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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

ISOLATION AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* AND OTHER SPECIES OF *LISTERIA* FROM CATTLE, SHEEP AND GOATS SLAUGHTERED AT ADDIS ABABA ABATTOIR, ETHIOPIA.

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Statistically no significant difference was observed among cattle, sheep and goats slaughtered at Addis Ababa abattoir in the isolation of *Listeria* species and *Listeria monocytogenes,* ($p > 0.05$) from Pearson's chi^2 in both cases.

The result of serotyping of the prevalent *Listeria monocytogenes* species (n=27) showed that, all the isolates were belonged to serotype 4b and 4e. In this study, antibiotic resistance was detected only for streptomycin by one of the isolate from goats' origin and two intermediate resistance for erythromycin by two isolates of sheep origin.

From the results of this study, it is possible to see that, the prevalence of *Listeria monocytogenes* in cattle, sheep and goats slaughtered at Addis Ababa abattoir and the emergence of drug resistance characteristic in these pathogenic species can be a potential risk to the public, because of the transmission of these bacteria through contaminated foods of animals origin to humans.

Further investigation on the role of ruminants and their foods in the ecology and transmission of these bacteria is to animals is important.

Key words: *Listeria moncytogenes, Listeria* species, prevalence, feces, Addis Ababa, abattoir, antimicrobial agent, susceptibility, serotype

For a long time, listeriosis was recognized only as a disease of animals. However, recently researchers from many countries reported that, it is also an important emerging food borne disease in humans (Low and Donachie, 1997). Animals that are stressed by adverse weather, poor housing and poor nutrition are more at risk to contract listeriosis, particularly if they are fed silage which contains about 10^6 *L. monocytogenes* cells per gram of silage (Radostits, *et al.*, 2000). Among the different species of the genus *Listeria*, *Listeria monocytogenes* has been known to cause listeriosis in humans and animals. The other pathogenic species is *L. ivanovii*, which causes abortion in animals (Schwarzkopf, 1996; Hitchins and Whitting, 2001).

The genus *Listeria* is ubiquitous in the environment and possess unique physiological characteristics that allow growth at refrigeration temperature those are usually adverse for most pathogenic food borne bacteria. The organism can tolerate a pH between 5.6 and 9.6 and the temperature variation between 4°C to 45°C (Rocourt and Cossart, 1997).

Now a day, no food borne pathogen has had more impact on the food industries than *Listeria monocytogenes*. The microorganism is widespread in the environment and has been isolated from soil, sewage sludge, vegetation and water (Frances *et al., 1993*). The prevalence of listeriosis in food animals has increased worldwide. This increase reflects changes in husbandry fluctuations, distribution of susceptible hosts and improved detection methods of the pathogen (WHO, 1988).

Ingestion of *Listeria monocytogenes* is a very common event, given the ubiquitous distribution of these bacteria (Farber and Peterkin, 1991). The trait that makes *Listeria monocytogenes* difficult to control during food processing is that it can multiply over a wide range of temperature, including at refrigerator temperatures (Salyers and Whitt, 2002).

The *Listeria monocytogenes* is a Gram-positive non-spore forming rod shaped bacteria are known human and animal pathogens frequently associated with food borne epidemics (Rocourt, 1996; Uyttendaele *et al.*, 1997; Farber, 2000). Highest risk foods are ready-to-eat foods stored at refrigerator temperatures for a long period and silage with higher pH (Rocourt, 1996).

in diverse environments. Hence, food can be contaminated at any stage of processing and refrigerated storage cannot stop the proliferation of the pathogen (Rocourt and Cossart, 1997)

Isolation of *Listeria monocytogenes* from swine and bovine carcass has revealed that the contamination of meat and meat products can occur in early stages of processing. In addition to this, some reports were also described that, *Listeria monocytogenes* was isolated from the feces of healthy warm-blooded animals (Autio *et al., 2000;* Karr *et al., 1996;* Saide-Albornoz *et al.,* 1995).

There is much variation in prevalence of listeriosis in ruminants. During an outbreak, up to 5-10% of goats and sheep exposed to *Listeria monocytogenes* showed clinical signs (Kimberling, 1988). Wesley (1999) reported that, up to 3-5% of abortion in cattle could occur due to listeriosis, while 1.2% of mastitis cases are due to listeriosis (Jensen *et al.*, 1996). However, healthy animals including farm ruminants (cattle, sheep and goats) as well as humans can also be asymptomaic carriers of *Listeria monocytogenes* and other species of *Listeria*, hence they often shed the organism in their fecal material (Husu, 1990).

In India, Girdhar and Garg (2002) observed that, fecal prevalence of *Listeria monocytogenes*, *Listeria ivanovii*, and *Listeria innocua* in healthy sheep was 10.5%, 5.2% and 7.8% respectively. Fecal prevalence of *Listeria monocytogenes* and other species in healthy goats is usually similar with that of in sheep (Nightingale *et al.*, 2004)

In Ethiopia only a few studies were undertaken on the status of *Listeria monocytogenes* and other species. According to (Molla *et al.,* 2004), of a total 316 food samples analyzed, 103(32.6%) were positive for *Listeria* species .In this study the level of contamination of food samples, by *Listeria* species was the highest in pork (69.8%) followed by minced beef (47.5%) and ice cream (43.5%). The dominant *Listeria* species was *Listeria innocua* (21.2%) followed by *Listeria monocytogenes* (5.1%).

In another study under taken by Mengesha (2005), from a total of 711 different food samples analyzed for the presence of *Listeria* species, 189(26.6%) of the samples were found to be

2. LITERATURE REVIEW

2.1. The Genus *Listeria*

2.1.1.Taxonomy and classification

The genus *Listeria* belongs to the Order Bacillales and family Listeriaceae. In the genus *Listeria*, there are six species, which include *L. monocytogenes, L. innocua*, *L. seeligeri, L. welshimeri, L. ivanovii and L. grayi* (James, 2000). The species *L. murrayi* has been now merged with *L. grayi* (Rocourt and Cossart, 1997; Scott, 2003).

Among the six species of *Listeria*, only *L. monocytogenes* and *L. ivanovii* are considered to be pathogenic. *L. monocytogenes* is pathogenic to both humans and animals while *L. ivanovii* is pathogenic to animals only (Rocourt and Cossart, 1997).

2.1.2. Morphology

Listeria species are medium sized, Gram-positive, non-spore forming and non acid-fast bacilli or cocco- bacilli measuring about 0.4 to 0.5μm in diameter by 0.5 to 2 μm in length (Quinn *et al.*, 2002). The bacterial colonies are small, smooth, slightly flattened and milky white by reflected light. But, when illumination is with obliquely transmitted light, the colonies exhibit a characteristic blue or green color. This method is known as Henry's lamp technique (Low and Donachie, 1997).

2.1.3. Growth requirements

2.1.3.1. Effects of temperature

Listeria monocytogenes is a psychrophilic bacterium capable of growing at refrigeration temperature. The optimal growth temperature is between 30°C and 37°C. But it can grow between 1°C and 50°C (James, 2000).

2.1.3.2. Effects of water activity (a_w) and salt solution

Listeria monocytogenes is tolerant to high concentration of sodium chloride and capable of growth in 10% Nacl, it can stay alive for a year in 16% of Nacl. The optimum water activity (a_w) for listerial growth is $\geq$ 0.97 *Listeria monocytogenes* is the second to staphylococci in being able to grow at water activity (a_w) less than 0.93 (James, 2000).

2.1.3.3. Effects of pH

Listeria monocytogenes and other species can grow best at the pH values that range from 4.5-9.6. The maximum growth pH of the bacterium depends on temperature of incubation, nutrient composition of growth substrate, water activity (a_w) and the presence of Nacl and other salts or inhibitors (James, 2000; Scott, 2003). Because of their decisive effect on the physiology of the bacterium the above-mentioned factors are known as growth conditions (Salyers and Whitt, 2002) (Table1).

Table 1 Growth conditions for *Listeria monocytogenes*

S.NO	Growth Factors	Lower limits	Optimum	Upper limits
1	Temperature (°C)	-0.4	37	45
2	pH	4.4	7	9.4
3	Salt tolerance (%)	-	10	25 at 4°C
4	O_2 Availability (%)	-	5%	-
5	Water activity (a_w)	0.9	0.92	0.97

Adapted from (Salyers and Whitt, 2002)

2.1.3.4. Growth and nutrition

Growth of *Listeria* spp. on semi-solid motility media is characterized by unusual umbrella-shaped growth in the surface. *Listeria* species grow on nutrient and blood agar but not on MacConkey agar. Small transparent colonies with smooth borders appear on blood agar in 24 hours becoming grayish white and 0.5 to 2 mm in diameter in 48 hours (Quinn *et* al., 2002). The bacterial colonies are small, smooth, slightly flattened and milky white by reflected light. Incubation of a 2 to 4 hour broth culture of *L. monocytogenes* at room temperature shows a tumbling motility, by hanging-drop method.

2.1.4. Biochemical characteristics

Listeria species are characterized by a wide range of biochemical characteristics most of which are used in the laboratory for the identification of the microorganism (Quinn *et al.*, 2002) (Table2).

Table 2: Biochemical characteristics of *Listeria* species

	Listeria species					
Characteristics	*L.monocytogenes*	*L.ivanovii*	*L.seeligeri*	*L.innocua*	*L.welshimeri*	*L.grayi*
Catalase test	+	+	+	+	+	+
Oxidasetest	-	-	-	-	-	-
Indol test	-	-	-	-	-	-
Hydrolysis of aesculin	+	+	+	+	+	+
Hydrolysisof urea	-	-	-	-	-	-
Voges-Prosakuer	+	+	+	+	+	+
Fermentation of: mannitol	-	-	-	-	-	+
Xylose	-	+	+	-	+	-
Rhamnose	+	-	-	V	V	V

+ =Positive, - = negative, V=variable

Source:(Scott, 2003; Quinn *et al.*, 2002; ISO11290-1,1996).

2.1.5. Typing of *Listeria*

Regulatory identification of *Listeria monocytogenes* does not require any specific sub typing of the isolates. However, sub-typing schemes can be useful in specific outbreaks, investigating, environmental tracking and public health surveillances (Graves *et al.*, 1999).

2.1.5.1 Serotyping

Strains of *Listeria* can be assigned into 17 different serotypes based on the combination of somatic (O) and flagellar (H) antigens. Although serotyping has poor discriminatory power, it can provide valuable information to facilitate the ruling out of isolates that are not parts of an outbreak (Graves *et al.*, 1999).

All serotypes are considered to be potentially pathogenic. However, only the three serovars,1/2a, 1/2b and 4b are involved in the majority of animal and human listeriosis cases (Low and Donachie, 1997). Among the human isolates, 95% belongs to serovars 1/2a, 1/2b and 4b (Rocourt and Bille, 1997). Serotype 4b has caused the majority of human epidemics, while serotype 4a and 4c are restricted to animals. Sporadic cases of listeriosis are caused in animals by 4b, 1/2a and 1/2b(Irene *et al.*, 2003) (Table3).

Table 3: Serovars of *Listeria* species

Listeria species	Sero types
L. monocytogenes	1/2 a, 1/2 b, 1/2 c 3a, 3b, 3c 4a, 4ab, 4b 4c, 4d, 4e, 7
L. ivanovii	5
L. innocua	4ab, 6a, 6b
L. welshimeri	6a, 6b
L. seeligeri	1/2 b, 4c, 4d, 6b

Source: FDA-CFSAN (2003).

2.1.5.2. Phage typing

Bacteriophage typing is a technique with a very good discriminatory power and that can be used to subtype a large number of isolates. However the current available phage sets are unable to type a high proportion of strains (20-51% in case of International phage typing set). Because of stringent standardization requirements and the biological nature of the reagents, this technique is performed at specialized National or International reference laboratories. Phage typing remains the most practical and convenient method for application in large and acute outbreaks (Graves *et al.*, 1999)

2.1.5.3. Genetic and molecular typing

DNA-based sub typing methods allow for differentiation of *Listeria monocytogenes* beyond the species and subspecies level. This sub typing method is improved not only the ability to detect and track animal and human listeriosis outbreaks but also provide tools to trace sources of *Listeria monocytogenes* contamination throughout the food chain (Irene *et al.*, 2003).

Genetic and molecular sub typing include Pulsed-field -gel electrophoresis (PFGE), ribotyping as well as PCR based formats such as randomly amplified fragment length of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) (Irene *et al.*, 2003). Pulsed-field gel - electrophoresis (PFGE) characterizes bacteria into subtypes by generating DNA banding patterns after restriction digestion of bacterial DNA with restriction enzymes. In PFGE sub typing method, restriction enzyme is used to cut the specific part of bacterial DNA into few large fragments while in ribotyping restriction enzymes that are used in initial digestion of DNA are used to cut the DNA into many different fragments (Bruce *et al.,* 1995).

2.1.6. Antimicrobial resistance

Due to the multiplicity of antibiotics, infections caused by drug-resistant bacteria did not represent a medical problem until the early 1980s. However, the selective pressure exerted by over prescription of drugs in clinical settings and their heavy use as growth promoters for farm animals has considerably accelerated evolution of bacteria towards resistance (Charpentier and Courvalin, 2002).

Today, it is the established fact that, several bacteria have a remarkable ability to develop resistance to every antibiotic. So, it can be expected that even the bacterial species such as *Listeria*, which are still considered susceptible to almost, all antibiotics, will evolve toward multi -resistance (Charpentier and Courvalin, 2002).

Listeria monocytogenes generally has been considered susceptible to antibiotics (Jones and MacGowan, 1995). For many years, there was no evidence of major changes in the susceptibility to antibiotics of *Listeria monocytogenes* in several countries (Heger *et al.*, 1997 and Larsson *et al.*, 1985).

But, recently several studies have described the transfer by conjugation of *streptococcal* plasmids and transposons carrying antibiotic resistant genes from *Enterococcus-Streptococcus* to *Listeria* and between *Listeria* species (Perez-Diaz *et al.*, 1982; Vicente *et al.*, 1998)

(Biavasco *et al.*, 1996; Charpentier *et al.*, 1994; Charpentier and Courvalin, 1997; McGowan *et al.*, 1990; Poyart-Salmeron *et al.*, 1990) have reported that, there were strains of *Listeria monocytogenes* that were resistant to such drugs like chloramphenicol, erythromycin, streptomycin, tetracycline, vancomycin and trimethoprim.

Resistant *Listeria monocytogenes* strains have also been identified in food samples (Slade and Collins – Thompson, 1990; Roberts *et al.,* 1996). It is therefore, important to be aware of the emergence and spread of resistant bacteria to antimicrobial agents in *Listeria monocytogenes* species

2.2. Epidemiology

Listeriosis has been reported world wide from almost all species of domestic animals as well as from many species of poultry, fish, wild animals and rodents (Malik *et al.,* 2002).

The wide distribution of environmental and animal associated occurrence of *Listeria* makes localizing the source of particular outbreak difficult (Dwight and Yuan, 1999). The main natural habitat for all *Listeria* species including *Listeria monocytogenes* is the natural environment (Rocourt and Seeliger, 1985). The organism is widespread and was isolated from surface soils, decaying vegetations, pasture, herbage, sewage sludge, effluents, silage and river water (Low and Donachie, 1997).

Epidemiological investigations of an outbreak of listerial abortions in sheep indicated that, long term confinement of animals in sheds in extremely cold and wet weather, precipitate outbreaks (Chand and Sadana, 1999). Poor nutrition, a sudden change in environment poor access to grazing pasture, overcrowding, unhygienic conditions and heavy burden o f parasitic infections increases the susceptibility of animals to infection. No breed difference has been noticed as far as the occurrence of the infection is concerned. However, it has been reported mainly from young or pregnant animals and also from animals with reproductive disorder (Malik *et al.,* 2002).

The high occurrence of the disease in young animals is related to their underdeveloped immune systems, while the fundamental physiological and immunological changes that occur in pregnant animals contributes to the susceptibility of such animals (Low and Donachie, 1997).

The reported incidence of human listeriosis has increased in developed countries. Factors such as increased awareness and better diagnostic methods are responsible for this increase. In humans, the prevalence of listeriosis is decidedly greater among immune-compromised individuals as a result of medication and or illness (WHO, 1988). The organism does not show any particular preponderance in any vertebrate species and *L. monocytogenes* infect almost all domestic animals (Cattle, buffalo, sheep, goats and pigs) as well as many species of rodents (guinea pigs, mice etc) and poultry (Malik *et al.*, 2002).

2.2.1. Prevalence of *Listeria* species in cattle, sheep and goats

There is a wide variation in prevalence of listeriosis among ruminants. Up to 5-10% sheep and goats exposed to *Listeria monocytogenes* during an outbreak showed clinical signs (Kimberling, 1988). In cattle, 3-5% of abortion is due to listeriosis (Wesley, 1999), while 1.2% of mastitis is due to listeriosis (Jensen *et al.*, 1996).

However, healthy animals including farm ruminants (cattle, sheep and goats) are carriers of *Listeria monocytogenes* and other species of *Listeria* and often shedding the organisms in their fecal material (Husu, 1990). Fecal carriage of *Listeria monocytogenes* by healthy animals was already reported in some of the earliest epidemiological studies of *Listeria* (Blenden and Szatalowicz, 1967; Donker-Voet, 1963).

In Japan, (Iida *et al.*, 1991), reported that, fecal prevalence of *Listeria monocytogenes* in healthy cattle was 1.9% while *Listeria innocua* was 3.2%. In another study in Finland, (Husu, 1990) observed that, fecal carriage of *Listeria monocytogenes* in healthy dairy cattle was 3.1%. According to the observation of Unnerstad *et al.*, (2000), fecal prevalence of *Listeria moncytogenes* and *Listeria innocua* in healthy cows was 6% and 2% respectively. In general,

fecal prevalence of *Listeria monocytogenes* in healthy cattle varies from 3.1% to 51% (Husu, 1990; Skovgaard and Morgen, 1988).

In India, Girdhar and Garg (2002) reported that, fecal prevalence of *Listeria monocytogenes, Listeria ivanovii*, and *Listeria innocua* in healthy sheep were 10.5%, 5.2% and 7.8% respectively. The fecal prevalence of *Listeria monocytogenes* and other species of *Listeria* in healthy goats were usually statistically similar with that of in sheep. (Nightingale *et al.*, 2004)

In Ethiopia, no study was conducted on fecal prevalence of *Listeria monocytogenes* and other species of *Listeria* in healthy cattle, sheep and goats.

2.2.2. Source of infection and mode of transmission

Listeria is widely distributed in animals and humans as well as in the environment. *Listeria monocytogenes* has been isolated from different mammalian and avian species and also from soil, plants, mud, pasture and streams. Cattle, sheep, goats and many other animals including humans discharge the agent in their feces by which feeds and the environment are contaminated, so that animals will be infected during feeding or grazing (Acha and Szyfres, 2001).

Soil contamination and ingestion of contaminated feed are the primary modes of transmission of *Listeria*. Poor quality silage is commonly implicated and accounts for listeriosis often being referred as " silage disease". Asymptomatic carriers can be a source for further contamination of the environment and therefore an indirect source of infection (Dwight and Yuan, 1999).

In humans, food borne transmission is the prominent means of infection. A wide variety of foodstuffs including raw milk, raw cheese, ice cream, raw meats and other ready-to- eat food items, poultry, unprocessed vegetables, raw fish products have also been shown to frequently contaminated with *Listeria monocytogenes*. Handling of calving, lambing and infected material also resulted in infection. Poor hygiene and environmental conditions or continuous exposure to domestic animals at abattoir can act as a source of infection (Barbudhe *et al.*, 1999).

2.2.3. Virulence factors

The virulent gene products of *Listeria monocytogenes* are involved in the intracellular life cycle of these bacteria. They are a cluster on the chromosome and are called the prfA –gene dependent virulent gene cluster (Kuhn and Goebel, 1999).

Host cell entry requires the presence of surface proteins Internalin A and B (InlAB). The synthesis of these proteins is encoded by InlAB gene (Ireton and Cossart, 1997). The surface proteins InlA and InlB mediate the adherence and invasion of the host cell by *Listeria moncytogenes*. After entry into the host cell, the bacteria will be encased in a vacuole. Listeriolysin O (LLO) mediates escape from a vacuole. LLO is a pore forming cytotoxin responsible for the zone of beta-haemolysis seen on blood agar plates. The synthesis of LLO is encoded by hly or haemolysin gene (Salyers and Whitt, 2002; Kuhn and Goebel, 1999).

Intra and intercellular movement of *Listeria monocytogenes* is mediated by the protein actA the synthesis of which is encoded by the actA gene. Also the enzymes catalase and super oxide dismutase are functioning as a secondary virulence factors by degrading toxic oxygen radicals produced during the respiratory burst following the macrophage phagocytosis (Ireton and Cossart, 1997).

2.2.3. Pathogenesis

Exposure to *Listeria* occurs via the oral route with contaminated feed. Internalin, a surface protein, and its interaction with the host cell receptors mediate entry into the intestinal epithelial cells. Passing the intestinal barrier, *Listeria* localizes in phagocytic cells within the lamina propria, and then dissemination occurs via the blood stream. An alternative route of entry has been through damaged oral, nasal, or ocular mucosal surfaces via the neural sheath of peripheral nerve endings, especially the trigeminal nerve into the central nervous system (Dwight and Yuan, 1999).



Penetration of the mucosa of the intestine by the a pathogen, leads to either an inapparent infection with prolonged fecal excretion of the organism, to bacteremia with localization in various organs or to the development of fatal septicaemia. Effect of the microorganism on the central nervous system mainly the brain stem results in encephalitis specially in ruminants, which is usually unilateral (Radostits *et al.*, 2000)

2.3. Clinical Features

2.3.1. In animals

Listeriosis is a severe disease associated with a high fatality rate. Nearly all domestic animals are susceptible to *Listeria* infections, but animal listeriosis is most commonly occurs in ruminants (Cooper and Walker, 1998).

2.3.1.1. Abortion

Listeriosis is a disease caused mainly by *L. monocytogenes* and *Listeria ivanovii* that are acquired by ingestion of contaminated feed with the organism (Quinn *et al.*, 2002). Oral infection does not consistently produce abortion. The pathogenesis of fetal infection is considered to be hematogenous spread of *Listeria* from the placenta to the fetus (Low and Donachie, 1997).

In cattle, listerial abortion occurs in the last trimester without any clinical signs of infection. Fetuses die *in-utero*. Stillbirths and neonatal deaths also occur. Retention of fetal membrane results in clinical illness with high fever. Cattle abortions are usually sporadic, but the rate of abortion may reach 15% in some herds. In sheep listerial abortion may occur after twelfth week of pregnancy. The fetal membrane is usually retained and a bloody vaginal discharge occurs for several days after abortion. Fetuses die *in-utero*. Ewes that retain the dead fetuses may die form septicemia. The ewe abortion rate in a flock is usually low but it may reach a rate of 20% in some outbreaks (Radostits *al.*, 2000). The clinical manifestation of listeriosis in goats closely resembles those in sheep (Low *et al.*, 1993).

2.3.1.2. Encephalitis

The organism *Listeria monocytogenes* can infect branches of cranial nerves specially the trigeminal nerve and then enters the brain stem and interior spinal chord. The most common lesion is a focal accumulation of mononuclear cells with or without neutrophils close to or around a blood vessel i.e. perivascular cuffing.

In other cases diffuse infiltration of inflammatory cells and micro abscesses may occur in the brain stem with minimal tissue necrosis and mononuclear meningitis. The affected animal shows a clinical signs that reflects the site of the brain stem inflammation. The clinical signs include walking in a circle ("circling disease"), head tilt, facial hypoalgesia (decreased sensitivity), facial paralysis with drooping of the ear, dropped jaw caused by paresis of jaw muscles, paralysis of lips and ptosis (drooping of upper eye lids). Ataxia with consistent falling to one side could also be observed (Rocourt and Cossart, 1997; Radostits *et al.*, 2000).

2.3.1.3. Septicaemia

Septicaemia is relatively uncommon and generally occurs in the neonates as an extension of intrauterine infection. The most consistent lesion is focal hepatic necrosis with pinpoint grayish white nodules throughout the liver. Lesions are also present in spleen. Histological appearance is of multiple focal areas of necrosis with invasion by polymorphs and mononuclear cells and generalized listeriosis most commonly occurs in new borne calves, lambs and monogastric animals including human infants (Low and Donachie, 1997).

The clinical signs associated with *L. monocytogenes* include septicaemia, non-specific fever, weakness, depression and emaciation. Septicaemia may cause retention of a dead fetus and fetal membranes, without the involvement of the nervous system. Corneal opacity, nystagmus and opistotonous can occur in septicaemic neonatal calves less than ten days old, with ophthalmitis and sero fibrinous meningitis at necropsy (Low and Donachie, 1997).

2.3.1.4. Other listeric infections

Iritis and kerato-conjunctivitis caused by *L. monocytogenes* have been recorded occasionally in both cattle and sheep (Walker and Morgan, 1993). Ovine mastitis has been reported but with few documented cases (Gitter *et al.,* 1980; Sharp, 1989). The condition may present as clinical or sub clinical mastitis. Main hosts and disease causing pathogenic *Listeria* species are shown in Table4.

Table 4: Main hosts and disease syndromes produced by pathogenic*Listeria* species

Listeria species.	Host	Disease syndromes
L.monocytogenes	Young animals of many animal species including lambs, calves and birds	Visceral (septicaemia) listeriosis, necrotic foci in the liver and other abdominal organs
	Sheep, goats and cattle Occasionally other species	Neural Listeriosis "circling disease" with micro abscesses in the brain stem and perivascular cuffing
	Sheep, goats and cattle	Abortion
	Cattle	Iritis with or without other signs often associated with feeding silage
L. ivanovii	Sheep and cattle	Abortion

Source: Quinn *et al,* (2002)

2.3.2. In humans

The majority of human listeriosis cases appear to be food borne. The primary manifestation of listeriosis in humans includes septicaemia, encephalitis and meningo–encephalitis, meningitis, usually preceded by influenza-like symptoms like fever. Although the morbidity of listeriosis is relatively very low, the mortality can reach about 30%. In pregnant women, infection may result in abortion, stillbirth or premature birth (Rocourt and Bille, 1997; Slutsker and Schuchat, 1999).

Unlike diseases caused by many other food borne pathogens, food borne listeriosis is characterized by a long incubation period (7 to 60 days). Only, a specific segment of the population like immunocompromised individuals, pregnant women, the elderly and the like are likely to develop clinical listeriosis after exposure to contaminated foods (Donnelly, 2000). Thus, listeriosis outbreaks that may occur over a wide geographical and temporal range may not be detected by classical epidemiological approaches.

The minimum human infectious dose for *L.monocytogenes* is not known and depends on many factors including strain variation, environmental factors and host susceptibility. In some countries, the presence of 100 colony forming unit (cfu) of *L.monocytogenes* per gram of food is considered to be within the regulatory limits for some ready-to-eat (RTE) foods whereas in other countries, such as United States enforces a "zero tolerance" of this organism in some or all RTE foods (Donnelly, 2002).

2.4. Public Health Imortance of Listeriosis

The *Listeria monocytogenes* is a bacterial food borne pathogen that causes severe invasive disease manifestations, including abortion, septicemia, and meningitis. Multiple large outbreaks have been recognized and most cases are sporadic (Slutsker and Schuchat, 1999). Human listeriosis is relatively rare, typically includes long incubation periods (7 to 60 days), usually results in hospitalization (85% to 90%), and often results in death ($\leq$ 30%) (Wallace *et al.*, 2000).

The majority of human listeriosis cases appear to be food borne. Various studies indicate *that from 1% to 5% of common ready- to-eat foods contain Listeria monocytogenes (*Soriano *et al.*, 2001; Pinner *et al.*, 1992), and these foods may widely distributed as a result of current marketing and distribution practices (Altekruse *et al.*, 1997; Tauxe, 1997).

In recent years, *L. monocytogenes* is considered to be one of the most important agents of food borne disease. The possible explanations for the emergence of human food borne listeriosis as a major public health concern include major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation means for foods, and changes in the eating habits of people, particularly towards convenience means for food, and an increase in the number of people considered to be at high risk for the disease (elderly, pregnant women, newborns, immune-compromised people) (Slutsker and Schuchat, 1999)

2.5. Economic Importance of Listeriosis

Listeriosis results in losses to the agricultural economy by increasing costs for production animals due to illness and increased infertility and abortion rates. Losses also occur when consumers' confidence in agricultural products is undermined because of *Listeria monocytogenes* regularly and decisively lead to the list of bacterial food contaminants (Orndorff *et al.*, 2006). The Economic Research Service (ERS) estimated that, each year in the United States alone, the costs of the acute illness from food borne *Listeria* are USD 2.3 billion (Buzby *et al.*, 1996).

2.6. Status of Listeriosis in Ethiopia

In Ethiopia only a few studies were undertaken on the status of *Listeria monocytogenes* and other species. According to Molla *et al.*, (2004) of a total of 316 food samples analyzed, 103(32.6%) were positive for *Listeria* species .In this study the level of contamination of food samples, by *Listeria* species was the highest in pork (69.8%) and followed by minced beef (47.5%) and ice cream (43.5%). The dominant *Listeria* species was *Listeria innocua* (21.2%) followed by *Listeria monocytogenes* (5.1%).

In another study under taken by Mengesha (2005), from a total of 711 different food samples analyzed for the presence of *Listeria* species, 189(26.6%) of the food samples were found to be positive for *Listeria* species with the dominant species being *Listeria innocua* (17.7%) and followed by *Listeria monocytogenes* (4.8%) (Table5).

Table 5: Distribution of *Listeria* species isolated from various types of food samples.

Identified *Listeria* species	Minced beef	Chicken	Cottage cheese	Fish	Ice cream	Pork	Total
L. monocytogenes	1	1	-	1	9	4	16 (15.5)
L. ivanovii	1	-	-	-	-	-	1 (0.9)
L. innocua	21	7	1	6	6	26	67 (65)
L. seeligeri	-	-	-	-	4	5	9 (8.7)
L. welshimeri	3	-	-	1	1	2	7 (6.8)
L.grayi	3	-	-	-	-	-	3 (2.9%)
Total	29(12.8)	8(7.7)	1(0.9)	8(7.7)	20(19.4)	37(35.9)	103(32.6%)

Source: Molla *et al.* (2004)

2.7. Diagnosis of Listeric Infections

Listeriosis is difficult to diagnose as it can easily be confused with other diseases such as ketosis, rabies, and encephalitis of viral cause. Since listeriosis is often a rapidly progressing disease, a rapid diagnosis and treatment initiation after the onset of clinical signs are necessary for successful treatment. However, early diagnosis prior to death is rarely achieved, so treatment is often unsuccessful (Low and Donachie, 1997). In many cases, definitive diagnosis of listeriosis cannot be accomplished in a live animal, but requires a post-mortem and histopathological examination, microbial culture or both (Ynte *et al.,* 2003).

2.7.1. Bacteriology

Bacteriological diagnosis can be done through isolation of the causal agent (Acha and Szyfres, 2001). Specimens are collected according to the form of disease from different organs (Quinn *et al.*, 2002).

Visceral form: Material from lesions in liver, kidneys or spleen.
Neural form: spinal fluid, brain stem tissue from several sites in the medulla Oblongata.
Abortion: Placenta (cotyledons), fetal abomasal and uterine contents.

2.7.1.1. Direct microscopy

Direct smears are not as useful in listeriosis as they are in some other diseases. Smears from lesions may reveal Gram-positive rods or bacilli (often coccobacillary) but isolation should always be attempted (Quinn *et al.,* 2002).

2.7.1.2. Isolation

Blood agar with antibiotic supplement can be used as selective media or blood agar containing potassium tellurite (inhibitory to Gram-negatives). Commercial selective and indicator medias are

also available, such as *Listeria* selective agar (Oxoid) and these are designed mainly for the isolation of *Listeria* from human foodstuffs (Quinn *et al.*, 2002).

2.7.1.3. Identification

The following are important criteria for the identification of *Listeria* species (Quinn *et al.*, 2002)

- Colonial appearance: Small transparent colonies with smooth borders appear on blood agar in 24 hours and becoming grayish white in 48 hours.
- Biochemical tests: All *Listeria spp*. hydrolyze aesculin, catalase positive, oxidase negative and reactions with carbohydrate fermentation such as xylose, rhamnose and mannitol are important.
- CAMP test: *Listeria monocytogenes* and *Listeria ivanovii* are CAMP-test positive with cultures of *S. aureus* and *R. equi* (Table 6).

Table 6: Tests used in the identification of *Listeria* species

Listeria species	Hemolyis test	CAMP test		Biochemical test		
		S.aureaus	*R.equi*	Mannitol	Rhamnose	Xylose
L.monocytogenes	+	+	-	-	+	-
L.ivanovii	+	-	+	-	-	+
L.innocua	-	-	-	-	V	-
L.welshimeri	-	-	-	-	V	+
L.seeligeri	(+)	(+)	-	-	-	+
L.grayi	-	-	-	+	V	-

V = Variable, - = negative, + = positive, (+)= weak reaction.

Source: (ISO, 11290-1, 1996).

2.7.1.4. Histopathology

Gross pathological lesions caused by listeriosis in ruminants are not normally noticeable. In fetuses and neonates granulomatosis is found in all organs, the liver being infiltrated with miliary pinpoint sized necrotic foci.

With central nervous system involvement, the cerebrospinal fluid (CSF) is cloudy and the meningeal vessels are congested. There are areas of softening (Dwight and Yuan, 1999).

2.7.1.5. Animal inoculation

This method can be used to evaluate the virulence of *Listeria s*pp., but is not employed routinely.

- Anton test: Inoculation within 24 hours broth culture into the conjunctiva of a rabbit or guinea pig. Only *L. monocytogenes* causes a purulent kerato-conjunctivitis within 24-36 hours (Quinn *et al.*, 2002).
- Intraperitoneal inoculation of mice with a 24 hour broth culture:
  Both *Listeria monocytogenes* and *Listeria ivanovii* are pathogenic for mice. They die with in 5 days with formation of necrotic lesions in the liver. (Quinn *et al.*, 2002).

2.7.1.6. Confirmation

According to ISO 11290-1(1996) and Quinn *et al.*, (1994), the confirmation of *Listeria* species by sub-culturing of the colonies on Tryptone Soya Yeast Extract agar (TSYEA) of presumptive *Listeria species* and conducting of morphological, physiological and as well as biochemical testing is essential.

The tests to be performed should include Gram-staining, catalase test, motility and haemolysis test. The *L. monocytogenes* is characterized by beta haemolysis, which is narrow, clear and light zone of haemolysis. A weak zone of haemolysis is produced by *L. seeligeri.*

Carbohydrate utilization test is also one of the essential tests. Rhamnose, mannitol, xylose are inoculated with a culture from TSYEA, and incubated at 35°C - 37°C for upto 5 days. The formation of acid (yellow coloration) within 24- 48 hours is considered to be an indication of a positive reaction.

CAMP test is also used to differentiate *Listeria* species. Cultures of the *Staphylococcus aureus* and *Rhodococcus equi* are streaked in single lines across sheep blood agar plate. The two cultures should be parallel and diametrically opposite. Consequently the obtained test strain is treated in the same way at right angles to these cultures, so as not to touch the test culture of *S. aureus* and *R. equi* but approaches at a distance of 1-2mm.

An enhanced zone of haemolysis at the intersection of the test strain with each of the culture of *S.aureus* and *R. equi* is considered to be a positive reaction. The positive reaction with *R. equi* characterized by wide zone of haemolysis with a diameter greater than 5 mm and an "arrow-head" is a peculiar reaction to *L. ivanovii*. Positive reaction with *S.aureus* appears as a small zone of enhanced haemolysis, which extends about 2 mm from the test strain and within the weak hemolytic zone due to growth of the *S. aureus* (ISO, 11290-1, 1996; Quinn *et al.*, 2002).

2.7.2. Rapid methods of detection of *Listeria monocytogenes* in clinical listeriosis

The *Listeria monocytogenes* is a zoonotic agent, and decisive role in the prevention of food-borne listeriosis in humans is the reduction of the presence of the organism in all the critical stages of the food production and distribution chain, including the epidemiological surveillance of the livestock (Anonymous, 2003a and b).

Bacteriological testing (Quinn *et al.*, 2002; Walker, 1999) and histological examinations are classical methods used for the laboratory diagnosis of listeriosis in animal specimen. These

procedures are usually considered the "gold standard" to which other methods are compared. However, these protocols are time consuming and need several manual steps to be completed (Gronstol *et al.*, 1986).

Isolation of *Listeria monocytogenes* can be unsuccessful even when adequate samples are examined and culture negative cases can be the result of few or no bacteria in the lesions examined and in case of antibiotic treatment (Barlow and Megorum, 1985; Johnson *et al.*, 1995)

Histological analysis also requires post-mortem clinical samples; therefore it depends on the availability of samples and the fatality rate in the course of disease. Therefore, rapid and sensitive methods for the diagnosis of animal listeriosis and the detection of subclinical infection are essential (Amagliani *et al.*, 2006).

The combined use of ELISA and PCR can enhance the overall sensitivity of the diagnostic procedures and could represent an effective approach to the epidemiological screening of the herd or flocks. These two methods are presented a significantly shorter analysis time and possibly a higher sensitivity. Moreover, their combined use counter balances their limitations. On top of these, these two procedures allow a rapid and effective detection in both early stage, before sero conversion and in a later phase even after antibiotic therapy (Amagliani *et al.*, 2006).

2.7.3. Treatment, prevention and control

2.7.3.1 Treatment

Practically all common antibiotics, except cephalosporins are effective against *L. monocytogenes in-vitro*. However, *in-vivo*, a low efficiency may be expected and is probably in part due to the organism's intracellular location. Currently only those cases which remain ambulatory are likely to recover (Scott, 1992).

Other forms of listeriosis are treated only occasionally as most animals make recoveries after abortion and individual cases of enteritis or septicaemia are seldom recognized. In humans, it is

suggested that treatment should only be initiated after studying the antibiogram of the isolates. Irrespective of the choice of antibiotics, treatment should be started before conception or during early pregnancy. The usual treatment of choice for human listeriosis is a combination of high doses of ampicillin or penicillin plus gentamycin administered intravenously at least two weeks, with symptomatic management (Gupta *et al.*, 1997)

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2.7.3.2 .Prevention and Control

The ubiquity and saprophytic nature of *Listeria* species makes them unlikely that they will be removed from the agricultural environment. However, control measures, in post- harvest monitoring and a better understanding of host and bacterial factors that influence disease pathogenesis can be combined to overcome the often severe and widespread effects of listeriosis (Orndorff *et al.*, 2006)

Control of contamination of human food is required at all stages of the food chain. Hazard Analysis and Critical Control Point (HACCP) procedures should be applied throughout the food chain. Listeriosis can be prevented in humans by taking care during handling of abortion cases in both humans and animals, avoiding consumption of contaminated food stuffs and avoiding cross-infections especially infants in the hospitals (Malik *et al.*, 2002).

The critical issues for *Listeria monocytogenes* are to control its survival and growth and to minimize the recontamination of processed foods from the environments. Survival and growth of *Listeria monocytogenes* is determined by food substrate, which includes pH, water activity (a_w) and salt concentration and also the time temperature relationship of a heating process and effectiveness of other listericidal processes. Contrary to most food pathogens, growth of *Listeria monocytogenes* is not completely inhibited at refrigeration temperatures (4-6°C). Hence, extended storage should be discouraged (WHO, 1988).

According to WHO (1988), the following control and prevention measures are recommended in relation to the appropriate environments:

In a processing plant environment:

- Separating non-contaminated foods from contaminated ones
- Limiting the potential for growth by elimination of unnecessary use of water and by application of adequate sanitation principles.
- Limiting suitable vectors such as drains, conveyor belts, and other equipments, water supplies, condensates, aerosols, insects and rodents.

In retail establishments and home environments:

- Cross contamination should be eliminated from the environment through suitable design of equipments and appropriate sanitations procedures and contamination from contact surfaces should be minimized. Listericidal processes such as pasteurization, cooking procedures and others if applied correctly, rid of *Listeria moncytogenes* and prevention of recontamination is achievable if adherence to good hygienic practices are maintained.

One of the feasible programs in the control of listeriosis is also to reduce exposure of animals and their environment to listerial contamination (Hird and Geniogeorgis, 1990). The production of good silage with minimal listerial growth is dependent upon early cutting of grass followed by its effective compaction by bailing in the clamp or bail. A heavy inoculation of *Listeria* from soil or feces should be avoided and in exceptional circumstances, it has been recommended that pastures identified for making silage should be kept free of grazing animals during the spring (Low and Donachie, 1997).

So far, no vaccine is available against listeriosis for human and animal use (Malik *et al.*, 2002).

3 .MATERIALS AND METHODS

3.1. Study Area

The present study was conducted at the public abattoir of the city of Addis Ababa, Ethiopia. Addis Ababa is the capital city and administration center for the Government of the Federal Democratic Republic of Ethiopia. Its location is in the central high lands of Ethiopia at an altitude of 2500 meters above sea level with an estimated human population of about 4.5 million. The average temperature and rainfall are 21^{0}C and 180 mm respectively and average humidity of Addis Ababa varies between 70% to 80% during the rainy season and 40% to 50% during the dry season (NMSA, 1999). . Microbiological analysis of the samples were performed at the Microbiology Laboratory of Faculty of Veterinary Medicine, Debre Zeit.

3.2. Study Population

The population under study was consisted of cattle, sheep and goats slaughtered at Addis Ababa public abattoir. The origin of cattle, sheep, and goats slaughtered at this abattoir is mainly from North Showa of Amhara and Oromia Regions, Jimma, Gojjam, Wollo, East Showa, Wellega, West Showa, Afar, Somali, Borana and Arsi areas. Sheep and goats usually brought to Addis Ababa market individually or in flocks on foot or by truck. The animals are slaughtered after antemortem examination within 1to 6 hours after their arrival to the abattoir. In this abattoir, an average of 250 sheep, 75 goats and 800 heads of cattle are slaughtered daily while only 50 swine are slaughtered per week (None published annual report of the abattoir). There were separate slaughtering sections for small ruminants, cattle and swine.

3.3. Study Type and Sample Size

A cross-sectional study was conducted at Addis Ababa Public abattoir from September 2005 to February 2006. On each sampling day, usually once in a week, 36 animals were sampled using repeated systematic random sampling and 12 animals from each group or species from amongst apparently healthy cattle, sheep and goats were selected. From the selected animals, rectal fecal sample was collected aseptically in a separate container and labeled.

The collected faecal samples with required supplementary informations like date of collection, species of animal, place of collection etc., were collected and transported in ice bath to the Microbiology laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit for microbiological analysis. The samples were kept at refrigeration temperature before processing for no longer than 24 hours. All samples were processed and analyzed separately.

3.3.1. Sample size

According to Thrusfield (1995), the required sample size for the study was determined by the following formula depending on the expected prevalence:

$$n = \frac{1.96^2 \, {}^{P}exp \, (1 - {}^{P}exp)}{d^2}$$

Where:

n = required sample size

$^{P}_{exp}$ = expected prevalence

d = desired absolute precision

Adjustment was necessary because no study was conducted in Ethiopia on the prevalence of *Listeria* in cattle, sheep and goats. Hence, 20% was considered as an expected prevalence and more samples were collected to have the maximum sample size for this study (Thrusfield, 1995). Considering a 95% confidence interval and 5% absolute precision the desired sample size at 20% expected prevalence were 246 cattle, 246 sheep, and 246 goats.

However, to increase the sample size, more samples i.e. 44 cattle, 34 sheep and 14 goats were additionally sampled based on the availability of animals at the abattoir. Therefore, the total sample size for the whole study period was cattle 290, sheep 280 and goats were 260 animals.

3.3.2. Sampling procedures

On each sampling day (usually once a week) from 36 slaughtered cattle, sheep and goats (12 animals from each group or species of animals) were sampled using a repeated systematic random sampling technique.

From each animal about 9 gm of fecal sample was collected separately in sterile containers. As soon as the abdomen of the animal was opened, the rectal part of the intestine was separated from the rest of the gastro intestinal part and kept in a separate clean container and then about 9 gm of faecal sample from rectum was collected aseptically from each animal and placed in a sterile universal bottle.

Each sample was labeled clearly and accompanied by the required identifying information, such as date of sampling, species of animal from which the sample was obtained and the identification codes. The samples were then transported in an ice bath to the laboratory for microbiological analysis. A total of 830 samples were collected and processed during the whole study period.

3.4 . Isolation and Identification

Listeria species were isolated and identified according to the method recommended by the International Organization for Standardization (ISO, 11290-1, 1996).

3.4.1. Primary selective enrichment (Half Fraser broth)

The initial suspension was prepared by adding 9 gm of feces to 90 ml of the primary selective enrichment medium (Half Fraser broth) which was composed of meat peptone, beef extract, yeast extract, sodium chloride, disodium hydrogen phosphate dehydrate, potassium dihydrogen phosphate, aesculin, lithium chloride, sodium salt of Nalidixic acid Acriflavin hydrochloride, Sodium hydroxide and water to get a ratio of test proportion to selective enrichmentmedium1/10 (mass by volume or volume by volume).Then incubated at 30°C for 24h±2h.

3.4.2. Secondary selective enrichment (Fraser broth)

After incubation of the initial suspension (primary enrichment) for 24h±2h, 0.1 ml of the culture obtained in 3.4.1 was transferred to a tube containing 10 ml of secondary enrichment medium containing full strength of selective agents. Then incubated for 48±2h at 37°C.

3.4.3. Identification on PALCAM and Oxford agars.

From the primary enrichment culture with the help of a loop, a portion of the culture was inoculated on to the surface of the first selective plating medium i.e. Oxford agar so that well separated colonies were obtained. The same procedure were followed with the second plating out agar plate i.e. PALCAM agar and incubated for 48±2h at 37°C.

After incubation for 24h and for an additional 18h if growth was slight or if no colonies were observed after 24h of incubation, plates were examined for the presence of colonies presumed to be *Listeria* species.

- On oxford agar: Typical colonies of *Listeria* species are small (with 1 mm diameter) grayish colonies surrounded by black halos. After 48 h colonies became darker, with a possible greenish sheen and about 2 mm in diameter, with black halos and sunken centers.

- On PALCAM agar: After 24 h *Listeria* species grow as small or very small grayish green or olive green colonies, 1.5 to 2 mm in diameter, some times with black centers, but always with black halos.
- After 48 h *Listeria* species appears in the form of green colonies about 1.5 to 2 mm in diameter, with a central depression and surrounded by blacks halos.

3.4.4. Confirmation of *Listeria* species

For confirmation, from each plate of each selective medium (Oxford and PALCAM agars), five colonies presumed to be *Listeria* species were taken. When on one plate there is fewer than five presumed colonies, all colonies were taken for confirmation. The selected colonies were streaked onto the surface of pre-dried plates of Tryptic Soya Yeast Extract agar (TSYEA) in a manner, which allowed well-separated colonies to develop. The plates were incubated at 37°C for 18 h to 24 h or until growth is satisfactory.

Typical colonies were 1 to 2 mm in diameter, convex, colorless and opaque with an entire edge. If the colonies were not well separated, another typical *Listeria* species colonies were inoculated on to another TSYEA plate. The following tests were carried out from colonies of a pure culture on the TSYEA.

All the listerial isolates were stored at 4°C in TSYEA (Difco, Detroit, USA)

3.4.4.1. Catalase reaction

An isolated colony obtained in Tryptone Soya Yeast Extract agar were taken and suspended in to a drop of 3% of hydrogen peroxide solution on a slide. The immediate formation of gas bubbles indicated a positive reaction.

3.4.4.2. Gram-staining

The Gram-stain was performed on a colony separated in 3.4.4. *Listeria s*pecies were revealed as Gram positive, slim, short rods.

3.4.4.3. Motility test

Isolated colonies obtained in 3.4.4 were taken and suspended in a tube containing Tryptone Soya Yeast Extract Broth (TSYEB) and incubated at 25°C for 8 h to 24 h until a cloudy medium is observed. A drop of the above culture was deposited using a loop on to a clean glass microscope slide.

A cover slip was placed on the top and examined under the microscope. *Listeria* species appeared as slim, short rods with tumbling motility. Cultures grown above 25°C may fail to exhibit this motion.

Rods with rapid swimming motility were not *Listeria* species. The *Listeria* species are motile, giving a typical umbrella like growth pattern, if growth is not sufficient, incubated for up to an additional 5 days and observed it again.

3.5. Confirmation of *Listeria monocytogenes*

3.5.1. Haemolysis test

When morphological and physiological characteristics and catalase reaction were indicative of the *Listeria* species, sheep blood agar plates were inoculated to determine the hemolytic reaction.

Colonies separated in 3.4.4 were streaked one space apart from each culture, using a loop. Simultaneously positive (*L. monocytogenes*) and negative control cultures of *L. innocua* were also streaked

After incubation at 37°C for 24h±2h, the test strains and controls were examined. *Listeria monocytogenes* showed narrow, clear light zones of β-haemolysis. Plates were examined in a bright light to compare test cultures with controls.

3.5.2. Carbohydrate utilization test

Each of the carbohydrate broths (rhamnose, mannitol and xylose) was inoculated with a culture from TSYEB (3.4.4.3) and was incubated at 37°C for up to 5 days. Positive reactions (acid formation) was indicated by yellow color production and occured mostly within 24h to 48h.

3.5.3. CAMP Test

- Each of the *Staphylococcus aureus* and *Rhodococcus equi* were streaked in a single lines across the sheep blood agar plate so that the two cultures are parallel and diametrically opposite. The test strains separated in 3.4.4 were streaked in a similar fashion at right angles to these cultures so that the test culture and *Staphylococcus aureus* and *Rhodococcus equi* cultures do not touch but at their closest and are about 1 to 2 mm apart.

- At the same time, control cultures of *L. monocytogenes, L. innocua* and *L. ivanovii* were also streaked in the same way as that of the test culture. The plates were incubated at 37°C for 18 h to 24 h. An enhanced zone of β-haemolysis at the intersection of the test strain with each of the cultures of *S. aureus* and *R. equi* were considered to be a positive reaction. The positive reaction with *R. equi* was seen as a wide (5-10 mm) "arrow-head" of haemolysis. The reaction was considered as negative when a small zone of weak haemolysis that extends only about 1 mm at the intersection of the test strain with the diffusion zone of the *R. equi* culture.

A positive reaction with *S. aureus* appears as a small zone of enhanced h aemolysis extending only about 2 mm from the test strain and within hemolytic zone due to growth of the *S. aureus* culture.

Table 7: CAMP test for *Listeria* species

	CAMP test with	
Listeria species	*Staphylococcus ureus*	*Rhodococcus equi*
Listeria monocytogenes	+	–
Listeria ivanovii	–	+
Listeria innocua	–	–
Listeria welshimeri	–	–
Listeria seeligeri	(+)	–
Listeria grayi	-	-

+ =Positive, - =negative reaction, (+)= weak reaction.

Source: (FDA- CFSAN, 2003).

3.5.4. Interpretation of results

All *Listeria* species are small, Gram-positive and catalase-positive and motile in wet mounts. They hydrolyses aesculin, utilize dextrose; maltose and some species utilize mannitol, rhamnose and xylose with the production of acid.

An isolate utilizing mannitol with acid production is *L. grayi*. *L. monocytogenes, L. ivanovii* and *L. seeligeri* produce haemolysis in sheep blood stabs and consequently are CAMP test-positive. Among the three species, only *L. monocytogenes* cannot utilize xylose and is positive for rhamnose utilization.

Differentiation of *L. ivanovii* from *L. seeligeri i*s made possible with the help of the CAMP test. *L. seeligeri* shows enhanced haemolysis at the *S. aureus* streak. *L. ivanovii* shows enhanced haemolysis at the *R. equi* streak. Of the non-hemolytic species, *L. innocua* cannot utilize rhamnose-xylose reaction as that of *L. monocytogenes*, but it is negative to the CAMP test. *Listeria innocua s*ometimes gives negative reaction to rhamnose utilization. The *Listeria monocytogenes* are distinguished from other species by β-haemolysis, CAMP test and fermentation of mannitol, xylose and rhamnose (FDA-CFSAN, 2003)

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3.6. Antimicrobial Susceptibility Test

In this study, *Listeria monocytogenes* species isolated from cattle, sheep and goats were tested for their antimicrobial susceptibility. For this, the disk diffusion susceptibility test was performed using Kirby – Bauer technique. The test result was interpreted according to the principle and guidance of National Committee for Clinical Laboratory standard reference procedure (NCCLS, 1997) using Mueller Hinton agar (Becton Dickinson). Throughout the testing, control was included with *staphylococcus aureus* ATCC 29213. The choice of specific antibiotics to be tested was based on the information obtained from (NCCLS, 1997) Performance Standards for antimicrobial disk susceptibility testing.

Antimicrobial paper disks (Oxoid, Hampshire, England) with contents or doses in the bracket were used to carryout the test. They include penicillin-G (10units), tetracycline (30µg), erythromycin (15µg), chloramphenicol (30µg), amoxicillin (10µg), kanamycin (30µg) streptomycin (10µg), vancomycin (30µg) and ampicillin (2µg).

Each bacterial isolate was grown in Tryptone Soya Yeast Extract broth(Difco) supplemented with Brain Heart Infusion broth (Difco) and prepared according to manufacturers' guideline. Incubation of the isolates were carried out at 37°C until it reached 0.5 McFarland turbidity standard in 2 to 6 hours according to Quin*n et al.* (2002). Then, the isolates were streaked on to

Mueller-Hinton agar with the sterile cotton tipped applicator (Hard Wood Products company L.P., Gulford Mass). Four antibiotic disks were placed on the surface of each inoculated plate with an Oxoid multidisk dispenser. Plates were incubated at 30°C for 18 hours.

After 18 hours, zones of inhibition were measured with the precision caliper to the nearest 0.1ml. A total of 9 antibiotics were tested. The results were interpreted according to the Manual of clinical microbiology standards (NCCLS, 1997) (Table8).

Table 8: Inhibitory zone diameter of antimicrobials for interpretive standards of *Listeria monocytogenes*

S.No	Antimicrobial Agent	Disk Content	Susceptible	Intermediate Resistance	Resistant
1	Ampicillin	10µg	≥ 20*	14 - 16	≤ 19
2	Penicillin-G	10µg	≥ 20	-	≤ 19
3	Vancomycin	30µg	≥ 12	10 - 11	≤ 9
4	Tetracycline	30µg	≥ 19	15 - 18	≤ 14
5	Erythromycin	15µg	≥ 23	14 - 22	≤ 13
6	Kanamycin	30µg	≥ 18	14- - 17	≤13
7	Amoxicillin	20/10µg	≥ 18	14 - 17	≤ 18
8	Chloreamphenicol	30µg	≥ 18	13 - 17	≤ 12
9	Streptomycin	10µg	≥15	12-14	≤ 11

*=Zone diameter in mm, - =Not applicable, Adapted from: (NCCLS, 1997).

3.7. Serotyping of *Listeria monocytogenes* Isolates

In the present study, the *Listeria monocytogenes* isolates were sent to French Authority for Food Safety (AFSSA), Laboratory, Ploufragan, France since, no serotyping facility is available in Ethiopia.

The *Listeria monocytogenes* isolates were inoculated in semi-solid media prepared from Tryptone Soya Yeast Extract agar (Difco, Detroit, USA) supplemented with Brain Heart Infusion media (Becton Dickinson, Maryland, USA) according to manufacturer's guidelines and incubated at 37°C for 18 hours. After 18 hours of incubation, the cultures were sent to the French Authority for Food Safety (AFSSA), Laboratory, Ploufragan for serotyping by air. The cultures were packed and sent as per the procedures and requirements of the AFSSA Laboratory of Ploufragan.

3.8. Data Management and Analysis

Microsoft Excel was utilized for data entry and storage. Calculation of descriptive statistics such as tables, proportions, percentiles (%) and graphs are applied to understand the nature and characteristics of the data, to see the rate of detection of *Listeria* species in each faecal sample collected from cattle, sheep and goats slaughtered at Addis Ababa abattoir and also to know the distribution of *Listeria monocytogenes* species.

The standard formula of the number of positive animals divided by total number of animals from which sample were collected were applied to estimate the prevalence of *Listeria* species in cattle, sheep and goats and also to obtain proportions of carriage of *Listeria* species.

Pearson's chi^2 test was applied to determine if there will be significant differences in *Listeria* isolation rates in faecal samples collected from apparently healthy slaughtered cattle, sheep and goats, in this, P-value<0.05 was considered significant, while P-value>0.05 was considered as non significant

4. RESULTS

Using a cross-sectional study design, study on *Listeria monocytogenes* and other species of *Listeria* was conducted on cattle, sheep and goats slaughtered at Addis Ababa abattoir.

The study was undertaken from September 2005 to February 2006 with the objectives of to determine the prevalence of *Listeria monocytogenes* and other species of *Listeria,* identification of serotype distribution of the prevalent *Listeria monocytogenes* species and also to determine the antimicrobial susceptibility of the *Listeria monocytogenes* isolates.

Bacteriological examination was done to isolate *Listeria monocytogenes* and other species of *Listeria* from *fecal* samples collected from a total of 830 animals, which includes cattle (n=290), sheep (n=280) and goats (n=260). The isolation of *Listeria moncytogenes* and other species of *Listeria* were followed as per the standard procedures of ISO 11290-1, 1996 and subsequently characterized by Gram-staining, motility at 25°C, haemolysis on 5% sheep blood agar, catalase test and CAMP (Christie, Atkins, Muench-Peterson) test. The isolates were stored at 4°C on Tryptone Soya Yeast Extract agar (TSYEA) (Difco, Detroit, USA) during the study period.

4.1. Prevalence and Distribution of *Listeria* Species in Cattle, Sheep and Goats

A total of 290 cattle, 280 sheep and 260 goats were sampled to know the prevalence of *Listeria* species in the feces of these animals by bacteriological examination. Accordingly, *Listeria* was isolated from a total of 63 (7.6%) animals, out of which cattle 22(7.6%), sheep 27(9.6%) and goats were 14 (5.4%) positive for *Listeria*.

Statistically there is no significant difference ($p > 0.05$) with *Listeria* isolation among cattle, sheep and goats slaughtered at Addis Ababa abattoir (Table 9).

Table 9: Isolation of *Listeria* species from cattle, sheep and goats slaughtered at Addis Ababa abattoir

Animals	Total number of animals examined	Number negative for *Listeria* species	Number positive for *Listeria* species	Positives (%)
Cattle	290	268	22	7.6
Sheep	280	253	27	9.6
Goats	260	246	14	5.4
Total	830	767	63	7.6

Pearson chi^2 (2)=3.4851, p=0.175

In this study, both pathogenic and non-pathogenic *Listeria* species were isolated from fecal samples of cattle, sheep and goats slaughtered at Addis Ababa abattoir.

A total of 110 *Listeria* species were isolated from 830 fecal samples collected from cattle, sheep and goats. The fecal samples from cattle and goats yielded *Listeria monocytogenes, Listeria innocua, Listeria welshimeri*, *Listeria grayi* whereas from sheep in addition to these, *Listeria ivanovii* also isolated. From all the three species of animals, *Listeria seeligeri* was not isolated.

From 290 fecal samples of cattle origin, the overall prevalence of *Listeria* species were 38(13.1%), out of which the prevalence of *Listeria monocytogenes* was 9(3.1%), *Listeria innocua* was 18(6.2%), *Listeria welshimeri* 4(1.4%), *Listeria grayi* 7 (2.4%) and no *Listeria seeligeri* and *Listeria ivanovii* were isolated.

Among *Listeria* species, isolated from fecal samples of cattle origin, *Listeria innocua* was higher in prevalence followed by *Listeria monocytogenes*, *Listeria grayi and Listeria welshimeri.*

A total of 280 fecal samples of sheep origin were examined for the prevalence of *Listeria* species in sheep slaughtered at Addis Ababa abattoir. It was observed that, the overall prevalence of *Listeria* species were 45(16%), out of which the prevalence of *Listeria monocytogenes* was 11(3.9%), *Listeria innocua* 6(2.1%), *Listeria ivanovii* 4(1.4%), *Listeria welshimeri* 10(3.5%), *Listeria grayi* 14(5%) and no *Listeria seeligeri* species was isolated.

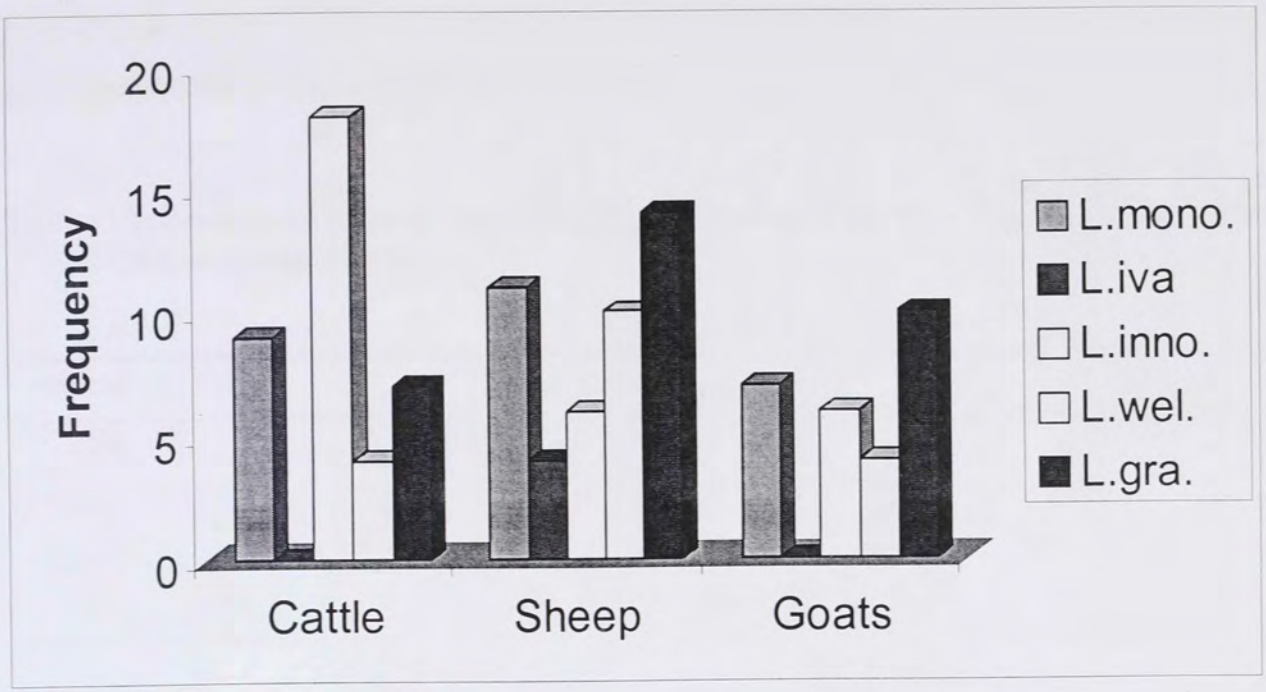
The result of bacteriological analysis indicated that, *Listeria monocytogenes* was the second in prevalence next to *Listeria grayi*, followed by *Listeria welshimeri*, *Listeria innocua* and *Listeria ivanovii.*

Incase of goats slaughtered at Addis Ababa abattoir, 260 fecal samples were examined for the isolation of *Listeria* species. The result of the analysis indicated that, the overall prevalence of *Listeria* species in goats slaughtered at Addis Ababa abattoir was 27(10.4%), of which *Listeria monocytogenes* was 7(2.7%), *Listeria innocua* 6(2.3%), *Listeria welshimeri* (4(1.5%) and *Listeria grayi* 10(3.8%). The *Listeria seeligeri* and *Listeria ivanovii* were not isolated.

The frequency of isolation of *Listeria* species from cattle, sheep and goats slaughtered at Addis Ababa abattoir are given in Table10 and Fig.1.

Table 10: The prevalence of *Listeria* species from fecal samples of cattle, sheep and goats slaughtered at Addis Ababa abattoir.

S. No	Observed *Listeria* Species	Cattle	Sheep	Goats	[95%ConfidenceInterval]		
					Cattle	Sheep	Goats
1	*L. monocytogenes*	9(3.1%)	11(3.9%)	7 (2.7%)	1.0-5.0	1.6-6.2	0.7-4.6
2	*L. ivanovii*	0	4(1.4%)	0	0	0.03-2.8	0
3	*L. innocua*	18(6.2%)	6(2.1)	6(2.3%)	3.4 -9.0	0.4 -3.8	0.47-4.1
4	*L. seeligeri*	0	0	0	0	0	0
5	*L. welshimeri*	4(1.4%)	10(3.5%)	4(1.5%)	0.03-3.8	1.8-6.7	0.03-3.0
6	*L. grayi*	7(2.4%)	14(5%)	10(3.8%	0.6-4.2	2.4 -7.5	1.5-6.2
	Total	38(13.1%)	45(16%)	27(10.4%)	5.03 -22	4.23 - 27	2.7 – 17.9



L.mono.=Listeria moncytogenes, L.iva=Listeria ivanovii, Linno.= Listeria innocua, L.wel.= Lsteria welshimeri, L.gra.= Listeria grayi.

Figure 1:Frequency of the isolated *Listeria* species in cattle, sheep and goats slaughtered at Addis Ababa abattoir.

From the result of this study it was observed that, statistically there is no significant variation, from (Pearson's chi^2 ($p>0.05$)) in prevalence of *Listeria monocytogenes* among cattle, sheep and goats slaughtered at Addis Ababa abattoir (Table 11).

Table 11:Prevalence of *Listeria monocytogenes* among cattle, sheep and goats slaughtered at Addis Ababa abattoir.

Animal	0(-)	1(+)	Total
Cattle	281	9	290
Sheep	269	11	280
Goats	253	7	260

Pearson chi^2 (2) = 0.6864 P = 0.710

4.2. Antibiogram of *Listeria monocytogenes* Isolates

The susceptibility of (27) *Listeria monocytogenes* isolates from cattle (9), sheep (11), and goats (7) to antibiotics currently in use in human and veterinary therapy was determined by the Disk diffusion method (Kirby – Bauer technique) (Quinn *et al.*, 2002).

The choice of specific antibiotics to be tested and interpretation of the test results were carried out according to the guidelines of NCCLS (1997) on Performance Standards for Antimicrobial Disk Susceptibility testing. The antibiotics used in the present study were: Penicillin-G, Tetracyclin, Ampicillin, Amoxicillin, Kanamycin, Vancomycin, Erythromycin, Streptomycin and Chloreamphenicol.

The result of this study showed that, all *Listeria monocytogenes* isolated from cattle slaughtered at Addis Ababa abattoir were sensitive to all the nine antibiotics used in the present study.

Two of the 11 isolates of *Listeria monocytogenes* from sheep showed intermediate resistance to erythromycin and the rest (9) were sensitive to all the nine antibiotics used in the study.

Among the isolates of *Listeria monocytogenes* from goats, one isolate was resistant to streptomycin and the remaining isolates were sensitive to all the antibiotics tested (Table13)

Table 12: Antibiogram of *Listeria monocytogenes* isolates

S.No	Antimicrobial agents used in the test	Number of isolates Tested	Number susceptible (%)	Number Intermediate resistant (%)	Number resistant(%)
1	Penicillin – G	27	27(100%)	0	
2	Tetracycline	27	27(100%)	0	
3	Ampicillin	27	27(100%)	0	
4	Amoxicillin	27	27(100%)	0	
5	Kanamycin	27	27(100%)	0	
6	Vancomycin	27	27(100%)	0	
7	Erythromycin	27	25(92.6%)	2(7.4)[a]	
8	Streptomycin	27	26(96.3%)	0	1(3.7)[b]
9	Chloramphenicol	27	27(100%)	0	

a-Intermediate resistant of two isolates from sheep origin to erythromycin.

b-One isolate from goats showed resistance to streptomycin

4.3. Distribution of *Listeria monocytogenes* Serotypes

The isolation of *Listeria monocytogenes* species from cattle (9), sheep (11) and goats (7) slaughtered at Addis Ababa abattoir were followed as per the standard procedures of ISO 11290-1, 1996.

Of the total of (n=27) isolates of *Listeria monocytogenes* in the present study, isolated from cattle, sheep and goats slaughtered at Addis Ababa abattoir, nine isolates were identified as 4b and eighteen isolates were identified as 4e serotypes. In this study, the result of serotyping showed that, all the nine *Listeria monocytogenes* isolates from cattle origin were identified as serotype 4e.

The *Listeria monocytogenes* isolates of sheep and goats origin were identified as serotypes 4b and 4e.

Among the eleven *Listeria monocytogenes* isolates from sheep, seven isolates were identified as 4b and the remaining four isolates as 4e serotype. Incase of seven isolates from goats, two were identified as 4b and five as 4e. In this study, other *Listeria monocytogenes serotypes* were not isolated (Table 13).

Table 13: The identified *Listeria monocytogenes* serotypes

Animals sampled	Number of faecal samples Examined	Number of *Listeria monocytogenes* Isolates	Serotypes identified	
			4b	4e
Cattle	290	9	0	9
Sheep	280	11	7	4
Goats	260	7	2	5
Total	830	27	9	18

5. DISCUSSION

For a long time listeriosis was recognized as a disease of animals only. However, it is now considered as an emerging food borne disease in humans (Wesley, 1999).

Although considerable amount of research has been carried out on listeriosis especially the isolation and identification of *Listeria* species, defined the magnitude of its public health importance, economic importance, identified the risk factors associated with the disease and its impact on food industries, and devised appropriate control strategies, etc., but a number of crucial questions remain incompletely elucidated until now (Rocourt, 1997).

Listeriosis is known to be one of the infectious diseases that is contracted from consumption of contaminated foods through the fecal-oral route (Jay, 2000). *Listeria monocytogenes* has been labeled as a leading cause of death due to food borne illness (Donnelly, 2002).

Healthy animals including ruminants often excrete the organism in their feces (Kampelmacher and Van Noorle Jansen, 1969; Gronstol, 1979; Skovgaard and Morgen, 1988; Fthenakis *et al.*, 1998). Fecal carriage of *Listeria monocytogenes* in healthy animals was already reported in some of the earliest epidemiological studies of *Listeria* (Blenden and Szatalowicz, 1967).

Healthy animals including farm ruminants, cattle, sheep and goats are asymptomatic carriers of *Listeria monocytogenes* and other species of *Listeria* and often shedding the organism in their feces (Husu, 1990).

Some of the studies have shown that, up to 50% of fecal samples collected from animals including cattle, sheep, goats, pigs and poultry with no clinical symptoms of listeriosis may contain *Listeria monocytogenes* (Nightingale *et al.*, 2004).

In the present study, prevalence of *Listeria* species in cattle slaughtered at Addis Ababa abattoir was 13.1% out of which *Listeria monocytogenes* was 3.1% and *Listeria innocua* 6.2%. Our finding of *Listeria monocytogenes* was in agreement with that of (Husu, 1990), who reported that

fecal samples from healthy cattle frequently contain *Listeria monocytogenes* with the prevalence ranging from 3.1% to 51%. Other researchers also reported its occurrence ranging from 1.9% to 33% (Iida *et al.*, 1991; Wesley, 1999; Unnerstad *et al.*, 2000).

In Ethiopia, there is no citable study conducted on the prevalence of *Listeria monocytogenes* and other species of *Listeria* in cattle, although its occurrence was reported in various food products of animal origin (Molla *et al.*, 2004 and Mengesha, 2005).

Occurrence of *Listeria monocytogenes* and other species of *Listeria* in poor quality silage was well documented. It was also reported that, there is fecal shedding of these pathogens by cattle, which are not silage fed. Both grazing animals and those fed with grass silage excreted *Listeria* species in their feces (Husu, 1990).

Besides clinical manifestations of listeriosis, sheep and goats are also asymptomatic carriers and shedding *Listeria monocytogenes* and other *Listeria* species in their feces and milk (Low *et al.*, 1993). In Brazil Antoniollo *et al.*, (2002) indicated that, prevalence of *Listeria* species in lamb was 28.6%. In India, Girdhar and Garg (2002) showed that prevalence of *Listeria* species in sheep was 31.4% out of which prevalence of *Listeria monocytogenes* was 11.4%, *Listeria ivanovii*, 5.7%, *Listeria innocua* 8.6%, *Listeria welshimeri* 2.8%.

In the present study, the overall prevalence of *Listeria* species in sheep and goats slaughtered at Addis Ababa abattoir was 16% and that of goats was 10.4% respectively out of which prevalence of *Listeria monocytogenes* and *Listeria innocua* in sheep was 3.9% and 2.1%, and in goats 2.7% and 2.3% respectively.

The results of our observation of prevalence of *Listeria monocytogenes* is in agreement with that of Garcia *et al.*, 1996, who reported that, prevalence of *Listeria monocytogenes* in ewes was 2.7%. Nightingale *et al.* (2004) reported that, prevalence of *Listeria monocytogenes* in goats were similar with that of sheep.

In Ethiopia, so far no work has been undertaken on listerial infection in domestic animals either in disease condition or in apparently healthy animals except some work on isolation of *Listeria monocytogenes* and other species of *Listeria* in ready-to-eat foods and raw foods of animal origin (Molla *et al.*, 2004; Mengesha, 2005).

Wesley (1999) observed that, the fecal shedding of *Listeria monocytogenes* in sheep was 8% while Garcia *et al., (*1996) reported that the prevalence of *Listeria monocytogenes* in ewes faecal sample was 2.7%.

From our study, it was observed that, statistically there was no significant difference ($p > 0.05$) in the prevalence of *Listeria monocytogenes* among cattle, sheep and goats slaughtered at Addis Ababa public abattoir.

Feed borne transmission of listeriae is considered to be the main mode of transmission in naturally occurring listeriosis in animals (Gitter *et al.*, 1986). Rates of fecal shedding of *Listeria* in cattle have varied from a few up to 52% and high rates of *Listeria* excretion was due to the common occurrence of these bacteria in silage (feed) in ruminants (Dijkstra, 1971).

In Ethiopia, there is no practice of silage feeding to the cattle, in the extensive or intensive management. But in the present study, *Listeria* species were isolated from faecal samples of cattle, sheep and goats slaughtered at Addis Ababa abattoir. These animals might have acquired the microorganism from the common grazing land, which is contaminated with faeces and might have become carriers to these organisms. A similar view was expressed by Acha and Szyfres (2001), where the animals acquired from feeding or grazing from the land contaminated with feces of carrier animals.

The entry of *Listeria* species especially *Listeria monocytogenes* which is pathogenic to humans and animals, into the abattoir environment and into the abattoir with asymptomatic carrier animals and other fomities, the method of slaughtering (the hygienic status of the abattoir), evisceration and preparation of the carcasses of both cattle and small ruminants inevitably leads this pathogen to contaminate the carcass which in turn becomes potentially risky to public health.

Unnerstad *et al.*, (2000) desribed that, the shedding of *Listeria monocytogenes* from clinically healthy cows showed that, cattle feces could be a source of contamination of carcasses and milk with this pathogen and thus contribute to the food borne listeriosis in humans. Other studies also have shown that food products can be contaminated with *Listeria monocytogenes* of cattle feces origin in the processing industry (Boerlin and Piffaretti, *et al.*, 1991; Rorvik *et al.*, 1995; Unnerstad *et al.*, 2000; Ericsson *et al.*, 1997).

In the present study, 7.6% of the fecal samples of the study animals i.e. cattle, sheep and goats tested were positive for listerial organisms. The *Listeria monocytogenes* were isolated from 3.25% of the sampled animals. This indicates that, there is a possibility of contamination of raw vegetables, raw meat etc. with *Listeria monocytogenes* organisms through feces and may pose a real threat to the food handlers, veterinarians, abattoir workers, and consumers who handle such foods. This view substantiates with the work of Molla *et al.*, (2004) and Mengesha (2005) where they reported the isolation of *Listeria* species including *Listeria monocytogenes* from various ready–to-eat and raw foods in Ethiopia.

There is ample circumstantial evidence, for the fact that only 3 of the 12 known serovars of *Listeria monocytogenes* 1/2a, 1/2b and 4b are accounting for more than 90% of human and animal cases of listeriosis while other serovars, such as 1/2c are often found as food contaminants (Farber and Peterkin, 1991; Low *et al.*, 1993).

Serotyping results of *Listeria monocytogenes* in this study revealed that, 9 of the 27 isolates were identified as 4b and the remaining 18 isolates as 4e. The 4b serotype is one of the 3 serotypes known for 90% of human and animal listeriosis (Farber and Peterkin, 1991; Low *et al.*, 1993).

The 4b, serotype of *Listeria monocytogenes* cause over 50% of listeriosis cases worldwide, but strains of antigenic group 1/2 (1/2a, 1/2b and 1/2c) predominate in food isolates (Rocourt, 1994). This fact suggests that serotype 4b strains are more adapted to mammalian host tissues than strains from serogroup 1/2.

It was also reported that 4b serotype was responsible for the occurrence of eight major outbreaks of human listeriosis which happened in America, Europe, Canada, and New Zealand, between the years 1995 and 1997 (Martin, 2003). Rocourt and Bille (1997) observed that, serotype 4b or related strains caused almost all recent major food borne listeriosis in the world supporting the notion that some clones of *Listeria monocytogenes* have better adaptations to wide environments and may be particularly pathogenic.

In the current study, 9 isolates (33.3%) of *Listeria monocytogenes* 4b serotype is an indication of the risk of food borne listeriosis in Ethiopia, which is explained in relations to the wide chance of the pathogen to enter the abattoir and other food establishments with carrier animals, humans and other fomites and also raw or undercooked meat consumption habit of the population as well as due to the adaptation of the pathogen to wide environment. Under this circumstances in Ethiopia, there may be possibility of incidence of listeriosis in population, there is no work or reported outbreak.

In this study it was observed that, out of 27 isolates of *Listeria monocytogenes*, only two isolates of sheep origin showed intermediate resistance to erythromycin and a goat isolate has shown complete resistance to streptomycin. The remaining 24 strains were found to be sensitive to all the antibiotics tested.

Despite the efficient antibiotic therapy, listeriosis represents a public health problem since it is fatal in up to 30% of cases (Jones and MacGowan, 1995; Farber and Peterkin, 1991). Multiple antibiotic resistances in *Listeria monocytogenes*, which appeared to be transferable to *Entercoccus*, *Streptococcus* and *Staphylococcus* was reported at least for three clinical strains among which two were isolated in France and the remaining one in Switzerland (Hadorn *et al.*, 1993; Quentin *et al.*, 1990).

The *Listeria monocytogenes* is frequently found in the digestive tract both in humans and animals where diverse species of *Entercoccus* and *Staphylococcus* harboring conjugative plasmids and transposons are also present at very high numbers. This observation reinforces the notion that the

intestinal tract represents an ecosystem favorable to the direct exchange of genetic information between these two bacterial genera (Doucet-Populaire *et al.*, 1991).

According to the National Committee for Clinical Laboratory Standards (NCCLS, 1997), isolates considered intermediately sensitive, have minimum inhibitory concentrations (MICS) that approach generally attainable blood and tissue levels and for which response rates may be lower than those for susceptible isolates. Isolates of *Listeria monocytogenes* resistant to low levels of streptomycin have been reported by Charpentier *et al.*, (1995).

The reason for emergence of the resistant *Listeria monocytogenes* species might be due to acquiring of the resistant characteristics through plasmids and transposon genes from other bacteria like *Enterococcus faecalis*, *Streptococcus* etc., which are constantly found in the digestive tract of animals together with *Listeria* species. Other researchers also expressed similar opinion confirming that, the digestive tract was the privileged site for acquisition by *Listeria* species of conjugative plasmids and transposons from Enterococcus-Streptococcus (Doucet-Populaire *et al.*, 1991).

In the present study also two strains of *Listeria monocytogenes* of sheep and one strain of goats origin were resistant to erythromycin and streptomycin respectively. Probably these strains might have acquired the antibiotic resistance in the gut of these animals.

6. CONCLUSIONS AND RECOMMENDATIONS

In the present study, from the fecal samples of cattle, sheep and goats slaughtered at Addis Ababa abattoir, both pathogenic and non-pathogenic *Listeria* species were isolated. The identified pathogenic *Listeria* species were *Listeria monocytogenes* isolated from c attle, sheep and g oats and *Listeria ivanovii* from sheep only. The none pathogenic *Listeria* species include *Listeria innocua*, *Listeria welshimeri* and *Listeria grayi* were isolated from the three species of animals.

The serotyping result indicated that, all the isolates of *Listeria monocytogenes* isolated from cattle ,sheep and goats slaughtered at Addis Ababa abattoir were belonged to serotype 4b and 4e.

Of the 27 *Listeria monocytogenes* isolates, one isolate of goats' origin showed resistance to streptomycin, while two isolates from sheep origin showed intermediate resistance to erythromycin and all the isolates from cattle were susceptible to all the antibiotics tested.

Recommendations:

- It is essential to ligate the esophagus and the gut during evisceration and immediate separation of the offal from the carcass to minimize contamination of the carcass with *Listeria monocytogenes*.

- Standard hygienic practice should be maintained by abattoir personnel to reduce contamination of meat and meat products with *Listeria monocytogenes*.

- The assumption that the species of *Listeria* are susceptible to many of the antimicrobials should be reassessed and antimicrobial agents should be used prudently both in human and animal therapy.

- Pastures used for making hay or silage should not be allowed for grazing of cattle, sheep and goats to prevent possible fecal contamination with *Listeria monocytogenes* species.

- Awareness to be created among public, food suppliers, retailers and consumers by veterinary and public health officials on the issues of hygienic way of food storage and handling and food borne diseases including the one due to *Listeria monocytogenes* and methods of prevention.

- Further research on investigation of the contamination level and survival of *Listeria monocytogenes* in ruminants, their feeds and environment is essential to know the ecology and transmission of these bacteria.

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8. ANNEXES

Annex 1: Sample collection format from study animals at Addis Ababa abattoir

S.No	Abattoir	Date	Animal Species	Animal code	Animal age	Anima sex	Sample type

Annex: 2 Format for laboratory analysis of *Listeria* species from fecal samples of study animals using oxford agar.

S.No	Spp.	Ani-mal Code	Age	Sex	Sam-ple type	Sample Source	Growth On Half Fraser Broth	Growth On Fraser Broth	Gro-wth On Oxford	Colony No.	Growth On TSYEA	Gram stain	Catalase Test	Motility test

Annex 3: Format for laboratory analysis of *Listeria* species from fecal samples using PALCAM agar

	Spp.
	Ani-mal Code
	Age
	Sex
	Sam-ple type
	Sample Source
	Growth On Half Fraser Broth
	Growth On Fraser Broth
	Gro-wth On PALCA-M
	Colony No.
	Growth On TSYEA
	Gram stain
	Catalase Test
	Motility

Annex: 4 Format for biochemical reactions of *Listeria* species

S. NO.	Colony From	Sample No.	Colony No.	Carbohydrate utilization test			CAMP test with	
				Rhamnose	Xylose	Mannitol	*S.aureus*	*R.equi*
1	PAL-CAM		1					
			2					
			3					
			4					
			5					
	Oxford		1					
			2					
			3					
			4					
			5					
	PALCA-M		1					
			2					
			3					
			4					

Annex 5 Composition and preparation of culture media used for the laboratory analysis of the study samples

a) Primary selective enrichment medium (Half Fraser broth) (AES Lab., Combourg, France)

Composition	(g/l).
Meat peptone (peptic digest of animal tissue)	5.0
Beef extract	5.0
Yeast extract	5.0
Sodium chloride	20
Disodium hydrogen phosphate dehydrate	9.6
Potassium dihydrogen phosphate	1.35
Aesculin	10
Lithium chloride	3.0
Acriflavin hydrochloride	0.0125
Nalidixic acid	0.01

Directions:

- Add 55.0 g of the powder into one litter of distilled water
- Mix carefully until complete dissolution
- Sterilize at 121°C for 15 minutes (N.B: Do not overheat)
- Aseptically add 0.5 g of ferric ammonium citrate after filter sterilization

b) Secondary selective enrichment medium (Fraser broth) (AES Lab., Combourg, France)

Composition	(g/l)
Meat peptone	5.0
Tryptone (peptic digest of casein)	5.0
Beef extract	5.0
Yeast extract	5.0

Sodium chloride	20.
Disodium hydrogen phosphate dehydrate	9.6
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Lithium chloride	3.0
Acriflavin hydrochloride	0.02
Nalidixic acid	0.02

Directions:

- Add 55.0 g of the powder into one litter of distilled water
- Mix carefully until complete dissolution
- Sterilize at 121°C for 15 minutes (N.B. Do not overheat)
- Aseptically add 0.1 g of ferric ammonium citrate after filter sterilization
- Dispense into sterile test tube

c) Polymixin Acriflavin Lithium chloride Ceftazidime Aesculin Mannitol (PALCAM): Listeria Selective Plating-out medium Agar base (AES Lab., Combourg, France)

Composition	(g/l)
Peptones	23.0
Starch	1.0
Sodium chloride	5.0
Yeast extract	3.0
Glucose	0.5
Mannitol	10.0
Aesculin	0.8
Ferric ammonium citrate	0.5
Phenol red	0.008
Lithium chloride	15.0
Agar	15.0

Supplement for 1000 ml medium (one vial) ((AES lab., Combourg, France)

Composition	(g/l)
Polymixin B sulfate (100,000 U)	0.1
Acriflavin hydrochloride	0.05
Sodium Ceftazidime Pentahydrate	0.116

Directions:

- Reconstitute the vial with 5 ml of sterile distilled water

iii) Complete medium

Directions:

- Add 73.9 g of powder into one litter of distilled water
- Mix carefully and heat with frequent agitation and boil till the powder dissolves completely
- Sterilize at 121°C for 15 minutes
- Cool it to about 45°-50°C and add the 2 impulse of the reconstituted PALCAM supplement
- Mix carefully and dispense into sterile petri-diishes

d) Oxford Listeria Selective Plating-out medium

i) Agar Base (AES Lab., Combourg, France)

Composition	(g/l)
Proteose peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar (depending on the gel strength of the agar)	9-8
Aesculin	1.0
Ammonium iron	0.5
Lithium chloride	15.0

ii) Supplement for 1000 ml medium (one vial) (AES Lab., Combourg, France)

Composition	(g/l)
Cyclohexamide	400.0
Colistin sulfate	20.0
Acriflavin hydrochloride	5.0
Cefotetan	2.0
Fosfomycin	10.0

Directions:

- Reconstitute the vial with 5 ml of 50% ethanol

iii) **Complete medium**

Directions:

- Add 57.5 g of powder into one litter of distilled water
- Mix carefully and heat with frequent agitation and boil until the powder dissolves completely
- Sterilize at 121°C for 15 minutes
- Cool it to about 45°-50°C and add 2 impulse of the reconstituted Oxford supplement
- Mix carefully and dispense into sterile petri dishes

e) Tryptone Soya Yeast Extract Agar (TSYEA) (Difco, Detroit, USA)

Composition	(g/l)
Tryptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Glucose	2.5
Agar	15.0
Yeast extract	6.0

Directions:

- Suspend 40 g of the powder in to one litter of distilled water
- Mix thoroughly
- Heat with frequent agitation and boil for one minute to completely dissolve the powder
- Autoclave at 121°C for 15 minutes
- Dispense into sterile petri dishes

f) Tryptone Soya Yeast Extract Broth (Merck, Darmstadt, Germany)

Composition	(g/l)
Tryptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Glucose	2.5
Yeast extract	6.0

Directions:

- Suspend 30 g of the powder in one litter of distilled water
- Mix thoroughly
- Autoclave at 121°C for 15 minutes
- Dispense into sterile test tubes

Directions:

- Suspend 30 g of powder in one litter of distilled water
- Mix thoroughly
- Autoclave at 121°C for 15 minutes
- Dispense into sterile test tubes

g) Blood Agar Base (Becton Dickinson, Maryland, USA)

Composition	(g/l)
Heart muscle infusion from (solids)	2.0
Pancreatic digest of casein	13.0
Yeast extract	5.0
Sodium chloride	5.0
Agar	15.0

Directions:

- Suspend 40 g of the powder in one litter of purified water
- Mix thoroughly
- Heat with frequent agitation and boil for one minute to completely dissolve the powder
- Autoclave at 121°C for 15 minutes
- Cool the base to 45°-50°C and add 5% sterile, defibrinated sheep blood
- Dispense into sterile petri dishes

h) SIM medium (Becton Dickinson, Maryland, USA)

Composition	(g/l)
Pancreatic digest of casein	20.0
Peptic digest of animal tissue	6.1
Ferrous ammonium iron sulfate	0.2
Sodium thiosulfate	0.2
Agar	3.5

Directions:

- Suspend 30 grams of the powder in one litter of purified water.
- Mix thoroughly
- Heat with frequent agitation and boil for one minute
- Autoclave at 121°C for 15 minutes
- Dispense into sterile test tubes

i) Carbohydrate utilization broths (rhamnose, xylose and mannitol)

ii) Phenol red (Merck, Darmstadt, Germany)

Composition	(g/l)
Peptone from casein	5.0
Peptone from meat	5.0
Sodium chloride	5.0
Phenol red	0.0180

Directions:

- Dissolve 15 g of powder in one litter of purified water
- Autoclave at 121°C for 15 minutes and cool it to about 60°C

ii) Carbohydrate solutions

Rhamnose (AES Lab., Combourg, France)

Xylose (AES Lab., Combourg, France)

Mannitol (Merck, Darmstadt, Germany)

Directions:

- Dissolve 5 g of each carbohydrate in 100 ml of water separately
- Sterilize by filtration

iii) Complete medium

Directions

- For each carbohydrate, add aseptically 0.5 ml of filter sterilized carbohydrate solution to 4.5 ml of phenol red solution prepared

k) Ferric ammonium citrate (Sigma, Steinheim, Germany)

Directions:

- Dissolve 5 gm of powder in 100 ml of distilled water
- Sterilize by filtration

l) Hydrogen peroxide (3%) (Sigma, Steinheim, Germany).

Mueller Hinton agar (Becton, Dickinson, Cockeys Ville, USA)

Composition	g/l
Beef extract	2.0
Acid hydrolysate of casein	17.5
Starch	1.5
Agar	17.0

Directions

Suspend 38 gm of the powder into 1 litter of purified water. Mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Sterilize it at 121°C for 15 minutes. Don't overheat it.

L) CAMP test (Christie, Atkins, Munch-Peterson) test strains

Known ß-hemolytic *Staphylococcus aureus* (CIP, Collection of Institute of Pasteur5710), *Rhodococcus equi* (CIP, 5869), *L. ivanovii* (CIP, 7842), *L. monocytogenes* and *L. innocua* (obtained from AFSSA) were used for the CAMP test. Stock cultures of these bacteria were maintained by inoculating onto TSYEA slopes incubating at 37°C for 24 hours and storing in a refrigerator at 4°C. They were frequently sub-cultured once in a month during the whole study period.

9. CURRICULUM VITAE

9.1. Personal data

NAME:	Belay Wagari Gondere
Sex:	Male
Date of birth:	20 June 1958 Eth. C.
Place of birth:	East Wollega.
Nationality:	Ethiopian.
Marital Status:	Married
Profession:	Veterinarian.

Home address:

Kimbibit district of Agriculture, Oromia Regional State, north Showa.

Language:

1/ Oromifa:	speaking and writing
2/ Amaharic:	speaking and writing.
3/ English:	speaking and writing.
4/ Russian:	speaking and writing.

Educational background

1) Primary school:	Hareto primary school, east Wollega
2) Junior secondary school:	Hareto junior secondary school, East Wollega.
3) Tertiary education:	Ambo Comprehensive senior secondary school 1978-1984 E.C.
4) University Education:	Kishinev Faculty of Veterinary Medicine, U.S.S.R. 1985-1990 E.C.

Degree awarded DV.M. (Doctor of Veterinary Medicine).

- Postgraduate education-Addis Ababa University Faculty of Veterinary Medicine 2004 - 2006.

Degree awarded-MSc Master of Science in Tropical Veterinary Medicine.

Employment Record:

Date of employment:	9 September 1990 E.C.
Position:	Field Veterinarian.
Employer:	Ministry of Agriculture.
Major task:	Coordinating and actively participating in Field Veterinary Service

9.3. RESARCH ACTIVITIES

- Isolation and Characterization of *Listeria monocytogenes* and other species of *Listeria* from apparently healthy cattle, sheep and goats slaughtered at Addis Ababa abattoir. Addis Ababa University, Faculty of Veterinary Medicine (MSc Thesis, 2006).
- Ovine Nematodirosis, Trichostrongylosis, Ostertagiosis, and their prophylaxis. Kishinev Faculty of Veterinary Medicine. U.S.S.R. (D.V.M.thesis, 1990).

9.4. REFERENCES

- Dr. Bayleyegn Molla (D.V.M., MSc., PhD), Associate professor, Department of Microbiology and Veterinary PublicHealth,Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.
- Professor A.R.S. Moorthy (B.V.Sc., M.V.Sc., PhD, F.N.A.V.S)

10. SIGNED DECLARATION SHEET

This thesis, which is my original work, has not been presented for a degree in any University and all sources of materials used in the thesis, have been given duly acknowledgements.

Name – Belay Wagari Gondere.

Signature_____

Date of submission_____

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

Professor A.R.S. Moorthy.

Signature_____

Date_____.

Dr. Bayleyegn Molla.

Signature_____

Date_____

1107/BEL/2006
AUTHOR Belay Wagary
TITLE Isolation & Characteriza
tion Of Listeria Monocytogenes.
DATE DUE | BORROWER'S NAME

1107
BEL
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Isolation & Characterization Of listeria Monocytogenes & Other Species Of Listeria From Cattle Sheep & Goats Slaughtered At Addis Ababa Abattor, Ethiopia

Belay Wagari

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