).

One way to become infected is to cut poultry meat on a cutting board, and then use the unwashed cutting board or utensil to prepare vegetables or other raw or lightly cooked foods. The *Campylobacter* organisms from the raw meat can then spread to the other foods (Stern and Kotula, 1982).

Although usually associated with a benign outcome in the developed world, the burden of illness posed by *Campylobacter* infections, especially in people with compromised immune systems, is

enormous (Friedman *et al.*, 2000). In patients with HIV, infections may be more frequent, may cause prolonged bouts of dirty brown diarrhea, and may be more commonly associated with bacteraemia and antibiotic resistance. The severity and persistence of infection in patients with AIDS and hypogammaglobulinemia indicates that both cell-mediated and humoral immunity are important in preventing and terminating infection (Wikipedia, 2010).

Adding to the human and economic costs are chronic sequelae associated with *C. jejuni* infection—Guillain-Barré syndrome and reactive arthritis. In addition, an increasing proportion of human infections caused by *C. jejuni* are resistant to antimicrobial therapy (Altekruse *et al.*, 1999).

A large animal reservoir is present as well, with up to 100% of poultry. Infected chicken feces may contain up to 10^9 bacteria per 25 grams, and due to the installations, the bacteria are rapidly spread to other chickens. This vastly exceeds the infectious dose of 1000-10,000 bacteria for humans (Jeurissen, 1998).

When assessing the likelihood of *Campylobacter* infection from foods, the comparative sensitivity of the organisms to the usual environmental influences must be balanced by the low infective dose. This is as low as 500 organisms making the risk for even lightly contaminated foods one that demands attention (Bourke *et al.*, 1998).

Most cases of human campylobacteriosis are sporadic. Outbreaks have different epidemiologic characteristics from sporadic infections. Many outbreaks occur during the spring and autumn. Consumption of raw milk was implicated as the source of infection in 30 of the 80 outbreaks of human campylobacteriosis reported to CDC between 1973 and 1992. Unpasteurized milk can become contaminated if the cow has an infection with *Campylobacter* in her udder or the milk is contaminated with manure. Surface water and mountain streams can become contaminated from infected feces from cows or wild birds. This infection is common in the developing world, and travelers to foreign countries are also at risk for becoming infected with *Campylobacter* (Friedman *et al.*, 2000).

The organism is not usually spread from person to person, but this can happen if the infected person is a small child or is producing a large volume of diarrhea. Larger outbreaks due to

Campylobacter are not usually associated with raw poultry but are usually related to drinking unpasteurized milk or contaminated water (Bourke *et al.*, 1998).

Occupational exposure may cause infection and disease in workers in poultry processing plants, animal health facilities, animal shelters, and animal agriculture and rendering plants. Family members of 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 farm. Risk of acquiring campylobacteriosis in developing countries include the presence of animals in cooking areas, uncovered garbage in cooking areas and the lack of piped waters (Reo *et al.*, 2001). 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; Pal, 2007).

Other risk factors accounting for a smaller proportion of sporadic illnesses include drinking untreated water, traveling abroad, eating barbecued pork or sausage, drinking raw milk or milk from bird pecked bottles, and contact with dogs and cats, particularly juvenile pets or pets with diarrhea (Altekruse *et al.*, 1999).

2.6 Clinical Manifestations of Campylobacteriosis

2.6.1 Clinical manifestations and pathological lesions in birds

Campylobacter infections in poultry usually produce no clinical signs of disease under natural conditions. However, it has been reported that in ostriches natural *Campylobacter* infection can cause clinical illness and pathological lesions in liver and intestines (Stephens *et al.*, 1998; Newell and Wagenaar, 2000). Vibrionic hepatitis was prevalent during 1950s and 1960s in commercial laying hens but is only occasionally reported nowadays. It was suspected that *Campylobacter* might be the cause of the disease, but the etiologic agent(s) for vibrionic hepatitis was not formally identified (Burch, 2005).

Some studies reported that experimental challenge of young chickens with *Campylobacter* can induce clinical diseases including watery/mucoid/ bloody diarrhea, weight loss, or even mortality (Ruiz-Palacios *et al.*, 1981; Welkos, 1984; Nesbit *et al.*, 2001). In an early report, 3-day-old chickens inoculated with a high dose of *C. jejuni* developed diarrhea within 72 hours, which lasted for 10 days and resulted in considerable weight loss as well as a mortality of 32% (Ruiz-Palacios *et al.*, 1981).

Welkos (1984) reported that almost one-third of the day-old-chicks and nearly all of the newly-hatched chicks, but none of the 3-day old chickens, developed signs of gastroenteritis after challenge orally with *C. jejuni*. Similarly, Nesbit (2001) observed watery/mucoid diarrhea in 81% of 36 to 72-hr-old birds 5 days after inoculation with *C. jejuni*, and also found that the Starbro strain of chickens was more likely to develop diarrhea than the white leghorns strain. *Campylobacter* infection in commercial broilers of less than 2 weeks of age (a rare event) was found to be associated with diarrhea, decreased weight gain, and excess mortality.

Another study using newly hatched or 4-day-old turkey poults also observed reduced weight gain and transient watery diarrhea in the birds after inoculation with *Campylobacter* (Lam *et al.*, 1992). Oral inoculation of 3-week-old Japanese quails with *C. jejuni* resulted in diarrhea that lasted for 2 weeks (Maruyama and Katsube, 1988). Despite these isolated reports, many other studies did not observe any clinical diseases associated with *Campylobacter* infections in poultry (Knudsen *et al.*, 2006).

Gross pathologic lesions associated with *Campylobacter* infection in experimentally infected chicks are minimal and mainly confined to the gastrointestinal tract. Due to accumulation of fluid, gas, or excess mucus, distention of intestines including ceca with watery/foamy material may be a common finding. Blood and mucus in the lumen of small intestine, and petechial hemorrhages in the gizzard mucosa of chicks can be seen occasionally (Welkos, 1984). There is a report that *Campylobacter* was isolated more frequently (21% of 223 livers) from broiler chicken livers with necrotic lesions than livers without lesions (12% of 50 livers) obtained from slaughter plants in Canada; however, there was no evidence that *Campylobacter* directly contributed to the lesion (Boukraa, 1991).

Microscopic lesions following experimental infection of chicks are mostly unapparent or minimal, but exceptions occur in birds with severe clinical and gross pathological signs. Usually examination of gastrointestinal tissue reveals no necrosis or invasion of the epithelium or any other pathological changes; however, a mild edema of the lamina propria and submucosa of the intestines, mostly in ceca, was reported with *Campylobacter* infections in chickens (Burch, 2005).

2.6.2 Clinical manifestations in humans

Infection in humans has an incubation period of two to seven days; and causes acute (bloody) diarrhea lasting about three to five days, accompanied by cramping, fever, abdominal pain, nausea, vomiting, head ache and muscle pain. The bacterial cells adhere to the walls of the intestinal tract where they damage the walls and produce toxins. The disease is usually self limiting and some people who are infected with *Campylobacter* do not have any symptoms at all (Bourke *et al.*, 1998). Most people who get campylobacteriosis recover completely within 2 to 5 days, although sometimes recovery can take up to 10 days (Friedman *et al.*, 2000).

Symptoms may also depend on route of transmission. In participants of anal intercourse, campylobacteriosis is more localized to the distal end of the colon and may be termed a proctocolitis. There are other diseases showing similar symptoms. For instance, abdominal pain and tenderness may be much localized, mimicking acute appendicitis. Furthermore, *Helicobacter pylori* is closely related to *Campylobacter* and causes peptic ulcer disease (Wikipedia, 2010).

Long term complications

Rarely, some long-term consequences can result from a *Campylobacter* infection. Some people may have bacteraemia, arthritis following campylobacteriosis, and some (1-2 in 100,000 cases) individuals develop Guillain-Barré syndrome (GBS) (Friedman *et al.*, 2000), in which the nerves that join the spinal cord and brain to the rest of the body are damaged, sometimes permanently resulting in acute, bilateral, ascending paralysis occurring typically 1-3 weeks following onset of diarrhea (ACMSF, 2004). This occurs only with infection of *C. jejuni* and *C. upsaliensis*; and occurs when a person's immune system is "triggered" to attack the body's own nerves. Approximately 20% of patients with GBS are left with some disability, and approximately 5%

die despite advances in respiratory care. The related Miller Fisher syndrome (MFS) is also documented (Friedman *et al.*, 2000).

Campylobacter has been associated with a range of rheumatological conditions, most commonly reported as Reiter's syndrome or reactive arthritis (ReA), defined here as an aseptic arthritis following an enteric infection. *Campylobacter*-induced ReA occurs an average of two weeks following onset of diarrhea. The condition typically affects more than one joint, most commonly the knees, ankles, wrists and lower back. The average duration of arthritic symptoms is approximately two months (Nachamkin *et al.*, 1998).

Other complications include toxic megacolon, dehydration and sepsis. Such complications generally form in little children (under 1 year of age) and immunocompromised people. A chronic course of the disease is possible; such form of the process is likely to develop without a distinct acute phase. Chronic campylobacteriosis features long period of sub-febrile temperature and asthenia; eye damage, arthritis, endocarditis may develop if infection is untreated. Occasional deaths occur in young, previously healthy individuals because of blood volume depletion, and in persons who are elderly or immunocompromised (Wikipedia, 2010).

2.6 Diagnosis of Campylobacteriosis

2.6.1 Direct examination

Gram stained smear from fecal material, carcass samples, fetal material or aborted fetal 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 campylobacters in smears (Hirsh and Zee, 1999). Impression smears of the intestines of swine, with proliferative enteritis, contain similar rods. A characteristic darting motility of *Campylobacter* spp. can be examined by dark field or phase contrast microscopy of fecal smear of acute stage diarrhea (Lofstedt, 1998; Pal, 2007).

2.6.2 Isolation and identification

Campylobacter spp. can be isolated from the feces, cecal and jejunal contents, liver tissue, bile, and blood in systemic infection of poultry (Calnek *et al.*, 1999). They can also be isolated from

fetal and abomasal contents of sheep (Smith and Sherman, 1994). In pigs, isolation of these organisms from feces is difficult due to intracellular localization of the bacteria (Quinn *et al.*, 2002).

2.6.3 Immunodiagnostics

Even though immunodiagnostics is not used for the diagnosis of intestinal disease, antibody response measured by enzyme linked immunosorbent assay (ELISA) has been applied for epidemiological purposes (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 (Quinn *et al.*, 2002). Assays using polymerase chain reaction (PCR) have been developed to amplify DNA from feces (Hirsh and Zee, 1999).

2.7 Treatment and Prevention of Campylobacteriosis

2.7.1 Treatment

Treatment is not generally indicated, except electrolyte replacement and rehydration. In more severe cases, antibiotics can be used, and can shorten the duration of symptoms if they are given early in the illness. Antibiotic treatment is controversial, and has only a marginal benefit (1.32 days) on the duration of symptoms, and should not be used routinely (Robinson and Jones, 1981).

Erythromycin can be used in children, and tetracycline in adults. Some studies show, however, that erythromycin rapidly eliminates *Campylobacter* from the stool without affecting the duration of illness. Nevertheless, children with dysentery due to *C. jejuni* benefit from early treatment with erythromycin (Butzler and Skirrow, 2009).

Treatment with antibiotics, therefore, depends on the severity of symptoms. Quinolones are effective if the organism is sensitive, but high rates of quinolone use in livestock means that quinolones are now largely ineffective. Antimotility agents, such as loperamide, can lead to

prolonged illness or intestinal perforation in any invasive diarrhea, and should be avoided. Trimethoprim-sulfamethoxazole and ampicillin are ineffective against *Campylobacter* (Wikipedia, 2010).

In the past, poultry infections were often treated by mass administration of enrofloxacin and sarafloxacin for single instances of infection. This practice was banned, as it promoted the development of fluoroquinolone-resistant populations. Currently growing resistance of the *Campylobacter* to fluoroquinolones and macrolides is of a major concern (Robinson and Jones, 1981).

2.7.2 Prevention of campylobacteriosis in poultry farms

Control of *Campylobacter* contamination on the farm may reduce contamination of poultry products at the retail level. Epidemiologic studies indicate that strict hygiene reduces, but do not eliminate, intestinal carriage in food producing animals (Altekruse *et al.*, 1999).

The control of *Campylobacter* spp. in broiler production is principally one of identifying ways by which the three-week *Campylobacter*-free period can be extended until slaughter age. Essentially, this means either preventing the entry of the bacteria into the flock and/or improving the resistance of the flock. In field studies, poultry flocks that drank chlorinated water had lower intestinal colonization rates than poultry that drank unchlorinated water (Altekruse *et al.*, 1999).

Advisory Committee on the Microbiological Safety of Food (ACMSF) recommends the following measures to control *Campylobacter* contamination on the farm.

- be species mono-specific (ie. farm only chickens);
- supply the birds with water of potable quality;
- properly clean and disinfect houses after flock removal, which should include disinfection of the water supply system;
- protect the house from entry by wild birds and rodents;
- supply feed which has received treatment sufficient to have eradicated *Salmonella* (and, hence *Campylobacter*), and protect it from re-contamination;

- only carry out thinning if done in association with proper crate washing (so that crates are not contaminated with *Campylobacter* spp. or other pathogenic microorganisms) and proper biosecurity measures, eg. clothing and footwear;
- ensure that transport crates and vehicles are cleaned and disinfected properly on every occasion;
- maintain general biosecurity and hygiene barriers at a high level, to prevent infection from the farm environment; and microbiological testing can be used to look for trends, with plants using the results to identify the need to make improvements in slaughter hygiene or cleaning and disinfection processes (ACMSF, 2004).

2.7.3 Prevention of campylobacteriosis in humans

Given the prevalence of *Campylobacter* in poultry, and knowing how easily pathogens can persist and spread in the domestic and catering environments, it is believed that reducing the level of the organism in poultry meat is likely to make a significant contribution to the battle against human food borne illness. Any reduction in the number of *Campylobacter* cells reaching the kitchen would enhance the effectiveness of normal hygiene measures (EFSA, 2006).

All poultry products should be cooked thoroughly until the meat is no longer pink and the inside is cooked to 74°C. Cross-contamination in the kitchen can be prevented by using separate cutting boards for foods of animal origin and other foods; or by carefully cleaning all cutting boards, countertops and utensils with soap and hot water after preparing raw foods. Avoid consumption of non-pasteurized milk and untreated surface water. Make sure that persons with diarrhea, especially children, wash their hands carefully and frequently with soap to reduce the risk of spreading the infection. Wash hands with hot, soapy water before and after handling raw foods of animal origin and after contact with pet feces (ACMSF, 2004).

The bacterium can also be present in water, particularly well water. Heating water at a full boil for 1 minute (3 minutes if you live in a high altitude) will kill *Campylobacter*. Boiled water should be stored in a clean container with a lid and refrigerated (EFSA, 2006).

Education in hygienic handling of foods for abattoir workers and those involved in the production of raw meat is essential to keep microbiological contamination to a minimum. Both professional and domestic food handlers should be vigilant during the preparation of food and should observe

hygienic rules of food preparation. Professional food handlers who suffer from fever, diarrhea, vomiting, or visible infected skin lesions should report to their employer immediately. In countries without adequate sewage disposal systems, feces and articles soiled with feces may need to be disinfected before disposal (ACMSF, 2004).

There is a view that vaccination may be a possible control option, as infected people mount a strong immune response. In addition, immunity against *Campylobacter* is possible, in the absence of acute infection, with many abattoir workers apparently being immune to infection after initial exposure. There have also been attempts to develop *Campylobacter* vaccines, whole-cell oral vaccine formulations having been tested with good results in primates, and a vaccine based on whole-cell formulations and a purified flagellin giving some protective immunity in the mouse model (ACMSF, 2004).

2.8 Status of Campylobacteriosis in Ethiopia

In recent years, reports show that *Campylobacter* species are becoming one of the major causes of childhood diarrhea (Gedlu and Assefa, 1996; Asrat *et al.*, 1997). However, little is known about the presence of these bacteria in various food animals as possible sources of infection to humans in Ethiopia (Tefera *et al.*, 2009).

Research and control efforts on the disease have been conducted more often in developed countries than developing countries. However, because of the increasing incidence, expanding spectrum of infections, potential of HIV-related deaths due to *Campylobacter*, and the availability of the complete genome sequence of *C. jejuni* NCTC 11168, interest in campylobacteriosis research and control in developing countries is growing (Akitoye *et al.*, 2002).

According to a study conducted in Jimma, *Campylobacter* spp. were isolated from 39.6% of 485 fecal specimens taken from various urban and rural farm animals. The highest isolation rate was recorded among chickens (68.1%), followed by pigs (50.0%), sheep (38.0%) and cattle (12.7%).

Among the 192 thermophilic campylobacters isolated, 70.3% were identified to be *C. jejuni*, 26.6% were *C. coli* and 3.1% were *C. lari*. *C. jejuni* was the most prevalent (80.8%) species in chickens (Tesfaye *et al.*, 2005). A related study carried out in Debre Zeit and Addis Ababa revealed a prevalence of 9.3% in different meat samples with highest prevalence in chicken meat (21.7%), followed by sheep meat, pork meat, goat meat and beef. Among the isolates, 78% were identified to be *C. jejuni*; while *C. coli* and *C. lari* were attributed to 18% and 4% of the isolates, respectively (Lema and Daniel, 2008).

In a study conducted in Dembia District, Mitike and others (2006) estimated the magnitude of *Campylobacter* enteritis in children below fifteen years of age reported that the prevalence of *Campylobacter* species was 10.5% and the frequency of isolation was twice as much as *Salmonella* or *Shigella* species. Contact with cats and diarrhea-sick person in the household was associated with isolation of *Campylobacter* species. Hospital-based studies conducted at Gondar and Addis Ababa teaching hospitals revealed slightly higher figures, 13.8% and 15.3%, respectively (Gedlu and Assefa, 1996; Asrat *et al.*, 1997).

3 MATERIALS AND METHODS

3.1 Study area

A cross-sectional study was conducted from October, 2010 to May, 2011 on apparently healthy broiler chickens (n=263) from six selected poultry farms situated in Debre Zeit, where broilers are raised and slaughtered. Debre Zeit is a town located 45 kilometers south east of Addis Ababa, the capital city of Ethiopia. The town is situated at 9⁰ N latitude and 40⁰ E longitude, and at an altitude of 1850 meters above sea level. The area has an annual rainfall of 866 millimeters with the rainy season extending from June to September; and the dry season extends October through February. The average temperature is between 14 and 26 degree centigrade with a relative humidity of 61.3% (CSA, 2001).

Based on figures from the Central Statistical Agency of Ethiopia (2005), Debre Zeit has an estimated total population of 131,159, of whom 64,642 are men and 66,517 women. The three largest ethnic groups reported for this town were the Amhara (42.86%), the Oromo (39.4%), and the Gurage (8.3%); all other ethnic groups made up 9.44% of the population. The 1994 census reported Debre Zeit had a total population of 73,372 of whom 35,058 were men and 38,314 were women.

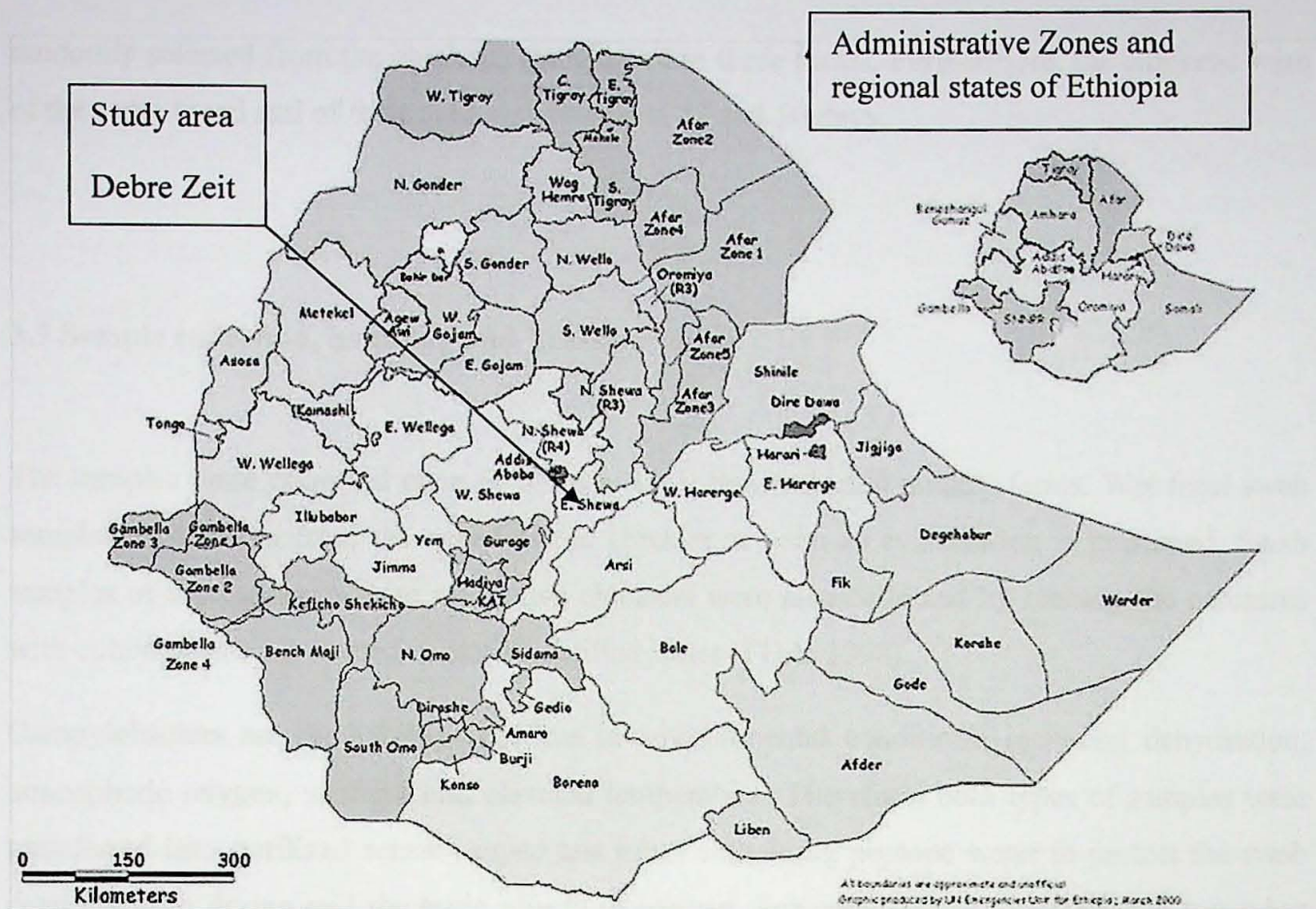


Figure 2: Location of the study area (UN Emergency Unit for Ethiopia, 2000).

3.2 Study design

During the aforementioned period, six broiler farms were studied. Two of the farms (farms 1 and 2) were considered as large farms with multiple broiler houses and enclosing 12,000 - 25,000 birds; and the other four farms (farms 3 to 6) were small farms with 1 or 2 houses and raised about 1000 to 3000 birds. The two large scale farms were selected because they were the only broiler farms which are currently supplying chicken meat in large amounts and carry out slaughter operations on an unremitting basis. The small scale farms were randomly selected. According to the formula recommended by Thrustfield (2005), the sample size was determined to be 262 for a 95% confidence interval and a predicted prevalence of 21.7%, according to a study conducted around the same area by Lema and Daniel (2008). A total of 526 samples (a pair of samples from each chicken) were collected and examined. All the chickens examined were

randomly selected from the chickens slaughtered in these farms. Furthermore, the chickens were of the same breed and of their ages were between 42 and 50 days.

3.3 Sample collection, handling and Transportation

The samples were collected once or twice a week from selected poultry farms. Wet fecal swab samples were taken from the cecae of the chicken as soon as evisceration is presumed. Swab samples of the carcasses from respective chickens were also collected by rubbing the carcasses with cotton swabs pre-wetted in sterile distilled water (FDA, 1998).

Campylobacters are remarkably sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Therefore, both types of samples were transferred into sterilized screw capped test tubes containing peptone water to protect the swab contents from drying and the toxic effects of oxygen. For convenience, the sterilized test tubes were labeled for farm code, sample number, code of sample source (fecal or carcass), and date just before the commencement of the sample collection. During collection, the samples from males and females were placed separately on racks. The sexes were labeled before they were transported to the laboratory.

The samples were transported in cold chain using ice box and ice packs to the Microbiology laboratory of Addis Ababa University School of Veterinary Medicine for microbiological analysis. The samples were kept under refrigeration temperature of $+4^{\circ}\text{C}$ until being processed within 24 hours of collection.

The samples were processed within 3 hours of collection and the samples were then analyzed microbiologically according to the standard set by the International Organization for Standardization (ISO 10272-1:2006).

3.4 Microscopic examination of morphology and motility

A pure culture is required for confirmatory tests, but a preliminary diagnosis was obtained by direct microscopic examination of suspect colony material. Material from a suspect colony was suspended in saline and evaluated by a dark field microscopy for characteristic “seagull” shaped, spiral or curved slender rods with a corkscrew-like motility (Corry *et al.*, 1995).

3.5 Plating and identification

For the isolation of *Campylobacter* from fecal/cecal or intestinal samples, no pretreatment is needed; samples can be plated on to selective medium or the filtration method on non-selective agar can be used. Enrichment is recommended to enhance the culture sensitivity of potentially environmentally stressed organisms or in the case of low levels of organisms in feces, for example from cattle, sheep or pigs. However, enrichment from the latter samples is not carried out routinely (Bolton *et al.*, 1988).

The media used in this study is *Campylobacter* selective agar base (CM 689, Oxoid, England) supplemented with 5% hemolysed horse blood (Oxoid, England), and Peston *Campylobacter* selective supplement (SR 0117E, Oxoid, England), which contain polymyxin B2, rifampicin, trimethoprim and cyclohexamide. All the swab samples were streaked on to petri dishes containing media within 3 hours after collection and were incubated at 42°C. As the additional number of positive samples obtained by prolonged incubation is very low, the samples were incubated for 48 hours. The required microaerobic atmosphere of 5–10% carbon dioxide was maintained by using an incubator fitted to a carbon dioxide gas tank. Once a week, the incubator was disinfected and water in the humidification pan was changed.

After incubation, colonies having mucoid grayish white or translucent white to spreading transparent colonies resembling droplets of water sprayed on the medium were further examined

based on the characteristic gram staining reactions, motility and positive tests for oxidase, catalase, hydrogen sulphide production and nitrate reduction.

When the cell morphology cannot be determined from the motility test, then a Gram stain method was performed. For the oxidase test, a piece of filter paper was moistened in a petri dish with 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride. The test bacterium was streaked firmly across the filter paper with a glass rod. A dark purple color along the streak line within 10 seconds indicated a positive reaction, Absence of color change or a purple color that develops later was considered to be a negative reaction. Whereas in catalase test, a loopful of the bacterial growth was taken from the top of the colonies and the bacterial cells were placed on a clean microscope slide and a drop of 3 percent hydrogen peroxide was added. An effervescence of oxygen gas, within a few second, indicated a positive reaction.

3.6 Species detection

All isolated strains identified as thermophilic *Campylobacter spp.* were tested for hippurate hydrolysis and suspension ability to nalixidic acid and cephalothin. These parameters can form the basis for identification of *C. jejuni*, *C. coli* and *C. lari*. For hippurate hydrolysis test, 2 or 3 colonies were taken from Colombia blood agar (DifcoTM, France) plate cultured for 48 hours. Then, the colonies were emulsified in 1% Sodium hippurate broth and incubated with loose caps at 42 degree centigrade for 48 hours in microaerobic conditions.

Following incubation the broth culture was centrifuged at 2500 rpm for 2 minutes and 0.8 ml of the supernatant was transferred to a sterile clean tube. The hydrolysis of hippurate was detected by addition of 0.2 ml 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 result.

3.6 Data management and analysis

The data collected were entered and managed in Microsoft Excel program. SPSS for windows version 15 (SPSS Inc., Chicago, Illinois, USA) for windows was used for data analysis. A Chi-Square test was applied to examine whether the differences between the values were significant. A p-value of less or equal to 0.05, and chi-square value greater or equal to 3.84 was considered indicative of a statistically significant difference.

3.7 Ethical Considerations

The research project has been approved by the Academic Commission of the School of Veterinary Medicine, Addis Ababa University. Consent from the poultry farms was acquired before the commencement of the study. Prudent care was taken not to disseminate microorganisms during collection of the samples.

4 RESULT

A total of 526 samples, 263 fecal swabs and 263 carcass swabs, taken from chickens slaughtered in 6 poultry farms in Debre Zeit were examined microbiologically for the presence of thermophilic *Campylobacter* species. The scale of production systems of the poultry farms, the sample type, and numbers of total, female and male chickens examined are summarized in table 3.

Table 3: Cross-tabulation of scale of production, farm code, sex of chickens, and sample type of samples collected from 6 poultry farms in Debre Zeit, Ethiopia (2010-2011).

Scale of Production	Farm Code	No. of chickens Examined	Sex		Swab sample type	
			Female	Male	Fecal	Carcass
Large Scale	1	79	68	11	79	79
	2	54	38	16	54	54
Small Scale	3	40	34	6	40	40
	4	28	22	6	28	28
	5	41	30	11	41	41
	6	21	16	5	21	21
Total		263	208	55	263	263

From the total of 526 fecal and carcass swab samples, thermophilic *Campylobacter* species were isolated in 284 (54%) samples. Out of these isolates, 274 (96.48%) were identified as *C. jejuni*, and the rest 10 (3.52%) isolates were *C. coli*. Of the examined 416 fecal and carcass swab samples from females (n=208), 53.13% were positive for campylobacteriosis; while the isolation rate in males (n=55) was found to be 56.36% of 110 samples (table 4).

Table 4: Distribution of isolation rates of thermophilic *Campylobacter* infection on the basis of sample type and species isolated from samples acquired from 6 poultry farms in Debre Zeit, Ethiopia (2010-2011).

Sample type	No. examined	Sex		Total Positives	Species Isolated	
		Female	Male		<i>C. jejuni</i>	<i>C. coli</i>
Fecal swab	263	208	55	149 (56.65%)	141 (56.61%)	8 (3.04%)
Carcass swab	263	208	55	135 (51.33%)	133 (50.57%)	2 (0.76%)
Total	526	416	110	284 (54%)	274 (52.1%)	10 (1.9%)

The overall isolation rates in the large and small scale farms, where the study is conducted, were 42.11% and 68.4%, respectively. There were higher incidences in small scale farms, with the highest incidence recorded in Farm 4 (73.21%), followed by Farm 6 (71.43%), Farm 3 (67.5%), and Farm 5 (56.1%). Slightly lower isolation rates (37.34% and 49.07%) were observed in the two large scale farms (Table 5). *C. coli* were isolated only from the two large scale farms. Out of the 10 *C. coli* isolates, Farm 1 attributed to 4 (40%) and the other 6 (60%) were isolated from samples taken from Farm 2.

Thermophilic *Campylobacter* spp. were isolated from 149 (56.65%) of the 263 fecal swab samples investigated from six poultry farms. Among the 149 viable isolates, 141 (94.63%) were identified as *C. jejuni* while only 8 (5.37%) were identified as *C. coli*.

135 (51.33%) of the carcass swab samples were found positive for thermophilic *Campylobacter* spp. 133 (89.26%) of these isolates were identified as *C. jejuni* and 2 (1.34%) were isolates of *C. coli*.

Table 5: Proportion of isolation of thermophilic *Campylobacter* species in six large and small scale poultry farms in Debre Zeit, Ethiopia (2010-2011).

Scale of production	Farm	No. of samples	Total Positives
Large scale	1	158	59 (37.34%)
	2	108	53 (49.07%)
Small scale	3	80	54 (67.5%)
	4	56	41 (73.21%)
	5	82	46 (56.1%)
	6	42	30 (71.43%)

Table 6: Potential risk factors associated with thermophilic *Campylobacter* infection in chickens slaughtered in 6 poultry farms in Debre Zeit, Ethiopia (2010-2011).

Category		No. examined	Positives (%)	X ²	P-value	OR (95% C.I)
Scale of production	Large	266	42.11	25.19	0.0001	-
	Small	250	68.4			0.404 (0.28-0.58)
Farms	1	158	37.34	38.98	0.0001	0.238 (0.11-0.50)
	2	108	49.07			0.385 (0.18-0.84)
	3	80	67.5			0.831 (0.37-1.88)
	4	56	73.21			1.093 (0.49-2.67)
	5	82	56.1			0.511 (0.230-1.14)
	6	42	71.43			-
Sex	Female	416	53.13	0.526	0.468	-
	Male	110	56.36			-
Swab type	Fecal	263	56.65	1.72	0.189	-
	Carcass	263	50.95			-

There was a statistically significant difference ($X^2 = 25.19$, $p = 0.0001$) in the isolation rates of *Campylobacter* species among the production scales. Production scale was found to be significantly associated ($p = 0.0001$) with isolation rate and the odds ratio (OR) was 0.404. Therefore, this indicates that small scale production is a risk factor for isolation rate. i.e. *Campylobacter* spp. were isolated 0.404 times (OR = 0.404, 95% CI = 0.28-0.58) more likely in small scale production system as compared to the large scale production system.

There was also a statistically significant difference ($p = 0.0001$) in the isolation rates of campylobacters among the broiler farms studied with isolation rate in Farm 4 (one of the small scale farms) being 1.093 (OR = 1.093, 95% CI = 0.49-2.67) times more than the rest of the farms. No statistically significant difference in the isolation rates of *Campylobacter* spp. between the sexes ($p = 0.468$) and between the types of swab samples ($p = 0.189$) taken.

5 DISCUSSION

Avian species, especially domestic poultry including chickens, turkeys, ducks and geese, are frequently infected with the members of *Campylobacter* species, primarily *Campylobacter jejuni* and *C. coli* (Stephens *et al.*, 1998; Sabin *et al.*, 2003). Foods of animal origin, especially poultry, have been incriminated as the main sources for *Campylobacter* infection in humans (Garcia *et al.*, 1983; NCCLS, 2000). Most human *Campylobacter* infections are caused by members of thermophilic *Campylobacter* spp., namely *C. jejuni*, *C. coli* and *C. lari* (Lema and Daniel, 2008).

It is now accepted that campylobacteriosis is acquired through the consumption of contaminated foods (Humphrey *et al.*, 2007). Raw meat, particularly from beef, is widely consumed in the country increasing the likelihood of pathogen transmission to humans. In Ethiopia, few published reports are available regarding the occurrence of *Campylobacter* in food animals (cattle, sheep, goats and chickens) (Asrat *et al.*, 1997) and their products (Gedlu and Assefa, 1996).

In the present study, the frequency of isolation of thermophilic *Campylobacter* species in chicken carcass was 51.33%. A study by Lema and Daniel (2008) around the same area revealed a lower incidence (21.7%), which could be attributed to the lower number of chicken carcasses examined (n=30). The very low occurrence of *C. coli* in the aforementioned study was similar to the current study; however, higher relative incidences of *C. coli* (up to 47.1%) have been observed in Iran (Rahimi and Tajbakhsh, 2008), Japan (Sallam, 2007), Korea (Kim *et al.*, 2010).

However, a higher prevalence of thermophilic campylobacters in chicken carcasses (68.1%) was recorded in another region of Ethiopia (Jimma) by Tesfaye and others (2005). Higher prevalence in carcasses have also been observed in Morocco, 62% (Jouahri *et al.*, 2007); Japan, 64.7% (Sallam, 2007); Senegal, 68.4% (Cardinale *et al.*, 2006); UK, 76.1% (Stern and Robach, 2003); and Korea, 78.95% (Kim *et al.*, 2010). Lower prevalences were reported from Egypt, 10% (Hassanain, 2011); Denmark, 16.28% (Heuer *et al.*, 2001); Thailand, 20.5% (Murphey *et al.*, 1996); Brazil, 38% (Dias *et al.*, 2004); and Italy, 46.3% (Di Giannatale *et al.*, 2009). Isolation rate of *campylobacter* spp. in a study in South Africa was lower (32.3%) than the current study

(van Nierop *et al.*, 2005). The isolation rate in chicken carcasses in this study was similar to the one recorded in Iran, 50.1% (Rahimi and Tajbakhsh, 2008).

Out of 135 viable isolates from the carcass swab samples, 133 (98.52%) were identified as *C. jejuni*, and the rest 2 (1.48%) were *C. coli* isolates. Skirrow and Benjamin (1982) reported *C. jejuni* to be recognized as the most important causal agent of human *Campylobacter* enteritis. Moreover, Calenk *et al.* (1991) reported that *C. coli* were occasionally involved in human infections. A review by Corry & Atabay (2001) reported a *C. coli* incidence of 6% to 50% on poultry carcasses. In another study, *C. coli* accounted for 17.6% of the cases of human infection in Ethiopia (Asrat *et al.*, 1997).

The prevalence of thermophilic campylobacters in fecal samples often ranges from around 30 to 100% (Jay, 2000). Other reported prevalences of *Campylobacter* spp. in broiler flocks ranged between 35-57% in Europe (van de Giessen *et al.*, 1996) and 64-100% in Africa (Kazwala *et al.*, 1990; Simango and Rukure, 1991; Osano and Arimi, 1999). In the current study, out of the total 263 fecal swab samples, 56.65% of the samples were positive for *Campylobacter* infection.

Furthermore, among the 149 thermophilic campylobacters isolated from the fecal samples, *C. jejuni* accounted for 141 (94.63%) and *C. coli* for 8 (5.37%) of the colonization rates. Similar results have also been reported in other countries indicating a higher prevalence of *C. jejuni* than *C. coli* in poultry farms compared to other *Campylobacter* species (Aho and Hirn, 1988; Humprey *et al.*, 1993; Steinhäuserova *et al.*, 2001; Padungton and Kaneene, 2003). *C. jejuni* is cause of “vibriotic hepatitis” in poultry and has been isolated from a wide range of animals and birds with or without the disease (Smith, 1996).

Although the prevalence in carcasses examined in Italy was lower, the prevalence in fecal samples, 63.9% (Di Giannatale *et al.*, 2009), was higher than the current study. The incidence in fecal swabs was also lower than other reports from Denmark (Heuer *et al.*, 2001), Northern Ireland (Oza *et al.*, 2003), Tanzania (Mdegela *et al.*, 2006), Cameroon (Wouafo *et al.*, 2010) and Malaysia (Saleha, 2002), which reported incidence rates of 60.0%, 67.7%, 69%, 71.5% and 72.6%, respectively.

This variation may reflect, at least in part, different sampling and isolation methods used. Zootechnical parameters, including the number of animals per farm, climatic conditions, and distance between farms, may all influence flock prevalence (Evans, 1997).

Sensitivity to nalidixic acid used to be one of the most commonly tested characteristics, but nowadays may give difficulties in interpretation, both due to an increase in nalidixic acid-resistant strains of *C. jejuni* and *C. coli* and to the isolation of nalidixic acid-sensitive genogroups of *C. lari* (Vandamme, 2000; On and Jordan, 2003). *Campylobacter* prevalence in poultry flocks is known to vary with season, showing a pronounced summer peak (Humphrey *et al.*, 1993). However, this study was not designed to investigate seasonality.

Poultry appeared to be significant sources of *Campylobacter*, and chickens were found to be healthy carriers of campylobacters, mostly *C. jejuni* (Padungton and Kaneene, 2003). Chicken products were found to be important sources of *Campylobacter* infection in humans in both Asia (Rasrinaul *et al.*, 1988) and Africa (Simango and Rukure, 1991). Although a higher prevalence of *Campylobacter* on poultry products has been reported in developing countries, local customs like eating well-cooked poultry are believed to reduce the risk of infection, compared to developed countries in Europe, where the consumption of undercooked poultry is more common (Padungton and Kaneene, 2003).

There were no statistically significant differences in the isolation rates of *campylobacter* spp. between the sexes ($p = 0.468$), and between the types of swab samples ($p = 0.189$) examined. However, a statistically significant difference ($p = 0.0001$) in the isolation rates of campylobacters among the broiler farms studied was indicated, with isolation rate in Farm 4 (one of the small scale farms) being 1.093 (OR = 1.093, 95% CI = 0.49-2.67) times more than the rest of the farms. Production scale was found to be significantly associated ($p = 0.0001$) with isolation rate and the odds ratio (OR) was 0.404, indicating small scale production is a risk factor for isolation rate. This finding is in consistence with a study in Morocco (Jouahri *et al.*, 2007).

The higher frequency of *Campylobacter* contamination in carcass is in proportion to higher slaughter capacity (Wagenaar *et al.*, 2006; Alleena *et al.*, 2007). Variation in the application of hazard analysis and critical control points and good manufacturing practice principles will influence product contamination (Humphrey *et al.*, 2007) and may thus explain the variation

found between the various poultry farms. In general, larger poultry farms are usually on top of implementing food safety regulations. Smaller companies, however, may lack trained technical employees to adopt good hygienic practices and may thus react slower to new regulations (Holt and Henson, 2000). Therefore, a large slaughterhouse (with high numbers of slaughtered animals) is assumed to give lower *Campylobacter* prevalences due to greater hygiene standards (Humphrey *et al.*, 2007).

The isolation rates vary from company to company, from farmer to farmer, and between flocks. Where numbers of colonized birds are lower than the average for housed poultry, it is likely that *Campylobacter* will only have become established towards the end of the commercial life of the flock. It is also possible that some strains of *Campylobacter* may spread more slowly in broiler flocks (ACMSF, 2004).

6 CONCLUSIONS AND RECOMMENDATIONS

In the current study, the overall prevalence of thermophilic campylobacters in broiler flocks and broiler carcasses in 6 large and small scale farms in Debre Zeit was 54%. *Campylobacter* species were isolated from 56.65% of the fecal swab samples and 51.33% of the carcass swab samples.

The results of this study indicate that thermophilic campylobacters are very frequent among poultry in Ethiopia. *Campylobacter* contamination of carcasses during processing constitutes a risk for consumers. This consequently suggests possible risks of infection to people through the consumption of contaminated poultry products or through contact with infected poultry. Raw poultry meat is and will continue for the foreseeable future to be a source of *Campylobacter* into domestic and catering premises. Such foods must therefore be recognized at all times as presenting a risk if not adequately cooked, or if they come into contact with ready-to-eat foods.

Since the available evidence shows that poultry constitute a major reservoir for these organisms, interruption of transmission to human beings from these sources should be given a high priority. Coordinated actions are needed to reduce or eliminate the risks posed by thermophilic *Campylobacter* species at a number of stages in the food chain. These include good agricultural practice, good manufacturing practice and hazard analysis critical control points (HACCP) at every stage of the meat supply chain, from the farm, through the abattoir, to the retailer, and those involved with the handling and processing of poultry. Awareness of the necessity for hand washing after contact with animals and their products and the importance of proper cooking and handling of foods of animal origin are probably as important in preventing *Campylobacter* infections.

Health authorities need to enforce legislative requirements aimed at making sure that the poultry farms operate to recognized hygiene standards. Failure to meet these requirements should result in enforcement action against premises, and this should ultimately lead to prosecution and suspension and/or revocation of their license to operate. Further studies are necessary to determine whether and how meat safety can be improved via elimination/ reduction of microbial contamination on poultry carcasses.

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ANNEX 1: Data collection sheet.

Sample code	Growth on media @ 42°C	Motility	Gram's reaction	Catalase	Oxidase	H ₂ S production	Nitrate reduction	Hippurate hydrolysis

ANNEX B: Bacteriological media and chemicals prepared and utilized in current study.

***Campylobacter* agar base (CM689, Oxoid, England)**

Mode of Action:

A nutrient-rich culture medium and an O₂-deficient, CO₂-enriched atmosphere ensure that *Campylobacter* grows well. The antibiotics which are added as a *Campylobacter* selective supplement largely inhibit the accompanying microbial flora.

Typical formula (gm/l):

‘Lab-Lemeo’ powder 10.0,

peptone 10.0,

Sodium chloride 5.0 and

Agar 12.0.

Directions: suspend 18.5 g of *Campylobacter* agar base (CM689) in 475 ml of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121⁰C for 15 minutes. Cool to 50⁰C. Aseptically add 25 ml of lysed (sheep) horse blood and 1 vial of Preston *Campylobacter* Selective Supplement (SR117) reconstitute as directed.

Peston *Campylobacter* selective supplement (SR 0117E, Oxoid, England)

Contain polymyxin B2, rifampicin, trimethoprim and cyclohexamide.

Tryptic Nitrate Medium (DEFCO, France)

Formula per liter:

Becton Tryptose 20.0 gm,

Becton Dextrose 1 gm,

Sodium phosphate 2 gm,

Potassium nitrate 2gm,

Becton Agar 1 gm.

Direction: to rehydrate the medium suspend 25 grams in 100 ml cold distilled water, heat to boil to dissolve completely. Sterilize in the autoclave for 15 minutes at 121°C . Inoculate with test organism and incubate at 42°C for 24 hours.

Test for nitrate reaction

Reagent A: sulfalinic 5 gm, acetic acid (5M)

Reagent B: N-N-dimethyl-naphthylamine 3 ml, acetic acid (5M) 500 ml.

Direction: add 3 drops of reagent A, then 3 drops of reagent B to the suspension of organism in broth. Wait for 30 minute for 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 color did not develop.

Difco™ Colombia Blood Agar Base (DIFCO, France)

Approximate formula per liter:

Pancreatic Digest of Casein, 10.0 gm

Protease peptone, 5.0 gm

Yeast extract, 5.0 gm

Agar, 12 gm

Direction: Suspend 39 gm of Colombia Blood Agar powder in 1 liter of distilled water and heat with frequent agitation and boil for 1 minute to completely dissolve and autoclave at 121°C for 15 minutes. For preparation of blood agar, cool the base to $45-50^{\circ}\text{C}$ and add 5% sterile, defibrinated blood, mix well and dispense an approximate of 12 to 15 ml into sterile Petri dishes.

Muller Hinton Agar (Oxoid, England)

Formula:

Beef infusion 300.0 gm,

Casamino Acid Technical 17.5 gm,

Starch 1.5 gm,

Agar 17.0 gm.

Direction: Suspend 38 gm of the powder in liter of purified water; mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave for 15 minutes at 121°C . Avoid over heating. Cool to 50°C and aseptically add 25 ml of horse/sheep blood.

SIM Medium (Becton, Dickinson, USA)

Formula per liter:

Pancreatic Digest of Casein 20.0 gm,

Peptic Digest of Casein 6.1 gm,

Ferrous Ammonium Sulphate 0.2 gm,

Sodium Thiosulphate 0.2 gm,

Agar 3.5 gm.

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

Sodium Hippurate Broth (Becton, Dickinson, USA)

Formula per liter:

Heart Muscle Infusion from (solids) 10.0 gm,

Peptic Digest of Animal Tissue 10.0 gm,

Sodium Chloride 5.0 gm,

Sodium Hippurate 10.0 gm.

Direction: Dissolve 10.0 gm in 1 liter of distilled water and distribute into final containers.
Sterilize by autoclaving at 121°C .

Ferric Chloride Test Reagent

Formula for 12% acid ferric chloride solution:

Ferric chloride 12.0 g,

Concentrated hydrochloric acid 5.4 ml,

Distilled water 94.6 ml.

Direction:

1. Add approximately 75 ml of distilled water to a 100 ml volumetric flask.
2. With transfer pipette add 5.4 ml HCL to flask, running the acid down the sides of the flask.
3. Add 12.0 g of ferric chloride.
4. Dissolve by warming gently, swirling contents to mix well.
5. Bring volume up to 100 ml 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 a non-inoculated tube as a negative control and a positive control (type cultures).

Incubate tubes with loose caps for 48 hours at $35 \pm 2^{\circ}\text{C}$ in a microaerobic atmosphere.

Following incubation, centrifuge all cloudy cultures and use the supernatant in the test.

Aseptically transfer its 0.8 ml supernatant to small test tubes using sterile pipette, add 0.2 ml 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.

9 SIGNED DECLARATION SHEET

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

Dr. Yohannes Semahegne

This thesis has been submitted for examination with my approval as a University advisor

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