ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

STUDY ON THE PREVALENCE OF THERMOPHILIC *CAMPYLOBACTER SPECIES* IN SHEEP AND GOAT CARCASSES AT HELIMEX EXPORT ABATTOIR

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Public Health

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ABBRIVATIONS

AAU	Addis Ababa University
CDSC	Center for Disease Surveillance and Control
CT	Cholera Toxin
DNA	Deoxyribonucleic acid
EC	European Commission
ELISA	Enzyme Linked Immuno Sorbent Assay
FVM	Faculty of Veterinary Medicine
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practices
GBS	Guillain-Barre' Syndrome
GC	Content of guanine (G) and Cytosine (C) in DNA
GM	Ganglion-side Membrane
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HL	Heat Labile
HS	Heat-Stable
ISO	International Organization for Standardization
IU	International Unit
LPSN	List of Prokaryotic Names with Standing in Nomenclature
LT	Heat-Labile Toxin
MIC	Minimum Inhibitory Concentrations
NAPRI	National Animal Production Research Institute
NMSA	National Meteorology Service Agency
PCR	Polymerase Chain Reaction
TMAO	trim ethylamine-N-oxide hydrochloride
VBNC	Viable but non-culturable campylobacters
VMAT	Vaginal mucus agglutination test
UNIDO	United Nations International Development Organization
WHO	World Health Organization of the United Nations

ABSTRACT

Campylobacter jejuni and *C. coli* are frequent worldwide causes of food-borne gastroenteritis in humans. A study on the prevalence of thermophilic C*ampylobacter* species from the carcasses of slaughtered sheep and goats was undertaken at Hashim Nuru Jiru Ethiopia livestock and meat import-exporter (HELMIX) export abattoir in Debre-Zeit, Ethiopia from November 2007 to April 2008. A total of 218 sheep and 180 goat carcasses (398 total carcasses) were examined from carcass swabs taken from crutch, abdomen, thorax and breast areas. From each slaughtered animal, carcass swab was taken only from one of these sites on the carcass but each swabbing site was swabbed for three different operations in the abattoir namely before evisceration, after evisceration and after washing. A total of 654 swab samples were collected from 218 sheep carcasses comprised of 56 crutch swabs, 49 abdomen swabs, 50 thorax swabs and 63 breast swabs before evisceration, after evisceration and after washing. Similarly, 540 swabs from 180 goat carcasses were collected consisting of 52 crutch swabs, 46 abdomen swabs, 42 thorax swabs and 40 breast swabs from each of the three slaughter operations. Thus from the three operations a total of 1194 swabs were analyzed. Bacteriological analysis of the samples was conducted in the Microbiology laboratory, Faculty of Veterinary Medicine, Debre-Zeit following the techniques recommended by the International Organization for Standardization (ISO, 2002).

From a total of 398 carcasses examined, 40 carcasses were positive for *Campylobacter* with contamination rate of 10%. Per contamination rate with either *C. jejuni* or *C. coli* were 10.6% (n=218) and 9.4% (n=180) for sheep and goat carcasses, respectively. However, statistically significant difference was not detected in the rate of carcass contamination between sheep and goat carcasses (p=0.72). The most prevalent thermophilic *Campylobacter* species recovered from the sheep and goat carcasses was *C. jejuni* accounting for 7.3% (n=398), followed by *C. coli* 2.7% (n= 398). Out of the 40 positive samples the proportion of the *Campylobacter species* was 72.5% and 27.5% for *C. jejuni* and *C. coli* respectively. This variation in the isolation rate between the two *Campylobacter species* was statistically significant (P=0.003). Though there was no statistically significant difference (P=0.57) in the rate of carcass contamination among the four swabbing sites, the highest contamination rate was observed in the breast area at a rate of 12.6% (n=103) followed by abdomen with contamination rate of 11.6% (n=95). Highest rate of carcass contamination was observed after evisceration as compared to prior evisceration and after

washing (p=0.000). Washing of the carcass did not reduce carcass contamination in the slaughtered sheep (χ^2=0.18; P=0.68), however there was a substantial reduction in the level of carcass contamination after washing in goat carcasses ((χ^2=10.72; P=0.001).

The present study revealed the existence of severe cross contamination during slaughter operations particularly during evisceration. Carcass contamination by *Campylobacter* can be reduced, and thus its public health impact, through good hygienic practices in the abattoir.

Keywords: Abattoir, C. *jejuni*, *C. coli*, Debre-Zeit, Ethiopia, Sheep and goat carcass.

1. INTRODUCTION

The genus *Campylobacter* is of great importance in human medicine and food safety, in addition to its veterinary importance. Since McFadyean and Stockman (1913) first isolated the organism from aborted ovine fetuses at the beginning of the 20th century, and Smith and Taylor (1919) proposed the name *Vibrio fetus* for the bacterium, a huge amount of knowledge has been discovered about this fascinating organism. Present knowledge now includes the complete genome sequencing of *C. jejuni* (Parkhill *et al.,* 2000), the most important species in human infection. Despite this, many details regarding the pathogenicity of the organism and the epidemiology of human campylobacteriosis remain unknown.

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

Campylobacter infects the gastrointestinal tract of food animals and man. *Campylobacter jejuni* and to a lesser extent *C. coli* are the major causes of diarrhea in humans. Human beings are a relatively minor source of infection, although person-to-person spread has been documented among young children (Skirrow, 1991). The most common causes of campylobacteriosis in food

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

Campylobacter enteritis in man has been diagnosed with increasing frequency over recent years, mainly due to *C. jejuni*, *C. coli* and *C. lari* (Davis *et al.*, 1990; Radostits, 2001). Thermophilic *Campylobacter* species, particularly *C. jejuni* and C. *coli* are recognized as one of the most frequent causes of acute diarrhea disease in humans throughout the world (Nachamkin, 1995; Taylor and Blaser, 1991). They are among the major microorganisms causing diarrhea in Europe, the United States and other industrialized countries. Besides, campylobacter infections are hyper endemic among the infant populations of underdeveloped countries (Jiménez, *et al*., 1994). *Campylobacter jejuni* is considered to be one of the principal bacterial agents causing enteritis and diarrhea in man, particularly in developed countries, where the incidence is similar to that of enteritis caused by *Salmonella*. Over 80% of human cases are caused by *C. jejuni* and about 10% of cases are caused by C. coli. Other *Campylobacter* species, such as *C. upsaliensis* and *C. lari*, may also be associated with human diarrhea (Lastovica and Skirrow, 2000). In susceptible humans, *C. jejuni* and *C.coli* infections are associated with acute enteritis and abdominal pain lasting for up to 7 days or more. Although such infections are generally self-limiting, complications can arise and may include bacteraemia, reactive arthritis, and abortion (Skirrow and Blaser, 2000). The infection is a very wide spread diarrhea disease in developing world commonly affecting babies and young children and in individuals with debilitating diseases such as HIV/ AIDS. *Campylobacter* infections usually occur following ingestion of improperly handled or cooked food. Campylobacteriosis is considered a zoonotic disease, and animals such as poultry, pigs, cattle, caprine and ovine may act as reservoirs for *Campylobacter (*Jimenez, *et al.*, 1994).

There are very few studies conducted on human campylobacteriosis in Ethiopia. A study conducted at Tikur-Anbessa and Ethio-Swedish children's hospitals indicated that

Campylobacter species are important causes of diarrhea both in adults and children in which *C. jejuni* accounted for 82-84% of the isolates. *Campylobacter* species were isolated from the stool of 60(13.8%) children out of the 434 samples investigated by cultural examination (Daniel *et al.*, 1999). However, no citable work has been done so far regarding the epidemiology, economic and health significance of campylobacteriosis both in animals and humans and the role of animals as the epidemiologic factor for human campylobacteriosis in Ethiopia.

Ethiopia has huge livestock population, which is not yet exploited its productions. In spite of this, the country gains negligible foreign exchange through export of chilled carcasses to the Middle East countries. Reduced export among other factors is attributed to prevalent livestock diseases and poor hygienic practices in the export abattoirs of the country. Meat export is a competitive business, which demands the supply of safe and hygienic meat to international markets. Hygienic meat production requires the control of bacterial contamination in general and that of campylobacter in particular that have impact on meat quality and/or public health.

The prime objective of this research was to study on the prevalence of thermophilic *Campylobacter species* in sheep and goat carcasses at HELIMEX export abattoir

2. LITERATURE REVIEW

2.1. Etiology

In 1963, Sebald and Véron proposed that *Vibrio fetus* and *Vibrio bubulus*, based on a considerably lower content of guanine and cytosine (GC) in DNA than other vibrios, should form a new genus, *Campylobacter* (from Greek καμπυλος (kampulos) = curved and βαχτηρς (baktron) = rod). Ten years later, Véron and Chatelain (1973) designated and described the neotype strain for the type species, *Campylobacter fetus*, of the new genus. They also proposed the transfer of *Vibrio jejuni* and *Vibrio coli* to the genus Campylobacter.

Since then, the genus *Campylobacter* has undergone several revisions, and species originally assigned to *Campylobacter* have formed new genera, the most notable being Helicobacter and Arcobacter (Vandamme *et al.,* 1991). The genus *Campylobacter*, *Arcobacter* and *Sulfurospirillum* belong to the family Campylobacteriaceae, which together with some other genera and unnamed *Campylobacter*-like organisms; form a separate phylogenetic branch, known as rRNA super family VI, within the class Proteobacteria (Vandamme *et al.,* 1991). According to the current version of the List of Prokaryotic Names with Standing in Nomenclature, (LPSN) (Euzéby, 2005), the genus *Campylobacter* consists of 17 species, whereof four are further divided into two subspecies each Of these 21 taxons, 15 were first described in 1981 or later.

According to the LPSN, *Campylobacter sputorum* is divided into two subspecies: *C. sputorum* subsp. sputorum and *C. sputorum* subsp. bubulus (Euzéby, 2005). However, On *et al.* (1998) proposed that the infrasubspecific divisions of *C. sputorum* should be revised to include three biovar of the species, based on their ability to produce catalase and urease: *C. sputorum* by sputorum (Catalase- and urease-negative), *C. sputorum* by faecalis (Catalase-positive, urease-negative) and *C. sputorum* by paraureolyticus (Catalase-negative, urease-positive). They recommended that strains previously assigned to *C. sputorum* subsp. bubulus should be redesigned as by sputorum (Roop *et al*., 1985; On *et al.*, 1998).

The family Campylobacteriaceae is classified under Phylum Proteobacteria, Class Epsilon Proteobacteria and Order Campylocacteriales, which were approved by (Vandamme and On, 2001). During the last 30 years differentiation of the *Campylobacter* genus from vibrio, groups of

Campylobacter-like organisms were detected, described and identified as new species, or as different biotypes of the existing species. Phylogenetic studies in late 1980's revealed genotypic heterogeneity among the species with three major clusters, rRNA homology groups, were formed (Vandamme *et al.,* 1991). *Campylobacter* was thus divided into three genera with revised genus descriptions, and new names were proposed for the remaining two genera: Arcobacter and *Helicobacter* (Schumacher *et al*., 1992). The genera *Campylobacte*r and *Arcobacter* formed a family of Gram-negative, nonsaccharolytic bacteria with microaerobic growth requirements and a low guanine and cytosine (G+C) content. Members of this family are encountered mainly as commensalisms or parasites 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). *Campylobacter* contains DNA with a G+C content between 29 and 36% (Veron and Chatelain, 1973). Most species are microaerophilic, although some show a range of oxygen tolerance, some are almost anaerobic, and others grow best in the presence of 5-10% oxygen (Doyle, 1986). The aerobic species of *Campylobacter* were reclassified as genus *Arcobacter* (Vandamme *et al.,* 1991). In 1963, when Campylobacter first described, the genus comprised of two species. At present the genus contains 16 species and six subspecies. The 16s rRNA gene, used to determine phylogenetic relationships among all living organisms, played a major role in all previous extensive rearrangement of *Campylobacter* taxonomy (Vandamme *et al*., 1991).

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

DNA: rRNA hybridization has demonstrated that *C. jejuni, C. coli, C. lari* and *C. upsaliensis* form a separate rRNA sub cluster within rRNA cluster I (constituting the genus Campylobacter) in rRNA super family VI (Vandamme *et al.,* 1991). *Campylobacter 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. lari* and *C. jejuni*, was described. *Campylobacter jejuni, C. coli, C. lari* and *C. upsaliensis* are often referred to as thermophilic or thermo tolerant 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 *Campylobacter* (Doyle and Roman, 1981). However, this terminology is not completely clear-cut, as neither *C. jejuni* subsp. *doylei* nor the recently described and genetically related *C. insulaenigrae* grows at 42°C.Moreover, several other *Campylobacter* species grow at 42°C and consequently may be called thermo tolerant *Campylobacter.* It is notable that the described use of "thermophilic" and "thermotolerant" is appropriate only in the strict context of Campylobacters, as thermophilic/thermo tolerant as general microbiological terms refer to microorganisms that thrive in or tolerate temperatures above 50°C. 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 *Campylobacter* 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). *C. jejuni* is further divided into two subspecies: *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. doylei differs from subsp. *jejuni* in that it does not reduce nitrate or grow at 42°C Hereafter in this thesis, *C. jejuni* refers to *C. jejuni* subsp. *jejuni*.

The second most common Campylobacter species isolated from humans is *C. coli*. The organism was first isolated from swine with swine dysentery (Doyle, 1944), and is the primary *Campylobacter species* 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). *Campylobacter Coli* differs phenotypically from *C. jejuni* primarily by its inability to hydrolyze hippurate. *Campylobacter lari* differs from other thermophilic campylobacters 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). It was first isolated in 1976 from human faeces, but most strains are found in wild birds (Benjamin *et a*l., 1983; Waldenström *et al.,* 2002).

During the 1980s, several authors reported the isolation of Catalase-negative or weakly positive thermo tolerant campylobacters from dogs; cats and humans. The organisms were called CNW

(Catalase-negative or weakly positive) campylobacters until 1991, when the new species *C. upsaliensis* was proposed.

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

Members of the genus *Campylobacter* are chemoorganotrophs, unable to ferment or oxidize carbohydrates. They obtain energy through the respiratory chain, from the metabolism of amino acids, and from the metabolism of intermediates of the tricarboxylic acid cycle (Amano and Shibata, 1992). *Campylobacter species* are oxidizing positive. Though they possess Catalase and super oxide dismutase, these enzymes are overwhelmed by the excess of hydrogen peroxide and super oxide anions formed when they are grown in the presence of atmospheric concentration of oxygen (Hirsh and Zee, 1999). Susceptibility or resistance to nalidixic acid or Cephalothin, hydrogen sulphide production, nitrate reduction growth at 25^oc or 45^oc and the Catalase reaction are some of the criteria on which a definitive identification of the *Campylobacter* species is based on (Quinn *et al.*, 2002).

2.1. 1. Morphology and biochemical characteristics

The genus *Campylobacter* comprises a number of species (Wallace *et al.*, 1998). Members of the genus are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria (0.2-0.8 µm wide and 0.5-5 µm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. These bacteria are microaerophilic, but some can also grow aerobically or anaerobically. They neither ferment nor oxidize carbohydrates. Some species, particularly *C. jejuni, C. coli* and *C. lari*, are thermophilic, growing optimally at 42°c (Hirsh and Zee, 1999). They can colonize mucosal surfaces, usually the intestinal tract, of most

mammalian and avian species tested. The species *C. jejuni* comprises two subspecies (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*) that can be discriminated on the basis of nitrate reduction (subsp. *doylei* does not reduce nitrate). Subspecies *jejuni* is much more frequently isolated than subspecies *doylei. Campylobacter jejuni* and *C. coli* are the major pathogenic species of interest because they are zoonotic agents (Skirrow and Blaser, 2000).

Their characteristic corkscrew-like motion is due to the presence of a single flagellum (occasionally multiple) at one or both cell poles. Nutrient limitation, aeration of the medium and the presence of free radicals affect the transition from spiral to coccoid morphology. This morphologic transition may be caused by certain changes in the structure of the peptidoglycans, mainly due to its enzymatic degradation (Amano and Shibata 1992; Wallace *et al.*, 1998). They survive for a longer time outside the host. The organisms are susceptible to drying, high oxygen conditions and low pH (Pattison, 2001).

Almost all pathogenic *Campylobacter* species are Oxidase and Catalase positive and unable to grow in 3.5% Nacl. Additionally, susceptibility to nalidixic acid and Cephalothin is important differential characteristics among species. Susceptibility is determined by inoculating a 5% sheep blood agar plates or Mueller-Hinton agar plate with a 0.5 McFarland turbidity suspension of the organism as for agar disk diffusion susceptibility testing placing 30μg disks on the agar surface and incubating micro-aerobically at 37^{o}c.

Cells exposed to oxygen or in old cultures may become coccoid (Ng *et al.*, 1985). The bacterium is motile in a characteristic corkscrew-like manner by means of a single unsheathed flagellum at one or both poles (Smibert, 1984). Most campylobacter are microaerophilic and require an oxygen concentration of 3–5% and a carbon dioxide concentration of 10–15%, but some species are anaerobic. Campylobacter are unable to utilize carbohydrates, instead they obtain energy from amino acids or tricarboxylic acid cycle intermediates (Smibert, 1984).

2.1.2. Genotypic properties

For most Campylobacter species, the DNA GC content is 30–36%, although for the entire genus it varies between 29% and 46%, since publishing of the complete genome sequence of *C. jejuni*

has increased rapidly. The chromosome of the sequenced strain is small compared to other prokaryotes, with a low GC content (30.6%), and an unusually high percentage protein coding sequences . In contrast to other sequenced prokaryotes, almost no insertion sequences or phage-associated sequences have been found, and very few repetitive sequences. Another property is the apparent lack of person organization of the genes. An important finding is the occurrence of hyper variable sequences, found mostly in genes coding for biosynthesis or surface structure modification, and closely linked genes .The hyper variable regions of the genome may be important for the adaptation and survival of *C. jejuni* in different environments, and for its pathogenic potential (Parkhill *et al.*, 2000).

2. 1. 3. Growth and survival

Microaerobic atmosphere

An optimal microaerophilic atmosphere for growth of campylobacters is one containing 5–7% oxygen, 10–15% carbon dioxide and 65–85% nitrogen or hydrogen (Butzler & Skirrow, 1979). This can be achieved by evacuating anaerobic jars and filling them with the above gas mixture (Luechtefeld *et al.*, 1982) or by using commercially available gas envelopes. A candle extinction jar is a cheap and simple alternative, although it gives a slightly higher oxygen pressure (Luechtefeld *et al.*, 1982).

Growth temperature and pH

All campylobacters grow well at 37°C. *C. jejuni* has been found to grow between 30°C and 45°C, with an optimal temperature range of 42°C to 45°C, and a pH range of 5.8 to 8.0. No growth occurs below pH 4.9, under otherwise optimal conditions (Doyle and Roman, 1981).

Survival in different environments

As thermophilic campylobacters do not multiply at normal room temperature, the role of *C. jejuni* as a food-borne pathogen is associated with its ability to survive in food during storage and handling. The temperature seems to be a key factor for the survival of *C. jejuni*, and one that determines which effect other adverse conditions will have on the decrease in viable cells. In

cattle slurry, *C. jejuni* survives 41 days at 4°C and 7.7 days at 37°C. Low pH (pH 3.0 to 4.5) results in a reduction in viable cells even in a growth-supporting medium, but the inactivation is much faster at higher temperatures. At 4°C and pH 4.5, more than 4 days is required for a 3-$\log_{10}$ decrease in the number of cells (Doyle and Roman, 1981). *Campylobacter jejuni* has been shown to survive for days or weeks in refrigerated foodstuffs (Zhao, Doyle & Berg, 2000). The number of *C. jejuni* on beef decreased during the first week of frozen storage and remained thereafter constant (Moorhead and Dykes, 2002). *Campylobacter jejuni* has been shown to survive in frozen chicken for more than 12 months (Beuchat, 1987). Recently, *C. jejuni* was shown to be infective for the protozoan Acanthamoeba polyphaga and to survive for longer periods when co cultured with amoebae. Protozoan hosts may play a role in the survival of campylobacters in the aquatic environment, although this remains to be elucidated.

Viable but non-culturable campylobacter (VBNC)

Campylobacters may go into a viable but non-culturable stage. VBNC, viable but non-culturable campylobacters, typically show a coccoid form with intact cell membranes. However, the coccoid form is not necessarily associated with non-culturability. The role of VBNC as infectious agents is still debated. In the study, VBNC were unable to revert to the viable Campylobacter form and colonize chicken gut with normal caecal flora (Ziprin and Harvey, 2004). In choosing a typing method, depending on the aims and settings, are the type ability, reproducibility, discriminatory power, ease of performance and interpretation, and availability, rapidity and cost of a method.

2. 2. Campylobacter in foods

2. 2. 1. Poultry products

The occurrence of *Campylobacter* in all parts of the broiler production chain is well documented all over the world. The *Campylobacter* prevalence in fresh and frozen poultry meat for human consumption varies from 7% to 83% in different countries and investigations (Park *et al*., 1981). Roasted chicken or other poultry products ready for consumption have sometimes been reported to be contaminated by campylobacters. Cross-contamination from raw poultry products due to poor hygiene practices is a suspected cause of this (Quinones-Ramirez *et al.*, 2000.

2.2.2. Other meats

In an investigation in Northern Ireland, no evidence of *Campylobacter* contamination was found on beef carcasses or retail beef or pork (Madden *et al*., 1998). Likewise, a Japanese investigation did not demonstrate any campylobacters on fresh meat from cattle or swine. In contrast, *Campylobacter* contamination of bovine, porcine and ovine liver was found in Britain (Moore and Madden, 1998). Lamb from halal butchers in Britain was found to be contaminated with *C. jejuni and C. coli*. Investigations where all lamb carcasses were *Campylobacter*-negative have been reported in Spain and Northern Ireland (Madden *et al*., 1998).

Hence, although campylobacters are frequently isolated from food-producing animals other than poultry, the prevalence in food products from these animals seems to be low. A possible cause of this difference may be the difference in slaughter procedures between poultry and larger animals.

2. 3. Campylobacteriosis in humans

Spiral-shaped bacteria in faeces from children with enteritis were described by Escherich already in 1886 (Escherich, 1886). However, for many years after 1906, the year of the first successful isolation of the organism that would later become *Campylobacter* (McFadyean and Stockman, 1913), diseases caused by these organisms were of exclusively veterinary concern. campylobacters were first isolated from humans during a milk-borne enteritis outbreak in 1938 and around 1960 a number of "related vibrios", apparently what we now know as *C. jejuni and C. coli*, were isolated from the blood of humans with diarrhea. Nevertheless, it was not until the breakthrough of the culture of campylobacters from faeces in the 1970s (Butzler *et al*., 1973) that the human pathogenic potential of *Campylobacter* was universally recognized. After Skirrow's medium for isolation of campylobacters from faeces was described diarrhea-associated occurrence of campylobacters in humans was reported from many countries, and during the 1980s the reported campylobacteriosis incidence increased rapidly.

The vast majority of campylobacteriosis cases in humans are gastrointestinal infections. The incubation time is usually 2 to 5 days, but may vary from 1 to 11 days (Black *et al*., 1988). Sudden onset of diarrhea, which may be watery or bloody, is the most common symptom. Other

symptoms are abdominal cramps, fever, myalgia, headache, nausea and vomiting. The onset of fever often precedes the onset of diarrhea by 12 to 24 hours (Black *et al.*, 1988). The illness is most often self-limiting, with symptoms diminishing after a few days up to two weeks. Faecal excretions of campylobacters usually continue for two to three weeks. Experimental studies have shown that an ingestion dose of 500 to 800 organisms may be sufficient to cause illness although the attack rate was higher among volunteers given higher doses (Black *et al*., 1988). The dose-response relationship of *Campylobacter* infections has recently been reconsidered (Teunis *et al*., 2005).

A few percent of patients develop reactive arthritis as a sequel to *Campylobacter* enteritis. The interval between the preceding infection and arthritis onset is up to four weeks. Individuals with human lymphocyte antigen B27 (HLA-B27) are more often affected (Peterson, 1994). A more infrequent sequel is the acute immune-mediated inflammation of peripheral nerves known as Guillain-Barré syndrome (Nachamkin, 2001).

2. 3.1. Seasonal variation

A striking feature of campylobacteriosis in temperate countries is the seasonal variation, with one or two incidence peaks occurring in spring, summer or early autumn The seasonal variations in nine 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* prevalence in broilers and other potential sources have been suggested to be related to the seasonal variations in humans. Others propose flies as important vectors for infection transmission during the summer (Nichols, 2005). In Wales, consumption or handling of milk contaminated by birds (magpies and jackdaws) picking at milk bottles was associated with *Campylobacter* infection during a spring incidence rise. Another suggested cause of the seasonal peaks is human behaviors that may be more common during the warmer season, such as barbecuing, camping, swimming in lakes and rivers, and drinking water from streams and lakes (Nylen *et al.*, 2002).

2.4. Epidemiology

Campylobacter species have been known as the cause of diseases in animals since 1913 (Blaser and Reller, 1981). Prior to the 1970s, *Campylobacter species* were known primarily to veterinary microbiologists as the organisms that caused spontaneous abortions in cattle and sheep and as the cause of other 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 the known reservoirs for *Campylobacter species.* The *Campylobacter species* are microaerophilic inhabitants of the gastrointestinal tract of various animals, including, cattle, sheep, goat, poultry and pig as well as the reproductive tract of various animal *species*, which acts as source of infection (Ellen *et al.*, 1994; Jay, 2000).

Campylobacter jejuni and *C. coli* are common worldwide causes of human gastroenteritis, in developed as well as developing countries. The reported incidence varies between countries, probably due to differences in surveillance systems as well as real differences in incidence. In 2004, 69 cases per 100,000 inhabitants were reported in Sweden, whereof 35% were reported to be domestically infected. The situation in other Scandinavian countries is similar, with a yearly incidence of about 50, 80 and 75 cases per 100,000 inhabitants in Norway, Denmark and Finland, respectively (European Commission, 2005). However, the proportion of infections acquired within the country is considerably higher in Denmark than Sweden, whereas a higher proportion of cases in Norway is acquired abroad. Hence, considering only domestic cases in each country reveals larger differences between countries.

Especially mild *Campylobacter* infections can be expected to be substantially underreported. Some efforts to estimate the true Campylobacteriosis incidence have been made. Assumed the quota reported: unreported cases of Campylobacteriosis in the United States to be 1:38, based on estimates from salmonellosis data. Others estimate the true incidence to be five to eight times higher than the reported incidence (Mafu, 2000).

2.4. 1. In cattle

Bovine genital campylobacteriosis is a true venereal disease that is characterized primarily by early embryonic death, infertility a protracted calving season, and abortion (Lofstedt, 1998). The disease is common in beef breeds worldwide. The causative agent of bovine abortion,

Campylobacter fetus subspecies venerealis survives on the penis and prepuce of bulls especially within mucosal crypts and within the female reproductive tract (Radostits, 2001). The *Campylobacter fetus subspecies fetus* causes sporadic abortion in cattle and is isolated from the reproductive tract of bull and cows, and there are some evidences that it may cause infertility as in the classical "bovine venereal Campylobacteriosis" syndrome. The *C. jejuni* is isolated sporadically from aborted bovine fetus (Acha and Szyfres, 2001). Abortion occurs in infected females 3% to 5% usually after 5 months of gestation but occasionally at 2 to 3 months (Ellen *et al.,* 1994). *Campylobacter jejuni* has been shown experimentally to be a potential cause of mastitis (Quinn *et al*., 2002).

Both C. *jejuni* and *C. fetus* subspecies *fetus* are incriminated as a cause of diarrhea in cattle (Quinn *et al.*, 2002). Experimentally the organism causes a mucoid diarrhea often with dysentery and fever in calves. The disease is mild and unapparent, without fever, and may be manifested by mild depression and soft feces with the occasional mucous. Most Campylobacter species are found in the alimentary system of healthy, carrier or diarrheic animal (Hirsh and Zee, 1999) the exception being *C .fetus subspecies Venereali*s, which is found only in the reproductive tract of cattle and *C.sputorum subsp. Bobulus* which is found only in the reproductive tract of cattle and sheep (Ellen *et al*., 1994) and also transmitted by contaminated instruments, bedding or by artificial insemination (AI) using contaminated semen. Bulls can also transmit the infection mechanically for several hours after mating with an infected cow (Lofstedt, 1998). The organism remains at the cervicovaginal junction until the end of estrus, then multiply at this site and when conditions are suitable move into the uterus. Further multiplication perhaps active invasion result in inflammation of the uterus with resultant endometritis and cessation of pregnancy and the animal will turn to estrus. In female animals, infected bull deposit *C. fetus subspecies Venerealis* in the vagina at coitus (Radostits, 2001).

2. 4. 2. In Sheep and Goats

Abortion, stillbirths and weak lambs characterize campylobacteriosis in sheep during late pregnancy. *Campylobacter jejuni* and *C. fetus subspecies fetus* are the 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 into a flock (Delong *et al.,* 1996). In Britain, it is the third most

common cause of ewes' abortion (Quinn *et al*., 2002). *Campylobacter fetus subspecies fetus* is one of the agents of ovine Campylobacteriosis that cause abortion in sheep. It is also incriminated as an enteric pathogen that causes enteritis and diarrhea in many species (Smith, 1996). Outbreaks of severe gastroenteritis in fattening lambs have been attributed to *C .jejuni* (Radostits *et al.*, 1994).

Isolation of *Campylobacter species* from rectal swabs or feces samples of healthy goats is being reported. The *Campylobacter species* were isolated from goats in different part of the world. . In Canada, 2.7% of the animals studied found positive for *Campylobacter jejuni* (Prescott and Bruin-Mosch 1981). Turkson *et al.*, (1988), in Kenya, found *Campylobacter species* in 6.3% of the goats sampled, Abrahams *et al.,* (1990) in Ghana detected *C. jejuni*, but not *C. coli*, in a high proportion (33.3%) of the goats tested. These different carriage rates could be attributable to contact of goats with other animal species. Thus, explored the prevalence of *Campylobacter species* in healthy goats kept under various management systems in Tanzania and observed that goats kept away from other farm animals, irrespective of whether the management system was good or poor, were negative for *Campylobacter species.*

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

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

2.4.3. In Pigs

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

The organism may cause diarrhea in nursing piglets and weaned pigs in certain circumstances with high rate of isolation from small intestine of such piglets (Radostits *et al.,* 1994). The *C. sputorum* subsp*ecies mucosalis* is frequently found in association with a group of enteric diseases in pigs called "porcine intestinal adenomatosis" complex. Recently *C. hyointestinalis* has also been isolated from the same lesion. Young adults show cutaneous pallor, weakness and black tarry feces (Radostits *et al.,* 1994). *Campylobacter coli* have been isolated from small intestine of diarrheic piglets. It has been also described to cause dysentery and narcotizing ileitis with mortality rate of more than 50%. Older animals suffer from diarrhea, rapid weight loss and mortality rate of 2-20% (Buxton and Fraser, 1977).

However, free-range pigs from a single organic farm seemed to be colonized with Campylobacter earlier in life than conventional pigs with respect to the occurrence of Campylobacter. For example, the lower animal density probably reduces the infection pressure and roughage stimulates the intestinal flora, which is likely to reduce the susceptibility to infections. Pigs acquire infection through ingestion of feed that has been contaminated with feces of reservoir host (Buxton and Fraser, 1977).

2. 4. 4. In birds

The thermophilic members of the genus *Campylobacter* attribute campylobacteriosis in birds to infections. The three species of significance for bird campylobacteriosis are *C. jejuni, C. laridis*

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

Poultry primarily serve as reservoir host 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, including partridges, pheasants and quails which act as reservoir of infection (Shani, 1998). Despite the fact that *C. jejuni* is the prevalent as an intestinal commensally in floor housed turkeys, broiler breeds 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).

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 the natural hosts for these organisms (Newell and Wagenaar, 2000). Campylobacters have been found in a great variety of bird species, both domesticated and wild. Among domesticated birds, a high prevalence of *C. jejuni and C. coli* is often found in broiler chickens as well as in older birds such as broiler breeder flocks and egg-laying hens. Furthermore, Campylobacter colonization is common in turkeys, geese, ducks, ostriches and quails (Wallace, *et al*., 1998; Cox *et al.*, 2000).

Campylobacters have been isolated from a wide range of wild bird species. However, they are unevenly distributed among species, and the feeding behavior of birds has been shown to influence the Campylobacter colonization rate (Waldenström *et al*., 2002). Campylobacter species 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*. Wild birds have been suggested to be important reservoirs for Campylobacters infecting broilers and humans. However, comparisons of *C. jejuni* subtypes from wild birds with subtypes from humans and chickens reveal only a few common subtypes (Broman *et al*., 2002).

2.4.5. Reservoirs

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

Campylobacter jejuni is not an environmental organism, but is associated with warm-blooded animals. A high percentage of all major meat producing animals have been shown to contain these organisms in their feces, with poultry being prominent. Its prevalence in fecal sample often ranges from around 30% to 100% (Jay, 2000).The *C. jejuni* and *C. coli* have been isolated from the intestine of healthy farm animals, poultry, pets, zoo animals and wild birds. *Campylobacter jejuni* and *C. hyointestinalis* can be found in the rumen and intestine of normal adult cattle and calves. *C. coli* and *C. jejuni* were isolated from rectal swabs taken from dairy cows. *Campylobacter jejuni* has been isolated from samples collected from various species of animals (Table 1).

Table 1: C. *jejuni* isolation rates from various samples taken from different food animals

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

Source: Jay, 2000

Campylobacter species are found on mucous membranes of the reproductive and gastrointestinal tracts and in oral cavities in a great variety of animals. Although the principal organ system in which bacteria are found is typical for most Campylobacter species, few species seem to be strictly host species-specific. *C. jejuni*, the leading cause of human campylobacteriosis, is frequently found in both birds and mammals. Although *C. jejuni* (as well as the classical causative agent of "vibrionic abortion", *C. fetus* subsp. fetus) causes abortion in sheep, and has occasionally been reported in association with various diseases in cattle, poultry, mink, goats (Anderson *et al.,* 1983), horses and dogs. Animals often carry the bacteria without any visible signs of disease or other harm (Davis *et al.,* 1984).

2. 4. 6. Other animals

Campylobacter jejuni and C. coli are often found in faeces from food-producing animals such as cattle, sheep and swine (Rosef *et al*., 1983; Stanley *et al.,* 1998). In cattle and sheep, *C. jejuni* is the most frequently isolated species, with only a small proportion of *C. coli* found. In swine, the ratio between the two species is the opposite, with *C. coli* accounting for the great majority of

isolates. A recent study reported the novel species *Campylobacter lanianae* being the most frequently found Campylobacter species in cattle. Horses and goats seem to be more rare carriers of Campylobacter spp. (Rosef *et al.,* 1983).

Dogs and cats, both with and without diarrhea, are frequent carriers of campylobacters . *C. upsaliensis* is the most frequently isolated species, but *C. jejuni and C. coli* account for a substantial proportion of the isolates. Also *C. helveticus* can be isolated from dogs and cats (Stanley *et al.,* 1992; Moser *et al.,* 2001).

Few studies have addressed the possible occurrence of campylobacters in wild mammals. However, *C. jejuni and C. coli* have been isolated from various wild mammals such as the hare, hedgehog, squirrel, deer, badger, fox, rodents and seal (Rosef *et al.,* 1983).

2.4. 7. Virulence factors

An important mechanism by which bacterial enteropathogenes induce diarrhea is through the production of potent toxins. They produce cytotoxin, enterotoxin or both. Cytolethal distending toxin activity causes certain cell types to become slowly distended, progressing to death. Bacterial toxins in general have been conveniently classified as either membrane damaging, such as haemolysins and phospholipases, or intracellular acting, such as the toxins produced by *Corynebacterium diphtheriae*, *Vibrio cholerae*, and *Shigella dysenteriae* (Radostitis *et al.,* 1994). The latter group is probably directly associated with the mechanisms for inducing diarrhea. These toxins are pro-enzymes that share several modes of action. They bind to specific receptors on the plasma membrane. The *Campylobacter jejuni* secretes toxin similar in activity to the enterotoxin of *Vibrio cholerae* (CT) and the heat-labile toxin (LT) of *Escherichia coli* by increasing intracellular cyclic adenosine monophosphate (cAMP) level and cytoskeletal rearrangement followed by cell death (Cytolethal distending toxin) a protein that was shown to induce hepatitis in mice (hepatotoxins) and protein that has hemolytic activity (hemolysin) (Hirsh and Zee, 1999). Both toxins are immunologic ally similar and bind to the same ganglioside on the target cells. *Campylobacter coli* and *C. lari* produce uncharacterized substances with cytotonic and cytotoxic activity (Jay, 2000).

Campylobacter jejuni secretes a toxin similar to a cholera toxin and heat labile toxin (HL) of *Escherichia coli* by increasing intracellular cAMP and cytoskeleton rearrangements. Both toxins are immunologic ally related and bind to the same ganglioside membranes (GM) on the surface of the target cell. It also produces a mannose-resistant adhesion that binds to a fructose-containing receptor on the target cell. In addition, it survives inside mononuclear phagocytes, implying the existence of other important as yet unidentified surface structure (Hirsh and Zee, 1999).

2. 5. Diagnosis

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

2.5. 1. Direct Examination

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

2.5. 2. Isolation and Identification

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

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

A great variety of selective media and procedures for the isolation of campylobacters exist. Since species differ in their resistance to antibiotics and other selective agents, no single medium is sufficient for the isolation of all *Campylobacter spp*. In most cases, selective media are necessary to enable isolation of the relatively slow-growing campylobacters in samples with a competing normal microbiological flora, for example faeces or food (Butzler and Skirrow, 1979). A prerequisite for recognizing the frequent appearance of *C. jejuni* as a human pathogen, and a starting point for the growing interest in Campylobacter was when developed a selective agar *for C. jejuni* and *C. coli* isolation from faeces. The Skirrow agar is based on blood agar supplemented with trimethoprim, polymixn B and Vancomycin. Many other media have since been developed according to the same principle: suppression of other micro flora by the addition of various selective agents. Both blood-containing media, e.g. Butzler agar and Campy-BAP (Blaser *et al.*, 1980), and charcoal-based media, e.g. mCCDA agar have been shown to be effective for the isolation of Campylobacters from human and animal faeces. Preston agar

(Bolton and Robertson, 1982) was developed for Campylobacter isolation from faeces as well as environmental samples. The membrane filtration technique utilizes the fact that campylobacters, in contrast to most other bacteria, easily pass through filters with a pore size of 0.45 µm). Filtration techniques are especially suitable for the isolation of *C. upsaliensis*, as this species is sensitive to most antibiotics used in other Campylobacter media. A pore size of 0.65 µm enabled a higher isolation rate of *C. jejuni and C. coli.* However, selective media with a high rate of isolation of *C. upsaliensis* as well as other thermophilic campylobacters have been developed (Bolton *et al*., 1988).

Recently, increased interest in Campylobacter species other than *C. jejuni and C. coli* as causes of human enteritis has occasionally resulted in the recommendation to routinely incubate specimens at 37°C instead of 42°C. This is aimed at increasing the probability of isolation of these other species, without any significant decrease in the isolation rate of *C. jejuni and C. coli*. However, a large British study with four participating laboratories found 42°C to be the optimal temperature for the isolation of *C. jejuni*, and that incubation at 37°C significantly reduced the isolation rate of this species (Gee *et al*., 2002). The incubation temperature (37°C or 42°C) had no effect on the isolation rates of Campylobacter from foodstuffs cultured on Preston agar after an enrichment step (Scates *et al*, 2003). However, each temperature was found to select for certain *C. jejuni* genotypes, which led to the recommendation to use both temperatures to detect the widest range of genotypes.

2. 5. 3. Species identification

Phenotypic tests used to differentiate between different Campylobacter species include growth at 25°C and 42°C or 43°C, Catalase production, nitrate and nitrite reduction, H_2 requirement for microaerophilic growth, Indoxyl acetate hydrolysis, growth in the presence of 3.5% Nacl, 1% glycine and 0.1% TMAO, and susceptibility to specific antibiotics such as nalidixic acid and Cephalothin (Skirrow and Benjamin, 1980). *C. jejuni* is the only Campylobacter species that hydrolyses hippurate (*C. jejuni* subsp. doylei may vary in its reaction). Therefore, hippurate hydrolysis has become the most widely used test to identify *C. jejuni*, and especially to differentiate it from the phenotypically and genotypically similar *C. coli* (Lior, 1984). In addition

to observed variability in hippurate reaction (Morris *et al.*, 1985), some strains of *C. jejuni* have eventually been shown to be hippurate-negative (Fermers and Engvall, 1999). This indicates the need for alternative or additional tests. A number of PCR (polymerase chain reaction)-based methods for identifying thermophilic campylobacters have been developed. PCR of the hippuricase gene identifies *C. jejuni* with higher sensitivity than the hippurate hydrolysis test. Other PCRs detect and differentiate all thermophilic species (Fermer and Engvall, 1999). Differentiation and species characteristics are summarized in tables 2 and 3.

Table 2: Differentiation of principal *Campylobacteter species*

Species	Growth at*		Catalase	Nitrate reduction	H_2S production		Susceptibility to (30 mg disc)	
	25°c	42°c			Lead acetate**	TSI	Nalidixic acid	Cephalothin
C. fetus subsp venerealis	+	_	+	_	_	_	R	S
C. fetus subsp fetus	+	_	+	_	+	_	R	S
C. jejuni	_	+	+	_	+	_	S	R
C. mucosalis	_	.	_	+	+	+	V	S
C. hyointestinalis	+	poor	+	+	+	+	R	S
C. coli	_	+	+	_	+	_	S	R
C. cryaerophilus	+	_	+	+	_	_	S	R
C. laridis	_	+	+	+	+	_	R	R

Source: Quinn *et al.*, 2002

Key: *= Tests carried out in thioglycollate medium; **= Lead acetate strips with inoculation of semisolid Brucella or brain heart infusion broth with 0.02% cysteine for 4-6 days; V=variable reaction; · = data not available; R = resistant; S = susceptible; TSI = triple sugar iron agar.

Table 3: Characteristics of selected thermophilic *Campylobacter* species

Characteristics	*C. Jejuni*	*C. Coli*	*C. laridis*	*C. upsaliensis*
Hydrolysis of hippurate	+	_	_	_
Catalase	+	+	+	_ or slight
Indoxyl acetate hydrolysis	+	+	_	+
Nalidixic acid	+	+	_	+
Cephalothin	R	R	R	S
Nitrate reduction	+	+	+	+

Source: Ellen *et al*., 1994

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

2. 5. 4. Immunodiagnostics

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

2. 5. 5. Sub typing of *C. jejuni* and *C. coli*

Methods of differentiating between bacterial strains below the species or subspecies level are generally known as bacterial typing or sub typing. The main purposes for bacterial sub typing are to evaluate taxonomy, evolutionary mechanisms and phylogenetic relationships, population genetics and bacterial epidemiology. Here, focus will be primarily on epidemiological typing. A basic assumption in epidemiological typing is that isolates from the same transmission chain, for example causing a disease outbreak, are clonally related, i.e. originate from a common ancestor. Some criteria that may be worth considering for many years, methods based on phenotypic traits formed the foundation for bacterial typing, and so also for *Campylobacter* typing. Several biotyping systems, i.e. typing based on biochemical tests, intended for *C. jejuni and C. coli*, have been described (Lior, 1984). Some of the individual tests included in these biotyping schemes are also utilized for species differentiation.

Resistotyping is typing based on an organism's sensitivity to selected antibiotics, and has been used for characterization of *Campylobacter* isolates, mostly in combination with other methods (Roop *et al*., 1985). With regard to the increasing prevalence of antibiotic-resistant Campylobacter strains in humans and food-producing animals, its greatest value may be as a monitoring tool, and as an aid in therapy choice. Other phenotypic methods used for Campylobacter sub typing are phage typing protein profiling and fatty acid methyl ester (FAME) analysis (Steele *et al*., 1998).

The most widely used phenotypic method for *C. jejuni and C. coli* is serotyping. Two serotyping systems have been extensively used: Lior and Penner serotyping. Performed slide agglutination of heat-labile antigens present in the bacterial cell. The antigen types are labeled with the prefix HL. Based their method on passive agglutination of heat-stable antigens on the cell surface, and theses types are given the prefix HS. The identified heat-stable (HS) antigens were initially thought to be lip polysaccharide (LPS) somatic O antigens, but have later been shown, at least in some cases, to be capsular antigens. Penner serotyping is labor-intensive, and modified protocols have been developed to make it simpler and more economic for use in the routine laboratory). Another shortcoming with both Lior and Penner serotyping is that they leave a substantial proportion of the strains untypical (Frost *et al*., 1998).

The research for thermophilic *Campylobacter spp.* necessarily involves examination of the colonies appearing in selective media after an incubation period of 48h at 42°c under microaerophilic conditions. Non-hemolytic grey or uncolored colonies, either plain aqueous with irregular edges, or round convex with regular round edges, should be investigated. Suspicious colonies are picked and subjected to at least three tests such as direct microscopic examination of motility and cell morphology, Gram-staining, which should be negative and Oxidase production, which should be positive. Most thermotolerant *Campylobacter spp.* involved in food-borne bacterial enteritis, except for *C. upsaliensis*, also produce Catalase. Skirrow and Benjamin introduced a scheme to distinguish *C. jejuni, C. coli* and *C. lari* based on the growth at 25°c and 43°c, susceptibility to nalidixic acid, hippurate-hydrolysis and hydrogen sulphide production in an iron-containing medium (Skirrow and Benjamin 1980). The scheme is accurate for routine work and is widely employed in microbiology laboratories. Another scheme, based on hippurate-hydrolysis, rapid production of hydrogen sulphide, and DNA hydrolysis were introduced by Lior (Lior, 1984). The classification of *Campylobacter* based only on biochemical tests is complex. However, the determination of biochemical features is the most widely employed identification strategy applied to *Campylobacter*, which justifies the optimization of phenotypic analysis for the differentiation of *Campylobacter species*. The application of numerical analysis of phenotypic features to Campylobacter was considered by Neill (Neill *et al.*, 1985). More recently, On and Holmes designed a scheme comprising the investigation of 67 phenotypic characters in the genera *Campylobacter, Helicobacter* and other related taxa, obtaining a final scheme that proved to be a valuable tool for identification at species and subspecies level in most strains studied and whose results coincided with previous results obtained by RNA and DNA sequencing (On and Holmes 1995). According to the study by On and Holmes, *C. coli, C. jejuni* subspecies *Jejuni* and *C. lari* are closely related, a similar result having been obtained by Vandamme *et al.,* from phylogenetic studies based on the analysis of 16S rRNA sequences (Vandamme *et al.,* 1991).

2.5. 6. Genotyping

Phenotypic methods are based on the detection of phenotypic properties, which depend on the organism's production of certain proteins. Gene expression may vary in the same bacterial strain, depending, for example, on the nutrients available in the medium or other culture characteristics,

and hence the phenotype may not be the same under different conditions. In contrast, genotyping is based on a more stable marker, DNA, and identifies the genotype regardless of gene expression.

Some genotypic methods employing different approaches are plasmid analysis DNA-DNA hybridization (Hernandez *et al*., 1991) and flagellum gene sequencing. Multilocus sequence typing, MLST, is based on sequencing of a set of so-called housekeeping genes, i.e. essential genes (mostly involved in the metabolism of the bacterium) that are present in all strains. An MLST system for *C. jejuni* has been developed and is being increasingly used to study epidemiology as well as the population structure of the bacterium. Micro arrays based on the sequencing of the entire genome of *C. jejuni* have been constructed, and may enable identification of variable markers of potential value in developing new typing techniques. Serotyping of *Campylobacter* is done as recommended by international committee for microbiology, and is base on isolation and typing of the organism. Serotyping of *Campylobacter* involve heamagglutination of heat-stable (HS or O) antigens, which were later confirmed to be the O somatic antigens. *C. jejuni* and *C. coli* have their own types of O antigen, although some cases (< 4.5%) of cross-reaction have been reported. Serotyping is also reported to be done based on the heat stable (HS) and heat labile (HL) antigens (Patton *et al*., 1985). Detection of O antigens by direct agglutination of *C. jejuni* and *C. coli* cells could able replace passive heamagglutination (Frost *et al.,* 1998).

In 1980, Penner and Hennessy described a passive heamagglutination procedure for serotyping *Campylobacter jejuni subsp. Jejuni* on the basis of soluble heat-stable antigens. Penner method uses passive heamagglutination, where by supernatant from a boiled cell suspension is used to sensitize erythrocytes that are later mixed with antiserum to demonstrate agglutination and this system recognizes 65 serotypes in total, and comprises 47 antisera for C .jejuni and 18 antisera for C.coli (Penner *et al.,* 1983). A comparative analysis of O and HL serogroup of human C. *jejuni* isolates has showed conserved associations between specific O and HL antigens (Jackson *et al.,* 1998).

2. 6. Public health significance of campylobacteriosis

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

Campylobacter is recognized as one of the principal causes of human acute gastro-enteritis worldwide (Allos, 2001). The only form of Campylobacteriosis of major public health importance is *Campylobacter enteritis* due to *C. jejuni* and *C. coli*. *Campylobacter lari* has also been implicated in two fatal cases of bacteraemia (Nachamkin, *et al*., 1984) and diarrhea in sporadic cases with gastrointestinal symptoms and in water-born out breaks. Human gastroenteritis caused by these organisms is frequently associated with consumption of red meat and poultry meat. (Beuchat, 1985)

The rate of Campylobacter infections worldwide has been increasing, with number of cases often exceeding those of salmonellosis and shigellosis. Campylobacter one of the most frequently isolated bacteria from stools of infants with diarrhea in developing countries a result of contaminated food or water.

There is a significant increase in incidence of acquired campylobacter infection both in industrialized and developing countries (Acha and Szyfres, 2001). In countries where records are kept, Campylobacter are now reported to be the leading cause of bacterial diarrhea in man and isolation and incidence rates in some developing countries have increased since their initial reports (Coker and Adefeso, 1994), with most being isolated from <2 year old children with diarrhea (Albert *et al* ., 1999). In some reports the isolation rates for symptomatic and asymptomatic children were not statistically significant. Campylobacter isolation rates in developing countries range from 5 to 20% (Oberhelman and Taylor 2000). Value as high as 14.9% in controls have been observed (Megraud *et al*., 1990).

However, Campylobacter is not frequently recovered from asymptomatic persons in developed countries, as observed in the Netherlands, where a 0.5% isolation rate has been reported (de Wit

et al., 2001). The infective dose of campylobacter ranges from $5*10^{2}$–10^{4} cells, depending on the strain, environmental stress and susceptibility of the host. These infections are manifested as meningitis, pneumonia and miscarriage (Wallace *et al*., 1998).

Campylobacteriosis affect all age groups, however, infections are recognized with increasing frequency in infants, children and in aged individuals suffering from deliberating disorder such as HIV/AIDS and in homosexual men, where, the infection is associated with prostitis (Davis *et al*., 1990). Campylobacter associated diarrhea and bacteraemia occur in HIV/AIDS patient's worldwide (Tee *et al*., 1995). For instance, *C fetus subspecies fetus* most often causes serious systemic infection in immunocompromised hosts (Davis *et al.*, 1990).

The pathogen because of poor sanitation and contact with animals early in life may explain the isolation the disease does not appear to be important in adults in developing countries. Acquisition of from healthy children in contrast, infection occurs in adults and children in developing countries. Poor hygiene and sanitation and the close proximity to animals in developing countries all contribute to easy and frequent acquisition of any enteric pathogen, including Campylobacter. Although infections in infants appear to decline with age, a comprehensive community-based cohort study in Egypt has shown that infection could be pathogenic regardless of the age of the child, underscoring the need for strengthening prevention and control strategies for Campylobacteriosis (Reo *et al*., 2001).

Human Campylobacteriosis is often acquired early in life in developing countries and thus disease is commonest in infants, and there are a relatively high proportion of asymptomatic carriers. In developed countries, infection generally occurs rather in the later age. The highest incidence of disease occurs in children and young adults and there are few asymptomatic carriers (Quinn *et al.*, 2002).

The disease is also an important cause of "travelers' diarrhea". Generally it is recognized when accompanied by predisposing debilitating factors such as pregnancy, premature birth chronic alcoholism, and neoplasia and cardio vascular disease (Ellen *et al.*, 1994). Fever malaise, headache and sometimes aching of limbs and 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 borne outbreaks have been recorded (Acha and Szyfres, 2001).

2. 6. 1. Sources of infection and mode of transmission of disease in man

The presence of *Campylobacter* in the migratory birds is the indication of the large distances that *Campylobacter* can be transferred (Humphrey and Muscat, 1995) and they are known reservoirs and responsible for shedding of the bacteria to cause contamination of the environment. *Campylobacter spp.* is widespread in the environment, where they are a sign of recent contamination with animal and avian feces, agricultural run off and sewage effluent. Intestinal carriage of *Campylobacter spp.* is ubiquitous in livestock, domestic animals, wild birds and poultry and depends on the environmental factors (Humphrey and Muscat, 1995). C*ampylobacter jejuni* commonly is found as a commensally in the gastrointestinal tract of wild and domestic ruminants, swine, dogs, cats, fowl, and rodents, and these reservoirs are the ultimate sources for most human infections. *C. jejuni* and *C. coli* have been isolated from chicken, goat, sheep and pigs in developing countries. Strains isolated from chicken and human were phenotypically and genotypically correlated, confirming that chickens are important source of human campylobacteriosis in developing countries. Disease caused by *Campylobacter jejuni,* now the leading cause of bacterial food poisoning, most often spread by contact with raw or undercooked poultry. A single drop of juice from a contaminated chicken is enough to make someone sick with campylobacteriosis. The organism present in about 15% of cattle at a time of slaughter. *Campylobacter coli* have been isolated from the intestinal content of 99% of pigs at slaughter (Radostits *et al.*, 1994). Six percent of fresh pork and liver has been found contained *Campylobacter* with *C. coli* being more abundant than *C. jejuni* (Jay, 2000).

A number of transmission vehicles, including food products have been implicated in the transmission of *Campylobacter spp.* to human (Jorgensen *et al*., 2002). The most significant risk factors identified include the consumption and/or handling of raw or undercooked poultry or other meats, raw milk and surface waters. Cross contamination of ready to eat foods during food preparation as well as direct contact with animals has been identified. Food animals may act as asymptomatic intestinal carriers of Campylobacter and animal food products can become

contaminated by this pathogen during slaughter and carcass dressing (Whyte *et al* ., 2003). Previous studies recorded infection rates in live broilers ranging from 0 to 100% (Moore *et al*., 2003) with prevalence up to 60% in cattle (Neilson *et al.,* 1997). Campylobacter prevalence up to 100% has also been reported on dressed poultry carcasses with significantly lower prevalence of the organism generally reported on beef carcasses (Madden *et al.,* 2001). Other foods from which *Campylobacter* has been recovered include raw milk and milk products (Lacerc *et al.,* 2002). It is now accepted that campylobacteriosis is predominantly acquired through the consumption of contaminated foods (Anonymous, 1995). *Campylobacter jejuni* is a frequent cause of diarrhea/dysentery in children, which is often related to pet keeping and chicken meat consumption as well as untreated drinking water (Ali *et al.,* 2003). Human beings in developed societies acquire *C. jejuni* from asymptomatic or symptomatic companion animals (dogs and cats) and from foods such as raw milk, water, and poultry products (Reo *et al.,* 2001). Contaminated, under cooked poultry is responsible for >50% of cases investigated in humans (Shani, 1998).

Occupational exposure may cause infection and disease in workers in animal health facilities, animal shelters, and poultry processing plants, animal agriculture, and rendering plants. Family members of aforementioned also are at increased risk of infection (Prescott and Munroe, 1982). It has also been reported that there is strong association between Campylobacter infection and residence on farm. Risks of acquiring campylobacteriosis in developing countries include the presence of an animal in cooking area, uncovered garbage in cooking areas and lack of piped waters (Reo *et al*., 2001). Poor hygienic and sanitation and close proximity to animals in developing countries contributes to the easy and frequent acquisition of any enteric pathogens including *Campylobacter* (Oberhelman and Taylor, 2000). The source of human campylobacteriosis is almost always food (Acha and Szyfres, 2001) and most sporadic cases probably arise from consumption of improperly handled poultry (meat) or contact with infected pets (Hirsh and Zee, 1999). Several research works so far conducted overseas, on various foods of animal origin indicated that different foods of animal origin particularly raw red meat are contaminated with enter pathogenic *Campylobacter species* as shown in Table 4.

These authors have reported thermophilic *Campylobacter spp.* at varying rate of recovery from different raw red meat samples collected from various sources.

Table 4: Prevalence of *Campylobacter species* in raw red meat overseas

Country	Product	Number samples tested	Number(%) Campylobacter	Reference
Australia	Beef carcasses	124	1(0.8)	Vanderlinde *et al.*, 1998
Australia	Sheep carcasses, domestic	140	3(2.1)	Vanderlinde *et al.*, 1999
	Sheep carcasses, export	330	3(0.9)	
Belgium	Pork carcasses	49	1(2.0)	Korsak
	Beef carcass	62	6(10.0)	*et al.*, 1998
Canada	Pork	463	78(12.1)	Lammerding, *et al.*, 1988
	Beef	598	135(14.7)	
	Veal	267	115(34.5)	
Canada	Pork carcass diaphragms	200	47(23.5)	Mafu, *et al.*, 1989
England	Minced meats	135	3(2.2)	Bolton, *et al.*, 1985

Continued on following page...

Table 4 Continued...

Country	Product	No samples tested	No,(%) Campylobacter	Reference
England	Beef	127	30(23.6)	Fricker and Park, 1989
	Pork	158	29(18.4)	
	Lamb	103	16(15.5)	
England	Raw sausages	1197	4(0.3)	Little and de Louvais, 1998
	Raw burgers	1015	10(1.0)	
England and Wales	Minced beef	2015	21(1.0)	Turnbull and Rose, 1982
	Minced pork	342	1(0.3)	
Ireland	Minced beef	20	4(20.0)	Cloak *et al.*, 2001
	pork	20	0	
Ireland	Raw beef	221	7(3.2)	Whyte *et al.*, 2004
	Raw pork	197	10(5.1)	
	Raw lamb	262	31(11.8)	
Italy	pork	27	1(3.7)	Zanetti *et al.*, 1996
Italy	Beef	151	2(1.3)	Pezzotti *et al.*, 2003
	Pork	175	18(10.3)	
Japan	Beef	94	2(2.1)	Tokumaru *et al.*,1990
	Pork	52	0	
Japan	Deer meat	30	0	Kanai *et al.*, 1997
Netherlands	Pig carcasses after evisceration	210	19(9.1)	Oosterom *et al.*, 1985

2.7. Status of campylobacteriosis in Ethiopia

There are very few studies conducted on human campylobacteriosis in Ethiopia. A study conducted at Tikur-Anbessa and Ethio-Swedish children's hospital indicated that *Campylobacter species* are an important cause of diarrhea both in adults and children in which *C. jejuni* accounted for 82-84% of the isolates (Asrat *et al*., 1999). A cross sectional study conducted on urban and rural farm animals indicated that thermophilic *Campylobacter spp.* are significantly identified with prevalence of 39.6% in the fecal sample (Kassa *et al*., 2005). The antimicrobial test conducted on these isolates indicates that some of the bacteria showed a range of resistance to certain antimicrobial agents (Asrat *et al.*, 1999; Kassa *et al.*, 2007). However, no citable work has been done so far regarding the prevalence of thermophilic *Campylobacter spp.* in foods of animal origin particularly in export sheep and goat carcasses which is one of the major source of the human infection and the role of animal products in the epidemiology of human Campylobacteriosis. Therefore this study was undertaken to determine the rate of isolation of these species from export sheep and goat carcasses.

2.8. Treatment and control

Campylobacter enteritis is a self-limiting disease, and antimicrobial therapy is not generally recommended. However, antimicrobial agents are recommended for extra intestinal infections such as Guillian-Barre' Syndrome (GBS) that is serious consequence of diarrhea disease characterized by polyneuritis of the peripheral nerves, which may lead to either a transient or long-term paralysis (Blazer *et al.*, 1983), for treating immuno-compromised persons.

Antimicrobial therapy is not required except in severe cases long-lasting *Campylobacter* enteritis and systemic infections (Aarestrup and Engberg, 2001). Erythromycin and ciprofloxacin are normally considered the drug of choice for *Campylobacter enteritis* (Engberg *et al*., 2001). Intravenous amino glycosides are recommended for the treatment of serious *Campylobacter* bacteraemia and other systemic infections (Tajada *et al.*, 1996). The rate of resistance to these drugs is increasing in both developed and developing countries, although the incidence is higher in developing countries. Use of these drugs for infections other than gastroenteritis and self-medication are often the causes of resistance in developing countries; in developed countries,

resistance is due to their use in food animals and travel to developing countries (Steinbrucker *et al.,* 2000; Feierl *et al*., 2001). There is growing scientific evidence that the use of antibiotics in food animals particularly in developed countries leads to the development of resistant pathogenic bacteria that can reach humans through food chain (Van Looveren *et al.,* 2001; Avrain *et al.,* 2003). The available information on antimicrobial susceptibilities of thermophilic *Campylobacter* has been found to differ in different countries (Van Looveren *et al.,* 2001; Avrain *et al*., 2003; Ishihara *et al*., 2004), but resistance has been reported to increasing particularly to macrolides and fluoroquinolones (Aarestrup and Engberg, 2001). The increase in erythromycin resistance in developed countries is often low and stable at approximately 1% to 2%; this is not true for developing countries (Steinbrucker *et al.,* 2000; Feierl *et al.,* 2001). For example, in 1984, 82% of Campylobacter strains from Lagos, Nigeria were sensitive to erythromycin; 10 years later, only 20.8% were sensitive (Coker and Adefeso, 1994). In addition resistance to another macrolides, erythromycin, was found in 7% to 15% 0f *Campylobacter* isolates in 1994 and 1995 in Thailand (Steinbrucker *et al.,* 2000). The increasing rate of resistance to the fluoroquinolones, ciprofloxacin limits its clinical usefulness. In Thailand, ciprofloxacin resistance among *Campylobacter spp.* increased from zero before 1991 to 84% in 1995 (Hoge *et al*., 1998). Recent data have shown a marked increase in resistance to quinolones in developed countries (Steinbrucker *et al.,* 2000; Feierl *et al.,* 2001).

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

3. MATERIALS AND METHODS

3.1. Study Area

The current study was carried out at Hashim Nuru Jiru Ethiopia Livestock and Meat export abattoir (HELIMEX) located in Debre-Zeit. HELIMEX abattoir is one of private slaughterhouses established according to export standard. The average numbers of animals slaughtered per day are around 1000 sheep and goats. But the full capacity of the abattoir is up to 2000 small ruminants. The abattoir does not slaughter large animals, but it has planned to slaughter large animals when it's new building will start working. Importing countries are Middle East countries such as Saudi Arabia, United Arab Emeriti, Yemen and others. The abattoir is in good hygienic condition except management problems. Debre-Zeit is a small town with human population of about 100,000. This town is located about 45 kms south east of Addis Ababa. The altitude is about 1,850 meters above sea level. It is an important small town where most governmental institutions, national and international research centers are located. The soil and climate are similar to those in many high land areas in Ethiopia. The main rainy season extends from June to September with an average rainfall of 800mm (of which 84% of rain is expected). There is also a short rainy season from March to May. The average minimum and maximum temperatures are 12.3^0c and 27.7^0c respectively (CSA, 2001). The average annual temperature and rainfall are 21^0c and 1,800mm, respectively. Debre-Zeit has a relative humidity varying between 70% and 80% during the rainy season and 40% to 50% during the dry season (NMSA, 2003). The microbiological analysis of the samples was done at the microbiological laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit and at Ethiopian Health & Nutrition Research Institute, Addis Ababa, Ethiopia.

3.2. Study animals

The study was conducted on apparently healthy animals slaughtered at HELIMEX export abattoir in Debre-Zeit, from October 2007 to March 2008. The animals slaughtered in the abattoir were originated mainly from markets around Awash Park in the rift valley area of Afar and Oromia regions. Some animals were also originated from other parts of eastern and southern regions of the country and were transported on double decked trucks made for animal

transportation or on open trucks made for transportation of goods. After arrival at the abattoir, the animals were kept for 24 to 72 hours in concrete floored roofed shades where they were fed and watered until they were inspected for slaughter. Animals to be slaughtered the next day were inspected by veterinary inspectors and moved into another lairage where they spend the night, feed being withheld since separation from other animals up to slaughtering.

3.3. Study design and sampling

A cross-sectional study has been conducted on randomly selected sheep and goat carcasses in the export abattoir. The sample size was based on the slaughterhouse practices for sheep and goats in the abattoir and the carcass sites for swabbing were chosen according to ISO, 2002.

Each carcass swabbing surface was swabbed (sampled) at three different operations i.e. before evisceration, after evisceration and after washing.

The sample size required for the study was determined based on expected prevalence of 50% with defined precision of 5 % and level of confidence of 95 % according to the formula described by Thrusfield, 1995. Accordingly a total of 398 sheep (mutton) and goat carcasses were sampled for isolation and identification of *Campylobacter* species. Therefore, 218 sheep carcasses (mutton) and 180 goat carcasses were sampled and analyzed for identification and isolation of *C. jejuni* and *C.coli* from four different sites on the carcasses (crutch, abdomen, thorax and breast) and three different operations (before evisceration, after evisceration and after washing). Each slaughtered animal was swabbed only for one swabbing site but once for each of the three slaughter operations. Thus one carcass was swabbed three times. Accordingly, 1194 swabs (654 from sheep and 540) were collected and analyzed from 218 sheep and 180 goats.

Each carcass was swabbed medially on sides (crutch) and lateral surfaces from the sides of abdomen (flank), thorax and breast. Samples were collected using standard swabbing techniques. Briefly, a sterile template (10cm, by 5cm) was placed on the swabbing sites of the carcass surface and the area delineated by the template was swabbed several times first with wet sterile cotton swab followed by dry sterile cotton swab. Sterile cotton swabs moistened with peptone water were first rubbed horizontally several times so as to pick as much sample

as possible. Following this the previously swabbed area was once again swabbed using dry sterile cotton swab and both swabs (wet and dry) were put into the same test tube. After completion of swabbing, the cotton swabs including the handle were put into a test tube containing 10ml of peptone water. The test tubes containing the samples collected following slaughter line operations were labeled for sample number, swabbing site on the carcass and operation type. Test tubes were then put into an insulated cool ice box filled with plastic ice bags and immediately transported to the laboratory for bacteriological processing.

3.4. Isolation and identification

3.4.1. Culture

Upon arrival at the laboratory, the swab was taken out of the test tube and streaked onto Campylobacter agar media (Oxoid CM689, England), which was prepared accordingly, to the manufacturer's descriptions. After setting aside for few minutes, the inoculated media plates were placed into an anaerobic jar (anaeropack system Mitsubishi gas chemical co). Then three pieces of CampyGen paper sachets were placed in the appropriate clip on the plate carrier in the jar. The jar lid was closed immediately and finally, the lid of the jar was sealed and incubated under microaerophilic condition at 42 ^{o}C for 48 hours. After this incubation period the petri dishes were taken out of the anaerobic jar and examined for the presence of presumptive *Campylobacter* colonies. Small, round, slightly raised smooth translucent colonies with "dewdrop" grey/white appearance with the tendency to spread along the inoculation track were considered to be *C. jejuni* and creamy grey colonies were considered to be suggestive of *C. Coli (*ISO/CD 10272-1 and 10272-2, 2002)

3.4.2. Confirmatory tests

Oxidase test

A piece of filter paper was moistened in a Petri dish with 1 % of aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride. The test bacterium was streaked firmly across filter paper

with glass rod. A dark purple color along the streak line within 10 seconds indicated a positive reaction. Absence of color change or a purplish color that develops later was considered to be negative reaction. Most *Campylobacter* spp. are Oxidase positive.

Microaerobic growth

The microaerobic growth test was performed by sub culturing the suspected colonies from the Campylobacter selective medium (Oxoid SR 117) to two blood agar plates (Oxoid, England). One of the plates was incubated in micro-aerobic conditions and the other plate aerobically at $41.5^{o}c \pm 1^{o}c$ for 22 ± 2 hours. *Campylobacter* species grew in micro-aerobic conditions but not under aerobic conditions. (ISO, 2002)

Cell morphology and motility

Cell morphology and motility test were performed by preparing a wet preparation as soon as possible after removal of the culture from micro-aerobic conditions. The suspected colony was emulsified in drop of broth medium (Oxoid, England) on a slide and covered with a cover slip and was examined immediately using phase contrast microscope. *Campylobacter* species are highly motile slender rods with curved or spiral morphology. Motility is characterized by darting and corkscrew like movements (ISO, 2002).

Gram stain

When sometimes the cell morphology cannot be determined from the motility test then a Gram stain method was performed. Young cultures appeared as small curved, Gram negative bacilli. Older cultures may appear as cocci (ISO, 2002).

Species identification

All isolates identified as thermophilic Campylobacters were tested for hippurate hydrolysis and susceptibility to nalidixic acid and Cephalothin. These parameters formed the basis for the identification of *C. jejuni*, *C. coli* and *C. lari*. For hippurate hydrolysis test, suspected colonies were taken from 48 hours Colombia blood agar plate culture (DEFCO, BECTON, DICKINSON France S.A) and were emulsified in 1% sodium hippurate broth (Becton, Dickinson, USA) and were incubated at $42^{o}c$ for 48 hours in a microaerobic condition. Following the incubation the

broth culture was centrifuged at 2500 rpm for 2 minutes and 0.8ml of the supernatant was transferred to sterile clean tube. The hydrolysis of hippurate was detected by addition of 0.2ml acidic ferric chloride (Oxoid, England) and gentle shaking. A dense brown persistent precipitate which indicates the presence of benzoic acid that results from hippurate hydrolysis was considered as positive test. *Campylobacter jejuni* is the only species that hydrolyses sodium hippurate (ISO, 2002).

3.5. Data management and analysis

The data collected were entered and managed in MS Excel program. SPSS version 12 for windows was used for data analysis. Results were summarized as a proportion of positive carcasses at least in one of the three operations divided by the total slaughtered animals examined (i.e. n=398). Proportion of contaminated carcasses per species of animals examined was expressed as the number of positive carcasses at least in one of the three operations divided by the total number of animals examined in each species (i.e. n=218 and 180 for sheep and goats respectively). Level of contamination per the carcass site was presented as a proportion of positive swabs in each site divided by total swabs examined in each site separately for each animal species and combining both species. Per operation contamination level was given as the proportion of positive carcasses in each operation divided by the number of carcasses examined (i.e. n=398) for overall level of contamination and n=218 and 180 of sheep and goats carcasses for species level contamination.

Difference in the isolation rate of the *Campylobacter* species between species of animals, different sampling sites and the different operations was assessed by Chi-square (X^2) test. Odds ratio was used to assess the degree of association for the species of animals and swabbing sites. Descriptive statistics such as percentages and frequency were used to present the data. A confidence level of 95% and a P value of less than 0.05 were used to examine the significance of the variables in relation to the level of carcass contamination.

RESULTS

A total of 398 carcasses consisting of 218 (54.8%) mutton and 180 (45.2%) goat carcasses from the four sites were swabbed for three different operations (Table 5).

Table 5: The number of carcasses (animals) swabbed according to swabbing site

Species	Swabbing sites				Total (%)
	Crutch	Abdomen	Thorax	Breast	
Sheep	56	49	50	63	218 (54.8)
Goat	52	46	42	40	180 (45.2)
Total	**108**	**95**	**92**	**103**	**398 (100)**

From 398 carcasses from both species 40 (10.1%) were positive for *C. jejuni* and *C. coli.* The highest positive carcasses were observed after evisceration accounting for 6.5% of all the carcasses examined. Only one carcass from the breast area was positive both after evisceration and after washing in one of the sheep carcasses (Table 6). Post evisceration had a significant contribution to the contamination of the carcasses as compared to pre-evisceration and post-washing operations in the abattoir (χ^2=19.72; p=0.000). Washing the carcasses of sheep did not reduce carcass contamination that has occurred during evisceration (χ^2=0.18; P=0.68). However washing goats carcasses caused a substantial reduction in the carcass contamination with campylobacter ((χ^2=10.72; P=0.001).

Table 6: Occurrence of *Campylobacter jejuni* and *C. coli* according to different operations

Species	Number examined	Proportion positive (n (%))		
		Before evisceration	After evisceration	After washing
Sheep	218	0 (0)	13 (6.0)	11 (5.0)
Goat	180	3 (1.7)	13 (7.2)	1 (0.6)
Total	**398**	**3 (0.8)**	**26 (6.5)**	**12 (3.0)**

The highest level of carcass contamination with Campylobacter was occurred in the breast region (Table 7). However there was no statistically significant variation in the rate of isolation of campylobacter species between the swabbing sites for the 398 carcasses examined ((χ^2= 2.02; P= 0.57). Similarly, there was no statistically significant difference in the proportion of positive swabs for Campylobacter species between sheep and goat carcasses (p=0.72) (Tables 7 and 8).

Table 7: Proportion of positive carcasses according to carcass swabbing sites in sheep and goat

Species	Crutch		Abdomen		Thorax		Breast		Total	
	Number examined	**Number positive (%)**	**Number examined**	**Number positive (%)**	**Number examined**	**Number positive (%)**	**Number examined**	**Number positive (%)**	**Number examined**	**Number positive (%)**
Sheep	56	4(7.1)	49	7(14.3)	50	5(10)	63	7(11.1)	**218**	**23 (10.6)**
Goat	52	4(7.7)	46	4(8.7)	42	3(7.1)	40	6(15)	**180**	**17(9.4)**
Total	**108**	**8(7.4)**	**95**	**11(11.6)**	**92**	**8(8.7)**	**103**	**13(12.6)**	**398**	**40(10.0)**

Table 8: Comparison of *Campylobacter* positive carcasses according to species of animals and swabbing site

Variables	Odds ratio (OR)	95% of CI for the OR	p-value
Species			
Goat	Ref	Ref	
Sheep	1.131	0.584---2.2	0.715
Swabbing sites			
Crutch	Ref	Ref	
Abdomen	1.64	0.63—4.26	0.312
Thorax	1.19	0.43—3.31	0.738
Breast	1.81	0.72---4.56	0.211

From the 40 positive carcasses for *Campylobacter* contamination, *C. jejuni* was isolated at higher proportion accounting for 7.0% (n=398) than *C. coli*, which was isolated at a rate of 2.7% (n=398) (Table 9). Taking only the positive isolates for *Campylobacter*, *C. jejuni* accounted 72.5% (n=40) and C. coli accounting for 27.5%. A statistically significant difference was observed between the two *Campylobacter* species identified (x^2= 8.53; p=0.003).

Table 9: Level of carcass contamination according to *Campylobacter* species in slaughtered sheep and goat carcasses

Species	**Number of car cases examined**	***Campylobacter* species isolated (Number (%))**		
		C. jejuni	***C. coli***	**Total**
Sheep	218	17 (7.0)	6 (2.8)	23 (10.6)
Goat	180	12 (6.7)	5 (2.8)	17 (9.4)
Total	**398**	**29 (7.3)**	**11 (2.7)**	**40(10.0)**

5. DISCUSSION

In the last decade, it has been shown that campylobacteriosis becomes the primary bacterial infections of humans. Foods of animal origin were incriminated as the main sources (Friedman *et al.,* 2000; Oberhelman and Taylor, 2000). Raw meat and its products are commonly consumed in traditional Ethiopian diets, but campylobacteriosis is rarely studied compared to other countries.

In the present study *C. jejuni* and *C. coli* were isolated from sheep and goat carcasses at different recovery rates. The 10.6% prevalence rate of thermophilic Campylobacter species from mutton samples is comparable to the prevalence rate of 10.5% from raw sheep meat examined from supermarkets in Ethiopia (Lemma, 2007). But it is lower than the 68.1% prevalence reported from food animals in Jima, Ethiopia by Kassa *et al*., (2005). The difference is attributed to the fact that Campylobacter is isolated at higher rate from fecal samples than carcass swabs. Our result is comparable with the 11.8% prevalence from raw lamb reported from Ireland (Whyte *et al*., 2004). However, it is higher than the 5.1% report from Pakistan (Hussein, *et al*., 2007), 8.1% from Norway (Rosef *et al.*, 1983) and (2.1%) from Australia (Vanderlinde *et al.*, 1999). The result of current study is relatively smaller than the 15% prevalence in lamb reported from Portugal (Cabrita *et al*., 1992), 15.5% prevalence in mutton recorded in England (Fricker and Park, 1989) and 20% in Brazil (Aquino *et al.*, 2002).

The prevalence of thermophilic *Campylobacter* species in goat carcasses in our study was 9.4%. In Canada, *C. jejuni* was identified at 2.7% of the animals studied (Prescott and Bruin-Mosch, 1981), which is less than the present finding. Turkson *et al* (1988) in Kenya have also recorded *Campylobacte*r species at the recovery rate of 6.3% in goat meat sample, which is similar to our result.

Campylobacter jejuni and *C. coli* are frequent worldwide causes of food-borne gastroenteritis. Slaughterhouse studies have shown that the main source of *C. jejuni* is the intestinal contents (Newell *et al*., 2001). In the present study the highest isolation rate was found in swabs collected from breast region (12.6%) (n=103) as compared to other sites but no statistically significant difference was detected (Table 9). This shows that during manual skinning, evisceration, washing and processing in the slaughter house there could be a contamination of carcasses with intestinal contents. The general bacterial contamination carried on operators' hands after making

skinning, a dressing procedure that necessitates direct hand contact with the hide, is very similar to that carried by the hide in that region. Therefore, contact between carcass and unclean operators' hands introduce comparable contamination to hide-carcass contact for those operations in which hide-hand contact is unavoidable. The intestinal tract could be the second major source of enteric pathogens during the slaughtering process. Leakage from gastrointestinal tract could cause widespread contamination.

In this study, it was demonstrated that there was a severe cross contamination of the carcass after evisceration. The level of contamination of the mutton has increased from 0% to 6% while in goat carcass it has increased from 1.7 to 7.2% from pre-evisceration to post-evisceration. This increased level of contamination was statistically significant (P>0.05). The preventive measures for reducing contamination (hazards) during evisceration are tying the esophagus to prevent escape of ingesta, enclosing the bung to prevent escape of faeces and the intact removal of the viscera. Cold-water carcass washes, although effective in removing macro contamination, are ineffective in removing microbial contamination. Trimming can reduce gross contamination at heavily and moderately contaminated sites and washing but they have no decontaminating effect on the carcass as a whole. This was evidenced by our study where practically there was no difference between post- evisceration and after washing in sheep carcasses though there was a substantial difference in the goat carcasses (Table 6).

The predominant species of *Campylobacter* from sheep (mutton) in this study was *C. jejuni* (7.3%). This result is less than the 59.3% *C. jejuni* recovery rate from sheep feces (Kassa *et al.*, 2007) and the 40.5% isolation rate from the intestines of sheep at slaughter in United Kingdom (Stanley *et al.*, 1998). The reason for this difference could be due to the difference in the nature of the samples examined because intestinal contents contain higher number of *Campylobacter* species than carcass swabs. Other differences could be variation in the number of samples examined, time, methodology and place of the study. Out of the 40 isolates, *C. jejuni* accounted for 72.5 %. *Campylobacter jejuni* was recovered with the isolation rate of 6.7% while *C. coli* accounted for 2.8% of goat carcasses, whereas *C. jejuni* in mutton was 7.8% and *C. coli* is 2.8%. The result was different from 33.3% prevalence of *C. jejuni* recorded in Ghana (Abrahams *et al.*, 1990). Generally the variation in the prevalence of *Campylobacter* isolation rate of carcasses

reported in other studies from the present may be the result of difference in sampling techniques, laboratory methodologies employed, place and time.

Absence statistically significant difference in level of carcass contamination between sheep and goats (P=0.72 indicates that species difference is not an epidemiologic risk factor for the rate of carcass contamination by *Campylobacter species* in this abattoir study.

Certain foods, principally raw meat, can have very high Campylobacter contamination levels (Jorgensen *et al*., 2002). These can lead to extensive cross contamination in commercial and household food preparation areas. Cross-contamination has been shown to be an important infection risk factor. Control can be particularly difficult in the household environment and there is a need to identify the best ways to advise consumers on this subject. Isolation of *C. jejuni* and *C.coli* from the intestinal contents of domestic livestock revealed considerable intra-and interspecies variation (Rosef *et al*., 1983). Campylobacters were isolated from the intestines of sheep at slaughterhouses in Preston, Lancashire, the United Kingdom (Stanley *et al.,* 1998). Although it is likely that intestinal infection is close to 100%, the shedding of Campylobacters in the faeces varies considerably with the time of the year (Stanley *et al.,* 1998). In some occasions, 100% of sheep were shedding Campylobacters and not shedding on other occasions. This may show seasonal patterns. The shedding of Campylobacters by sheep has potential to contaminate pastures and surface waters. Contamination of surface and subsurface waters may transmit *Campylobacter* within herds and between farms and other livestock groups). The role of *C. jejuni* as a primary pathogen in farm animals is uncertain however its role is as the main food borne human pathogen (Padungton *et al*., 2003).

6. CONCLUSIONS AND RECOMMENDATIONS

The study of *Campylobacter* in export standard abattoir showed 10% contamination level of the carcass. There was no species difference in the level of carcass contamination. No statistically significant variation was observed in the isolation rate of *Campylobacter* species between breast, abdomen, thorax and crutch areas. A total of 40 *Campylobacter* isolates consisting of two species were identified from the carcasses examined. The dominant isolate was *C. jejuni*). Our study also indicated that there was severe cross contamination during evisceration. Washing did not reduce the level of contamination after washing in mutton but it had considerably reduced the level of carcass contamination in the slaughtered goats. The existences of thermophilic *Campylobacter* species in export mutton and goat carcasses highlight the threat to our export trade. This poses risk to public health particularly in importing countries and who have direct contact with food animals and raw animal products (farmers, abattoir workers and animal health personnel) and immunocompromised individuals. To control and prevent *Campylobacter* infection and contamination in live animal and animal products, it is crucial that risk reduction strategies: ensuring safety of food, implementing good hygiene practices, good management practices and HACCP be used throughout the food chain i.e. from farm to fork.

Based on the findings of the present study the following are recommended:

- The presence of cross contamination of carcasses with *Campylobacter* found in this study has indicated the need to apply good manufacturing practice, and hygienic standards in the slaughter house during the whole slaughtering process
- Standard abattoir and personnel hygiene should be employed to control contamination of carcasses.
- Further studies should be undertaken to establish the critical control points in the production chain so that corrective measures can be taken.
- Since the breast region was the most contaminated part care should be taken during evisceration/skinning and animals intended for slaughter should be free of any dirt on their skin
- The highest rate of carcass contamination after evisceration warrants that extra care should be exercised during evisceration to reduce cross contamination.

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8. ANNEXES

Annex 1. Sample collection sheet for bacteriological analysis

No	Date	No of carcasses	Type of animals	Sample sites	Operation time(after)	results	Remark

Figure: 1.Carcass swabbed Goats before slaughter in the lairage

Figure: 2. **C**arcass swabbed Sheep before slaughter in the lairage

Figure: 3 Swabs collected from abattoir slaughter hall

Figure: 4 during swab collection

Annex 2. Microbiological activities in isolation and identification of thermophilic C. spp

Sample code	Growth On selective Medium at 42^{o}_{C}		Morph-ology		Motility test		Grams reaction		Catalase test		Oxidase test		H2S Production		Nitrate Reducti-on		Hippur-ate Hydrol-ysis	
	P	N	P	N	M	NM	P	N	P	N	P	N	P	N	P	N	P	N

Key:-P = Positive, N =Negative, M= Motile, NM=Non-Motile

Annex 3. Samples types, sites, period and number of samples used for study

Sample type	Sampling sites	period	Total number of samples	Results		
				N	P	%

KEY: n=negative, P= positive

Annex 4: Bacteriological media and chemicals prepared and utilized in current study

Campylobacter agar base (CM689, OXIOD, ENGLAND)

Typical formula (g/l): 'Lab-Lemco' powder 10.0; peptone 10.0, Sodium chloride 5.0 and Agar 12.0.

Directions: suspend 18.5g of Campylobacter agar base (CM689) in 475 ml of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121^0c for 15 minutes. Cool to 50^0c. Aseptically add 25 ml of lysed (sheep) horse blood (SR48) and 1 vial of Preston Campylobacter Selective Supplement (SR117) reconstitute as directed.

Tryptic Nitrate Medium (DEFCO, France)

Formula per liter: Becton Tryptose 20.0g, Becton Dextrose 1g, Imodium phosphate 2g, potassium nitrate--, Becton Agar 1g.

Direction: to rehydrate the medium suspend 25grams in 100ml, cold distilled water heat to boil, to dissolve completely. Sterilize in the autoclave in the autoclave for 15 minutes at 121^0c. Inoculate with test organism and incubate at 37^0c for 24 hours.

Test for nitrate reaction:

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

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

Add 3 drops of reagent A, then 3 drops of 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 red color did not develop.

Difco™ Colombia blood Agar Base

Approximate Formula per liter: pancreatic Digest of Casein, 10.0g, Protease peptone, 5.0g, Yeast Extract, 5.0g, Agar, 12g.

Difco^{TM} Colombia blood Agar Base (DEFCO, BECTON, DICKINSON France S.A.).

Direction: suspend 39g 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 121^0c for 15 minutes.

For preparation of blood agar, cool the base to 45-50^0c and add 5% sterile, defibrinated blood, mix well and dispense an approximate of 12 to 15ml into sterile Petri dishes.

Mueller Hinton Agar II (DEFCO, BECTON, DICKINSON, France S.A.)

Formula: Beef infusion 300.0g, Casamino Acid Technical 17.5g, starch 1.5g, Agar 17.0g

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

SIM Medium (BBL, Becton Dickinson Microbiology System)

Formula per liter: Pancreatic Digest of Casein 20.0g, Peptic Digest of Casein 6.1g, Ferrous Ammonium Sulphate 0.2g, Sodium Thiosulfate 0.2g, Agar 3.5g

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

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

Formula per liter: Heart Muscle, infusion from (solids) 10.0g, Peptic Digest of Animal Tissue 10.0g, Sodium Chloride 5.0g, Sodium Hippurate 10.0g

Direction: Dissolve 10.0g in 1liter of distilled water and distribute into final containers. Sterilize by autoclaving 121^oc

Ferric Chloride Test Reagent

Formula for acid ferric chloride solution 12%, Ferric chloride 12.0g, concentrated hydrochloric acid 5.4ml, Distilled water 94.6ml

Direction:

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

   Procedure:

   Inoculate tubes with one to two isolated colonies from pure culture plate.

   Include an un inoculated tube as a negative control and a positive control (type cultures).

   Incubate tubes with loosed caps for 48 hours at 35 ± 2^{o}c in a microaerobic atmosphere.

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

   Aseptically transfer its 0.8ml supernatant to small test tubes using sterile pipette, add 0.2ml of 12% acidic ferric chloride and gently shake.

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

   Tryptone Soya Broth (CM0129, OXOPID, ENGLAND)

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

Direction: Dissolve 30g in 1 liter of distilled water and distribute into final containers. Sterilize by autoclaving at 121^0c

Brucella Broth (B2900 -02, Oxoid, England)

Formula per liter : Casein Digest Peptone 10.0g, yeast extract 2.0g, sodium chloride 5.0g, sodium bisulfate 0.1g, peptic digest of animal tissue 10.0g and Dextrose 1.0g.

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

Annex 5: Curriculum vitae

Biodata

Name…Tefera Woldemariam Agago

Profession…Veterinarian

Nationality…Ethiopian

Date & place of birth…February 20th 1960, Wolayta, SNNPR

Languages…Wolayta, Amharic, Oromiffa, English and Russian

Permanent Address…P.O. Box 837, Debre-Zeit, Ethiopia

Tel. (251-1), 011-433-91-56 (Res)

Mobile: (251-1) 0911-66-04-67

Marital Status…Married and have two children

Education / training

1974... Elementary and Junior Secondary School, Dubbo Catholic Mission Wolayta, Ethiopia

1978…Comprehensive High School, Wolayta Soddo, Ethiopia

1980…Diploma in Animal Health, Institute for veterinary Assistant Debre-Zeit,

Ethiopia

1988…Doctor of Veterinary Medicine (DVM), Kishinov Agricultural Institute

Kishinov, Moldova

1991…Certificate on Veterinary Public Health, A.A.U FVM Debre-Zeit, Ethiopia

2002…Computer Diploma, Access Computer Center, Debre-Zeit, Ethiopia

2003…Certificate on Veterinary Public Health, Shefayim, Israel.

2005…Certificate on Risk Analysis in Animal Health, Addis Ababa, Ethiopia.

2007…SPSS training program, Civil Service College, Addis Ababa, Ethiopia.

2007…Meat Inspection training, A.A.U FVM, Debre-Zeit, Ethiopia.

2008…Master's of Science in Veterinary Public Health (MSc), A.A.U. FVM, Debre-Zeit, Ethiopia.

Work experience

Professional

- ❖ 1981-1982….. Assistant Veterinarian and Head of Woreda Agricultural Office, Goffa Woreda
  - o Participate in the overall Supervision of Animal Health
  - o Undertook work in Immunization, Deworming and Ectoparasite Treatment and Control
  - o Maintained Data on Animal Health, which gives Indication on Seasonal Occurrence of Disease and yearly Drug Consumption
- ❖ 1988-2007…Veterinary Inspector and Team Leader of Meat Inspection Service

ELFORA Export Abattoir

- ❖ Since 2007… Veterinary Inspector in HELMEX Export Abattoir
  - o Collect and Evaluate Information that might have Influence on Ante-mortem and Post-mortem Inspection in Export Abattoir
  - o Carry out the Ante-mortem Examination of all Animals intended to Slaughter for Export
  - o Ensure that General Hygiene rules for Facilities, Equipments used during Slaughter and Personnel are at Required Standard

- Carry Post-Mortem Inspection in a Systematic Manner and Ensure that Meat passed for human consumption is Safe and Wholesome
- Ensure that Refrigeration, Handling and Transport of Carcasses are in the Required Range
- Certify meat and meat products that are fit to be exported for human consumption and ensuring the disposal of all condemned organs and carcasses
- Contributing and influencing on HACCP implementation in export abattoir

Teaching

- ✓ Since 1996 give lecturer to assistant meat inspectors training MOARD, Debre-Zeit

Professional Association

- ✓ 1990-to-date Member, Ethiopian Veterinary Association
- ✓ 2003-to-date Member, HACCP team

Conferences/ Workshops Attended

1991……Veterinary Public Health, A.A.U FVM, Debre-Zeit, Ethiopia

1922……Food Safety Assurance, Addis Ababa, Ethiopia

2003……Veterinary Public Health, Shefayim, Israel

2005……Risk Analysis in Animal Health, Addis Ababa, Ethiopia

2007……SPSS training program, Civil Service College Addis Ababa, Ethiopia

2007 ……...Meat Inspection training A.A.U FVM, Debre-Zeit, Ethiopia

Research Experiences:

. Have gathered knowledge and data on:

- Treatment of cows with Acute Post-Parturient Endometritis (DVM thesis)

- The impact of disease/ with infection and others / to lower Livestock Production
- Parasitic infection and their contribution to Condemnation of Livestock Production
- Cases of Tuberculosis and their frequency at Debre-Zeit ELFORA Export Abattoir
- Occurrence of Campylobacteriosis in Mutton in Export Abattoirs (Seminar presented)
- Prevalence of *C. jejuni* and *C. coli* in Export Sheep(Mutton) and Goat Carcasses (MSc thesis conducted)

Summary of professional skills/ expertise

- Knowledge and Understanding of Complex Lab Techniques and their Application
- Ability to Use Complex Scientific Instruments
- Skills of Leader-ship, Team building and Interpersonal Skills
- Computer Knowledge
- Third Grade Driving License

Hobbies:

- Playing Table & Ground Tennis
- Watching TV- Different Programs
- Reading Books

9. SIGNED DECLARATION SHEET

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

NAME: Tefera Woldemariam Agago Signature---------- Date of submission ----------

This thesis has been submitted for examination to advisors:

Advisors	Signatures	Date of submission
Dr. Girma Zewde	----------	----------
Dr. Daniel Asrat	----------	----------
Dr. Getahun Ejeta	----------	----------