THERMOTOLERANT CAMPYLOBACTER SPECIES IN FARM ANIMALS AS A POTENTIAL RISK TO PUBLIC HEALTH IN JIMMA ZONE, SOUTHWEST ETHIOPIA

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ABBREVIATIONS

C. coli        Campylobacter coli
C. jejuni     Campylobacter jejuni
C. lari       Campylobacter lari
GBS           Guillain Barré Syndrome
HL            Heat-Labile
HS            Heat-Stable
Ig            Immunoglobulin
JDDE          Jimma Dairy Development Enterprise
JUCA          Jimma University College of Agriculture
LOS           Lipo-oligosaccharide
mCCDA         modified Charcoal Cephoperazone Deoxycholate Agar
SOD           Super Oxide Dismutase
Spp.          Species
Subsp.        Subspecies
ABSTRACT
Thermotolerant campylobacters particularly *C. jejuni* and *C. coli* have been established as causative agents of diarrhoeal diseases in humans worldwide. These organisms are widely distributed and present in most warm-blooded domestic and wild animals. The aim of the study was to determine the prevalence of thermotolerant *Campylobacter spp.* in various farm animals and to analyze the antimicrobial susceptibility patterns of *Campylobacter* isolates. A cross sectional study was conducted from rural setting in peri-Jimma town area (Merrewa peasant association) and in Jimma town (Jimma University Agriculture College and Jimma Dairy Development Enterprise). Fecal specimens from four hundred eighty five were collected on a systematic sampling technique from cattle (n=205), poultry (n=191), sheep (n=71), and pigs (n=18) during the period of January and February 2004. All specimens were cultured on selective media and isolated strains of *Campylobacter spp.* were tested for antibiogram activity using standard disk diffusion techniques. *Campylobacter spp.* were isolated from 192 (39.6%) of the 485 fecal specimens investigated [130/192 (67.7%) were from poultry, 27/192 (14.1%) from sheep, 26/192 (13.5%) from cattle and 9/192 (4.7%) from pigs]. Of the isolates that were identified to species level, *C. jejuni* accounted for 70 %, *C. coli* 27 %, and *C. lari* 3 %. The antimicrobial susceptibility pattern for 192 strains of the isolates showed 100% resistance to cephalothin followed by, trimethoprim-sulfamethoxazole (37.0%), ampicillin (20.0%), nalidixic acid and streptomycin (6.3 % each). All strains were sensitive to ciprofloxacin and chloramphenicol. More than 95% of the strains were sensitive to erythromycin, tetracycline, clindamycin, gentamicin, and norfloxacin. Multi-resistance to two or more antimicrobial agents was observed in 14.6% of all campylobacter strains tested. This
study indicates that farm animals could serve as a potential source of human infections. Thus, hygienic steps should be followed during handling of food of animal origin. Moreover, if extensive veterinary use of antimicrobial drugs continues, there is a considerable risk that the spread of resistant organisms will seriously limit the future usefulness of these antimicrobial agents in the treatment of \textit{Campylobacter} infections in humans.
CHAPTER - I

INTRODUCTION

1.1. General Introduction

*Campylobacter* is a Gram-negative slender, spiral-curved rod having a dimension of 0.2-0.9µm width and 0.5-5µm length. It is a microaerophilic and motile bacterium that is becoming the most frequently diagnosed bacterial cause of human gastroenteritis worldwide (Allos, 2001). It is found mainly in the intestines of many birds and mammals, including notably in poultry and cattle.

Most campylobacters have been isolated since the 1970s from humans, but by far four groups of campylobacters (*C. jejuni, C. coli, C. lari,* and *C. upsaliensis*) cause human enteritis. Again among these, only two are responsible for the majority of cases: *C. jejuni*, accounting for 98% of cases in Canada and the United Kingdom, and *C. coli* accounting for 40% in Poland and parts of central Africa. *C. upsaliensis* is the most important *Campylobacter* species after *C. jejuni* and *C. coli*, but in Europe, it has an isolation rate of about 1 to 2% (Skirrow, 2002). *C. lari* accounts for less than 1 percent of *Campylobacter* isolations. According to the study done by Asrat and colleagues (1997) from human patients, *C. jejuni* accounted for 82.4 % and *C. coli* for 17.6 % of all the isolates at Tikur Anbassa Hospital in Ethiopia.

Diarrhea is one of the most prevalent problems throughout the world and the leading cause of morbidity and mortality (Huttly *et al.*, 1997; WHO, 1990). According to WHO (1990), over 1.4 billion episodes of diarrhoea occur each year in children under five years of age in Asia (excluding China), Africa, and Latin
America, and that 4 million children in this age group die annually from diarrhea. Eighty percent of these deaths occur in the first two-year of life (WHO, 1990). In Ethiopia, studies have demonstrated that about 10% of infants died before their first birthday and more than 15% of the children died before their fifth birthday, which primarily was due to diarrhea (Tesfaye, 1998).

Campylobacter is only one of many causes of acute diarrhea. The past three decades have witnessed the rise of campylobacter enteritis in man from virtual obscurity to notoriety. The present isolation rates of Campylobacter spp. is superseding those of other enteric pathogens such as diarrhoeagenic E. coli O157:H7, Salmonella spp. and Shigella spp. in most developed and developing countries (Tauxe, 1992; Asrat et al., 1999). Campylobacter enteritis has been increasing in many countries: Australia; Queensland (Stafford et al, 1996), England (Tauxe, 1992), United States of America (Tauxe et al., 1988), Norway (Kapperud et al., 1992), and Thailand (Bodhidatta et al., 2002).

Campylobacter, as causes of acute diarrhea, produced up to 20% of all infectious diarrhea in developing countries (WHO, 1990). Since, the disease transmission is generally believed to be food borne, via poultry, undercooked meats, and meat products, as well as raw or contaminated milk and contact to animals, campylobacteriosis is considered as zoonosis. One of the major gaps in our knowledge at present is the relative contribution of animals and their products to the overall burden of disease in man. Estimation of the importance of all known sources is therefore extremely important.
Among campylobacters, the themophilic campylobacters are now recognized as the most common cause of bacterial gastroenteritis in humans in many developed and developing countries (Atabay and Corry, 1998). Diseases caused by other species, such as *C. upsaliensis* and *C. hyointestinalis*, have also been described at much lower frequency (Saleha et al., 1998). *Campylobacter* spp. is very common in domestic and wild animals from which they are rather often and easily isolated (Steinhauserová and Fojtíková, 1999). Several countries supervise and control the likelihood of food animal colonization and contamination of animal products before it reaches to the consumer. However, how frequent this bacterium exists in various farm animals in Ethiopian settings was not performed.

Very little research has been done on the epidemiology of campylobacteriosis in food animals in developing countries. Because of the difference in the rearing systems and the slaughtering processes, risk factor for *Campylobacter* contamination in food animals in developing countries may be significantly different from those in developed countries. It may be logical to view and consider the peculiar situation in Ethiopia where the greater proportion of the people live together with domestic farm animals. Furthermore, along with the huge number of farm animals in this country and the literacy status of the community, the danger of *Campylobacter* infection in human, especially in children of under five, might be high.

In fact, there are few studies on enteric campylobacteriosis in hospital of major cities in Ethiopia (Gedlu and Assefa, 1996; Asrat et al., 1999; Awol et al., 2002). There are no studies specifically performed on farm animals, and/or domestic animals in rural settings for the isolation of *Campylobacter* strains. In addition, the
sensitivity of the bacteria from farm animal sources in the country to antimicrobial agents was not known and evaluated. As a result, with the aim to determine the prevalence of thermotolerant *Campylobacter* in various farm animal groups, this research was designed to realize the potential implication of food animal products. Furthermore, the study aimed to determine the antimicrobial susceptibility of *Campylobacter* isolates from animals to available antimicrobial drugs that are mainly used in human medicine, and come up with results, if any, to show the risk of animal husbandry to the public at large.

### 1.2. Review of Literature

#### 1.2.1. Historical Background

In 1886, Theodore Escherich observed organisms resembling campylobacters in stool samples of children with diarrhea (Nachamkin *et al.*, 2000). According to Skirrow (2000), campylobacters were first isolated in 1906 from aborting sheep in the UK. Another literature depicted that the first *Campylobacter* might have been isolated in 1913 in fetal tissues of aborted sheep by McFaydean and Stockman (Allos, 2001). However, in any case, the bacterium was known as *Vibrio fetus*. Later in 1957, King (1957) reported a thermophilic vibrio-like bacterium from blood samples of children with diarrhea associated with human acute enteritis but subsequently was classified as *Vibrio* spp.

The naming of the bacteria as ‘related’ *Vibrio* or *Vibrio* spp. was continued until the genus name *Campylobacter* was established in 1963 (Vandamme, 2000). The name *Campylobacter* is derived from the Greece ‘*campylos*’ meaning ‘curved’ and ‘*baktron*’ meaning ‘rod’ (Blaser, 2000). In 1972, Butzler and other clinical
microbiologists in Belgium first isolated camplobacters from stool samples of patients with diarrhea (Butzler et al., 1973).

The development of selective growth media in the 1970s permitted more laboratories to test stool specimens for Campylobacter (Dekeyser et al., 1972; Butzler et al., 1979). Soon Campylobacter species were established as common human pathogens. Two species, C. jejuni and C. coli, are now among the commonest identified causes of enteritis in many countries.

During the last 25 years, new pathogenic species have been assigned to the genus. Although a number of these have been implicated in human enteritis, the most common by far are the so-called ‘thermophilic’ or ‘thermotolerant’ campylobacters by which, more than 95% of campylobacter enteritis was caused worldwide. Those thermophilic or thermotolerant species will grow at 42°C-43°C and also at 37°C but not at 25°C. This group includes C. jejuni, C. coli, C. lari, and C. upsaliensis (Padungton and Kannen, 2003).

1.2.2. Taxonomy

Bacterial taxonomy is changing rapidly (Holt et al., 1994). According to the second edition of Bergey’s manual of systematic Bacteriology, an edition that was published, Campylobacter belong to proteobacteria kingdom (Prescott et al., 1999).

The kingdom proteobacteria is a large and extremely complex group that currently contains over 1,300 species in 332 genera. Even though, they are all related, the group is quite diverse in morphology, physiology, and life-style. Many species important in medicine, industry, and biological research are proteobacteria. The
kingdom is divided in to five groups based on rRNA data. These are $\alpha$-, $\beta$-, $\delta$-, $\epsilon$-, and $\gamma$- proteobacteria. The group $\epsilon$-proteobacteria is the smallest and composed of one class, Campylobacteres, one order Campylobacterales, and two families, Campylobacteraceae and Helicobacteraceae (Prescott et al., 1999).

The family Campylobacteraceae includes more than 18 species and subspecies with in the genus Campylobacter and four species in the genus Arcobacter. Two species that were formerly classified in the genus Wolinella (W. curva and W. recta) are reclassified as Campylobacter (C. curva and C. recta) (Nachamkin, 1999). The continual progress and developments in the criterion of taxonomy may refine the number of Campylobacter species.

Recently all the species and subspecies of Campylobacter include C. jejuni (subsp. jejuni and subsp. doylei), C. coli, C. fetus (subsp. fetus and subsp. venerealis), C. lari, C. upsaliensis, C. hyointestinalis (subsp. hyointestinalis and subsp. lawsonii), C. sputorum (biovar sputorum, biovar bubulus, and biovar fecalis), C. helveticus, C. mucosalis, C. concisus, C. curvus, C. rectus, C. hominis, and C. showae (Collee et al., 1996; Nachamkin, 1999).

The genus Campylobacter contains both non-pathogens and species pathogenic to humans and animals (Padungton and Kannen, 2003). Campylobacter spp. are Gram negative, slender, non-spore forming, curved, ‘S’-shaped, or spiral bacteria, which are 0.2 to 0.9$\mu$m wide and 0.5 to 5 $\mu$m long. They are motile by means of polar unsheathed flagella at one or both ends and grow under microaerophilic conditions (Padungton and Kannen, 2003; Blaser, 2000).
1.2.3. Laboratory Identification Methods

Campylobacteriosis may be established by demonstration of the organisms by different methods. Specimen collection and processing requires special attention: feces in plain container, refrigerated, or examined within few hours. Rectal swabs must be in semisolid transport medium (e.g., Cary-Blair transport medium; Wang’s medium).

a) Direct examination of faeces under Microscopy

Examination of diarrhoeal fecal specimens stained by Gram’s stain for the presence of Gram-negative ‘Gull-wing’ appearance under microscope is a very specific diagnostic feature, although the sensitivity ranges from 66 to 94% (Nachamkin, 1999; Blaser, 2000). Examination of fecal specimens by dark field or phase contrast microscopy is also of value within two hours of passage for the characteristic darting motility in wet mount preparation. Fecal leukocytes are commonly present and can be readily visualized with methylene blue staining of wet or dried fecal smears. However, examination for leukocyte is not recommended as a test for predicting bacterial infection or for selective culturing for campylobacter pathogens as the absence of fecal leukocytes does not rule out disease (Collee et al., 1996; Nachamkin, 1999).

b) Culture

Diarrheic stools should be routinely cultured for Campylobacter in most clinical laboratories. Special media supplemented with blood or other nutrients and antibiotics to inhibit normal microbial flora must be used to culture the organism.
Different manufacturers formulate selective media such as Butzler's agar; Skirrow's agar; and Campy-BAP. Isolation requires growth in microaerophilic atmosphere (5-7% oxygen, 5-10% carbon dioxide, balanced nitrogen). For thermophilic species, (e.g. *C. jejuni*; *C. coli*) incubation at 42-43°C for 24-72h is recommended. For non-thermophilic species, (e.g. *C. fetus*; *C. hyointestinalis*) 37°C is optimal.

Enrichment broth has been formulated to enhance recovery of campylobacters from stool samples. These include: Campy-thioglycollate, Preston selective enrichment, Campylobacter Enrichment Broth, etc. Enrichment media are useful for healthy animal faeces, environmental and food samples where organisms may be stressed or in low numbers (*Stanley et al.*, 1998).

Selective isolation of *Campylobacter* by filtration of stool specimen can be used based on the principle that campylobacters, compared to other enteric bacteria, can pass through 0.45 µm, 0.65 µm, or 0.8 µm pore-size filter membranes, while most other enteric microorganisms are retained. Filtrate can then be grown out on nonselective agar though the method has a lower sensitivity than direct culture onto selective media. Therefore, filtration method should be used to complement direct culturing on selective plating media and not as replacement. The temperature for thermotolerant campylobacters i.e. 42-43°C allows the growth of *C. jejuni*, *C. coli*, and *C. lari* on selective media. *C. uplasiensis* also grows well at 42°C but usually not recovered on selective media. Plates should be incubated for 72h before being reported as negative (*Collee et al.*, 1996; Nachamkin, 1999).

Because of the selective media and incubation conditions for growth, an abbreviated set of tests is usually all that is necessary for identification. Thermophilic
Campylobacters are oxidase and catalase positive. Gram stained preparation demonstrating distinctive morphologic appearance (Gram negative ‘S’ shaped rods) can be reliably reported as *Campylobacter* spp. Primary isolates can be checked for carbohydrate fermentation or oxidation which are negative; nitrate reduction test, hydrogen sulfide production test, hippurate hydrolysis test (*C. jejuni* is positive), and susceptibility to nalidixic acid and cephalothin to determine the species of *Campylobacter* (Table- 1.1).

PCR and DNA probes are on the horizon although the method is expensive and complex. Antigen detection methods are described below in an antigen and strain typing of campylobacters. Antibody detection correlates of protection ("markers" that predict protection of host from disease) have not been established as a routine technique. However, serodiagnosis may be important in the immunological sequelae such as reactive arthritis or Guillain-Barrè syndrome (*Collee et al., 1996*).
Table-1.1. Distinguishing character of thermotolerant *Campylobacter* species (Adapted from Collee *et al.*, 1996; Nachamkin, 1999).

<table>
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<tr>
<th></th>
<th>Catalase</th>
<th>Oxidase</th>
<th>H₂S (TSI)</th>
<th>Hippurate hydrolysis</th>
<th>Growth at 42°C</th>
<th>Susceptibility to Nalidixic acid</th>
<th>Susceptibility to Cephalothin</th>
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<tr>
<td><em>C. jejuni</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>C. upsaliensis</em></td>
<td>W*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*W= weak reaction; S= Susceptible; R= Resistant; TSI= Triple Sugar Iron agar.*
1.2.4. Antigen and Strain Typing of Campylobacters

Campylobacters possess two classes of antigen: heat (thermo)-stable lipo-polysaccharide antigens (HS), which form the basis of the Penner serotyping scheme (65 serotypes), and heat (thermo)-labile surface and flagellar protein antigens (HL), which form the basis of the Lior serotyping scheme (160 serotypes) (Asrat et al., 1997; Skirrow, 2000). Serotyping can be used in concert with biotyping or phage typing to give finer discrimination (Frost, 1999). Molecular ‘fingerprinting’ methods are gaining favors in epidemiological studies (Ribot, 2001).

Means and routes of Campylobacter spp transmission have been known to occur through various ways. Serotyping is one of the possibilities to identify and evaluate the epidemiological relation of strains isolated from different sources and patients. Numerous methods such as tests directed to polysaccharides, lipopolysaccharides, or proteins have been described (Nachamkin, 1999).

1.2.5. Epidemiology of Enteric Campylobacteriosis

Campylobacters are commonly found as commensals in the gastrointestinal tract of wild or domestic cattle, sheep, swine, goats, dogs, cats, rodents, and all varieties of fowls (Blaser et al., 1983; Simbert, 1984). The bacteria, some times, cause gastroenteritis in these animals. Due to this, campylobacteriosis is considered as a worldwide zoonosis. C. jejuni has a very varied reservoir, but C. coli is, most commonly, isolated from swine, C. upsaliensis from dogs and cats, and C. lari from poultry, birds, dogs, cats, monkeys, and horses (Simbert, 1984; Skirrow et al., 2002).
The vast reservoir in animals is probably the ultimate source for most enteric 
*Campylobacter* infections of humans.

Most cases of human campylobacteriosis are sporadic. Outbreaks have different epidemiological characteristics from sporadic infections (Tauxe, 1992). Outbreaks of *Campylobacter*, especially in developed countries, are usually associated with raw milk consumption (Schmidt *et al.*, 1987; Kalman *et al.*, 2000; Pederson, 2003), whereas sporadic illnesses are often associated with consumption of poultry (Deming *et al.*, 1987; Effler *et al.*, 2001). Contaminated poultry is responsible for about or more than half of all *Campylobacter* infections. Other risk includes contact with cats (Deming *et al.*, 1987) and dogs (Kapperud *et al.*, 1992), eating pork, living or working on farms, working in slaughter houses, and traveling abroad from developed to developing countries (Nielsen *et al.*, 1998). Fecal-oral transmission from person to person may also occur, but it is uncommon for the disease to be transmitted by food handlers (Nachamkin, 1999).

Various molecular identification techniques are now available for epidemiological typing of *Campylobacter* spp. These techniques, along with other methods mentioned above in antigen and serotyping of campylobacters, have been used to trace outbreaks back to the source animal or rarely to food handlers (Pearson *et al.*, 2000; Olsen *et al.*, 2001). A recent molecular study suggested a link between *Campylobacter* spp. found in farm environments and those causing diseases in local communities’ (Fitzgerald *et al.*, 2001). Overlap is reported between serotypes of *C. jejuni* found in humans, poultry, and cattle, indicating that foods of animal origin may play a major role in transmitting *C. jejuni* to humans (Nielsen *et al.*, 1997).
There have been several studies on the epidemiology of *Campylobacter* in poultry in developed countries. The intestine of poultry is easily colonized with *C. jejuni*. Most chickens in commercial operations are colonized by four weeks of hatching (Humphrey *et al*., 1993). Sources of infection were more likely to be horizontal contamination from the environment or water system rather than from direct flock-to-flock transmission (Sjögren, 1988; Jacob-Reitsma, 1997).

Additional risk factors for *Campylobacter* colonization in poultry include housing with no air circulation, batch depletion of the flock (not all in/ all out) and the presence of other animals (pig, cattle, sheep) on the farm (van de Giessen *et al*., 1996). *Campylobacter* spp. have been found with prevalence up to 50% in wild birds; not associated with poultry sources (Chuma *et al*., 2000).

A longitudinal study that was done on flocks of chickens from farm to processing plant and market, in developed country, showed the highest prevalence of *Campylobacter* at the processing plant (52%), suggesting that contamination of carcasses most likely occurred at the processing plant (Jones *et al*., 1991). Another series of case control studies identified some risk factors for sporadic campylobacteriosis, particularly handling raw poultry (Hopkins and Scott, 1983) and eating under cooked poultry (Kapperud *et al*., 1992). Other risk factors accounting for a smaller proportion of sporadic campylobacteriosis include drinking untreated water (Blaser, 2000).

Campylobacters are found in natural water sources throughout the year. Survival in cold water is important in the life cycle of campylobacters. In various studies done, serotypes found in water were similar to those found in humans (Bolton...
et al., 1987; Peterson, 2003). Unlike salmonellae, campylobacters do not multiply in foods; so explosive food poisoning outbreaks are rare. Most infections are sporadic or confined to one household (Skirrow, 2000).

Campylobacters demonstrate a ‘viable but non-culturable state’ characterized by uptake of amino acids but inability to grow on selective media; such organism, however, can be transmitted to animals and may gain capacity to infect and cause disease (Stern et al., 1994; Lazaro et al., 1999).

Although C. jejuni is among the most commonly recognized bacterial pathogen in man that often exceeds the combined total for diarrheal illnesses caused by Salmonella spp., Shigella spp., and E. coli O157:H7 (Tauxe, 1992; Asrat et al., 1999), many laboratories do not routinely do culture for this organisms (Tauxe, 1992). In addition, it is more expensive and more difficult to do culture for campylobacter than for ordinary Salmonella and Shigella species; thus, infection with C. jejuni is considered to be substantially underreported (Allos and Blaser, 1995).

1.2.6. Pathogenesis of Campylobacter Infection in Human

Little is known bout the pathogenesis of Campylobacter infection. In one study, volunteers become ill after ingesting as few as 500 organisms (Black et al., 1988). Among exposed persons who become ill, the incubation period varies from 1 to 7 days. Most infections occur 2 to 4 days after exposure. Rate of infection increases with the ingested dose. The pathogenesis of C. jejuni infection involves both host- and pathogen-specific factors.
Efforts to define the role of pathogen-specific virulence factors in *Campylobacter* disease have been impeded by lack of an animal model to study the disease. Comparatively *C. jejuni* is the best-studied species. Although, adhesins, cytotoxic enzymes, and enterotoxins have been detected in this species, their specific role in disease remains poorly defined. However, patients with hypogammaglobulinemia have prolonged severe disease with *C. jejuni* (Nachamkin, 1999).

Many pathogen-specific virulence determinants probably contribute to *C. jejuni* infection though not well proven (Ketleyse, 1997). Suspected determinants of pathogenecity include chemotaxis, motility, and flagella, which are required for attachment and colonization of the gut epithelium (Ketleyse, 1997; Blaser, 2000). Once colonization occurs, other possible virulence determinants are iron acquisition, host cell invasion, toxin production (enterotoxin or cytotoxin), inflammation and active secretion, and epithelial disruption with leakage of serosal fluid (Ketleyse, 1997; Nachamkin, 1999).

*C. jejuni* expresses, unique surface sugars that do not exist in mammals, and are likely to be important for the virulence of the bacteria. The importance of catalase in *Campylobacter* survival inside the host has also recently been investigated. Evidence indicates that production and secretion of catalase and super oxide dismutase (SOD) aid *Campylobacter* survival inside infected cells (Day et al., 2000), by making them resistant to the initial respiratory burst that occurs when a cell is infected.

*C. jejuni* like *Vibrio cholerae* is susceptible to hydrochloric acid conditions (pH ≤5). Rates of illness appeared to increase when the bacteria were ingested in a
suspension buffered to reduce gastric acidity such as milk, fatty foods, and water (Blaser, 2000). The sites of tissue injury include the jejunum, ileum, and colon with similar pathologic features in each. The mucosal surface appears ulcerated, edematous, and bloody, with crypt abscesses in the epithelial glands and infiltration of the lamina propria with neutrophils, mononuclear cells, and eosinophils. This inflammatory process is consistent with invasion of the organisms into the intestinal tissue (Blaser, 2000).

*Campylobacter* is known to cause inflammatory enteritis. During the course of disease, it can penetrate the epithelial barriers and may interact with leukocytes. The extent of *Campylobacter* to stimulate proinflammatory cytokines suggests that monocytes could significantly contribute to inflammation and disease pathology (Jones *et al*., 2003).

Ganglioside mimicry by *C. jejuni* and *C. upsaliensis* lipo-oligosaccharide (LOS) has been implicated in production of anti-ganglioside antibodies, which play a role in Guillain Barré Syndrome (GBS). The pathogenesis of this disease believed to be related to antigenic cross-reactivity between oligosaccharides of *Campylobacter* and glycosphingolipides present on the surface of neural tissues (Weinberg *et al*., 2001; Nachamkin *et al*., 2002). Thus, antibodies directed against specific strains of *Campylobacter* (particularly from the strains of *C. jejuni*) can damage neural tissue in the peripheral nervous system.
1.2.7. Clinical features of Campylobacter Infection

a) In humans

*C. jejuni, C. coli* and *C. upsaliensis*, all of which are thermotolerant, have been recognized as important etiologic agents of gastrointestinal infection since the 1970s (Nachamkin *et al.*, 2000). Moreover, the clinical feature of *Campylobacter* enteritis in humans caused by *C. jejuni* and *C. coli* are indistinguishable from each other and from acute bacterial diarrhea caused by other pathogens like *Salmonella* enteritis (Boyed, 1995; Skirrow *et al.*, 2002). *Campylobacter* may cause mild or severe diarrhea, bloody diarrhea, nausea, and stomach pain, often with fever.

In developed countries, typical *Campylobacter* spp. infection includes acute self-limiting gastroenteritis, characterized by diarrhea, nausea, and abdominal cramps often with fever. Symptoms usually start after 2 to 5 days of incubation period and mostly last for less than ten to fourteen days, and sometimes a relapse can occur in 5 to 10% of untreated patients after recovery from symptoms (Kaminstein, 1999; Blaser, 2000). Diarrhea is initially watery, which may last for more than two weeks in traveler’s diarrhea (Gallardo *et al.*, 1998) or may become bloody because of diffused inflammatory colitis and enteritis (Blaser, 1997).

In developing countries, the clinical picture of *Campylobacter* infection characterized by a milder form of gastroenteritis (Oberhelman and Taylor, 2000), and symptomatic infection is uncommon in adults (Blaser, 1997). Severe diarrhea requiring hospitalization is usually the result of co-infection with other virulent bacteria or viruses. *C. jejuni* infection is much more common in developing than in
developed countries, but many characteristics are different (Table-1.2). In developing countries, symptomatic infection predominantly affects young children.

Campylobacters may be excreted in the feces for several weeks after clinical recovery, although the rate of excretions falls exponentially with time (Skirrow, 2002). The incidence of campylobacter infection is increased in patients with AIDS (Nachamkin, 1999). Long-term carriage of campylobacters can occur, sometimes associated with recurrent attacks of enteritis and bacteremia.

The most common clinical features in children are diarrhea, fever, abdominal pain, and vomiting. Blood in the stools may be present in more than one half of children with Campylobacter gastroenteritis. Severe abdominal pain arising before the onset of diarrhea can mimic acute appendicitis and result in unnecessary surgery. The severity of clinical symptom and fecal excretion of campylobacters in children was inversely related to age (Taylor et al., 1993). In developing countries, where infection is hyper-endemic, children become repeatedly infected early in life and often associated with diarrhea (Gedlu and Assefa, 1996; Bodhidatta et al., 2002; Aboderine et al., 2002).

Extra-intestinal infection and chronic sequelae of infection did occur in smaller proportion of patients (Nachamkin, 1999). Bacteremia has been noted in less than 1% of patients with C. jejuni infection. This appears to be more common in persons at the extremes of age (Friedman et al., 2000). Meningitis and endocarditis are rare manifestation of C. jejuni infection. There have been infrequent reports of C. jejuni infections manifested as septic abortion, acute cholecystitis, pancreatitis, and cystitis (Nachamkin, 1999).
Table 1.2. Comparison of the feature of *C. jejuni* infections in developed with those in developing countries (adapted from Blaser, 1997).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Developed countries</th>
<th>Developing countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of infections per life time</td>
<td>0-1</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>Principal age group affected</td>
<td>Young adults</td>
<td>Children &lt; 2 years old</td>
</tr>
<tr>
<td>Principal manifestation of illness</td>
<td>Inflammatory diarrhea</td>
<td>Simple (watery) diarrhea</td>
</tr>
<tr>
<td>Wide spread immunity among adults</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Principal vehicle</td>
<td>Poultry</td>
<td>Unknown (probably contact to animal and ingestion of contaminated animal products).</td>
</tr>
</tbody>
</table>
Campylobacters have also recently been linked to some autoimmune diseases such as Reactive Arthritis and Guillain-Barrè Syndrome (GBS). These two major late onset complications of Campylobacter are estimated at one case per 2000 infections (Boyed, 1995; Altekruse et al., 1999). Campylobacter infection is recognized as the most commonly identified antecedent event in GBS (40-60% of all cases), also known as post-infective polyneuropathy. The main lesions are acute inflammatory demyelinating polyradiculo-neuropathy that results in a flaccid paralysis (Weinberg et al., 2001). Reactive arthritis occurs in approximately 1% of patients with Campylobacter enteritis (Skirrow, 2002). The manifestations are similar to those associated with Salmonella, Shigella, and other bacterial diarrheas (Peterson et al., 1994).

On the other hand, particularly in patients with Campylobacter-associated disease, acute axonal degeneration may happen in some proportion of patients. These changes may be caused by cross-reacting antibodies to GM1 ganglioside formed in response to similar epitopes expressed by the infecting Campylobacter strain (Aspinall et al., 1994; Tsang et al., 2001; and Nachamkin et al., 2002). A particular C. jejuni clone marked by lipopolysaccharide (O) type 19 is over-represented among persons who develop GBS (Kuroki et al., 1993). O type 41 also has been implicated (Prendergast et al., 1998).

The clinical manifestations of infection due to other campylobacters overlap substantially with those of C. jejuni infection. On average, C. coli may produce more mild diseases. Among immunocompromised patients, especially those with AIDS,
bacteremia from the ‘atypical’ campylobacters appears relatively common. Deaths attributable to *C. jejuni* infection are uncommon but do occur (Nachamkin, 1999).

b) **In Food or Farm Animals**

*Campylobacter* species cause enteritis, abortions, and infertility in various species of animals. The role of *C. jejuni* as primary pathogen in farm animals is uncertain (Padungton and Kaneene, 2003). *C. jejuni* and occasionally *C. coli* cause enteritis in dogs, cats, calves, sheep, mink, poultry and some species of laboratory animals. The clinical signs may be more severe in young animals.

In dogs, symptoms can include diarrhea, decreased appetite, vomiting, and sometimes fever. The feces are usually watery or bile-streaked, with mucus and sometimes blood. Symptoms generally last 3 to 7 days, but some animals may have intermittent diarrhea for weeks and occasionally for months. Calves typically have a thick, mucoid diarrhea with occasional flecks of blood, either with or without fever. Mucoid, watery, and sometimes bloody diarrhea is also seen in cats, primates, and ferrets. Newly hatched chicks and fowl develop acute enteritis, with rapid onset of diarrhea and death. Gastrointestinal campylobacteriosis is usually self-limiting in animals; however, up to 32% mortality may be seen with highly pathogenic isolates in chicks (Garcia *et al*., 1983).

Bovine venereal campylobacteriosis is a chronic infection of the female genital tract, characterized by mild endometritis and transient infertility (Garcia *et al*., 1983). Reproductive symptoms in cattle due to *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* can cause bovine genital campylobacteriosis. This disease is characterized by infertility, early embryonic death, and a prolonged calving season.
Abortions are uncommon but are occasionally seen as sporadic abortion in cattle. Infected cows may develop a mucopurulent endometritis but do not usually have other systemic signs (Garcia et al., 1983; Aiello and Mays, 1998).

*C. fetus* subsp. *fetus* and *C. jejuni* can cause enzootic abortion that can result in late term abortions, stillbirths, and weak lambs in sheep. Infections in sheep are sometimes followed by endometritis and occasionally deaths. Morbidity may be up to 90% in outbreaks in sheep but is usually around 5 to 50%. Morbidity in sheep can result in prolonged lambing, and reduction in milk output. Recovery, with immunity to re-infection, is typical. Sheep can become persistently infected and continue to shed bacteria in the feces (Aiello and Mays, 1998). Infection in bulls is not accompanied by histological changes or modification in the characteristic of semen. Bulls are frequently asymptomatic (Blaser, 2000). Young lambs and newborn calves rapidly acquire the organism from the farm environment via horizontal not vertical transmission (Aiello and Mays, 1998).

Other *Campylobacter* infections including *C. lari*, *C. hyointestinalis* and *C. upsaliensis* can cause disease but seem to be of minor importance (Saleha et al., 1998). Communicability of *Campylobacter* species readily occurs between animals. Organisms are present in feces, vaginal discharges, and the products of abortions and can be spread by direct contact, on fomites and by arthropods acting as mechanical vectors. Contaminated food and water is often the source of infections (Aiello and Mays, 1998).
1.2.8. **Immunity**

Campylobacteriosis demonstrate a different pattern in developing countries. High transmission rates, unlike the developed world, mean that children become repeatedly infected early in life, which often is associated with diarrhea. However, as immunity is gained, subsequent infections are increasingly likely to be asymptomatic (Blaser *et al.*, 1986). Thus, the disease is virtually unknown in older children and adults.

Patients infected with *Campylobacter* develop specific IgG (immunoglobulin G), IgM, and IgA antibodies in serum and IgA antibodies in intestinal secretion (Black *et al.*, 1988). However, in conditions, such as in patients with congenital or acquired hypogammaglobulinemia, failures to produce a humoral response to infection have been observed (Perlman *et al.*, 1988; Boyed, 1995). In HIV infected patients as well, failure of C. jejuni infection to respond to antimicrobial therapy has been correlated to produce a humoral response to infection (Perlman *et al.*, 1988). On the other hand, the value of cell-mediated immunity is indispensable in preventing and terminating infection.

An effective vaccine search against campylobacteriosis in humans and animals is on trial in various animals and *in-vitro* tests. Candidate antigens include primarily flagellin protein, and others include live sub lethal campylobacter infection, and bivalent anti Salmonella/Campylobacter chicken vaccine–an antigen derived from *Campylobacter* inserted in *Salmonella* vector, (Lis *et al.*, 2003).
1.2.9. Treatment

Most patients with diarrhea due to campylobacteriosis require simple supportive treatment in the form of oral fluid and electrolyte replacement. Dehydrated patients should receive intravenous fluids (electrolyte in water) for rapid volume expansion (Nachamkin, 2002; Altekruse et al., 1999). The value of antimicrobial therapy in Campylobacter enteritis is limited by the fact that most patients recover by the time a laboratory report has been received.

When antimicrobial agents are required, various antimicrobial agents including fluoroquinolones, aminoglycosides, tetracycline, chloramphenicol, macrolids and nitrofurans can be used to which C. jejuni and C. coli are normally sensitive (Blaser, 1997; Brooks et al., 1998; Skirrow, 2002).

The conditions for treatment include patients who have high fever, bloody diarrhea, or more than 8 stools in 24h; immune suppressed patients; patients with blood stream infection; and those whose symptoms worsen or persist for more than one week. Campylobacters are inherently resistant to trimethoprim and most beta-lactam antibiotics, including penicillins and cephalosporins. However, amoxicillin or ticarcillin plus clavulanic acid appear to be universally effective (Lachance et al., 1993).

Erythromycin was the first antibiotic to be used for Campylobacter enteritis; it is still the agent of first choice when the diagnosis has been confirmed. Studies in children with dysentery due to C. jejuni showed a clear benefit from early treatment with erythromycin. The use of anti-motility agent appears to prolong duration of symptoms and has been associated with fatalities. The necessity for treating
bacteremic episodes with agents other than erythromycin has not been established (Blaser, 2000).

An alternative agent is ciprofloxacin, which has activity across a broad spectrum of bacteria causing diarrheal illness as well as against campylobacters. Another alternative agent is tetracycline except in children under 9 years of age. In this aged children, clindamycin may be used (Blaser, 2000; Skirrow, 2000). In few countries, resistance rates of campylobacters seldom exceed 5 percent for macrolids while in some parts of the developing world they are much higher (Blaser, 1997). A higher prevalence of macrolide resistance has been found for *C. coli* than *C. jejuni* both in animals and humans (Nachamkin, 2000). Strains of *Campylobacter* acquired from developing countries, especially *C. coli*, are more likely to be resistant to erythromycin and tetracycline.

The other aspect of antimicrobial use is the problem of resistance that has become a major public health concern in both developed and developing countries in recent years (Witte, 1998; Padungton et al., 2003). The emergence of antibiotic resistance as a serious problem in human medicine has provoked concerns about the public health implications of antibiotic use in agriculture. Those antibiotics have been used for various reasons such as to treat an identified illness in animals caused by bacteria, to prevent illness in advance, and to animal performance enhancement in order to increase yield.

The practices of using antimicrobial drugs are known to facilitate the emergence of resistant *Campylobacter* strains. There has been speculation that the use of antimicrobial agents in food animals resulted in increasing the prevalence of

For instance, in the United States the approval of sarafloxacin in 1995 and enrofloxacin in 1996 for use in poultry flocks contributed to an increase in the number of domestically acquired fluoroquinolone-resistant Campylobacter infections in Minnesota (Smith et al., 1999). In that state, fluoroquinolone resistance among Campylobacter isolates from humans increased from 1.3% in 1992 to 10.2% in 1998. The increasing resistance of campylobacters was also observed in many parts of the world (Arestrup et al., 1997; Bodhidatta, et al., 2002; Gaudreau and Michaud, 2003; Pedersen and Wedderkopp, 2003). Elsewhere in Europe, resistance rates of quinolones are closer to 5 to 10%, and many of the resistance strains are acquired abroad (Guant and Paddock, 1996; Sjögren et al., 1997; Rauteline et al., 2003; Iovine and Blaser, 2004).

Campylobacter with resistance to antimicrobial agents have been reported in both developed and developing countries, and the situation seems to deteriorate more rapidly in developing countries, where there is a widespread and uncontrolled use of antibiotics (Guant and Paddock, 1996; Hart and Kariuki, 1998; Shapiro et al., 2001; Bodhidatta, et al., 2002; Padungton and Kannen, 2003). To the knowledge of this writer, there is no recent indicator in Ethiopia for the pattern of Campylobacter susceptibility to antimicrobials with particular emphasis to animal isolates. However, a study conducted on human patients of Tikur Anbassa Specialized Hospital, in Ethiopia showed that about 60% of Campylobacter strains were found resistant against ampicillin and trimethoprim-sulfamethoxazole (Asrat et al., 1999). However,
all *Campylobacter* isolates were sensitive to erythromycin, nalidixic acid, norfloxacin, chloramphenicol, gentamicin, sulfonamide, and tetracycline

**1.2.10. Control and Prevention of Campylobacter Infection**

a) **On the Farm**

Epidemiological studies indicate that strict hygienic steps reduce intestinal carriage in food producing animals (*Humphrey et al.*, 1993). In field studies, poultry flocks that drank chlorinated water had lower intestinal colonization rates than poultry that drank unchlorinated water (*Gregory et al.*, 1997). *Campylobacter* is thought of as a commensal of the avian host. The struggle to develop methods such as treatment of chickens with commensal bacteria other than campylobacter, which is called competitive exclusion regimens (*Stern et al.*, 1994); and flock vaccination (*Lis et al.*, 2003) starts to show green light in minimizing excretion rate. Nevertheless, elimination of intestinal *Campylobacter* carriage from food producing animals may not be an easy task and thus the risk of infection from these sources will remain.

b) **At Processing of Food Animals**

Bacterial counts on carcasses increase during slaughter and processing steps. In studies of chickens and turkeys (*Mead et al.*, 1995) at slaughter, bacterial counts increased by approximately 10 to 100 fold during defeathering and reached the highest level after evisceration. However, bacterial counts on carcasses could decline through hygienic slaughter and processing steps such as forced-air blast chilling of carcasses, scalding or mild heat treatment, use of chlorinated sprays, maintenance of
clean working surfaces, lactic acid spraying of swine carcasses, and terminal radiation (Skirrow, 2002).

c) **At Home**

Proper preparation of food, avoidance or heating of unpastuerized dairy products, avoidance of eating raw meat, travel to underdeveloped countries (holds true for developed people moving to hyper-endemic *Campylobacter* transmission area), and exposure to animals such as pet animal with diarrhea (particularly puppies and kittens) should be avoided (Skirrow, 2002).

The wide distribution of campylobacters in nature constitutes an irreducible reservoir. For the prevention of campylobacteriosis, methods should direct at reducing or minimizing infection in reservoir animals and interrupting the transmission of campylobacters from animals to humans. Moreover, control measures at all stages of the food chain from agricultural production are the primary tool to reduce the transmission to humans.

1.2.11. **Summary**

Awareness of the public health implication of campylobacter infections has evolved for several decades. *Campylobacter* spp. is Gram-negative non-spore forming curved or spiral bacilli, which require a microaerophilic conditions. The “thermotolerant” species are so named for their ability to grow at higher temperatures while unable to grow below 30°C. Thermotolerant campylobacters are the most common species associated with gastrointestinal illness in human, and are part of the natural intestinal flora of a wide range of birds and mammals. Transmission to
humans is usually by contamination of food and water although unpasteurized milk and animal contact are implicated. Poultry and other foods of animal origin are the most likely sources of infection in developed countries.

A variety of *Campylobacter* spp. have been isolated from healthy and diseased domesticated farm animals. Among them, the classical thermophilic *Campylobacter* spp. are recognized as the most common cause of bacterial gastroenteritis in humans in many countries worldwide. Several studies evidenced, through direct epidemiological studies and molecular techniques, the link between human infection with antimicrobial resistant pathogens and use of such drugs in farm or food animals.

In general, the wide distribution of campylobacters in nature constitutes an irreducible reservoir from which domestic and food producing animals may become infected. Thus, prevention must be directed at interrupting transmission from animals to humans by simple hygienic measures at home and at processing plants. However, the prevalence of these bacteria in farm animals has not been determined in any place in Ethiopia.
1.3. **Objectives of the Study**

1. To determine the prevalence of enteric *Campylobacter* spp. in various farm / food animals in Jimma area, southwest Ethiopia

2. To identify the species of *Campylobacter* isolates from infected animals in order to provide additional epidemiological markers for future studies

3. To determine the resistance pattern of *Campylobacter* spp. isolated from various farm animals to selected antimicrobial agents.
CHAPTER- II

MATERIALS AND METHODS

2.1. Study Area

All the study areas were in Jimma zone and included urban and rural farm animal settings, Jimma University College of Agriculture (JUCA) animal science center and Jimma Dairy Development Enterprise (JDDE) both of which are located within Jimma town. The other site was, a rural peasant association setting located in around Jimma town area called Merrewa 12 Km away from the town on the way to Addis where the university has run community based training program (CBTP).

Jimma town is stated in the southwestern Ethiopia about 345 km far from Addis Ababa. Jimma Aba Jiffar founded the town approximately at the same age to Addis in the 19th century. Its mean altitude is 1,780 m above sea level. The mean annual temperature is 24.9°C. The town has an estimated population size of over 110,000 with relatively equal male to female ratio. Seventy-eight percent of the town has access to potable tap water while the remaining 22 percent use different water sources, mainly wells (personal communication with Jimma Zonal Development Bureau).

2.2. Study Design

To study for the carriage of *Campylobacter spp.* in the rectal content or faeces of diverse farm animals, a cross-sectional study design was conducted during the period from January 1 to February 27, 2004 on chickens, cattle, sheep, and pigs of Jimma University College of Agriculture (JUCA) animal science center, Jimma
Dairy Development Enterprise (JDDE), and in Merrewa peasant association of Jimma zone.

In addition, a structured questionnaire formats was prepared and administered to the rural household heads and respective professional persons of the urban setting. The questionnaire touched various issues to gather information including the usage of antimicrobial agents in the treatment or performance enhancement of farm animals, the situation of animal pens (enclosures) existing separately or together with humans in the same space, the number and types of herds, the tradition of raw milk and meat consumption, the presence of pit latrine, etc (see Appendix-I).

2.3. Sample Size

The sample size considerations were based on the assumption that the mean prevalence of campylobacters carrier positive numbers would be 65% or higher. Accordingly, 485 fecal samples were collected based on proportional allocation to all sample sources during the period of January and February 2004.

Fecal samples (n= 485) from the two sites: one from Merewa rural setting (n₁=277) a combination of 112 cattle, 71 sheep, and 94 chickens; and the other from urban setting (n₂= 208) a combination of 93 cattle, 97 chickens and 18 pigs were included in the study. No goats were included and obtained from both sites. Description of animals surveyed and the farm animal locations were summarized in Table 3.1.
2.4. *Collection, Handling and Transport of Specimens*

Cloacal swabs were taken from each randomly selected urban and rural household live adult chickens. Samples were collected from each chicken using sterile cotton-wool headed swabs moistened in nutrient broth. The swabs with rectal content were immersed in well-labeled Carry-Blair semi-solid medium (Oxoid Ltd, Basingstoke, Hampshire, England) prepared in screw-capped tubes as described by Sjögren *et al.* (1988).

Fecal samples of approximately one to five grams amount were obtained by direct rectal retrieval system from adult cattle and sheep and placed into clean and dry containers. Separate sterile disposable gloves were used for each farm animal. In addition, while we were there for collection, fresh fecal manures passed were obtained from pen floors at the adult cattle location. Rectal swab collection was employed using sterile cotton-wool headed swabs for calves, young borne sheep lambs, adult pigs, and piglets. Rectal swabs collected were immersed in labeled Carry Blair transport medium.

All fecal samples from various animals were transported from fields and in-town area to the laboratory in ice-cold box with in a maximum of four hours after collection. Immediately on arrival, aliquots of approximately one-gram faeces mixed in 9 ml buffered peptone water were prepared and the suspension became ready for culturing. Cloacal or rectal swabs collected from chickens, lambs, calves, and pigs were ready to culture without any treatment directly as described elsewhere (*Collee et al.*, 1996; Atabay and Cory 1998).
2.5. **Isolation and Identification of Campylobacter spp.**

*Campylobacter* spp. were recovered from different farm animals by plating the respective specimens directly on to *Campylobacter* blood free selective agar base (*mCCDA*-Preston *modified Cephoperazone Charcoal Deoxycholate Agar*; from Oxoid Ltd). Streaked plates were incubated at a temperature ranges between 42-43°C in anaerobic jar under a microaerophilic atmosphere produced from gas generating sachets (Campy-Gen™; Oxoid Ltd) for 48h and plates with no growth were incubated in a microaerophilic condition for additional 24h.

Suspected colonies on each *mCCDA*-Preston plate were sub-cultured on Colombia agar (Oxoid Ltd) with 5% sheep blood and incubated in a microaerophilic environment at 37°C for 24 to 36 h. suspect colonies were noted for absence of hemolysis on blood agar, shiny, convex, colorless to grayish colony characteristics with irregular or round edged nature. Microscopy was done to see characteristic darting motility with the iris diaphragm closed effectively to contrast the field. Gram stained morphology showed a Gram negative organism with an ‘S’-shaped appearance. Positive results with oxidase and catalase tests identified thermotolerant *Campylobacter* genera. The entire above laboratory finding were recorded (Appendix-II)

Additional tests used for identification of *Campylobacter* isolates were the following:

Hippurate hydrolysis test: - a 0.5 ml sterile sodium hippurate solution in screw capped tubes was inoculated with a colony from Colombia (Oxoid Ltd) blood agar and incubated in a water bath at exactly 37°C for 3 to 4h. Overlaying with
ninhydrin solution without disturbance allowed the development of deep purple color within five to ten minutes indicating a positive result. Development of no color or slight greenish is regarded as negative. *C. jejuni* is positive for hippurate hydrolysis test.

Hydrogen sulfide (H$_2$S) production test: - Triple sugar iron agar (TSI) was inoculated and incubated at 37°C to observe the production of hydrogen sulfide as a blacking around the bacterial mass to be regarded as positive but none of the isolates produced the reaction in this study.

Susceptibility to cephalothin (30 µg) and nalidixic acid (30 µg) disk was evaluated and this test interpreted zones of any size of inhibition as sensitive. (Collee et al., 1996; Medeiros and Hoffmann, 1998).

2.6. *Antimicrobial Susceptibility Testing*

Antimicrobial sensitivity testing was performed on *Campylobacter* isolates using the standard disc diffusion methods recommended by Bauer and colleague (1966). Suspensions of campylobacter colonies were prepared by transferring a loopful of the organism to a tube containing buffered saline. The approximate turbidity of the suspension was adjusted visually to the optical density of 2 McFarland suspensions. Then using sterile swab, the bacterial suspension was spread gently over the surface of Mueller-Hinton agar plate supplemented with 5% sheep blood as described by Janosi and Kaszanyitzki (2003).

Inoculated plates were engrossed with the different impregnated antimicrobial discs and then incubated at 37°C for 48h under microaerophilic atmosphere. Each
plate was examined under an indirect light source from lamp to see the growth and inhibition zone around the disks. The inhibition zones were measured to the nearest millimeter using a sliding caliper. The zone diameters measured around the disks were interpreted on the bases of the manufacturer instructions along the disk packages to classify as sensitive, intermediate, or resistant. Control strains were included on each procedure date and on each batch of cartridges of antibiotic disks: *E. coli* ATCC 25922 was the reference strain.

The following selected antimicrobial agents were included in the study. Ampicillin (AM) 10µg, Streptomycin (S) 10µg, Chloramphenicol (C) 30µg, Trimethoprim-Sulfamethoxazole (SXT) 25µg, and Tetracycline (Te) 30µg (Becton, Dickinson & Company, Sparks, Maryland, USA); Ciprofloxacin (CI) 5µg, Clindamycin (CM) 2µg, Erythromycin (E) 15µg, Gentamicin (J) 10µg, and Nalidixic acid (NA) 30µg (Span Diagnostics Ltd, Sachin, India); Cephalothin (KF) 30µg and Norfloxacin (NOR) 10µg (Oxoid Ltd).

2.7. **Statistical Methods**

Data were organized and summarized in simple descriptive statistics methods. Moreover, all components of the data entered and analyzed using EPI-INFO 2000 in computer software. Chi-square test ($\chi^2$) results were used and a *p*-value of less than 0.05 was considered statistically significant.
CHAPTER- III

RESULTS

Questionnaire information form for Merewa peasant association setting of 64 households showed that average family size was 5.22 per household; households who have pit latrine were 52%; all the heads of the households responded that 100 percent of them consume raw milk or meat. Likewise, none of the studied group had a separate enclosure for animals. Though, nearly all farmers do not have knowledge of antibiotic enhancement performance activity, 82% of them have bought drugs without consultation of veterinarians (data not shown). The drugs were mainly for treatment of cattle and as it has been demonstrated from the package on request, some of them were antihelmintic drugs. However, none of them have shown me antimicrobial drugs. For the institutionalized, Jimma urban-based, farm animals, antimicrobial agents including penicillins, oxytetracyclines, sulfad drugs, or streptomycin have been prescribed by veterinarians primarily for cattle upon illness, and for chickens upon sign of outbreaks within flocks.

3.1. Isolation Rate of Campylobacter spp. from Different Farm Animals

A total of 485 various farm animal fecal specimens were examined, of which 208 (42.9%) were from urban and the remaining 277 (57.1%) from rural setting. Table-3.1 shows different animal sources examined in both settings from which Campylobacter strains were isolated. Campylobacter spp. were isolated from 192 (39.6%) of 485 fecal specimens investigated from various farm animals. The distributions of campylobacters isolated across the various farm animals investigated
in both settings were as follows: in chickens 130/485 (26.8%); in cattle 26/485 (5.4%); in sheep 27/485 (5.6%); and in pigs 9/485 (1.9%). The highest number of \textit{Campylobacter} spp. isolation come from chickens’ 130/191 (68.1%), followed by 27/71 (38.0%) from sheep, 26/205 (12.7%) from cattle, and 9/18 (50.0%) from pigs.

The rate of \textit{Campylobacter} spp. isolated in all animals was higher in urban (118/208=56.7%) than in rural animals (74/277=26.7%). This prevalence rate shows significant difference between urban and rural setting animals (p< 0.05).

The estimation of prevalence within chicken flock was based on the proportion of positive samples against the total samples collected in each flock. Accordingly, rural chickens from mainly a free-range rearing system have a rate of 44.7% (42/94) positivity for \textit{Campylobacter} isolation unlike 90.7% (88/97) of urban chickens, which belonged to an intensive indoor poultry flocks (p< 0.05). The average flock-to-flock variation across the poultry houses in the urban setting (JUCA), where there were four houses with an average of 450 chickens per house, did not show significant difference in the rate of positivity for \textit{Campylobacter} strain (p > 0.05).

There was a significant higher prevalence in cattle of the urban than in cattle of the rural setting (p-value < 0.05). Based on the results of the examination of these 205 cattle, the prevalence of \textit{Campylobacter} spp. in cattle from urban area was 22.6% (21/93) compared with 4.5% for the rural setting. However, there was no significant difference in the prevalence rates of \textit{Campylobacter} carriage within the urban JUCA and JDDE cattle (p> 0.05).
Table 3.1. Different farm animal sources (n = 485) examined in the urban and rural setting for the tolerant *Campylobacter* spp. in Jimma zone, 2004.

<table>
<thead>
<tr>
<th>Farm Animals</th>
<th>Urban</th>
<th>Rural</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimen</td>
<td><em>Cam. spp.</em> d No. (%)</td>
<td>No. of specimen</td>
</tr>
<tr>
<td></td>
<td>JUCA a</td>
<td>JDDE b</td>
<td>JUCA</td>
</tr>
<tr>
<td>Cattle</td>
<td>42</td>
<td>51</td>
<td>10 (23.8)</td>
</tr>
<tr>
<td>Chicken</td>
<td>97</td>
<td>-</td>
<td>88 (90.7)</td>
</tr>
<tr>
<td>Pigs</td>
<td>18</td>
<td>-</td>
<td>9 (50.0)</td>
</tr>
<tr>
<td>Sheep</td>
<td>- c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td></td>
<td>118 (56.7)</td>
</tr>
</tbody>
</table>

a = Jimma university college of agriculture.  
b = Jimma dairy development enterprise.  
c = No farm animal of that type exists in all ‘-’ sign.  
d = *Campylobacter* species.
3.2. *Species Distribution*

The species distribution of 192 *Campylobacter* spp. isolated from different farm animals was as follows: *C. jejuni* made of 135/192 (70.3%) of the isolates, *C. coli* 51/192 (26.6%), and *C. lari* 6/192 (3.1%). *C. jejuni* was predominant in chickens 105/135 (77.8%), followed by cattle (10.4%), and sheep (11.8%). It was not recovered from pigs. *C. coli* was also predominant among isolates from chickens 21/51, and also recovered in lesser proportion from sheep 11/51, cattle 10/51 and pigs 9/51; whereas *C. lari* isolates were recovered in much smaller numbers from chickens (4/130) and cattle (2/26) (Table-3.2).

The species distribution of all *Campylobacter* strains between the two rearing systems (urban and rural) were as follows: of the urban (JUCA) chickens, 90.9% had *C. jejuni* and 9.1% *C. coli*, while the proportion for rural (Merrewa) chickens was 59.5%, 31.0%, and 9.5 % for *C. jejuni*, *C. coli*, and *C. lari*, respectively (Table-3.3a). In all cattle of urban (both JUCA and JDDE) sources, *C. jejuni* accounted for 57.1%, *C. coli* for 33.3%, and *C. lari* for 9.6%; while in rural cattle *C. jejuni* was recovered from 40.0% and *C. coli* 60.0% (Table-3.3b). *C. jejuni* was generally less prevalent in cattle of rural compared with the other two farms (p< 0.05). Campylobacter isolate distributions in sheep of the rural setting were 59.3% *C. jejuni* and 40.7% *C. coli*; while in pigs of urban sources, all the isolates were *C. coli* (100%).
### Table-3.2. The distribution of all *Campylobacter* spp isolated from farm animals in Jimma zone, 2004.

<table>
<thead>
<tr>
<th>Animals</th>
<th><em>C. jejuni</em></th>
<th><em>C. coli</em></th>
<th><em>C. lari</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle (n = 26)</td>
<td>14 (53.8)</td>
<td>10 (38.5)</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>Chickens (n=130)</td>
<td>105 (80.8)</td>
<td>21 (16.2)</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>Pigs (n= 9)</td>
<td>-</td>
<td>9 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Sheep (n= 27)</td>
<td>16 (59.3)</td>
<td>11 (40.7)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total (N =192)</strong></td>
<td><strong>135 (70.3)</strong></td>
<td><strong>51 (26.6)</strong></td>
<td><strong>6 (3.1)</strong></td>
</tr>
</tbody>
</table>

### Table-3.3a. The distribution of *Campylobacter* spp. isolated from chickens in Jimma area, 2004.

<table>
<thead>
<tr>
<th>Setting</th>
<th><em>C. jejuni</em></th>
<th><em>C. coli</em></th>
<th><em>C. lari</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Merrewa rural sources n₁= 42</td>
<td>25 (59.5)</td>
<td>13 (31.0)</td>
<td>4 (9.5)</td>
</tr>
<tr>
<td>Urban (JUCA) n₂= 88</td>
<td>80 (90.9)</td>
<td>8 (9.1)</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL (n= 130)</strong></td>
<td><strong>105 (80.8)</strong></td>
<td><strong>21 (16.2)</strong></td>
<td><strong>4 (3.1)</strong></td>
</tr>
</tbody>
</table>

s = Jimma university college of agriculture
**Table-3.3b.** The distribution of *Campylobacter* spp. isolated from cattle in Jimma area, 2004.

<table>
<thead>
<tr>
<th>Source</th>
<th><em>C. jejuni</em></th>
<th><em>C. coli</em></th>
<th><em>C. lari</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Merrewa rural sources n1= 5</td>
<td>2 (40.0)</td>
<td>3 (60.0)</td>
<td>-</td>
</tr>
<tr>
<td>Urban (JUCA / JDDE ¹) n2= 21</td>
<td>12 (57.1)</td>
<td>7 (33.3)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>TOTAL n= 26</td>
<td>14 (53.9)</td>
<td>10 (38.5)</td>
<td>2 (7.7)</td>
</tr>
</tbody>
</table>

¹ = Jimma dairy development enterprise

### 3.3. **Susceptibility Testing of Campylobacter Isolates**

One hundred ninety two *Campylobacter* spp. were subjected to antimicrobial susceptibility tests using disk diffusion method. The highest level of resistance of the *Campylobacter* isolates was recorded to cephalothin (100%), followed by trimethoprim-sulfamethoxazole (37.0%), ampicillin (20.0%), streptomycin (6.3%), and nalidixic acid (6.3%) (Table-3.4).

The higher resistance frequencies of *C. jejuni* were observed against trimethoprim-sulfamethoxazole (34.1%) and ampicillin (17.0%) although there were significant differences when compared with those of *C. coli* (p < 0.05) (Table-3.5). Resistance of the *C. coli* isolates was shown to trimethoprim-sulfamethoxazole (45.1%), ampicillin (27.5%), streptomycin (9.8%), and nalidixic acid and tetracycline.
The resistance frequencies of six *C. lari*, though with limited isolate, were observed against cephalothin and nalidixic acid (each 100%); then three of the six (50%) isolates were resistant to trimethoprim-sulfamethoxazole, followed by two of the six (33.3%) strains to streptomycin, clindamycin, and erythromycin.

Multi-resistance to two or more antimicrobials was seen in 14.6% (28/192) of *Campylobacter* strains. Twelve of 135 (8.9%) *C. jejuni* isolates and 11 of 51 (21.6%) *C. coli* isolates were resistant to two or more antimicrobials tested. Five *C. lari* isolates were also multi-resistant (Table-3.6). Cephalothin was not considered as a multi-resistant variant property as campylobacters are inherently resistant to these groups.

Recovery rate of resistant campylobacter strains across the various farm animals studied didn’t show statistically significant difference (data not shown), except that *C. coli*, recovered from pigs, tended to demonstrate higher resistance rates to antimicrobial agents tested.
Table 3.4. Antimicrobial susceptibility pattern of *Campylobacter* strains (n= 192) against 12 antimicrobial agents in Jimma zone, 2004.

<table>
<thead>
<tr>
<th></th>
<th>AM</th>
<th>C</th>
<th>KF</th>
<th>CI</th>
<th>CM</th>
<th>E</th>
<th>J</th>
<th>NA</th>
<th>NOR</th>
<th>Te</th>
<th>SXT</th>
<th>S$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
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</tr>
<tr>
<td>Urban</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>62(52.5)</td>
<td>118(100)</td>
<td>-</td>
<td>-</td>
<td>118(100)</td>
<td>116(98.4)</td>
<td>110(93.2)</td>
<td>117(99.2)</td>
<td>111(94.1)</td>
<td>117(99.2)</td>
<td>109(92.4)</td>
<td>48(40.7)</td>
<td>108(91.5)</td>
</tr>
<tr>
<td></td>
<td>131(68)</td>
<td>192(100)</td>
<td>-</td>
<td>-</td>
<td>192(100)</td>
<td>186(96.9)</td>
<td>182(94.5)</td>
<td>190(98.9)</td>
<td>180(93.7)</td>
<td>186(97.9)</td>
<td>181(94)</td>
<td>95(49.5)</td>
<td>176(91.7)</td>
</tr>
<tr>
<td>Rural</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>69(93.2)</td>
<td>74(100)</td>
<td>-</td>
<td>-</td>
<td>74(100)</td>
<td>70(94.6)</td>
<td>72(97.3)</td>
<td>73(98.7)</td>
<td>69(93.2)</td>
<td>69(93.2)</td>
<td>72(97.3)</td>
<td>47(63.5)</td>
<td>68(91.9)</td>
</tr>
<tr>
<td></td>
<td>131(68)</td>
<td>192(100)</td>
<td>-</td>
<td>-</td>
<td>192(100)</td>
<td>186(96.9)</td>
<td>182(94.5)</td>
<td>190(98.9)</td>
<td>180(93.7)</td>
<td>186(97.9)</td>
<td>181(94)</td>
<td>95(49.5)</td>
<td>176(91.7)</td>
</tr>
<tr>
<td>All strain</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>131(68)</td>
<td>192(100)</td>
<td>-</td>
<td>-</td>
<td>192(100)</td>
<td>186(96.9)</td>
<td>182(94.5)</td>
<td>190(98.9)</td>
<td>180(93.7)</td>
<td>186(97.9)</td>
<td>181(94)</td>
<td>95(49.5)</td>
<td>176(91.7)</td>
</tr>
<tr>
<td></td>
<td>131(68)</td>
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<td>192(100)</td>
<td>186(96.9)</td>
<td>182(94.5)</td>
<td>190(98.9)</td>
<td>180(93.7)</td>
<td>186(97.9)</td>
<td>181(94)</td>
<td>95(49.5)</td>
<td>176(91.7)</td>
</tr>
</tbody>
</table>

$^4$AM=Ampicillin  
C=Chloramphenicol  
CI = Ciprofloxacin  
KF = Cephalothin  
S=Sensitive  
$^5$Te=Tetracycline  
E = Erythromycin  
S = Streptomycin  
J = Gentamicin  
NOR = Norfloxacine  
SXT = Trimethoprim-Sulfamethoxazole  
NA = Nalidixic Acid  
R= RESISTANT  
CM = Clindamycin
Table 3.5. The antimicrobial susceptibility pattern of *C. jejuni*, *C. coli*, and *C. lari* isolated from farm animals in Jimma area, 2004.

<table>
<thead>
<tr>
<th></th>
<th>AM (%)</th>
<th>C (%)</th>
<th>KF (%)</th>
<th>CI (%)</th>
<th>CM (%)</th>
<th>E (%)</th>
<th>J (%)</th>
<th>NA (%)</th>
<th>NOR (%)</th>
<th>Te (%)</th>
<th>SXT (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>96 (71.1)</td>
<td>135(100)</td>
<td>-</td>
<td>-</td>
<td>135(100)</td>
<td>132(97.8)</td>
<td>129(95.6)</td>
<td>133(98.5)</td>
<td>132(97.8)</td>
<td>133(98.5)</td>
<td>130(86.3)</td>
<td>67(49.6)</td>
</tr>
<tr>
<td>I</td>
<td>16 (11.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2(1.5)</td>
<td>5(3.7)</td>
<td>1(0.7)</td>
<td>-</td>
<td>-</td>
<td>1(0.7)</td>
<td>3(2.2)</td>
</tr>
<tr>
<td>R</td>
<td>23 (17.0)</td>
<td>-</td>
<td>-</td>
<td>135(100)</td>
<td>-</td>
<td>1(0.7)</td>
<td>1(0.7)</td>
<td>1(0.7)</td>
<td>3(2.2)</td>
<td>1(0.7)</td>
<td>2(1.5)</td>
<td>46(34.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>31(60.8)</td>
<td>51(100)</td>
<td>-</td>
<td>-</td>
<td>51(100)</td>
<td>50(98.0)</td>
<td>49(96.1)</td>
<td>51(100)</td>
<td>48(94.1)</td>
<td>50(98.0)</td>
<td>45(88.2)</td>
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</tr>
<tr>
<td>I</td>
<td>6(11.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>3(5.9)</td>
<td>4(7.8)</td>
</tr>
<tr>
<td>R</td>
<td>14(27.5)</td>
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<td>-</td>
<td>1(2.0)</td>
<td>2(3.9)</td>
<td>-</td>
<td>-</td>
<td>3(5.9)</td>
<td>1(2.0)</td>
<td>3(5.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>4(66.7)</td>
<td>6(100)</td>
<td>-</td>
<td>-</td>
<td>6(100)</td>
<td>4(66.7)</td>
<td>4(66.7)</td>
<td>6(100)</td>
<td>-</td>
<td>-</td>
<td>3(50.0)</td>
<td>6(100)</td>
</tr>
<tr>
<td>I</td>
<td>1(17.7)</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(17.7)</td>
<td>-</td>
</tr>
<tr>
<td>R</td>
<td>1(17.7)</td>
<td>-</td>
<td>-</td>
<td>6(100)</td>
<td>-</td>
<td>2(33.3)</td>
<td>2(33.3)</td>
<td>-</td>
<td>-</td>
<td>6(100)</td>
<td>2(33.3)</td>
<td>-</td>
</tr>
</tbody>
</table>

*AM = Ampicillin  C= Chloramphenicol  KF = Cephalothin
CI= Ciprofloxacin  CM = Clindamycin  E = Erythromycin
J = Gentamicin  NA = Nalidixic acid  NOR = Norfloxacin
Te = Tetracycline  SXT = Trimethoprim-Sulfamethoxazole  S = Streptomycin
Table 3.6. Multi-resistant property of campylobacter strains isolated from farm animals to two or more antimicrobial agents in Jimma zone, 2004.

<table>
<thead>
<tr>
<th>Combinations of drugs #</th>
<th>All Campy(^\xi). Strains</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. lari</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-SXT</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>NA-Te</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>NA-NOR</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>AM-SXT-E</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>AM-SXT-NA</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>AM-SXT-S</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>NA-Te-S</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AM-SXT-Te-NA</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>AM-SXT-Te-NA-S</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>SXT-CM-S-NA</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

**TOTAL** 28 12 11 5

# AM = Ampicillin        C = Chloramphenicol        KF = Cephalothin
CI = Ciprofloxacin      CM = Clindamycin         E = Erythromycin
J = Gentamicin          NA = Nalidixic acid      NOR = Norfloxacin
Te = Tetracycline       SXT = Trimethoprim-Silfamethoxazole
\(\xi\) = Campylobacter

S = Streptomycin
CHAPTER- IV

DISCUSSION

Campylobacter spp., especially thermotolerant campylobacters the most common species associated with gastrointestinal illness, are part of the natural intestinal flora of a wide range of birds and animals. The most important risk factor for human Campylobacter infection is widely held to be the handling and consumption of raw poultry and cross contamination to uncooked products (Tauxe, 1992).

This study proved the presence of thermotolerant Campylobacter spp. in about 40 percent of various farm animals included in this project and showed the role of the animals as potential reservoir hosts to risk the public. The special characteristic of the bacteria across the various farm animal species is an important one to describe systematically.

The overall results on chickens indicate that the prevalence rate was 68.1%. This finding is consistent with many of the studies conducted in developed countries ranging from 45 to 100 percent (Sjögren, 1988; Jones et al., 1991; Jacob-Reitsmal et al., 1995; Van de Giessen et al., 1996). As a commensal organism in poultry, campylobacters colonize the intestinal mucus layer in the crypts of intestinal epithelium (Sjögren, 1988). Chickens can harbor very high level of Campylobacter in the gut without symptoms and the bacteria can be rapidly transmitted among birds within a flock (AlteKruse et al., 1999). Moreover, the colonization of hens often goes with the age of the birds that usually occur at the
end of the first week after hatching and continues for many weeks (Sjögren, 1988). There is an associated reduction in prevalence, as the birds get older. In general, the higher prevalence in chickens may play in the transmission of *Campylobacter* to humans.

The study in chickens of free-range rearing production provides a low prevalence of *Campylobacter* compared with the intensive indoor rearing system of the urban (44.7% vs. 90.7%). Although there is limited information on *Campylobacter* spp. of farm animals from developing countries, a high prevalence of *Campylobacter* in chickens reared under free-range conditions has been reported in the ranges between 64 to 100% (Kazwala *et al.*, 1993; Simango and Rukure, 1991). In a study from Tanzania, 76.5% of samples of droppings from indigenous free-range poultry were *Campylobacter* positive (Kazwala *et al.*, 1993), and in a study of *Campylobacter* in free-range chickens and confined commercial chickens in Peru, the isolation frequencies were 54% and 35%, respectively (Tresierra-Ayala *et al.*, 1995). The differences in prevalence in the free-range rearing chickens of this study may be due to the husbandry practices and geographic circumstances. Moreover, a distinct seasonal variation was observed in several studies conducted in developed world (Anon, 2001).

The limitation of this study to assess seasonal variations is because the study was performed cross-sectionally over about one and half months. As for instance, the prevalence in hens ranged from 3.6% in April to 87.1% in August with a mean of 37.8% that has been studied in Denmark by the year 2000 (Anon, 2001).
Therefore, the time of the study is so important to define or the study might have been conducted during relatively lower prevalence point in time.

On the other hand many studies have shown that once Campylobacter is introduced in to a broiler shed, birds rapidly excrete high numbers in their faeces and the organism spreads rapidly so that 100 percent of birds may be colonized within a few days. The results from the urban poultry houses suggest that if a chicken flock is Campylobacter infected, a large proportion of the birds within the flock are infected (Gregory et al., 1997). Similarly in our study, higher prevalence rate (90.7%) was found in intensive in-door rearing chickens than free-range rearing (44.7%) production of the rural setting (p value < 0.05).

The fact that the greatest proportions of Campylobacter strain (130 Campylobacter strain out of 192) were contributed from chickens that once again probably suggest the importance of avian species. Chickens and other fowl are supposed to be the natural hosts of thermophilic campylobacters because they have a core temperature of 41°C, which is the optimum growth temperature of C. jejuni and other thermotolerant campylobacters (Stanley and Jones, 2003). Thus, the epidemiological significance of these groups can be underlined and predicted.

The prevalence of thermophilic Campylobacter spp. in overall dairy and beef cattle in this study was 12.7%. This finding is comparable with the finding of Atabay and Corry (1998) in UK. Compared to the rural prevalence in the carriage rate of Campylobacter strain (4.5%) in healthy cattle, there was significant difference from that of the urban (22.6%) (p-value=0.006). Over the last decades, overall prevalence of thermophilic campylobacters in adult cattle had been estimated at a comparable
reading to the above rate such as 19.5% in Portugal (Cabrita et al., 1992), 23% in Denmark (Nielsen, 2002), and 5% in the USA (Hoar et al., 2001) while a higher rate of *Campylobacter* prevalence has been found in Japan (46.7%) (Giacoboni et al., 1993), and 37% in the United States of America (Wesley et al., 2000).

Regardless of this, a direct comparison of reports on *Campylobacter* prevalence from different countries will be strongly biased by, isolation procedures, sample material used, the rearing system, and differences in sample size. The method used in this study was based on direct plating of samples on to *m*CCDA- Preston selective media alone. According to a study conducted by Stanley and colleagues (1998), direct plating of intestinal content to isolate *Campylobacter* from cattle on *m*CCDA- Preston alone isolated from 26.7% while by including an enrichment broth (Preston selective enrichment broth) a further 62.7% of samples were found to be *Campylobacter* positive giving a total of 89.4%. Thus, an enrichment step has often been found to increase the recovery of *Campylobacter* from cattle and sheep. This may be due to the lower number of the bacteria in healthy cattle and sheep compared to chickens.

There was a significantly higher rate of prevalence in sheep (38.0%) as compared to cattle (12.7%) of this study (p < 0.05). Although there are no literatures found to compare continentally, the prevalence report from the European union (Anon, 2001) has indicated 24.8% in Denmark, 1999; 17.0% in UK-England, 2000; and 13.0% and 20.7% in Netherlands, 2000 and 2001, respectively. The results found in the sheep were higher compared to the above areas probably due to geographic and sheep strain variation. In addition, the shedding of campylobacters was shown to be
intermittent and seasonal. Thus, the relatively higher rate of shedding may be coincided with peak point of time.

The significance of *Campylobacter* colonization of sheep and cattle relates to the potential for contamination of milk at the farm and the carcass at slaughter places. Gross microbial contamination of the carcass with gut contents may occur during evisceration or during removal of the hide or from cross contamination from hide to carcass via hands and instruments of slaughter men (Gannon, 1999). Since 100 % of the populations in Merrewa peasant association consume raw milk or raw beef, the epidemiological and clinical significance can be drawn to risk the public particularly children of under two.

This study found 50% carriage rate of thermophilic campylobacter in pigs. According to report on trends and sources of zoonotic agents in European union and Norway (Anon, 2001), food animals besides poultry are often surveyed and monitored for thermophilic *Campylobacter* (Anon, 2001). For instance, a nationwide survey in Denmark found prevalence rate of 76.9% for pigs in 2000; and in Germany, 47.2% of pig herds were positive in 2001. In general, though the greater proportion of our population is not at risk of these animals, pigs probably contribute as source of infection to other animals.

Poultry appear to be the main reservoir of *C. jejuni*. In this study the contribution of chickens accounted for more than three fourth of all *C. jejuni* strains isolated from all farm animals (105 from a total of 135). *C. jejuni* with the highest isolation rate (80.8%) was found from chickens. In the UK, up to 100% of chickens and turkeys have been shown to harbor this organism (Blaser and Reller, 1981). The
observed species distribution was in agreement with results of previous studies of *Campylobacter* in developed (Heuer *et al.*, 2001; Jacob-Reitsma *et al.*, 1995) and developing countries (Simango *et al.*, 1991; Kazwala *et al.*, 1993). Thus, the results found here for the chickens probably justified the possible contribution of these group in sporadic campylobacteriosis that mainly occur following handling of raw poultry and eating undercooked poultry (Hopkins and Scott, 1983; Deming *et al.*, 1987)

Overall, *C. jejuni* was detected in 6.8% of all cattle faecal samples (i.e. 14/205). *C. jejuni* was generally less prevalent on cattle of rural compared with the other two farms (p< 0.05). Previous estimates of *C. jejuni* in dairy cows range from 0.8 to 100%, depending on season, age of animal analyzed, number of animals surveyed, and isolation methods (Wesley *et al.*, 2000). For these reason, explanation of the two setting differences may be difficult except the observed difference for the cattle species i.e. Abyssinian cows of the rural versus cows coming from abroad or hybrid of them.

In pigs, as it was found from this study confirmed 100% *C. coli* positivity from faecal samples (Padungton and Kannen, 2003). Horizontal transmission from the environment has been put forward as a likely route of *Campylobacter* infection in various animals. Therefore, pigs probably act as source of *C. coli* for other farm animals in the vicinity. Pork may not be public hazard as there is no tradition of consuming pork in the locality and may be true in Ethiopia at large.

The development of antimicrobial resistance in pathogenic bacteria is a matter of increasing concern. The thermophilic *Campylobacter* spp resistant to antibiotics
(C. jejuni, C. coli, and C. lari) can be isolated from different animal sources and might transfer from animals to humans. The susceptibility pattern of all Campylobacter strains isolated from farm animals against 12 chosen antimicrobial agents was studied. The agar disc diffusion method was used, as it is simple and inexpensive to perform in our setup. Moreover, Janosi and colleague (2003) have obtained comparable results between disk diffusion assay and Epsilometer test (E-test). Alternatively, several studies demonstrated that the results found for E-test compared with broth micro dilution and agar dilution methods had a high degree of agreement and correlation (Luber et al., 2003). Nevertheless, standardized antimicrobial susceptibility testing methods were not found to Campylobacter spp. using impregnated disks. For the moment, most researches or researchers in these days utilize the agar dilution as a reference technique.

All of the strains tested were sensitive to ciprofloxacin and chloramphenicol, while all were resistant to cephalothin. Studies from other countries have reported various levels of resistance. For example, in Spain, the proportion of broiler and pig isolates of Campylobacter resistant to quinolones (ciprofloxacin) was found to be 99%, and on the other hand, all the strains were sensitive to chloramphenicol (Saenz et al., 2000); isolates from the Swiss poultry flocks were all sensitive to quinolones (ciprofloxacin) while 8% of Campylobacter isolates were resistant to chloramphenicol (Frei et al., 2001).

The result to cephalothin in this study found 100% resistance to all isolates recovered from all farm animals. This is because most Campylobacter species are resistant to cephalothin (Allos, 2001). Moreover, this cephalothin, an agent to which
most other stool flora are susceptible, are used as a selective complement added to medium for isolation of campylobacters from stool samples. (Skirrow, 2002)

It is often proposed that the indiscriminate use of antimicrobial agents in the veterinary fields leads to increased resistance in bacteria pathogenic to humans. Although for human medicine there is some potential danger behind the use of antimicrobial agents in animals, very few species isolated in animals, which are also pathogenic to man, have been mentioned in the literature (Witte, 1998; Padungton and Kannen, 2003). Information on the antibiotic sensitivity of C. jejuni from animal is rather scarce.

A low level of antimicrobial resistance was found among Campylobacter isolates from the free-range rearing systems of the rural setting than urban. Particularly significant differences were observed for the resistance pattern between the two setting for ampicillin and trimethoprim-sulfamethoxazole (p value < 0.005 for both). Moreover, there was significant difference observed across the two species of Campylobacter which is consistent with most literature which describe difference in the level of resistance between C. jejuni and C. coli (Nachamkin, 1999). Relatively high levels of resistance to streptomycin among isolates from cattle have been reported in other studies (Wesley et al., 2000). Among C. coli isolates, especially from pigs, there is a high level of resistance to streptomycin, macrolids, and other.

Only six C. lari isolates were recovered among the isolates examined in the present study. These isolates were susceptible to most antimicrobial agents tested, except cephalothin and nalidixic acid, which demonstrated 100% resistance.
Considering the limited number of C. lari isolates, it is not possible to compare levels of resistance with other Campylobacter spp. or among from different animal sources.

The antimicrobial sensitivity findings from previous studies conducted in Ethiopia showed about 60% of Campylobacter strains isolated from humans were resistant to ampicillin and trimethoprim-sulfamethoxazole (Asrat et al., 1999); and another recent study conducted in south west Ethiopia of under-fifteen children indicated 60% resistance level against trimethoprim-sulfamethoxazole, 50% to ampicillin, 14% to tetracycline, and 10% to erythromycin (Personal communication, 2004). This study, though done in farm animal isolates, found some how comparable resistance pattern to trimethoprim-sulfamethoxazole, and ampicillin taking into account the results of intermediately sensitive values, 13.5%, and 12% for the two drugs mentioned, respectively.

Conclusion

In conclusion, this study showed that farm animals of Jimma local could serve as reservoir of thermotolerant Campylobacter that probably risk the public at large. On the other hand, antimicrobial resistance is found only at relatively low frequencies among isolates of Campylobacter spp from various farm animals. The results of this investigation indicate that resistant strains of campylobacters have existed in the farm animals in the study area that probably emerged from human and transferred to farm animals through environmental contamination or vice versa. Whatever the case may be this study indicated the requirement of surveillance at farm or food animal
processing centers in the country to minimize the risk of infection due to campylobacters in human.

**Recommendations**

Based on this study the following recommendations are made: -

1) Farm animals will play a significant role as an epidemiological source of infection to humans; thus, a larger epidemiological survey should be done with an enrichment broth in order to reflect the actual condition.

2) The intimation of antimicrobial resistance in campylobacters derived from farm animals justified rational use of antimicrobial agents based on defined guidelines in the field of animal as well as human medicine.

3) Both domestic and professional food handlers should be aware of these groups of bacteria (thermotolerant campylobacters) to follow hygienic rules of food preparation particularly of foods of animal’s sources.

4) The problem of campylobacter in variety of farm animals should be conscientiously evaluated in the field of veterinary medicine by establishing diagnostic facilities.
REFERENCES


Personal profile

Name of the household_________________________ Age_______  Sex = M □  F □

Family size_________________________ Income ______________________________

Literacy statuses of the head of the household

☐ 1. Read and Write  ☐ 2. Primary  ☐ 3. Secondary

☐ 4. University /College  ☐ 5.None

Are there domestic /wild animals in your household? _ Y □  /N □

If yes, circle them and mention the numbers.

• Cattle-----------------------  Sheep-----------------------

• Goats-----------------------  Pigs-----------------------

• Dog-----------------------  Cat -----------------------

• Chickens---------------------  Donkey/horse-----------

• Others (please specify)---------------------------------- ----

Do the animals have their own enclosure to pass the night alone or together with humans in one roof?  ☐ Their own  ☐ Together with the family

Have you ever used antimicrobial drugs:

• For treatment purposes? Y □  /N □
• Animal enhancement purposes?  Y□  /N□

• Have you consulted veterinarians before administration to animals?  Y□  /N□

• What was the drug, (at least confirm looking to the package)?  -------------------------

Have you use to consume raw milk?  Y□  /N□

• Raw meat?  Y□  /N□

Do you have latrine?  Y□  / N□__________________________

Name of the person who filled__________________________

Sig. ___________________________

Date_____________________________
APPENDIX-II

LABORATORY FORM

Code no ____________________

For Laboratory Use Only

The sample collected is of: ________________


Suspected growth of *Campylobacter* spp. from the excreta

A) Positive/present = ☐          B) Negative/absent = ☐

Identification steps for suspected colonies

• Motility under microscopy with the iris diaphragm closed__ Y ☐ /N ☐

• Gram stain result________________________

• Catalase test__________________________

• Oxidase test___________________________

• Hydrogen sulfide production______________

• Hippurate hydrolysis test ________________

• *Cephalothin susceptibility result______________

• *Nalidixic acid susceptibility result______________

• * Any size of inhibition around the disks will be considered ‘Sensitive’.
Based on reading of the biochemical test, the species of the bacteria is of ____________

A) C. jejuni  B) C. coli  C) C. lari  D) C. upsaliences

Antimicrobial susceptibility results (Resistant [R], Intermediate [I], or Sensitive [S])

Ampicillin (AM) 10µg, ____________  Chloramphenicol (C) 30µg, _________

Cephalothin (KF) 30 µg _________  Ciprofloxacin (CI) 5µg, ______________

Clindamycin (CM) 2µg, ____________  Streptomycin (S) 30µg, ____________

Erythromycin (E) 15µg, ____________  Gentamicin (J) 30µg, ______________

Tetracycline (Te) 30µg, ____________  Nalidixic-Acid (NA) 30µg, __________

Norfloxacin (NOR) 10µg. ____________  Trimethoprim-Sulfamethoxazole (SXT) 25µg

____________________________________

Sig.____________________

Date____________________
DECLARATION

I, the undersigned, declare that this M.Sc thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc candidate:    Tesfaye Kassa, B.Sc

Signature:    ______________________

Date and place of submission:    ______________________

Addis Ababa, Ethiopia.

Supervisor:    ______________________

Signature:    ______________________

Date:    ______________________

Addis Ababa, Ethiopia