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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES IN MILK AND MEAT PRODUCTS IN RETAIL MARKETS OF ADDIS ABABA, ETHIOPIA

MSc Thesis

By

DESALEGNE MENGESHA DEGEFAW

JUNE 2005
DEBRE ZEIT, ETHIOPIA

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

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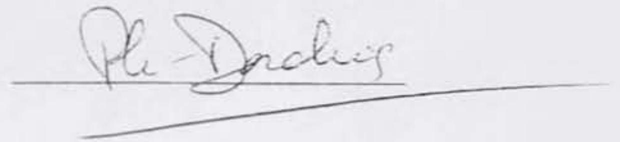
1. Prof. Ph. Dorchies National Veterinary School of Toulouse, France	
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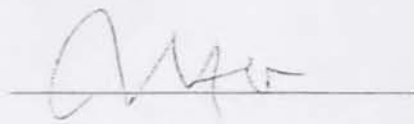
4. Dr. Mohammed Abdella Faculty of Veterinary Medicine Alemaya University, Ethiopia	

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To my mom, Seferie Tassew

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LIST OF ABBREVIATIONS

AFSSA	Agence Francaise Securite Sanitaire des Aliments
AIDS	Acquired Immuno-deficiency Syndrome
CAMP	Christie, Atkins, Munch-Peterson medium
CFSAN	Center for Food Safety and Applied Nutrition of the United States
CFU	Colony Forming Units
CIP	Collection of Institute of Pasteur
CSA	Central Statistical Authority
^{0}C	Degree Celsius
FDA	Food and Drug Administration of the United States
HACCP	Hazard Analysis Critical Control Point
HIV	Human Immunno-deficiency Virus
ISO	International Organization for Standardization
LEB	*Listeria* enrichment broth
NMSA	National Metrological Services Agency
OIE	Office International des Epizooties
PALCAM	Polymixin Acriflavin Lithium chloride Ceftazidime Aesculin and Mannitol
RTE	Ready-to-eat
spp.	Species
TSYEA	Tryptone Soya Yeast Extract Agar
TSYEB	Tryptone Soya Yeast Extract Broth
WHO	World Health Organization

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ACKNOWLEDGEMENTS

It is not easy to express my sincere thanks to my supervisor, Dr. Bayleyegn Molla, Associate Professor of Microbiology and Veterinary Public Health, who has always been following my work in the laboratory throughout my study period. He is also appreciated for provision of important articles and reference materials and for rectifying this manuscript so that it is timely and effectively finalized. He deserves a lot of acknowledgements for he has thought me the way to be strong and this is a good opportunity for me to express my great respect and appreciation to him.

I am also grateful to Dr. Daniel Alemayehu at the North Carolina State University who has always been providing me important materials throughout my stay in the Faculty of Veterinary Medicine. Mrs. Marie Thresse Toquin in Agence Francaise Securite Sanitaire des Aliments (AFSSA), Ploufragan, France also shares special thanks for serotyping *L. monocytogenes* isolates.

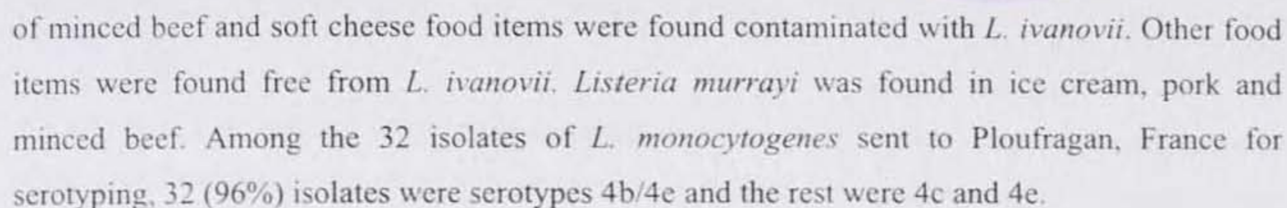
I will never forget those times we spent in the laboratory with my colleagues Dr. Kassaye Aragaw, Dr. Messele Abera, Dr. Assefa Wakwoya and externship students Dagnachew Demissie, Ayalew Berhanu and Tekleselassie Ayalew. Tsehay Tsegaye is also greatly acknowledged for her unreserved effort in making working materials ready for use in our daily activities.

Using this opportunity, I would like to express my heartfelt thanks to Estie Office of Agriculture for giving me the opportunity to attend postgraduate study and for securing my monthly salary throughout my study period. Ato Fentie Bishaw, Ato Destaw Mekuanent, and Ato Zenbaba Telila share special acknowledgements for their individual and collective contributions during my study. Ato Aschalew Kassie is also credited for his lovely friendship and advice for all the times we spent together.

It is my pleasure to express my great love and respect to all of my family members especially, my father Ato Mengesha Degefaw, my mother W/O Seferie Tassew, my brothers Mersha Mengesha, Bewketu Mengesha, Aderaw Mengesha and my sister Tigist Mengesha who have always been

eager to see the fruits of my efforts. My lovely sister, Sr. Yemesrach Mallede deserves a lot of love and respect for she has always been my footsteps since my childhood; I am really privileged to be her brother.

Melaku, Atsede, Habtu, Sefiager and Betty, do you think that I can forget your moral and material support and your everyday wishes for my success? Never.



of minced beef and soft cheese food items were found contaminated with *L. ivanovii*. Other food items were found free from *L. ivanovii*. *Listeria murrayi* was found in ice cream, pork and minced beef. Among the 32 isolates of *L. monocytogenes* sent to Ploufragan, France for serotyping, 32 (96%) isolates were serotypes 4b/4e and the rest were 4c and 4e.

The microbiological examination in our current study suggested that *L. monocytogenes* is found in the different fooditems examined with the exception of pasteurized milk and cottage cheese. This indicates the possible risk of foodborne listeriosis particularly for the pregnant women, the elderly and immuno-compromised individuals in Ethiopia. The responsible veterinary and public health authorities should make efforts to raise the awareness of the food producers, suppliers, retailers and the public at large through various ways about the methods of safe food production, storage and handling. Awareness should also be created about the risks of foodborne diseases and the methods of preventing such diseases so that a considerable part of the population can be protected from many foodborne diseases including listeriosis.

Key words: *Listeria monocytogenes*, *Listeria* species, serotypes, milk and meat products, prevalence, Ethiopia

ABSTRACT

A cross sectional study of *Listeria monocytogenes* and other *Listeria* species was undertaken in major supermarkets, pastry shops and restaurants in Addis Ababa, Ethiopia from September 2004 to March 2005. The study was conducted on milk and milk products (pasteurized cows' milk, cottage cheese, soft cheese, creamed cakes, and ice cream) and meat products (minced beef, chicken carcass, and pork). A total of 711 food samples consisting of 50 pasteurized milk, 101 soft cheeses, 80 cottage cheeses, 103 ice creams, 107 creamed cakes, 109 minced beef, 80 pork and 81 chicken carcasses were collected for analysis during the study period. *Listeria monocytogenes* and other *Listeria* species were identified and isolated according to the techniques recommended by the International Organization for Standardization (ISO 11290-1, 1996). Serotyping of *L. monocytogenes* strains was carried out at the French Authority for Food Safety (AFSSA), Ploufragan, France.

Out of the total of 711 food samples examined in this study, 189 food samples (26.6%) were positive for *Listeria* species. Of the samples examined, pork was the most contaminated food item with *Listeria* species with an overall prevalence of 62.5%. Of the examined samples, 52 (47.7%) minced beef, 44 (42.7%) ice cream, 17 (16.8%) soft cheese, 13 (16.0%) chicken carcasse and 13 (12.1%) cake samples were also positive for *Listeria* species. All pasteurized milk and cottage cheese samples were negative for all *Listeria* species in this study. *Listeria innocua* was the predominant species (17.7%) isolated from the food samples examined in the current study. Its prevalence was higher in pork with a prevalence of 53.8%, followed by 31.2% in minced beef and 25.2% in ice cream. Chicken, soft cheese and cakes were also found contaminated with this species with a prevalence of 11.1%, 9.9% and 3.7% respectively. *Listeria monocytogenes* was the second most predominant *Listeria* species isolated (4.8%) in the food items examined. Considering the prevalence of *L. monocytogenes* in each food item, ice cream was the most contaminated food item with a prevalence of 11.7% followed by cakes (6.5%) and pork (5.0%). Soft cheese, chicken carcass and minced beef were also found contaminated with *L. monocytogenes* with a prevalence of 3.9%, 3.7% and 3.7% respectively. Other *Listeria* species isolated from the different food samples examined include, *L. welshimeri* (1.8%), *L. murrayi* (0.8%), *L. seeligeri* (0.7%), *L. ivanovii* (0.6%) and *L. grayi* (0.1%). Only two samples from each

1. INTRODUCTION

Several types of foodborne diseases affect human health following consumption of contaminated foods of animal origin among which listeriosis has been said to be one of the most important emerging diseases (Vitas *et al.*, 2004). Human and animal listeriosis is a rare but severe disease associated with infection by the Gram-positive facultative intracellular bacterium, *Listeria monocytogenes*. The other pathogenic species is *Listeria ivanovii*, which causes abortion in animals (Quinn *et al.*, 1994), particularly sheep (Axelsson and Sorin, 1998). There are seven species in the genus *Listeria*: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayi* and *L. murrayi*. However, *L. murrayi* is currently merged with *L. grayi* (Rocourt and Cossart, 1997; Martin, 2003).

Listeria are widely distributed in nature and can be found on decaying vegetation and in soils, animal feces, sewage, silage, and water (James, 2000; Acha and Szyfres, 2001). In their study of the carriage rate of *Listeria* in different kinds of materials, G'rdhar and Garg (2002) showed that silage had the maximum prevalence rate of the organism (75%), followed by fodder (43.5%), feed (33.5%), fecal matter (20.5%), sewage (16.6%) and soil (4.8%). Humans and animals can be asymptomatic excretors, thus introducing *L. monocytogenes* into the environment (Uyttendaele *et al.*, 1997).

Listeria monocytogenes does not show any particular preponderance in any vertebrate species and it has been reported to infect almost all domestic animals (cattle, buffalo, sheep, goats and pigs) as well as many species of rodents (guinea pigs, mice, etc) and poultry. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products (Malik *et al.*, 2002). Abortion is a common clinical manifestation of listeriosis in sheep. *Listeria monocytogenes* is transmitted to the fetus via the placenta, leading to septic infection of the fetus. Clinical signs in the ewe are resolved following abortion of the fetus, which usually occurs as a stillbirth in the third trimester of pregnancy (Schukken *et al.*, 2003). Encephalitis is the most common manifestation of listeriosis in ruminants. In sheep and goats, the disease has a hyperacute course, and mortality may vary from 3% to more than 30% (Low and Donachie, 1997).

It is well established that any fresh food product of animal or plant origin may harbor varying numbers of *L. monocytogenes*. Because it can grow over a temperature range of about 1 to 45° C and a pH range of 4.1 to around 9.6. *Listeria monocytogenes* may also be expected to survive in foods for long periods of time, and this has been confirmed (James, 2000). In general, the organism has been found in raw milk, soft cheeses, fresh and frozen meat, poultry and seafood products and on fruits and vegetables (WHO, 1988; Rocourt and Cossart, 1997; James, 2000; Katharìou, 2003). Asymptomatic fecal carriage of *L. monocytogenes* has been studied in a variety of human populations, including pregnant women, healthy people, patients undergoing renal transplantation or hemodialysis and patients with symptoms of gastroenteritis. It was isolated from 2-6% of fecal samples from healthy people (Rocourt and Cossart, 1997).

Food has been identified as the vehicle of several major outbreaks of listeriosis investigated since 1981. The first confirmed foodborne outbreak of listeriosis occurred in 1981 in Nova Scotia, Canada, and involved 41 patients. Cabbage fertilized with manure from sheep suspected to have had *Listeria* meningitis was the probable source (Rocourt and Cossart, 1997). The second outbreak of human listeriosis was documented in Boston in 1983 and included 49 cases over a two-month period where a case control study implicated pasteurized milk as a vehicle (Kathariou, 2003). An outbreak of 11 cases occurred in Western Australia between March and September 1990. Among 11 fetuses or infants affected, there were six stillbirths or mid-trimester miscarriages with a case fatality rate of 55% (WHO, 1992).

The data obtained during the past years regarding the sources of outbreaks suggest that some foods are more hazardous than others. Highest risk foods include (i) ready-to-eat and stored at refrigerators temperatures for a long period of time, thereby enabling *Listeria* to grow, and (ii) contaminated with a high population of *L. monocytogenes* (>100 cfu/g or ml). The various ways in which the bacterium can enter a plant, its tenacity in the industrial environment, its ability to grow at refrigeration temperatures and to survive in the food for prolonged periods under adverse conditions have made it one of hottest topics for the food industry. Entry of *L. monocytogenes* into the food processing plants is often primarily due to animals which excrete the bacterium, raw foods of animal origin and possibly healthy human carriers (Rocourt and Cossart, 1997).

Listeria monocytogenes is the etiological agent of about 98% of human listeriosis but at least 3 human cases have been known to be caused by *L. ivanovii* and one by *L. seeligeri* (James, 2000). In human beings, listeriosis is characterized by a variety of several syndromes. Pregnant women are said to be most affected in the third trimester. Infection of the mother may be asymptomatic or characterized by a flu-like illness with fever, myalgia, or headache. Consequences for the fetus or infant are more serious, including spontaneous abortion, fetal death, stillbirth, severe neonatal septicemia, and meningitis. After delivery, the mother shows no disease symptoms, but *L. monocytogenes* can be isolated from the vagina, cervix, or urine. If the child is borne alive, it dies shortly afterwards from listerial septicemia. Some children borne apparently healthy fill ill with meningitis a few days to 3 weeks later; in these cases the infection was probably acquired *in utero* or during birth. Meningitis or meningo-encephalitis is the most common clinical form in non-pregnant adults especially in those over 50 years of age. Listerial meningitis will often occur as a complication in debilitated persons, alcoholics, diabetics, or cancer patients (Acha and Szyfres, 2001).

Most human cases of listeriosis occur in individuals who have predisposing diseases which lead to impairment of T-cell mediated immunity. The most affected group is newborn children (making 50% of cases in France and 39% in the United States), followed by persons over 50 years of age. The disease is rare among those between 1 month and 18 years of age (Acha and Szyfres, 2001). The percentage of patients suffering from a known underlying condition varies greatly among studies, ranging from 70 to 85% of the cases in some surveys to nearly all cases in others. The most commonly affected populations include neonates and the orderly, pregnant women, and those who are immuno-suppressed by medication (such as corticosteroids), especially after organ transplantation or illness (hematological malignancies such as leukemia or lymphoma) (Rocourt and Cossart, 1997) and HIV/AIDS patients (WHO, 1992; Rocourt, 1996). A cutaneous eruption has also been documented among veterinarians who handled infected fetuses (McLauchlin and Low, 1994).

Listeria monocytogenes is best considered as an environmental contaminant whose primary means of transmission to humans is through foodstuffs contaminated during production and processing (WHO, 1988). The emergence of listeriosis is the result of complex interactions

between various factors reflecting changes in social patterns. These factors include: (i) medical progress and consequent demographic changes, such as the increasing proportion of immuno-compromised people and the elderly, (ii) changes in primary food production (large- scale production of raw materials, modifications in food processing technology, expansion of the agricultural food industry and development of cold storage systems) and (iii) changes in food habits (increased consumer demand for convenience food that has a fresh cooked taste, can be purchased ready-to-eat, refrigerated, or frozen, can be prepared rapidly, and requires essentially little cooking before consumption) and changes in handling and preparation practices (WHO, 1988; Rocourt and Cossart, 1997).

The capability of *L. monocytogenes* to contaminate food and cause severe foodborne illness has created substantial challenges both to public health and to the food industry. The organism is ubiquitous and can colonize the food-processing environment. It is also capable of growing at low temperature, thus becoming a problematic contaminant of cold-stored foods. In heat-processed foods in which competing microflora have been reduced or eliminated, environmental contamination by *Listeria* and subsequent refrigeration of the product can reach numbers sufficient for infection. Although the infectious dose remains undetermined, it is now clear that food vehicles most likely to be commonly involved in human listeriosis are ready-to-eat foods and foods that have been refrigerated for varying lengths of time and that are commonly consumed without substantial heating (Kathariou, 2003). Milk and dairy products were the first and are among the most extensively studied foods. A wide variety of meats and meat products, including beef, pork, minced meat, chicken, ham, smoked and fermented sausages have also been associated with *L. monocytogenes* contamination, most of which is surface contamination (Rocourt and Cossart, 1997).

Listeriosis is recognized and studied to any extent mainly in industrialized countries and several outbreaks of listeriosis have been recorded since the foodborne nature of the disease has been confirmed (WHO, 1988; Martin, 2003). While sporadic cases and occasional outbreaks of human listeriosis and examples of food contamination are detected in other countries, reported prevalences in Africa, Asia and South America are non-existent or low. Whether this is a result of different consumption pattern and dietary habits or represents a lack of available reference

facilities (whether because of their absence or because of their dedication to other public health priorities) is not known but human listeriosis remains a worldwide problem, with increasing urbanization, social evolution and changes in dietary habit (WHO, 1988).

In Ethiopia, Molla *et al.* (2004) examined 53 pork, 61 minced beef, 46 ice cream, 52 chicken, 43 fish and 61 cottage cheese samples. In this study, *L. monocytogenes* was isolated from 5% of the total samples. It was isolated from 9 (19.6%) ice cream samples followed by 4 (7.5%) pork samples. Ashenafi (1994), on his study of microbial flora and some foodborne pathogens in fresh raw beef in butchers' shops in Awassa, reported that no *Listeria* species were found from a total of 102 fresh raw beef samples examined in that study. Apart from these reports, published reports on the epidemiology of *L. monocytogenes* and the status of foodborne listeriosis in Ethiopia are very rare. Therefore the objective of this study was to determine the occurrence and distribution of *Listeria monocytogenes* and other *Listeria* species in milk and meat products obtained from retail markets of Addis Ababa, Ethiopia.

2. LITERATURE REVIEW

2. 1. Milk and meat products commonly implicated in foodborne listeriosis

Prior to the 1970s, *Listeria* species were mainly associated with encephalitis and abortion in farm animals feeding on silage. However, during the 1980s, *L. monocytogenes* rapidly became recognized as a foodborne human pathogen following several epidemics of listeriosis. During the last 15-20 years there has been an increasing concern worldwide about *L. monocytogenes* and its implications for food safety. Several large well documented foodborne outbreaks and sporadic cases have been described and *L. monocytogenes* has been isolated from a wide range of raw and ready-to-eat meats, poultry, dairy products, seafoods and vegetables and from various food processing environments (European Commission, 1999).

In 1983, the American dairy industry was seriously discredited when epidemiological data showed that 14 out of 49 people in Massachusetts died after drinking a specific brand of pasteurized milk, supposedly contaminated with *L. monocytogenes* (Axelsson and Sorin, 1998). In the following years two listeriosis outbreaks: one in California in 1985 and one in Vaud, Switzerland, between 1983-87, involving over hundred cases each, were directly linked to contaminated soft cheeses. These outbreaks finally established human listeriosis as a foodborne illness and have led to widespread concern regarding the prevalence of *L. monocytogenes* in various raw and processed foods. Foodborne listeriosis occurs worldwide, either as part of an outbreak of variable size and duration or as sporadic cases, with an annual incidence of about 2-15 cases per million (Axelsson and Sorin, 1998). In both cases listeriosis is severe with case fatality rates reaching up to 50%. Different types of ready- to- eat foods have been incriminated for a number of outbreaks of listeriosis recorded in Europe and USA since 1976 (European Commission, 1999), Table 1.

Table 1: Outbreaks of foodborne listeriosis in humans

Year	Country	Food item implicated	No. of cases	No. of deaths	*L. m*/g
1976	USA	raw salad*	20	5	
1980	New Zealand	shell or raw fish*	20	5	
1981	Canada	coleslaw	41	18	
1983	USA	milk*	49	14	
1985	USA	soft cheese	142	48	10^3-10^4
1983-87	Switzerland	soft cheese	122	34	10^4-10^6
1987-89	UK	pâté	>350	-	10-10
1989-90	Denmark	hard and blue cheese	26	6	
1990	Australia	pâté	9	6	10^3
1991	Australia	smoked mussels	4	-	10^7
1992	New Zealand	smoked mussels	4	2	10^3
1992	France	pork tongue in aspic	279	85	10^4-10^6
1993	France	Pork rillettes	33	-	10^2-10^4
1993	Italy	rice salad	18	-	
1994	USA	chocolate milk	45	-	10^9
1994-95	Sweden	smoked fish	8	2	10^2-10^6
1995	France	soft cheese	33	4	
1996	Australia	cooked chicken	4	1	
1997	Italy	corn meal	748	-	10^6
1998-99	USA	hot dogs and deli meats	100	>10	
1998-99	Finland	butter	18	4	10^1-10^4

L. m/g - *L. monocytogenes* per gram of food sample   * = epidemiological association only

Source: European Commission (1999)

2.1.1. Milk and dairy products

2.1.1.1. Milk

Milk and dairy products were the first and are among the most extensively studied foods in relation to foodborne listeriosis (Rocourt and Cossart, 1997). *Listeria monocytogenes* has been isolated from raw milk. In some surveys up to 5% of samples contained the organism, at levels of ≤ 10 cells per ml. Milk can be contaminated by environmental sources including cow dung, soil, straw and, rarely, by mastitis (Rocourt and Bille, 1997). The origin of *L. monocytogenes* in milk is mainly from fecal contamination. Several studies have also confirmed a link between fecal excretion of *L. monocytogenes* and the condition of silage fed to the cows (WHO, 1988). *Listeria monocytogenes* populations in bulk tank raw milk are usually low (<1 to 10 cfu/ml) (Rocourt and Cossart, 1997).

Cows suffering from mastitis caused by *L. monocytogenes* are very rare but in such cases upto 10^3 cfu/ ml may be excreted (Rocourt and Bille, 1997). Raw goat and ewe milk are frequently used for cheese production but there are only limited data on the occurrence of *L. monocytogenes* in these types of milk (WHO, 1988). In bulk tank raw milk from 260 farms in Scotland examined over a year, 25 out of the 160 had been found to be positive for *L. monocytogenes* once, but 7 were found positive for three or four times (Eilertz *et al.*, 1993). The process of milk harvesting is probably one of the most important steps in reducing the contamination risk of this product (Schukken *et al.*, 2003).

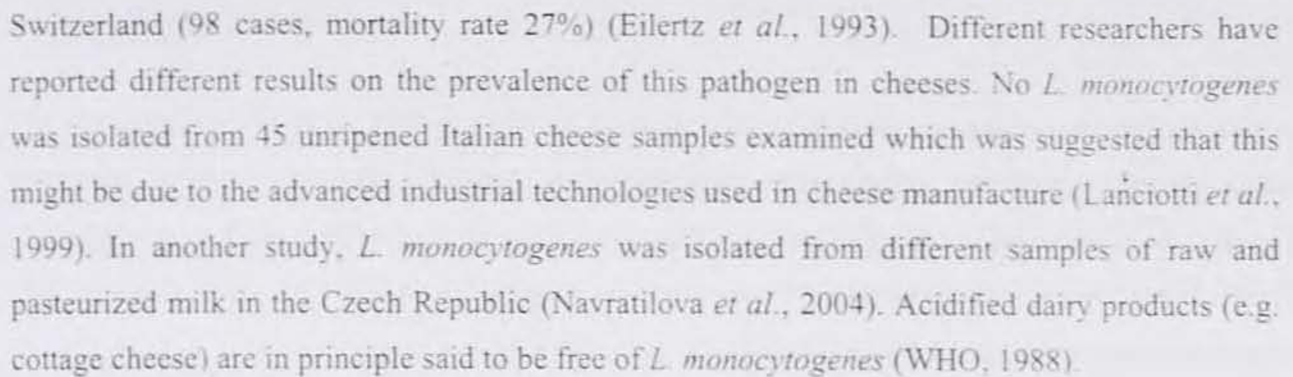
Pasteurization was confirmed to be a safe process, which reduces the number of *L. monocytogenes* occurring in raw milk to levels that do not pose an appreciable risk to human health (WHO, 1988), however, post pasteurization contamination in processing plants has been documented (Schukken *et al.*, 2003). So efforts to ensure that milk is safe from *L. monocytogenes* contamination should focus on identifying and eliminating sources of post-pasteurization contamination (Rocourt and Bille, 1997).

2.1.1.2. Soft and cottage cheese

The prevalence of *L. monocytogenes* in milk and dairy products received much attention because of early outbreaks (Eilertz *et al.*, 1993). The reported incidence of *L. monocytogenes* contamination of cheeses varies greatly between different surveys (WHO, 1988). Large surveys including hundreds of samples suggest that 1 to 10% of cheeses (especially soft cheeses) are contaminated with *L. monocytogenes* (Rocourt and Bille, 1997). Other surveys indicate that 1-15% of cheeses are contaminated with the organism (Axelsson, and Sorin, 1998). Of all foods, cheeses have been found to be frequently contaminated with *L. monocytogenes* and associated with human disease listeriosis suggesting this type of food to be particularly suitable as a vehicle for infection by *L. monocytogenes* (WHO, 1988; Axelsson and Sorin, 1998).

Soft ripened cheeses appear to be most suitable both to contamination and growth of *L. monocytogenes*. This may be due to the higher pH of these cheeses in the later stages of ripening (WHO, 1988). Studies on the behavior of *L. monocytogenes* in various artificially contaminated cheeses showed that substantial numbers of *L. monocytogenes* cells survive the manufacture and ripening of various types of cheeses including cottage cheese. There is a highly significant correlation between listerial growth and cheese pH values >5.5 and the absence of starter cultures during manufacturing. The concentration of *L. monocytogenes* in cheeses is usually low but levels higher than 1000 cfu/g may occur in up to 5.5% of samples (Rocourt and Bille, 1997). When contaminated, certain cheeses are found to be capable of supporting outgrowth of *L. monocytogenes* to populations of 10^{4}-10^{7} cfu/gm. Variations in manufacturing practices result in opportunities for post-process contamination. In theory, cheeses manufactured from *L. monocytogenes* contaminated raw milk would be more likely to be contaminated, but only a low percentage of contaminated raw milk has been reported. Surveys in Germany, Switzerland and France strongly suggest that cheeses made from pasteurized milk are as frequently contaminated with *L. monocytogenes* as cheeses made from unpasteurized milk, which might be due to contamination during manufacturing processes and handling (WHO, 1988).

Cheeses have been the vehicle in at least the two major outbreaks of listeriosis, one in the USA, associated with Mexican-style cheese (142 cases, mortality rate 34%) and the other in



Switzerland (98 cases, mortality rate 27%) (Eilertz *et al.*, 1993). Different researchers have reported different results on the prevalence of this pathogen in cheeses. No *L. monocytogenes* was isolated from 45 unripened Italian cheese samples examined which was suggested that this might be due to the advanced industrial technologies used in cheese manufacture (Lanciotti *et al.*, 1999). In another study, *L. monocytogenes* was isolated from different samples of raw and pasteurized milk in the Czech Republic (Navratilova *et al.*, 2004). Acidified dairy products (e.g. cottage cheese) are in principle said to be free of *L. monocytogenes* (WHO, 1988).

There are two main ways for *L. monocytogenes* to find its way to cheeses. This pathogen might be present in the raw milk delivered to the dairy or it might also be a part of the resident bacterial flora of the processing plant or ripening room of the dairy and thus constitute a risk of contamination for curd and cheeses. The organism might originally have been introduced not necessarily by means of contaminated raw milk but also by soil, water or air (Eilertz *et al.*, 1993). Cloet *et al.* (1989) traced the source of *L. monocytogenes* following four occurrences of contamination of cheese with this bacterium through the bulk milk tank to a particular farm, and eventually to a particular cow which was found to be excreting the organism in its milk from one quarter. The organism was also isolated from the feces of the same animal.

2.1.1.3. Ice cream and cakes

Listeria often lives in the cold, moist environment found in refrigerators. Even if a *Listeria*-free product is placed in a refrigerator, if it is improperly handled or stored, bacteria in the refrigerator can contaminate products (WHO, 1988; Axelsson and Sorin, 1998). Ice cream, which is commonly stored in refrigerators, is frequently found contaminated with *L. monocytogenes* (0.3 to 2% of samples tested) (Rocourt and Cossart, 1997). Other data from surveys indicate prevalence from zero to approximately 5.5% of products tested (WHO, 1988). Some instances of ice cream contamination have been attributed to post process contamination. Quantitative data are limited, but suggest contamination levels of from less than 1 to 15 *L. monocytogenes* per gram. As *L. monocytogenes* is found in a variety of foods of both animal and plant origin, it is certain to occur in cakes and pastry products with high content of cream, eggs, milk and fruits (Uhitil *et al.*, 2004).

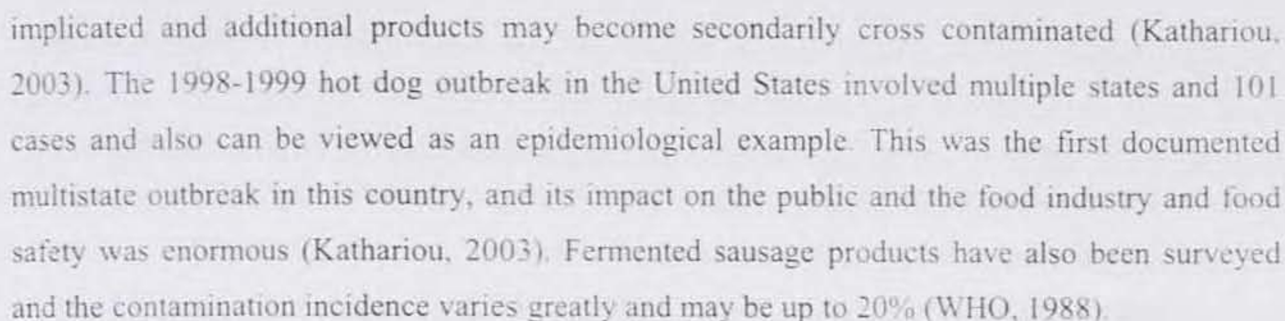
2.1.2. Meat and poultry

The reported ability of *L. monocytogenes* to grow and proliferate on many refrigerated raw, processed and ready-to-eat products, including meat and poultry products, suggests that consumption of these products could play a role in the spread of human listeriosis. Studies have also found a high prevalence of *Listeria* in these foods (Axelsson and Sorin, 1998). The prevalence of *L. monocytogenes* contamination of raw and processed meat products can be high (from < 1 to 70%) (Rocourt and Cossart, 1997), but the levels of contamination in meat and poultry products, however, are usually low, with 80-90% of samples below 10-100 cfu/g (Axelsson and Sorin, 1998). The presence of *Listeria* on carcass is usually attributed to contamination by fecal matter during slaughter. The proportion of healthy carriers harboring *Listeria* in their intestines ranges from 11 to 52% (Rocourt and Bille, 1997).

Listeria monocytogenes was detected in many types of processed meat products: cooked meats (4.9%), raw cured meats (13.7%), mayonnaise-based salads (21.3%), and prepared meals (11.7%). It was noted that raw cured meat products were significantly contaminated with *L. monocytogenes* than cooked meat products, 13.7% and 4.9%, respectively (Uyttendaele *et al.*, 1999). *Listeria monocytogenes* has been isolated from raw beef and pork, lamb, ground and/or minced meat and various poultry. Up to 30% of minced meat has yielded *L. monocytogenes* in some surveys with reported numbers ranging from < 20 to 10^3 per gram. Post processing manipulations, such as slicing, appear to be responsible for contamination of these products with *L. monocytogenes* and freezing appears to have no detrimental effect on *L. monocytogenes* (WHO, 1988).

Meat had not been implicated in human listeriosis until 1988, when investigation of one case in Oklahoma led to the identification of contaminated turkey franks as the vehicle of infection. In the 1990's, however, contaminated ready-to-eat meat products led to several outbreaks, some of which were highly publicized and had profound epidemiological impact (Kathariou, 2003).

The jellied pork tongue outbreaks in France can be seen as paradigms for the challenges associated with epidemiological investigations for listeriosis outbreaks, a specialty food item is



implicated and additional products may become secondarily cross contaminated (Kathariou, 2003). The 1998-1999 hot dog outbreak in the United States involved multiple states and 101 cases and also can be viewed as an epidemiological example. This was the first documented multistate outbreak in this country, and its impact on the public and the food industry and food safety was enormous (Kathariou, 2003). Fermented sausage products have also been surveyed and the contamination incidence varies greatly and may be up to 20% (WHO, 1988).

Listeria monocytogenes has been recovered from 15-80% of retail poultry depending on the sampling method (surface, whole carcass wash or swab) and the numbers of this bacterium have been observed to increase during product storage under refrigeration to appreciable numbers within few days (WHO, 1988). Uyttendaele *et al.*, (1997) demonstrated that *L. monocytogenes* was isolated from all categories of poultry and poultry products investigated. With respect to the group of raw products, the incidence of *Listeria* contamination was high (76.3%) in poultry in the same study. Similarly, a higher incidence of *L. monocytogenes* (36.1%) was detected in poultry in Navarra, Spain (Vitas *et al.*, 2004). Poultry and poultry products are often implicated in foodborne infections and this can be ascribed to the intensity of broiler production, the possibility of cross-contamination during the slaughter process and the mishandling of the food product during storage and preparation (Uyttendaele *et al.*, 1997).

2.2. Bacteriological techniques used in the diagnosis of *L. monocytogenes* and other *Listeria* species in foods

It is generally believed that consumption of contaminated foods is the main transmission route for human listeriosis. Therefore retrospective study of *L. monocytogenes* in foods is an important component of investigation of sources outbreaks of human listeriosis. There are a variety of conventional methods currently available for the detection and identification of *L. monocytogenes* and other *Listeria* spp. in food samples. Conventional bacteriological methods are important for various reasons. Their use results in a pure culture of the organism, which is useful for regulatory purposes. They remain the 'gold standards' against which other methods are compared and validated. These methods are usually very sensitive and they do not require sophisticated and expensive equipments. The isolation and identification of *L. monocytogenes* and other *Listeria*

spp. from food and environmental samples require the use of selective agents and enrichment procedures that limit the levels of contaminating microorganisms to reasonable numbers and allow multiplication of *L. monocytogenes* to levels that are enough for detection of the organism (OIE, 2004).

In the early days of listerial clinical bacteriology, cold enrichment was regularly used, exploiting the ability of the organism to multiply at refrigeration temperatures, whereas contaminating bacteria could not multiply under these conditions. However, this procedure requires very long incubation times, often months, making it unacceptable for current investigations of food-borne outbreaks and sporadic cases, as well as for the implementation of effective hazard analysis critical control point (HACCP) programmes in food production and processing establishments. A number of selective compounds that allow growth of *L. monocytogenes* at normal incubation temperatures have been incorporated into culture media, thus shortening the time required for selective growth of the organism. Examples of these selective compounds include cycloheximide, colistin, cefotetan, fosfomycin, lithium chloride, nalidixic acid, acriflavine, phenylethanol, ceftazidime, polymixin B and moxalactam (Oxoid, 1998; OIE, 2004).

In spite of advances made in the selective isolation of *L. monocytogenes* from food, there is still a room for improvement in a number of areas. No single procedure can be credited with being sensitive enough to detect *L. monocytogenes* and other *Listeria* spp. from all types of food. In addition, sublethally injured *L. monocytogenes* cells can be found in processed food due to freezing, heating, acidification and other types of chemical or physical treatment. These sublethally injured bacteria require special culture conditions for damage repair, before being able to be detected in culture (ISO, 1996; FDA – CFSAN, 2003; Merck, 2004; OIE, 2004).

Conventional methods for the isolation of *L. monocytogenes* and other *Listeria* spp. from food that have gained acceptance for international regulatory purposes include the following (Merck, 2004; OIE, 2004):

- The United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA–CFSAN) method,

- The Association of Official Analytical Chemists (AOAC) official method,
- The International Organization for Standardization (ISO 11290) standard,
- The International Dairy Federation (IDF) method,
- The United States Department of Agriculture (USDA)-Food Safety and Inspection Service (FSIS) method
- The French Standards (Association Francaise de Normalization, AFNOR) method and
- Health Canada method for foods and environmental samples.

Listeria species may be present in small numbers and are often accompanied and overgrown by considerably larger numbers of other genera, therefore all efforts should concentrate on suppressing possible accompanying flora using selective enrichment. It is also necessary to detect injured *Listeria* species using a primary selective enrichment medium. Therefore, whatever method is used in the diagnosis of *L. monocytogenes* in foods, for the best possible recovery of all injured cells, a three-stage procedure is recommended (ISO, 1996; FDA–CFSAN, 2003; Merck, 2004):

- Primary selective enrichment
- Secondary selective enrichment and
- Selective plating followed by
- Biochemical tests

All culture media prepared should be subjected to quality control and must support growth of the suspect organism from a small inoculum. The reference strain should also be cultured in parallel with the suspicious samples to ensure that the tests are working correctly (FDA-CFSAN, 2003; OIE, 2004). Purification is a mandatory step in the conventional analysis because isolated colonies on selective agar media may still be in contact with an invisible weak background of partially inhibited competitors (FDA–CFSAN, 2003).

The FDA-CFSAN method utilizes *Listeria* enrichment broth (LEB), which contains nalidixic acid and acriflavin, as a primary selective enrichment medium. After this step, plating is done on lithium chloride-phenylethanol-moxalactam medium (LPM) and Oxford agar plates. Further

biochemical tests are done from colonies taken from tryptone soya yeast extract agar (TSYEA) plates (FDA-CFSAN, 2003).

The USDA-FSIS method uses two enrichment steps: The 'primary' selective enrichment is done in University of Vermont medium (UVM I), containing nalidixic acid and acriflavine; the 'secondary' selective enrichment is carried out either in UVM II or in Fraser broth, containing nalidixic acid, lithium chloride and acriflavine. This method utilizes lithium chloride-phenylethanol-moxalactam medium (LPM) as a selective plating medium and further biochemical tests are carried out on colonies taken from tryptone soya yeast extract agar (TSYEA) (Merck, 2004; OIE, 2004).

The IDF method is recommended to detect *L. monocytogenes* in milk and milk products. Modified *Listeria* enrichment broth (LEB) is used to enrich the sample and this is followed by streaking on PALCAM (Polymixin acriflavin lithium chloride ceftazidime, aesculin and mannitol) and/or Oxford selective agar plates. Five presumptive colonies (or all of the colonies if there are less than five) are then sub-cultured on TSYEA for further biochemical confirmation (IDF, 1990, Merck, 2004).

In the AFNOR method, Demi-Fraser broth is used as a pre-enrichment medium and this is cultured onto Oxford or PALCAM agar plates. Fraser broth is used as a secondary selective enrichment medium where Oxford or PALCAM plates are again used as selective plating media. Further biochemical tests are used for confirmation (AFNOR, 1993).

The Health Canada method for foods and environmental samples uses *Listeria* enrichment broth (LEB) as a primary selective enrichment medium. Modified Fraser broth is used as a secondary selective enrichment. Oxford agar, lithium chloride-phenylethanol-moxalactam (LPM), modified Oxford or PALCAM agar plates are used as selective plating media and further biochemical tests are done (AFNOR, 1993).

The International Organization for Standardization (ISO) uses half Fraser broth as a primary selective enrichment medium and Fraser broth as a secondary selective enrichment. PALCAM

and Oxford agar plates are used as selective plating media. TSYEA is used for purification of colonies for further biochemical tests used in the differentiation of *L. monocytogenes* from other species of *Listeria* (ISO, 1996; Merck, 2004). The International Organization for Standardization Technical Committee ISO/TC 34, Agricultural Food Products, Subcommittee SC 9, Microbiology, claims that the ISO Standard 11290, parts 1 and 2 can be used for the detection of *L. monocytogenes* in a large variety of food and feed products. Although they recognize that this standard might not be appropriate in every detail in certain instances, they recommend that every effort should be made to apply this horizontal method as far as possible (OIE, 2004).

The two selective plating media used in the ISO method, Oxford and PALCAM agar plates, contain a number of selective agents which can inhibit the growth of most other bacteria and differential agents are incorporated as indicator system (Oxoid, 1998). *Listeria* selective medium (Oxford formulation) is recommended for the detection of *L. monocytogenes* from clinical and food specimens. This medium utilizes the selective inhibitory components lithium chloride, acriflavin, colistin sulphate, cefotetan, cyclohexamide and fosfomycin, and the indicator system aesculin and ferrous iron for the isolation or differentiation of *L. monocytogenes (*Oxoid, 1998).

Listeria monocytogenes hydrolyses aesculin, producing black zones around the colonies due to the formation of black iron phenolic compounds (Figure 1). Gram-negative bacteria are completely inhibited while most unwanted Gram-positive species are suppressed. Typical *Listeria* colonies are almost always visible after 24 hours, but incubation should be continued for further 24 hours to detect slow growing strains (Oxoid, 1998).

PALCAM medium is recommended for the isolation of *L. monocytogenes* and other *Listeria* spp. from foods. This medium is highly selective due to the presence of polymixin, acriflavin hydrochloride, lithium chloride and ceftazidime. It allows easier differential diagnosis of *L. monocytogenes* by utilizing the double indicator system (Oxoid, 1998): aesculin and ferrous system and mannitol and phenol red.

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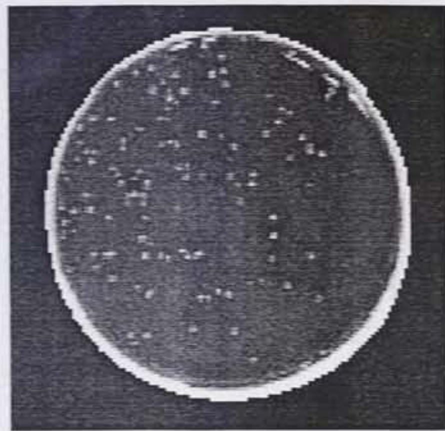


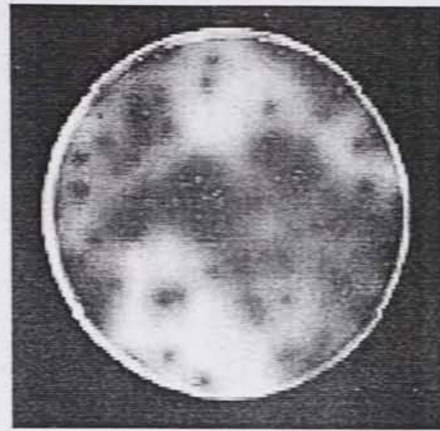


Figure 1: Colonies of *L. monocytogenes* on PALCAM and Oxford agar

Source: Merck (2004)

Listeria spp. hydrolyse aesculin resulting in the formation of a black halo around the colonies (Figure 1). *Listeria monocytogenes* does not ferment mannitol so easy differentiation from contaminants such as enterococci and staphylococci can be made, as these will ferment mannitol and produce a change from red to yellow in the pH indicator phenol red (Oxoid, 1998).

Various biochemical tests are used by different laboratories for the differentiation of *Listeria* including nitrate reduction, urea hydrolysis, methyl red, Voges-Proskaur, dextrose, maltose, aesculin hydrolysis and other tests (Table 2).

Table 2: Biochemical reactions of *Listeria* species used in different bacteriological methods

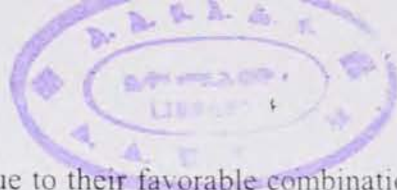
Species	*L. monocytogenes*	*L.innocua*	*L. ivanovii*	*L. seeligeri*	*L. welshimeri*	*L.grayi*	*L. murrayi*
Growth (at 35°C)	+	+	+	+	+	+	+
β-hemolysis	+	-	+	(+)	-	-	-
Catalase	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	+
Urea hydrolysis	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+
Voges-Proskaur	+	+	+	+	+	+	+
TSIA	A/A[a]	A/A	A/A	A/A	A/A	A/A	A/A
SIM motility[b]	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-	-
Dextrose	+	+	+	+	+	+	+
Aesculin	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	+	+
Rhamnose	+	V[c]	-	-	V	-	V
Xylose	-	-	+	+	+	-	-

[a]Acid slant/acid butt; [b]At 20-25°C; [c]Variable reaction; (+): Weak haemolysis; Source: Merck (2004)

2.3. Serotypes of *Listeria*

Serological typing is a widely used method among microbiologists to characterize or fingerprint species or strains of foodborne pathogens. A typical serotyping scheme makes use of specific antibodies (antiserum) that agglutinate with specific antigens located on the bacteria. The *Listeria* species can be subdivided into serotypes by the presence of their somatic (O) and flagellar (H) antigens. Under the currently accepted Seeliger/Donker-Voet scheme (Wesley *et al*., 2003), there are 16 serotypes defined by 15 O antigens and 5 H antigens. *Listeria monocytogenes* is represented by 13 serotypes some of which are shared by *L. innocua* and *L. seeligeri* (Table 3). In general, all nonpathogenic species except *L. welshimeri*, share one or more somatic and flagellar antigens with *L. monocytogenes*.

Studies have also indicated that immunological cross reactions occur between strains of *Listeria* species and *Streptococcus*, *Staphylococcus*, *Escherichia* and some *Corynebacterium* species. Serotyping alone, without biochemical characterization, is therefore not adequate for identification purposes of *L. monocytogenes* and species distinctions. Serotyping is also of limited value in epidemiological studies of listeriosis as 92% of isolates from man and animals belong to only three serotypes: 1/2a, 1/2b and 4b (Martin, 2003). Serotype 4b has caused the majority of human epidemics. Sporadic cases of listeriosis are caused by serotypes 4b, 1/2a, and 1/2b. Serotypes 4a and 4c may be restricted to animals (Wesley *et al*., 2003).



Vegetables and soft cheese are suitable for *L. monocytogenes* due to their favorable combination of conditions including pH, moisture, salt concentration, and nutrients. The surfaces of meats are also suitable substrates to support the growth of the organism after contamination from the environment. Contrary to most foodborne pathogens, growth of *L. monocytogenes* is not completely inhibited at refrigeration temperature (4-6°C). Hence, extended storage should be discouraged (WHO, 1992).

As much of the human cases arise from consumption of contaminated foods, the use of high temperature to eliminate cells of *L. monocytogenes* can be employed but the heat resistance of *L. monocytogenes* is in question. Some studies suggest that the pathogen can survive exposure to High -Temperature-Short - Time (HTST) pasteurization of milk at 71.7°C for 15 seconds but they do not survive heating at 60°C for 30 minutes, so that a higher temperature and/or a longer exposure time will be more effective in eliminating *L. monocytogenes* (Martin, 2003). However, James (2000) indicated that HTST protocol for milk (71.7°C for 15 seconds) is adequate to reduce normally existing numbers of *L. monocytogenes* below detectable levels. Low - temperature - long-time (LTLT) pasteurization protocol (62.8°C for 30 minutes) is said to be even more destructive (James, 2000). Implementation of an effective Hazard Analysis Critical Control Point (HACCP) program by food processors may help to reduce the total level of contamination by *L. monocytogenes* (Martin, 2003). Cross-contamination should be avoided through suitable design of equipment and appropriate sanitation procedures, and contamination by food handlers, contact surfaces and other transmission vectors (WHO, 1992).

Table 3: Serotypes of *Listeria* species

Listeria species	Serotypes
L. monocytogenes	1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, "7"
L. ivanovii	5
L. innocua	4ab, 6a, 6b, Un[a]
L. welshimeri	6a, 6b
L. seeligeri	1/2b, 4c, 4d, 6b, Un

[a] Un, undefined

Source: FDA-CFSAN (2003)

2.4. Control of *Listeria* in foods

The complete elimination of *L. monocytogenes* from all foodstuffs is impractical approach to the control of this organism. The critical issues are to control its survival and growth, and to minimize the recontamination of processed foods from the environment (WHO, 1988). Most researchers concerned with food safety agree that a multifaceted approach to minimize the risk of foodborne listeriosis is required. At least four factors should be considered in the overall control of the organism:

- laboratory monitoring of risk products,
- the use of hazard analysis critical control point (HACCP) approach for controlling *Listeria* during the food process,
- the use of 'hurdles' (e.g. temperature treatments), and
- educating individuals at risk (Axelsson and Sorin, 1998).

Survival and growth of *L. monocytogenes* are determined by the food substrate (including pH, water activity and salt concentration), the time temperature relationship of a heating process and effectiveness of other listericidal processes (Martin, 2003).

3. MATERIALS AND METHODS

3.1. Study area

A cross sectional study of *Listeria* species was undertaken in major supermarkets, pastry shops and restaurants in Addis Ababa, Ethiopia from September 2004 to March 2005. Addis Ababa is the capital city and administration center for the Federal Democratic Republic of Ethiopia comprising about 10 sub-cities. It lies in the central highlands of Ethiopia at an altitude of 2500 meters above sea level and has an estimated human population of about 3 million (AACA, 2004). The average annual temperature and rainfall are 21°C and 1800 mm respectively. Addis Ababa has relative humidity varying between 70% to 80% during the rainy season and 40% to 50% during the dry season (NMSA, 1999). Microbiological analysis of the samples was undertaken at the Microbiology Laboratory of Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit.

3.2. Origin and type of study samples

The study was conducted on milk and milk products (pasteurized cows' milk, cottage cheese, soft cheese, creamed cakes, and ice cream) and meat products (minced beef, chicken carcass, and pork). The beef, chicken carcasses, pork, soft cheese, cottage cheese and milk were purchased as sold for consumers (usually in a refrigerated display at 4°C) from 37 supermarkets under six different sub-cities. Cakes and ice creams were purchased in 78 pastry shops and restaurants found in six different sub-cities and data were recorded accordingly (Annexes 1- 4). The Addis Ababa Abattoirs Enterprise supplies beef and pork for the supermarkets, while milk, soft cheese and cottage cheeses were supplied by some commercial dairy processing plants such as Sebeta Agro-industry, Shola Dairy processing Enterprise and Adaa Dairy Processing Unit. Dressed chicken carcasses were supplied by some poultry farms in and around Addis Ababa such as Almaz Poultry, ELFORA Agro-industry, ALEMA Farm and others.

3.3. Study type and sample size

A cross sectional study of *Listeria* species was undertaken in pastry shops and supermarkets in Addis Ababa from September 2004 to March 2005. Each sample was collected aseptically and transported in icebox packed with ice to the microbiology laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit for analysis. A total of 711 food samples (Table 4) were collected for analysis during the study period. The samples were stored overnight in a refrigerator at 4°C until processed in the morning of the next day following the standard laboratory procedures set by the International Organization for Standardization (ISO 11290-1: 1996) for the detection of *L. monocytogenes*.

Table 4: Type and number of samples collected and analyzed during the study period

Food items	No. of samples examined
Pasteurized cows' milk	50
Soft cheese	101
Cottage cheese	80
Ice cream	103
Cakes	107
Minced beef	109
Pork	80
Chicken carcass	81
Total	711

3.4. Isolation and identification

Listeria species were identified and isolated according to the techniques recommended by the International Organization for Standardization (ISO 11290-1: 1996), Figure 2. The detection of

Listeria necessitated different successive stages. The bacteriological media used in different stages were prepared in accordance with the manufacturer's recommendations (Annex 5).

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3.4.1. Primary selective enrichment (Half Fraser broth)

Half Fraser broth (AES Lab., Combourg, France) was used as a primary selective enrichment medium to prepare the initial suspension with ferric ammonium citrate (Sigma, Steinheim, Germany) added as a supplement. Half Fraser contains one volume of lithium chloride (3g/l of distilled water) and half a volume of both acriflavin hydrochloride (0.0125 g/l of distilled water) and sodium salt of nalidixic acid (0.01 g/ l of distilled water) (Annex 5). A test portion of 25gm (25ml for pasteurized milk) was added to a Stomacher bag (Bag Filter®, Interscience, France) and 225ml of the selective primary enrichment medium, half Fraser broth was added into the test portion to obtain a ratio of test portion to selective primary enrichment medium of 1:10 (mass to volume or volume to volume). The mixture was homogenized using a laboratory blender, Stomacher-400™ (Seward Medical, London, UK) at a high speed for two minutes and then incubated at 30°C for 24 hours (Figure 2).

3.4.2. Secondary selective enrichment (Fraser broth)

After incubation of the initial suspension (primary selective enrichment) for 24 hours, 0.1 ml of the culture was transferred to a tube containing 10 ml of Fraser broth (containing acriflavin hydrochloride, 0.025 g/l of distilled water and nalidixic acid, 0.02 g/l of distilled water) (AES Lab., Combourg, France) with ferric ammonium citrate added as a supplement. The inoculated medium was incubated at 37°C for 48 hours (Figure 2).

3.4.3. Plating out and identification (PALCAM and Oxford agar plates)

From the secondary enrichment culture, Fraser broth, incubated for 48 hours at 37°C, a loopful inoculum was taken and streaked onto pre-dried sterile plates of each of PALCAM (polymixin acriflavin lithium chloride ceftazidime aesculin mannitol) and Oxford agar plates (AES Lab.,

Combourg, France) in such a way that isolated colonies are obtained. The plates were then incubated at 37°C for 24 to 48 hours based on the growth of colonies and examined for the presence of colonies presumed to be *Listeria* species. On PALCAM agar, after 24 hours *Listeria* species grow as small or very small grayish green or olive green colonies, 1.5 mm to 2 mm in diameter, sometimes with black centers, but always with black halos. After 48 hours *Listeria* species appear in the form of green colonies about 1.5 to 2 mm in diameter, with a central depression and surrounded by a black halo. Typical colonies of *Listeria* species grown on Oxford agar plates for 24 hours are small (1 mm) grayish colonies surrounded by black halos. After 48 hours colonies become darker, with a possible greenish sheen, and are about 2 mm in diameter, with black halos and sunken centers (ISO 11290-1,1996), Figure 1.

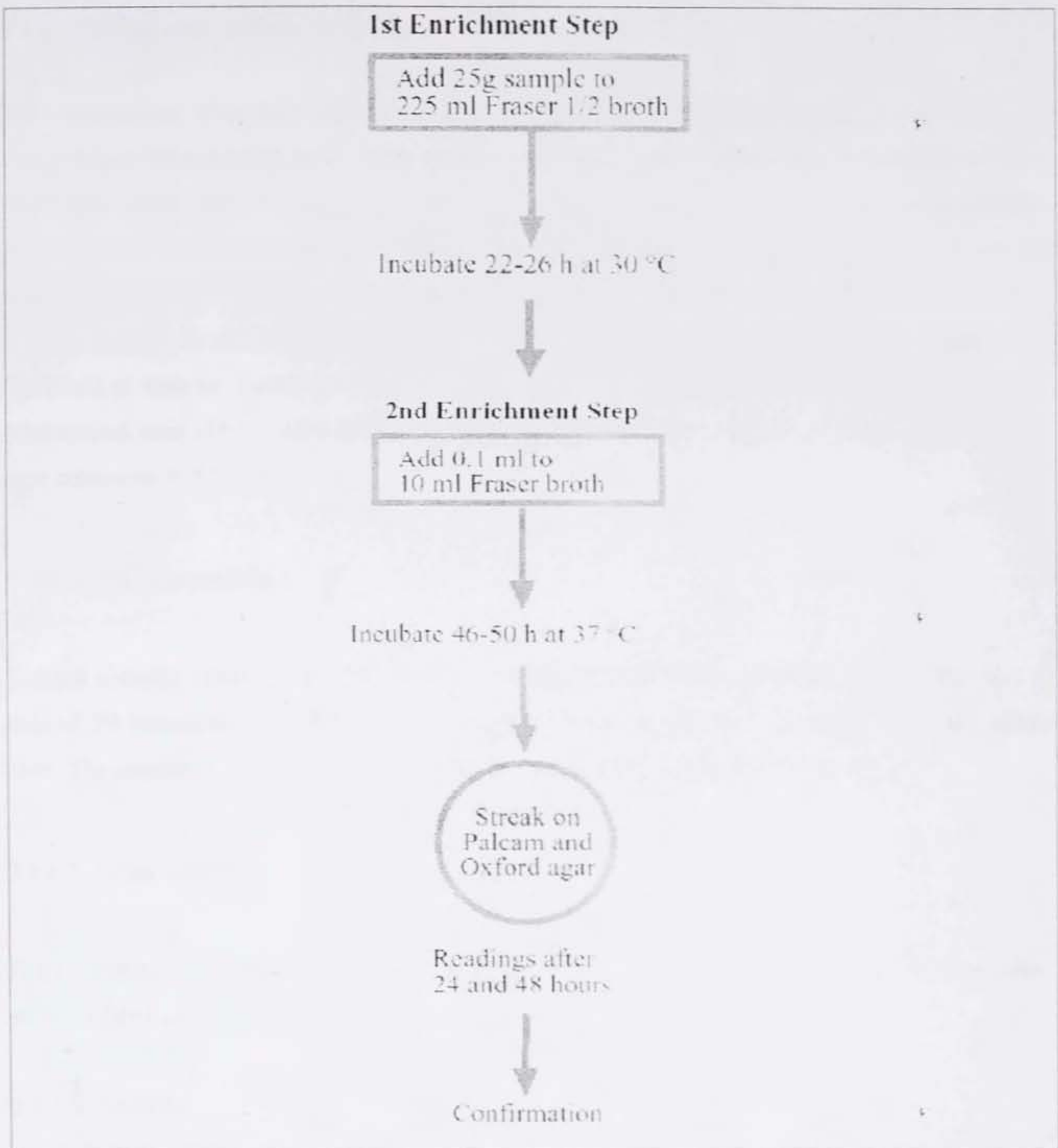


Figure 2: Conventional enrichment method used for the isolation and detection of *Listeria*

Adapted from ISO 11290-1 (1996)

3.4.4. Confirmation of *Listeria* species

For confirmation, from each plate of each selective medium (Oxford and PALCAM agar plates), five colonies presumed to be *Listeria* species were taken and streaked onto the surface of pre-dried plates of tryptone soya yeast extract agar (TSYEA) (Difco, Detroit, USA) in a manner which allowed well separated colonies to develop. All colonies were taken for confirmation when there are fewer than five presumed colonies. The plates were then incubated at 37°C for 24 hours or until growth is satisfactory. Typical colonies grown on tryptone soya yeast extract agar (TSYEA) (1 mm to 2 mm in diameter, convex, colorless and opaque), were used for further biochemical tests (ISO 11290-1,1996). The following tests were carried out from colonies of a pure culture on the TSYEA.

3.4.4.1. Catalase reaction

Isolated colonies obtained in TSYEA were transferred onto clean and sterile glass slides and a drop of 3% hydrogen peroxide (Sigma, Steinheim, Germany) solution was added onto each glass slide. The immediate formation of gas bubbles was recorded as a positive reaction.

3.4.4.2. Gram staining

Gram staining was performed on separated colonies taken from TSYEA and *Listeria* species were revealed as Gram-positive short rods.

3.4.4.3. Motility

Isolated colonies from TSYEA were taken and inoculated into SIM (hydrogen sulphide, indol and motility) medium (Becton Dickinson, Maryland, USA) using a straight inoculating needle and were incubated for 48 hours at 25°C. *Listeria* species were observed as motile, giving a typical umbrella-like growth pattern under the sub-surface (ISO 11290-1, 1996).

3.4.5. Confirmation of *L. monocytogenes*

3.4.5.1. Hemolysis test

Sheep blood agar (Becton Dickinson, Maryland, USA) plates were inoculated with isolated colonies taken from TSYEA using an inoculating loop to determine the hemolytic reaction. The colonies were examined after incubation at 37°C for 24 hours. *Listeria monocytogenes* showed narrow, clear, light zones of β-hemolysis. *Listeria innocua* showed no clear zone around the colony. *Listeria seeligeri* showed a weak zone of hemolysis and *Listeria ivanovii* showed wide, clearly delineated zones of β-hemolysis around the colonies. Control cultures of these species (*L. monocytogenes and L. innocua* obtained from the French Authority for Food Safety, Ploufragan, France and *L. ivanovii* (CIP, 7842)) were also used to compare the results of the hemolytic reaction of the test cultures. The hemolytic reactions of the isolates were recorded on the workbook and were subjected to further carbohydrate utilization and CAMP tests.

3.4.5.2. Carbohydrate utilization test

Tryptone soya yeast extract broth (TSYEB) was inoculated with a culture from TSYEA and incubated for 24 hours. Each of the carbohydrate utilization broths (rhamnose, xylose (AES Lab., Combourg, France) and mannitol (Merck, Darmstadt, Germany)) prepared using phenol red (Merck, Darmstadt, Germany) as an indicator were inoculated with a culture from TSYEB (Merck, Darmstadt, Germany) and incubated at 37°C for up to 5 days (ISO 11290-1, 1996). Positive reactions (acid formation) were indicated by a yellow color and occurred mostly within 24 h to 48 hours.

3.4.5.3. CAMP (Christie Atkins Muench-Peterson) test

Each of known β-hemolytic *Staphylococcus aureus* (CIP: Collection of Institute of Pasteur, 5710) and *Rhodococcus equi* (CIP, 5869) cultures were streaked in single lines across the sheep

blood agar plate (Becton Dickinson, USA) so that the two cultures were parallel and diametrically opposite. The test strains were then streaked in a similar fashion at right angles to these cultures so that the test cultures and *Staphylococcus aureus* and *Rhodococcus equi* cultures do not touch but at their closest were about 1 mm to 2 mm apart. Simultaneously, control cultures of *L. monocytogenes and L. innocua* (obtained from the French Authority for Food Safety (AFSSA), Ploufragan, France) and *L. ivanovii* (CIP, 7842) were streaked on the blood agar in the same manner as the test cultures (Figure 3). The plates were then incubated at 37°C for 18-24 hours. An enhanced zone of β-hemolysis at the intersection of the test strain with each of the cultures of *S. aureus* and *R. equi* is considered to be a positive reaction. *Listeria monocytogenes* showed an enhanced zone of hemolysis near the *Staphylococcus aureus* streak while *L. ivanovii* formed a wide (5 mm to 10 mm) and clear "arrow-head" hemolysis towards the *R. equi* streak. *Listeria seeligeri* showed a weak enhanced hemolysis around the *Staphylococcus ahreus* streak while *L. innocua, L. welshimeri, L. grayi* and *L. murrayi* were observed as non-hemolytic and were considered as CAMP test negative.

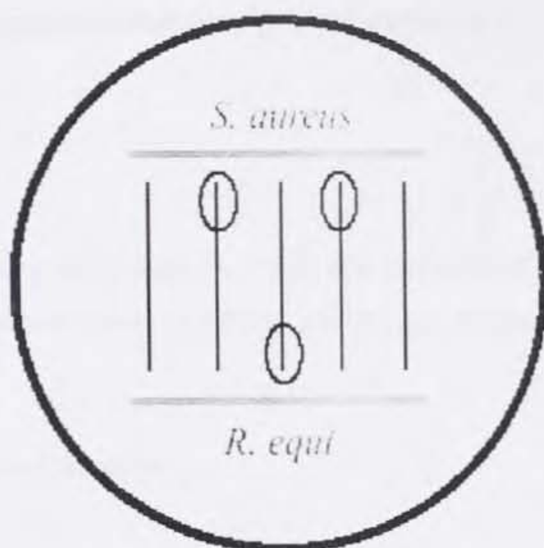


Figure 3: CAMP test for differentiating *Listeria* species. Horizontal lines represent streaks of *S. aureus* and *R. equi*. Vertical lines represent streaks of *Listeria* species. Oval areas represent typical hemolytic reactions of *L. monocytogenes, L. seeligeri* (both towards *S. aureus)* and *L. ivanovii* (towards *R. equi*).

3.4.6. Interpretation

Table 5 shows the various reactions used to differentiate *Listeria* species. All *Listeria* species were examined as small, catalase-positive, Gram-positive rods and motile. Some species utilize rhamnose, xylose and mannitol with production of acid. Isolates utilizing mannitol with acid production were referred as *L. grayi* and *L. murrayi*. Those isolates negative for rhamnose and positive for mannitol were recorded as *L. grayi*. *Listeria monocytogenes*, *L. ivanovii*, and *L. seeligeri* produce hemolysis on sheep blood stabs and consequently were CAMP test-positive. Of the three, only *L. monocytogenes* fails to utilize xylose and was positive for rhamnose utilization. The difficulty in differentiating *L. ivanovii* from *L. seeligeri* was resolved by the CAMP test. *Listeria seeligeri* shows enhanced hemolysis at the *S. aureus* streak while *L. ivanovii* showed enhanced wide zone of hemolysis at the *R. equi* streak. Of the non-hemolytic species, *L. innocua* showed the same rhamnose-xylose reactions as *L. monocytogenes* but it was found negative on the CAMP test. *Listeria innocua* sometimes gives negative results for utilization of Rhamnose. *Listeria monocytogenes* was distinguished from other species by β-hemolysis, CAMP test and fermentation of sugars (mannitol, xylose and rhamnose).

3.4.7. Serotyping

Serotyping of *L. monocytogenes* strains (n = 34) was carried out following standard methods at the French Authority for Food Safety (AFSSA), Ploufragan, France.

3.5. Data management and analysis

Microsoft Excel was employed for data entry, computation of descriptive statistics and drawing graphs. Descriptive statistics such as percentages and proportion were applied to compute some of the data. Prevalence of *Listeria* species in each food items was computed as the number of food samples positive for *Listeria* species divided by the number of samples examined in each food item. The overall prevalence of *Listeria species* was computed as the number of food samples positive for *Listeria* divided by the total number of samples examined.

Table 5: Reactions for the identification of *Listeria* species

Species	Hemolysis	Carbohydrate utilization test			CAMP test with		Gram stain	Catalase	Motility
		Rhamnose	Xylose	Mannitol	*S. aureus*	*R. equi*			
L. monocytogenes	+	+	-	-	+	-	+	+	+
L. innocua	-	V	-	-	-	-	+	+	+
L. ivanovii	+	-	+	-	-	+	+	+	+
L. seeligeri	(+)	-	+	-	(+)	-	+	+	+
L. welshimeri	-	V	+	-	-	-	+	+	+
L. grayi	-	-	-	+	-	-	+	+	+
L. murayi	-	V	-	+	-	-	+	+	+

V: variable reaction

(+): Weak reaction

+: >90% of positive reactions

-: no reaction

Source: (ISO 11290-1,1996; FDA - CFSAN, 2003)

4. RESULTS

4.1. Description of food samples, supermarkets and pastry shops

It has been said that the number of supermarkets that serve the population through the supply of milk and meat products in Addis Ababa, is on the increase in the last few years. The number of people using these supermarkets as food shopping centers is also increasing. It is also obvious that a number of people visit pastry shops and restaurants in Addis Ababa. Based on these observations it would be logical to include such supermarkets, pastry shops and restaurants in the epidemiological studies of food contaminating bacteria such as *L. monocytogenes*.

In our current study, thirty-seven supermarkets under six sub-cities were visited to collect minced beef, pork, chicken, soft cheese, pasteurized milk and cottage cheese. Seventy-eight pastry shops and restaurants under six sub-cities were also sampled for cakes and ice cream samples. All pork, cottage cheese and chicken carcass samples purchased in the supermarkets were sold either deep frozen (mostly chicken and pork) or refrigerated (mostly beef, soft cheese and milk). Chicken carcasses are sold as wrapped by polyethylene plastic bags. Cottage cheese or "Ayib" is an Ethiopian traditional dairy product made from sour milk after the removal of the fat by churning and cooking the curd to a temperature of 40°C to 70° C. It comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash 3.1 % soluble milk constituents (O'Mahony, 1988).

Ice creams and cakes sold in the pastry shops were mostly displayed in display freezers. Cakes were of various types but only those containing creams were sampled. Ice creams were predominantly made of vanilla, pineapple, banana, orange, strawberry, lemon, chocolate, milk, eggs and other sweeteners.

4. 2. Prevalence and distribution of *Listeria* species in food items examined

Table 6 shows the occurrence and distribution of *Listeria* species in the different food items examined in the current study. Out of a total of 711 food samples examined in this study, 189 food samples (26.6%) were contaminated with *Listeria* species.

Of all food items examined, pork was the most contaminated food item with *Listeria* species with an overall prevalence of 62.5% (50/80). Of the examined samples, 52 (47.7%) minced beef, 44 (42.7%) ice creams, 17 (16.8%) soft cheeses, 13 (16.0%) chicken carcasses and 13 (12.1%) cakes were positive for *Listeria* species.

All pasteurized milk and cottage cheese samples were negative for *Listeria* species in this study. The overall prevalence of *Listeria* species in the food items examined is displayed in Figure 4.

Table 6: Distribution of *Listeria* species in the different food samples examined

Listeria species isolated	Number and percentage of *Listeria* species isolated from the different food items examined									
	Minced beef	Pork	Chicken carcass	Pasteurized milk	Cottage cheese	Soft cheese	Cakes	Ice cream	Total	**Prevalence (%)**
L. monocytogenes	4	4	3	-	-	4	7	12	34	**4.8**
L. ivanovii	2	-	-	-	-	2	-	-	4	**0.6**
L. innocua	34	43	9	-	-	10	4	26	126	**17.7**
L. seeligeri	3	-	-	-	-	-	1	1	5	**0.7**
L. welshimeri	8	-	1	-	-	1	1	2	13	**1.8**
L. grayi	-	-	-	-	-	-	-	1	1	**0.1**
L.murrayi	1	3	-	-	-	-	-	2	6	**0.8**
Total positive	52	50	13	-	-	17	13	44	189	**26.6**
Samples examined	109	80	81	50	80	101	107	103	711	
Prevalence (%)	**47.7**	**62.5**	**16**	**0.0**	**0.0**	**16.8**	**12.1**	**42.7**	**26.6**	

The overall prevalence of *Listeria* species in the food items collected from the thirty-seven supermarkets (minced beef, pork, chicken carcass, soft cheese, pasteurized milk and cottage cheese) was 26.3%. The prevalence of *Listeria* species was found similar among the six sampled sub-cities except that it was found higher in Lideta sub-city (41.1%), Table 7. The prevalence of *L. monocytogenes* in these food items in the same supermarkets and sub-cities ranged from 0% to 4.1% with an overall prevalence of 3%.

Table 7: Prevalence of *Listeria* species and *L. monocytogenes* in food items sampled from supermarkets (minced beef, pork, chicken, milk, cottage cheese and soft cheese)

Sub-city	Number positive / number tested (% positive)	
	Listeria species	*L. monocytogenes*
Arada	19/73 (26.0)	1/73 (1.4)
Bole	39/152 (25.7)	7/152 (4.6)
Kirkos	37/161 (22.9)	5/161 (3.1)
Lideta	17/41 (41.4)	1/41 (2.4)
Nifas silk Lafto	16/62 (25.8)	1/62 (1.6)
Yeka	4/12 (25.0)	0/12 (0.0)
Total	**132/501 (26.3)**	**15/501 (3.0)**

The prevalence of *Listeria* species in cakes and ice cream samples collected from 78 pastry shops and restaurants under six sub-cities was found to be 12.1% and 42.7% for cakes and ice cream respectively. This prevalence in cakes in the sub-cities ranged from 0% to 14.1% while it ranged from 0% to 54.5% in ice cream samples (Table 8).

Table 8: Prevalence of *Listeria* species in cakes and ice cream in the sampled sub-cities of Addis Ababa

	Number positive / Number tested (% positive)		
Sub-city	Cake	Ice cream	Total
Arada	5/38 (13.2)	12/26 (46.2)	17/64 (26.6)
Bole	2/14 (14.3)	9/29 (31.0)	11/43 (25.6)
Guelele	0/1 (0.0)	1/3 (33.3)	1/4 (25.0)
Kirkos	6/48 (12.5)	18/33 (54.5)	24/81 (29.6)
Nefas Silk Lafto	0/2 (0)	0/4 (0)	0/6 (0)
Yeka	0/4 (0)	4/8 (50.0)	4/12 (25.0)
Total	**13/107 (12.1)**	**44/103 (42.7)**	**57/210 (27.1)**

Other *Listeria* species were also isolated from the different food samples examined. These include *L. welshimeri* (1.8%), *L. murrayi* (0.8%), *L. seeligeri* (0.7%), *L. ivanovii* (0.6%) and *L. grayi* (0.1%), Table 6. *Listeria welshimeri* was isolated with a relatively higher prevalence in minced beef (7.3%) than in other food items. Only two samples from each of minced beef and soft cheese were contaminated with *L. ivanovii*. *Listeria murrayi* were found in ice cream, pork and minced beef (Table 5).

Figure 4: Prevalence of *Listeria* in the different food items examined

Comparing the proportion of each of the *Listeria* species isolated to the total number of isolates in the current study (189), *L. innocua* was dominant with a total of 126 isolates comprising 66.7% of the total isolates of *Listeria* species followed by *L. monocytogenes*, 18% (34 / 189) and *L. welshimeri*, 6.9% (13 / 189). *L. murrayi*, *L. seeligeri*, *L. ivanovii*, and *L. grayi* comprised 3.2%, 2.6%, 2.1% and 0.5% of the total *Listeria* isolates respectively (Figure 5).

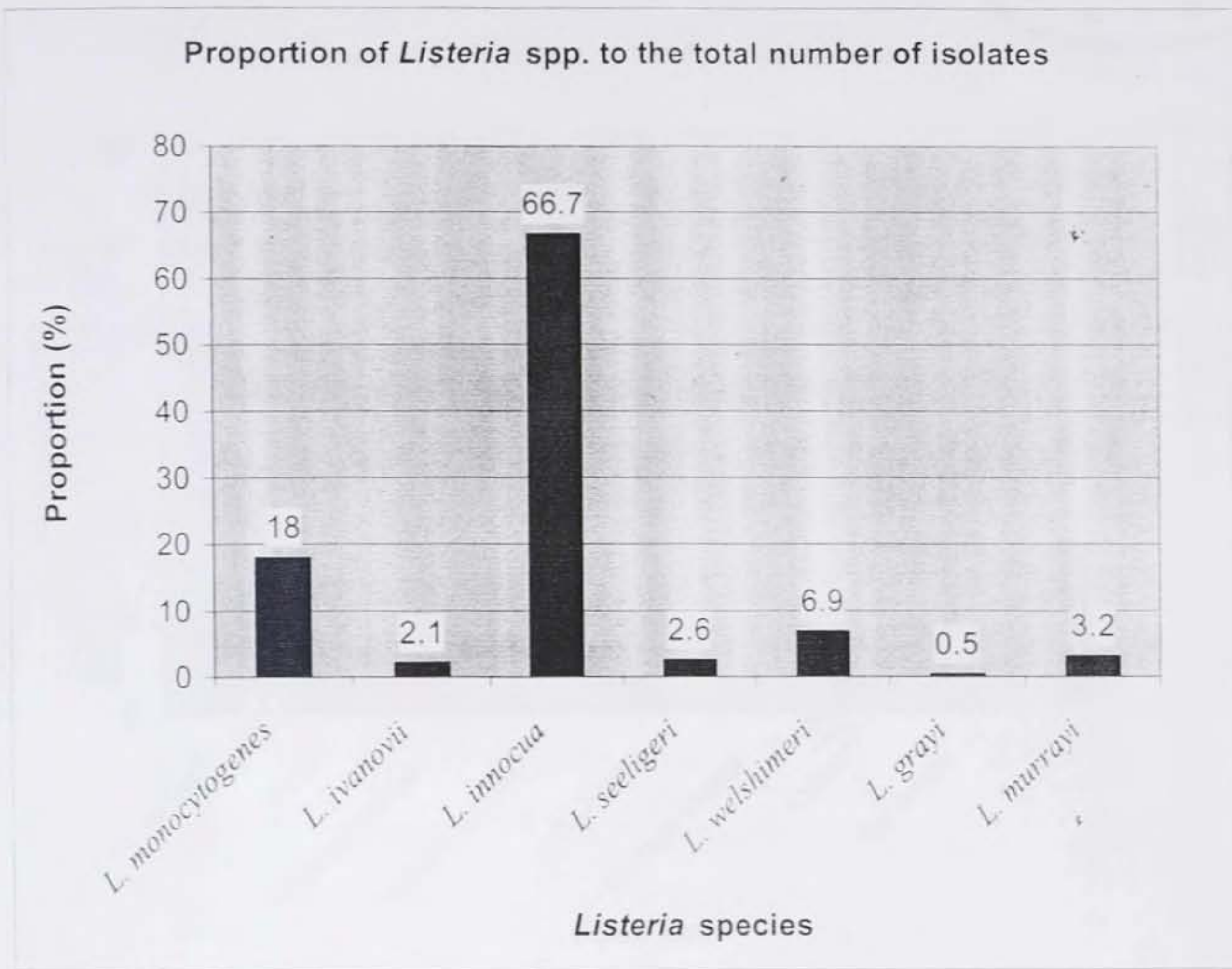


Figure 5: Proportion of *Listeria* species as compared to the total number of *Listeria* isolates

In the current study, *L. innocua* was the predominant species isolated (17.7%) from the food samples examined. Its prevalence was found to be highest in pork (53.8%) followed by 31.2% in minced beef and 25.2% in ice cream. Chicken, soft cheese and cakes were also found contaminated with *L. innocua* (Figure 6).

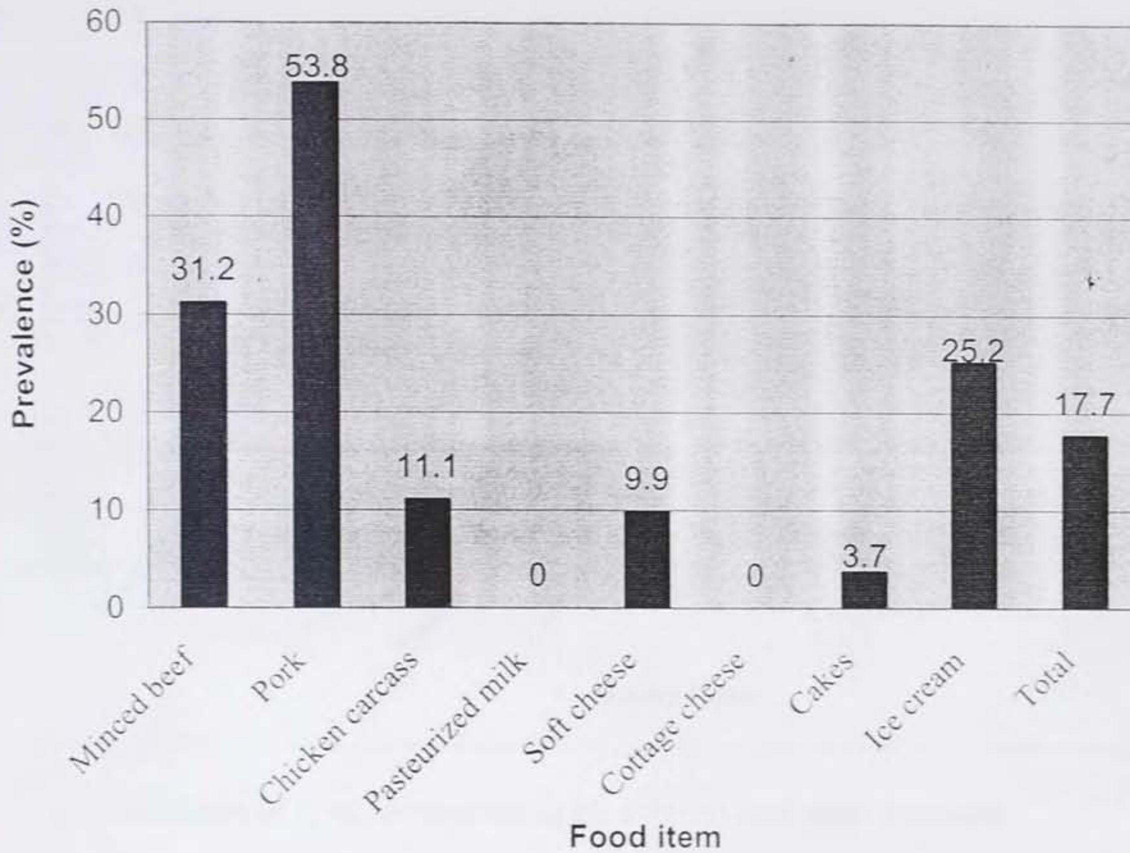


Figure 6: Prevalence of *L. innocua* in the different food items examined

Listeria monocytogenes was the second most predominant *Listeria* species isolated in the food items examined with a total of 34 isolates comprising an overall prevalence of 4.8%. Ice cream was the most contaminated food item with *L. monocytogenes* with a prevalence of 11.7% followed by cakes (6.5%) and pork (5.0%). *L. monocytogenes* was also detected in soft cheese, chicken carcass and minced beef samples (Figure 7).

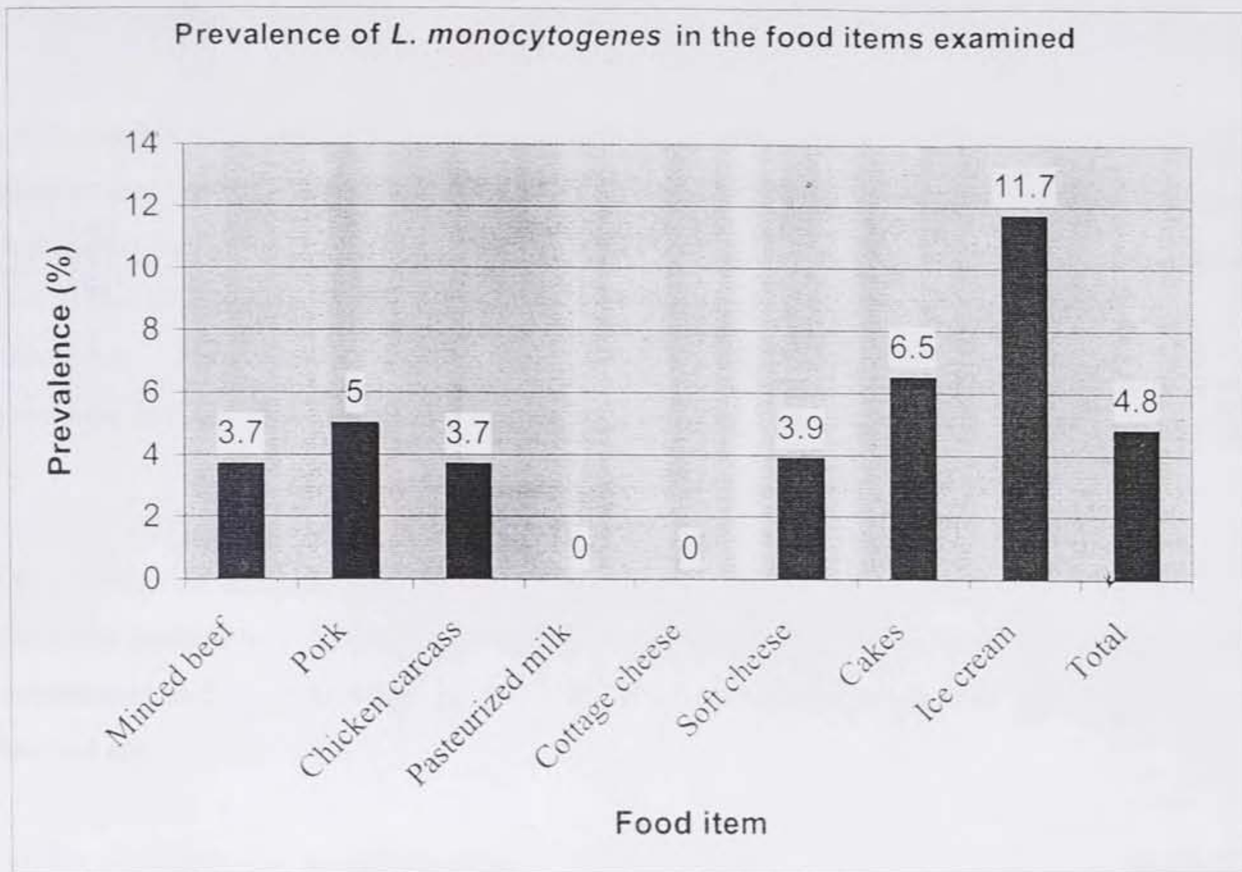


Figure 7: Prevalence of *L. monocytogenes* in the different food items examined

From a total of 34 isolates of *L. monocytogenes* serotyped at the French Authority for Food Safety (Ploufragan, France), 32 (94%) belonged to 4b/4e serotypes while the rest were identified as 4c and 4e (3% each). Serotype 4b/4e was identified from each food item where *L. monocytogenes* was isolated while 4c was isolated from cake sample and 4e from chicken carcass.

5. DISCUSSION

Foodborne transmission of listeriosis was suggested early in medical literature but was first demonstrated in 1981 during an outbreak in Canada (Axelsson and Sorin, 1998). Since then epidemiological investigations have repeatedly indicated that the consumption of contaminated food is the primary vehicle of transmission of listeriosis and food has been identified as the vehicle of several major outbreaks of listeriosis investigated. The significance of *L. monocytogenes* as a foodborne pathogen is complex. Listeriosis is said to be an emerging disease with a low incidence, but with a high fatality rate especially in immuno-compromised individuals (Norrung, 2000). The severity and case-fatality rate of the disease require appropriate preventive measures, but the characteristics of the microorganism are such that it is unrealistic to expect all food to be *Listeria* free. This dilemma has generated various researches in various areas, such as conventional and rapid methods of detection of *L. monocytogenes* and its behavior in foods (Rocourt and Cossart, 1997).

Listeria organisms are present in many foodstuffs, both raw and processed and it is able to survive in several foodstuffs during storage. A survey by the Public Health Laboratory Service, London, England (McLauchlin and Gilbert, 1990) indicated that 6% of 18,000 food samples were contaminated with *L. monocytogenes* and in another study, 11% of foods sampled from the refrigerators of patients suffering from listeriosis in the United States were found positive for *L. monocytogenes* (Pinner *et al.*, 1992). In addition to the above reports, Rorvik and Yndestad (1991) found that out of a total of 460 different retail food items investigated in Norway, 78 (16.9%) were contaminated with *L. monocytogenes*. Choi *et al.* (2001) also found that from a total of 410 domestic Korean food samples analyzed for the presence of *Listeria* species 8 (1.95%) food samples were contaminated with *L. monocytogenes*.

In Ethiopia, Molla *et al.* (2004) reported an overall prevalence of 5.1% for *L. monocytogenes* from a total of 316 different retail food samples examined. In our current study, from a total of 711 different food samples examined, 189 samples except for pasteurized milk and cottage cheese, were contaminated with *Listeria* species with an overall prevalence of 26.6% where *L. monocytogenes* was recorded to be the second predominant species with an overall prevalence of

4.8%. The difference in the prevalence of *L. monocytogenes* in foods in different countries might be attributed to the difference in food item composition or the difference in the level of hygiene and sanitation of slaughterhouses and food processing plants. Furthermore the probable difference in the sensitivity of bacteriological detection methods should also be emphasized. The prevalence of *L. monocytogenes* in the current study lies in the range of the different findings recorded in various countries and this suggests that there is a potential risk of contracting *L. monocytogenes* through consumption of these food items in Ethiopia. A number of outbreaks and sporadic cases of listeriosis have been recorded through consumption of different kinds of food items contaminated with *L. monocytogenes* in other countries where the presence of this pathogen in foods was frequently reported.

The overall prevalence of *Listeria* species in minced beef samples in our study was 47.7% of which 34 isolates (31.2%) being *L. innocua*. In minced beef, a prevalence of 3.7% was recorded for *L. monocytogenes*. Our findings of *L. monocytogenes* was in line with the findings of Baek *et al.* (2000) who reported a prevalence of 4.3% in domestic and imported foods in Korea. In Ethiopia, Molla *et al.* (2004) reported a prevalence of 1.6% of *L. monocytogenes* in 61 minced beef samples collected form supermarkets in Addis Ababa. In Poland, Kwiatek (2004) reported a prevalence of 8.5% in beef from a total of 317 samples examined. Inoue *et al.* (2000) reported a prevalence of 12.2% of *L. monocytogenes* in minced beef samples collected from retail markets in Japan. In other studies, Wong *et al.* (1990) isolated *L. monocytogenes* in 24% of beef steaks in Taiwan. *Listeria monocytogenes* was also isolated from 34% of beef samples collected from local retail stores in Australia (Ibrahim and MacRae, 1991). Iida *et al.* (1998) and MacGowan (1994) also indicated *L. monocytogenes* contamination rate of 34.2% and 34.6% respectively in retail sliced beef and beef samples.

Even though, a prevalence of 3.7% in our study seems to be relatively lower than most of the reports indicated above, it indicates that there is still a risk of acquiring *L. monocytogenes* through consumption of minced beef in Ethiopia. The habit of consumption of raw and undercooked beef in the country could increase the risk of acquiring this and other foodborne pathogens. In those countries with no habit of consumption of raw beef whatever the prevalence

might be higher, heat treatments have been found to sufficiently reduce the level of contamination of different foods items including beef (WHO, 1988).

Entry of *L. monocytogenes* into the food processing plants might occur through soil on workers' shoes and clothing and on transport equipment, animals which excrete the bacterium or contaminated hides or surfaces and possibly through healthy human carriers (Rocourt and Cossart, 1997). In our study, the organism isolated might have come from the animals slaughtered or it might have come from the abattoir environment including abattoir personnel. Contaminated equipments or personnel from supermarkets could also be the possible sources of contamination of minced beef sold to consumers.

It has been reported that *Listeria innocua* shares similar ecological niches with *L. monocytogenes* (Lanciotti *et al.*, 1999). A relatively high prevalence of *L. innocua* in minced beef (31.2%) in this study might be associated with a possible chance of increase in the prevalence of *L. monocytogenes* in this food item in the supermarkets. It has been indicated that at least three human cases have been recorded to be caused by *L. ivanovii* and one by *L. seeligeri* (James, 2000).

Buncic (1991) reported that 45% of all pigs examined in Yugoslavia harbored *L. monocytogenes* in the tonsils, and 3% were found to be fecal excretors. The isolation of *L. monocytogenes* from the rectal contents and body surface of live pigs suggested sources of carcass contamination (Sunaga *et al.*, 1991). These reports indicate that the prevalence of *L. monocytogenes* might be higher in pork because contamination can occur during skinning, evisceration or carcass splitting in slaughterhouses. The prevalence of *L. monocytogenes* in pork samples reported from different countries varies. Kwiatek (2004) isolated *L. monocytogenes* in 45 (9.41%) pork samples from a total of 478 pork samples examined. In Italy, Lanciotti *et al.* (1999) indicated that pork revealed the highest incidence of *L. monocytogenes* (17.6%) among poultry, beef, pork and cheese samples analysed. Baek *et al.* (2000) also reported a prevalence of 19.1% of *L. monocytogenes* in pork.

Duffy *et al.* (2004) indicated that out of 384 samples collected from 24 stores in six cities in the United States to determine the microbiological contamination of pork, 19.8% retail pork samples were contaminated with *L. monocytogenes*. In other studies, Inou *et al.* (2001), MacGowan *et al.* (1994), Iida *et al.* (1998) and Wong *et al.* (1990) also reported a prevalence of 20.6%, 28.1%, 36.4% and 58.8% respectively of *L. monocytogenes* in pork samples. In our study, the prevalence of *L. monocytogenes* in pork was found to be 6.3% which is comparable with the findings of Molla *et al.* (2004) who reported a prevalence of 7.5% in pork samples collected from supermarkets in Addis Ababa. The prevalence of *L. monocytogenes* in our study seems to be lower than the above reports from different countries. It should be recalled that the magnitude of prevalence should not directly be interpreted as a risk to consumers because pork is mostly consumed as cooked or thermally treated at temperatures where *L. monocytogenes* could not survive or be reduced to levels which could not pose problems to consumers. However, the possibility of cross-contamination of other foods by contaminated pork should be emphasized in addition to possible contamination of kitchen utensils and hands of food handlers both in supermarkets and households.

Mickova (1992) reported a prevalence of 10% of *L. monocytogenes* in chicken samples. Marinsek and Grebenc (2002) isolated *L. monocytogenes* in 15.78% of 57 mechanically deboned chicken carcass samples in Slovenia. In Turkey, Akpolat *et al.* (2004) also reported that *L. monocytogenes* was isolated most frequently from raw chicken samples with a prevalence of 18%. In Korea, Baek *et al.* (2000) indicated a prevalence of 30.2% of *L. monocytogenes* in chicken. Vitas *et al.* (2004) also reported a prevalence of 36.1% of *L. monocytogenes* from a total of 158 chicken samples examined in Navarra, Spain. In another study conducted in Japan, *L. monocytogenes* was isolated in 17 (37.0%) from a total of 46 minced chicken samples (Inoue *et al.*, 2000). In Taiwan, Wong *et al.* (1990) isolated *L. monocytogenes* in 50% of chicken carcasses. Pini and Gilbert (1988) reported a contamination rate of 60% in raw fresh and frozen chickens of *L. monocytogenes* while MacGowan *et al.* (1994) indicated that chicken was found as a major source of *L. monocytogenes* (65.6%, 21/32) among pork, beef, lamb, chicken and sausage samples. These findings indicate that chicken carcasses can be contaminated with *L. monocytogenes* with a wide range of prevalences in different countries. These differences in the prevalence of *L. monocytogenes* might be attributed to the differences in the level of hygiene in

the poultry plants. However, the sensitivity of the different bacteriological culture methods used by the different researchers should also be underlined as another factor.

In Ethiopia, Molla *et al.* (2004) reported a prevalence of 1.9% of *L. monocytogenes* from a total of 52 retail chicken carcasses in Addis Ababa. In our study, the prevalence of *L. monocytogenes* in chicken carcasses was 3.7%, which is relatively similar to the findings of Molla *et al.* (2004). Different factors might be considered for the difference in the prevalences of *L. monocytogenes* in the various countries starting from animal farms through slaughterhouses to carcass retail markets. Whatever the magnitude of the prevalence might differ, one can understand from the above reports that there is always a risk of contamination of chicken carcasses by *L. monocytogenes*. In Ethiopia chicken carcasses are thoroughly cooked or treated to temperatures at which *L. monocytogenes* could not possibly survive or be reduced to levels that it will no more be a risk to consumers. It should however, be emphasized that contaminated chicken carcasses can directly cross contaminate other foodstuffs parallely on preparation in the kitchen which do not need further heat treatment. It is also to be underlined that contaminated kitchen utensils could further be sources of contamination later to other foodstuffs and food handlers. Usually the same individuals were observed serving customers in the sale of meat products in the sampled supermarkets. This might create a chance of transferring *L. monocytogenes* to other food items on sale by same individuals.

Recent outbreaks of listeriosis in humans have confirmed the potential for milk and milk products to act as transmission vehicle of *L. monocytogenes* (Lanciotti *et al.*, 1999). Pasteurized milk was also responsible for several epidemics of listeriosis (Jay, 1996). Milk can be contaminated by environmental sources including cow dung, soil, straw and more rarely, by mastitis. Cows suffering from mastitis caused by *L. monocytogenes* are very rare, nevertheless, raw milk must be considered by the dairy processor as a source of contamination coming into the plant (Rocourt and Bille, 1997). On the other hand, a World Health Organization (WHO, 1988) informal working group on foodborne listeriosis indicated that the origin of *L. monocytogenes* in milk is mainly from fecal contamination. Fedio and Jackson (1992) also indicated that a comparative study of 3 farms with no history of *L. monocytogenes* in raw bulk tank milk revealed the organism in 13.2% rectal fecal samples.

Pasteurization is a safe process, which reduces the number of *L. monocytogenes* occurring in raw milk to levels that do not pose an appreciable risk to human health (WHO, 1988). However, Schukken *et al.* (2003) indicated that post-pasteurization contamination in processing plants has been documented. Navratilova *et al.* (2004) also reported *L. monocytogenes* in 5% of a total of 20 samples of pasteurized milk collected from a milk processing plant in the Czech Republic while about 2.1% from a total of 278 raw bulk milk samples collected in the same study were found positive for *L. monocytogenes*. These researchers concluded that contamination after pasteurization or faults of technology during pasteurization (inadequate temperatures or duration of pasteurization, technical faults) are responsible for the presence of *L. monocytogenes* in pasteurized milk. No single isolate of *Listeria* species was identified from 50 pasteurized milk samples examined in the current study. This finding is in line with the report of the WHO (1988) and the report by Mickova (1992) who indicated that pasteurized milk did not contain any strains of *Listeria* species. This might indicate that the current dairy processing plants in and around Addis Ababa are following effective pasteurization and post-pasteurization procedures which keep ready-to-drink packed milk free from *Listeria* species or reduced the levels of the bacteria to undetectable levels insuring food safety to consumers.

Soft cheeses have been incriminated as causes for several outbreaks of listeriosis than other milk products since the foodborne nature of the organism has been documented (Rocourt and Cossart, 1997). Soft ripened cheeses appear to be the most suitable to both to contamination and growth of *L. monocytogenes*, which is said to be attributed to the higher pH of cheeses in the later stages of ripening (WHO, 1988; Rudol and Scherer, 2001). The sources of contamination might vary according to the procedures of preparation. Pak *et al.* (2002) indicated that the highest proportion of positive samples for *L. monocytogenes* (9.5%) was observed in water samples used for cheese washing, followed by cheese-surface swabs (5.0%) in the study of risk factors for the contamination of dairy products in Switzerland.

Variations in manufacturing practices could also result in opportunities for post-process contamination. In theory, cheeses manufactured from *L. monocytogenes* contaminated raw milk would be more likely to be contaminated, but only a low percentage of contaminated raw milk has been reported. Surveys in Germany, Switzerland and France strongly suggest that cheeses

made from pasteurized milk are as frequently contaminated with *L. monocytogenes* as cheeses made from unpasteurized milk, which might be due to contamination during manufacturing processes and handling (WHO, 1988). Rudol and Scherer (2001) also recorded a higher incidence of *L. monocytogenes* in European cheeses made from pasteurized milk (8.0%) than in cheeses manufactured from raw milk (4.8%). On the contrary, Cheeses made from raw milk were found more frequently contaminated with *L. monocytogenes* (42%) than cheeses made from heat-treated milk (2%) in another study (Loncarevic *et al.*, 1995). These conflicting reports indicated that there is always a chance of soft cheeses to be contaminated by *L. monocytogenes* whether the milk used to make the cheese has been pasteurized or not.

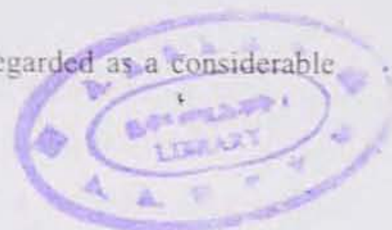
The same study by Rudol and Scherer (2001) also reported different incidences of *L. monocytogenes* in cheeses from various countries: Italy 17.4%, Germany 9.2%, Austria 10%, and France 3.3%. In another study, Copes *et al.* (2000) reported a prevalence of 11.4% of *L. monocytogenes* in soft paste cheeses. A prevalence of 4.9% was also reported by Pinto and Reali (1996) in Italian - made soft cheeses purchased from retail markets. *Listeria monocytogenes* was also isolated from 6% of cheese samples in Sweden (Loncarevic *et al.*, 1995).

In our study, a prevalence of 3.9% was found for *L. monocytogenes* in soft cheeses, which is relatively lower than most of the prevalences from various reports in Europe. This comparatively lower prevalence might indicate good preparation and proper storage and handling of these products by the dairy processing plants and supermarket personnel. *Listeria monocytogenes* was identified as a contaminant in soft cheese which is a RTE food item in the study area and should always be considered as a risk to consumers in assessing food safety issues. Acidified dairy products such as cottage cheeses are, in principle said to be free of *L. monocytogenes* (WHO, 1988). It should be mentioned that this product is prepared boiled which might be the major reason for our finding of zero prevalence in this product apart from its acidity. The present finding was also similar with the findings of Molla *et al.* (2004) who reported only a single isolate of *L. innocua* with no other species of *Listeria* being isolated from a total of 61 cottage cheese samples examined in the same study area. This zero prevalence in our study might be an indication of effective heat treatment and post pasteurization hygienic procedures in the dairy plants in the study areas.

Pearson and Marth (1990) indicated that milk and dairy products under low temperatures, constitute a potential risk for listeriosis. In our study, where all pastry shops were observed using cold storage systems for ice creams, the prevalence of *L. monocytogenes* in ice cream samples was high (11.7%) and this was recorded as the highest prevalence among the different food items examined in the same study. Molla *et al.* (2004) reported a prevalence of 19.6% of *L. monocytogenes* in ice cream samples in the same study area. The prevalence of *L. monocytogenes* in ice cream samples in our study is comparatively higher than most other reports documented. Farber *et al.* (1989) in a total of 394 ice cream samples examined, found a prevalence of 0.3% of *L. monocytogenes*. Cordano and Rocourt (2001) also reported a prevalence of 3.5% from a total of 603 ice cream samples examined in Santiago, Chile through the years 1990-1997. Casedei *et al.* (1998), Laciar *et al.* (1999); Dhanashree *et al.* (2003) and Akman *et al.* (2004) were unable to isolate *L. monocytogenes* in ice cream samples examined. The high prevalence of *L. monocytogenes* in ice cream samples in our study might be an indication of poor preparation and handling practices in the pastry shops. This further warrants that consumption of ice creams in our study areas has a risk of acquiring *L. monocytogenes* and listeriosis because this product is produced as ready-to-eat food item. The risk could be considered as significant where the number of immunocompromised people especially HIV patients is very high. This is because these groups of people are considered as one of the risk groups for infection with *L. monocytogenes* (WHO, 1988).

Pastry products have been observed to be most suitable environments for the growth of *L. monocytogenes* with respect to favorable pH, water activity, nutrient availability and storage conditions (Uhitil *et al.*, 2004). Contamination of cakes with this pathogen occurs as a result of inadequate preparation process or recontamination (Uhitil *et al.*, 2004). In this study, the cakes sampled were made up of milk, creams, eggs, different fruits and sweeteners. All ingredients should always be considered as possible sources of contamination as well as dishes, environment and working staff in the pastry shops (Uhitil *et al.*, 2004). The prevalence of *L. monocytogenes* in cakes in our study was found to be 6.5% which is comparable with the results of Uhitil *et al.* (2004) who reported a prevalence of 4.3% from a total of 283 cakes examined in Croatia. As to any other pastry shops in other studies, the contamination of cakes in our study might start from the components, preparation procedures, storage and handling activities and equipments. As

cakes are ready-to-eat food products, the prevalence (6.5%) can be regarded as a considerable risk to consumers especially to high risk groups.



Serotypes usually involved in cases of listeriosis are 4b, 1/2a and 1/2b (Vitas *et al.*, 2004). Different researchers have reported serotype 4b in their respective studies of *L. monocytogenes* in foods (Pini and Gilbert, 1988, da Silva *et al.*, 1998, Iida, 1998, Choi *et al.*, 2001, Fantelli and Stephan, 2001). In Ethiopia, Molla *et al.* (2004) reported serotypes, 1/2b, 4b and 4e among 16 isolates of *L. monocytogenes* isolated from a total of 316 different food items examined in Addis Ababa. Martin (2003) reported that serotype 4b had been the cause of at least eight major outbreaks which occurred in USA, Canada, New Zealand, Switzerland, United Kingdom and France, which have been linked with the consumption of different contaminated foodstuffs with *L. monocytogenes* between the years 1979 and 1995. Rocourt and Bille (1997) also indicated that of the 13 commonly described serotypes of *L. monocytogenes*, serotype 4b (or 4b related) strains have caused almost all recent outbreaks of foodborne listeriosis, posing the question of an increased virulence of this serotype, a better adaptation to foods or to humans, or a broader distribution in the environment.

The food items incriminated as the causes for these outbreaks included, pasteurized milk, soft cheeses and pork products among others. Especially this serotype (4b) was recorded as the cause of an episode in Southern California, which occurred in 1985 in USA (Martin, 2003). Following consumption of soft Mexican style cheese, 142 cases of listeriosis were recorded in this outbreak. Among 93 pregnant women infected, 29 lost their babies; of 49 immuno-compromised adults, 18 died. An overall case fatality rate of 33% was recorded in this outbreak. The same serotype (4b) was also responsible for the outbreaks which involved 300 cases of listeriosis in the UK between the years 1989 and 1990 and for that which occurred in France in 1992 which involved 279 cases with a total of 85 recorded deaths (Martin, 2003).

These reports indicate that serotype 4b is the most common serotype, which is responsible for most cases of outbreaks of listeriosis in the world. The finding of 32 isolates of 4b/4e among 34 isolates of *L. monocytogenes* indicates the risk of foodborne listeriosis in Ethiopia. Different

clinical forms of listeriosis might occur due to this serotype because it has been said that no clear cut association seems to exist between a given serotype and a particular clinical form of listeriosis (bacteremia versus meningitis or meningoencephalitis) or a particular host (Rocourt and Bille, 1997). There are no published reports of outbreaks or sporadic cases of listeriosis, which can be attributed to this serotype or any other serotype of *L. monocytogenes* in Ethiopia. However, this should not be interpreted as if foodborne listeriosis is not a risk in the country where there are millions of high risk groups including HIV/AIDS patients.

6. CONCLUSIONS AND RECOMMENDATIONS

The complete elimination of *L. monocytogenes* on the farm level, as indicated by different researches, has been said to be very difficult, if not impossible because *Listeria* are ubiquitous in the environment. The elimination of the pathogen from food processing facilities is also generally assumed to be a very hard-to-achieve goal for food manufacturers. The microbiological examination of food samples particularly those RTE ones in our study suggested that *L. monocytogenes* is present in the different food items examined with the exception of pasteurized milk and cottage cheese with considerable prevalence suggesting the possible risk of foodborne listeriosis. It has also been generally accepted that raw meat products (beef, pork and poultry) cannot be free from *L. monocytogenes* because of the methods of slaughter, evisceration and sample preparation (Vitas *et al.*, 2004). This has been witnessed in our study. These meat products are commonly treated with heat before consumption which can kill cells of *L. monocytogenes* or reduce them to undetectable levels.

The possible risks of cross-contamination of other foods which do not require further heat treatments and household and kitchen utensils including hands of food handlers, however, should not be underestimated. In addition, these meat products are stored under refrigeration temperatures in the sampled supermarkets and the suitable water activity and appropriate pH values of these products might allow the growth of *L. monocytogenes* to higher infectious levels without considerable competition with other contaminating bacteria.

The prevalence of *L. monocytogenes* in cakes, ice creams and soft cheeses, however, can directly be considered as a considerable risk to consumers. This study has also indicated us that the current dairy processing plants are following appropriate hygienic procedures, which enabled them to produce microbiologically safe pasteurized milk and cottage cheese products with special reference to *L. monocytogenes*.

Different researches on *L. monocytogenes* and foodborne listeriosis have been done in various countries among which most of these reports are coming from developed countries while reports from Africa are lacking. We believe that research results like ours could be good assets to the

veterinary and public health organizations in the country in their effort in undertaking further investigations. Such studies should be promoted to collect baseline information on the distribution and foodborne nature of *L. monocytogenes* and other foodborne pathogens in Ethiopia in order for public health authorities to design appropriate monitoring and surveillance programs and to ensure microbiological food safety to the consumer with special reference to foodborne diseases including listeriosis. Further detailed researches should be made with the aim of collecting comprehensive data of this pathogen in the different food items commonly consumed by the population at national level. Such researches should also be extended to clinical studies both in human beings and animals and in identifying the common serotypes in these hosts and in the food chain.

The presence of *L. monocytogenes* has been demonstrated in a variety of milk and meat products in Ethiopia in our study and previously by Molla *et al.* (2004). Responsible veterinary and public health authorities should educate food producers about foodborne diseases and the methods of production of microbiologically safe food to the consumer. The food producers should be made aware of the principles of HACCP so that they take the principles as the major tools in their effort in producing safe food to the consumer.

Food suppliers and retailers also need to be educated about the risks of foodborne diseases and about the sanitary and hygienic methods of food storage and handling especially about the principles and applications of HACCP. They should also be monitored and inspected regularly by the responsible institutions so that they could supply safe food to the consumer.

It will also be very important to raise public awareness through various ways about the methods of safe food handling, about the risks of foodborne diseases, and the methods of preventing such diseases so that a considerable part of the population can be protected from many foodborne diseases including listeriosis.

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8. ANNEX

Annex 1: Sample collection sheet for bacteriological analysis

Serial Number	Source supermarket	Type and number of samples collected						Total	Remark
		MB	SC	CC	C	P	M		
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

MB = minced beef, SC = soft cheese, CC = cottage cheese, C = chicken carcass, P = pork, M = milk

Annex 2: Sample collection sheet for bacteriological analysis (cakes and ice creams)

Serial Number	Pastry Shop	Type and number of samples		Remark
		Cake	Ice cream	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

8. ANNEX

Annex 1: Sample collection sheet for bacteriological analysis

Serial Number	Source supermarket	Type and number of samples collected						Total	Remark
		MB	SC	CC	C	P	M		
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

MB = minced beef, SC = soft cheese, CC = cottage cheese, C = chicken carcass, P = pork, M = milk

Annex 3: Location of sampling supermarkets, type and number of samples used for the study

Code	Source	Name	Type of sample	TNS	Sub-city
1	Supermarket	Abadir	M, CC, SC, MB & C	13	Arada
2	Supermarket	Abebe Abshir	M, CC, SC, MB & C	12	Arada
3	Supermarket	Abrico-1	M, CC, SC, MB & C	13	Bole
4	Supermarket	Abrici-2	M, CC, SC, MB & C	13	Bole
5	Supermarket	Addis Ababa	M, CC, SC, MB & C	12	Yeka
6	Supermarket	Almaz poultry	C	4	Kirkos
7	Supermarket	Bambis	M, CC, SC, MB, P & C	18	Kirkos
8	Supermarket	Belonias	M, CC, SC, MB, P & C	18	Arada
9	Supermarket	Berta	M, CC, SC, MB, P & C	18	Kirkos
10	Supermarket	Central	M, CC, SC, MB & C	12	Bole
11	Supermarket	Dalol	M	2	Arada
12	Supermarket	Ethio	M, CC, SC, MB & C	11	Kirkos
13	Supermarket	Ethiovision	M, CC, SC, MB & C	13	Kirkos
14	Supermarket	Eyo-ta-1	M, C & MB	6	Kirkos
15	Supermarket	Fantu - 1	M, CC, SC, MB, P & C	20	Bole
16	Supermarket	Fantu - 2	M, CC, SC, MB, P & C	20	Nifas silk lafto
17	Supermarket	Fantu -3	M, CC, SC, MB, P & C	19	Bole
18	Supermarket	Fantu-4	M, CC, SC, MB, P & C	20	Kirkos
19	Supermarket	Felix	M, CC, SC, MB, P & C	19	Kirkos
20	Supermarket	Hadiya	M, CC, SC, MB, P & C	16	Kirkos
21	Supermarket	Leonardo	M, CC, MB & C	9	Kirkos
22	Supermarket	Loyal	M, CC, SC, MB, P & C	15	Arada
23	Supermarket	Meskerem	M & C	3	Bole
24	Supermarket	Negash	M, CC, SC, MB & C	13	Lideta
25	Supermarket	New York	M, CC, SC, MB, P & C	18	Bole
26	Supermarket	Novis - 1	M, CC, SC, MB, P & C	19	Bole
27	Supermarket	Novis -2	M, CC, SC, MB, P & C	19	Nifas silk lafto
28	Supermarket	Populare	M, CC, SC, MB, P & C	16	Lideta

29	Supermarket	Savemore	M, CC, SC, MB, P & C	17	Bole
30	Supermarket	Scandic foods	M & SC	4	Kirkos
31	Supermarket	Shisolomon -1	M, CC, SC, MB, P & C	17	Kirkos
31	Supermarket	Shisolomon-2	M, CC, SC, MB, P & C	19	Kirkos
33	Supermarket	Shopper's mart	M, CC, SC, MB & C	14	Nifas silk lafto
34	Supermarket	Solsis	M, CC, SC, MB & C	12	Lideta
35	Supermarket	Summer	M	1	Nifas silk Lafto
36	Supermarket	Supersave	M, SC, MB	8	Nifas silk lafto
37	Supermarket	The twins	M, CC, SC, MB, P & C	18	Bole

MB = minced beef, SC = soft cheese, CC = cottage cheese, C = chicken carcass, P = Pork, M = Milk, TNS = total number of samples taken in each supermarket

Annex 4: Location of sampling pastry shops, type and number of samples used for the study

Code	Source	Name	Type of sample	TNS	Sub-city
1	Pastry shop	Abyssinia cafe	Cake	2	Kirkos
2	Pastry shop	Addisu	Ice cream and cake	3	Arada
3	Pastry shop	Addisu cafe	Ice cream and cake	4	Arada
4	Pastry shop	Aled cafe	Cake	2	Kirkos
5	Pastry shop	Alem coffee	Cake	2	Kirkos
6	Pastry shop	Arat killo mini	Cake	2	Arada
7	Pastry shop	Bekele Molla	Ice cream and cake	4	Bole
8	Pastry shop	Betelehem	Ice cream and cake	3	Yeka
9	Pastry shop	Big Burger	Ice cream and cake	3	Yeca
10	Pastry shop	Big mak	Ice cream and cake	4	Kirkos
11	Pastry shop	Blue Tops	Ice cream and cake	4	Arada
12	Pastry shop	Bole mini	Ice cream and cake	4	Bole
13	Pastry shop	Bow cafe	Cake	2	Kirkos
14	Pastry shop	Bridge	Cake	2	Arada
15	Pastry shop	Brown forest	Cake	2	Kirkos
16	Pastry shop	Café Denebil	Cake	2	Kirkos
17	Pastry shop	Café Miru	Cake	2	Kirkos
18	Pastry shop	Capitol cafe	Ice cream and cake	4	Kirkos
19	Pastry shop	Carem	Ice cream and cake	3	Bole
20	Pastry shop	Chintro	Ice cream and cake	4	Arada
21	Pastry shop	Christina	Ice cream and cake	2	Kirkos
22	Pastry shop	City cafe	Ice cream and cake	4	Bole
23	Pastry shop	Classic	Ice cream	2	Bole
24	Pastry shop	Cordial	Cake	2	Arada
25	Pastry shop	Crocodile cafe	Cake	2	Kirkos
26	Pastry shop	Dandi	Ice cream	2	Kirkos
27	Pastry shop	Denver cafe	Ice cream and cake	4	Kirkos

28	Pastry shop	Diana	Cake	2	Arada
29	Pastry shop	Dondor	Cake	2	Arada
30	Pastry shop	Enaria	Ice cream and cake	4	Bole
31	Pastry shop	Entermezo	Cake	2	Arada
32	Pastry shop	Eyob	Cake	2	Kirkos
33	Pastry shop	Fasika	Ice cream and cake	4	Gulele
34	Pastry shop	FG cafe	Cake	2	Kirkos
35	Pastry shop	Florida	Cake	2	Arada
36	Pastry shop	Fuge cafe	Cake	2	Kirkos
37	Pastry shop	Genet	Cake	2	Kirkos
38	Pastry shop	Geyus cafe	Ice cream and cake	2	Bole
39	Pastry shop	Ghion	Ice cream and cake	3	Kirkos
40	Pastry shop	Henok cafe	Ice cream and cake	3	Kirkos
41	Pastry shop	Kalids cafe	Ice cream and cake	3	Kirkos
42	Pastry shop	Lal	Cake	2	Kirkos
43	Pastry shop	Lavera cucina	Cake	2	Arada
44	Pastry shop	Lidia	Ice cream and cake	3	Yeka
45	Pastry shop	Lions' cage	Ice cream and cake	3	Arada
46	Pastry shop	Londo cafe	Ice cream and cake	4	Kirkos
47	Pastry shop	London café	Ice cream and cake	3	Bole
48	Pastry shop	Maleda	Ice cream and cake	4	Arada
49	Pastry shop	Median cafe	Ice cream and cake	3	Bole
50	Pastry shop	Meseret cafe	Cake	2	Arada
51	Pastry shop	Mimmo	Cake	1	Arada
52	Pastry shop	New York	Ice cream and cake	4	Bole
53	Pastry shop	Omega	Cake	2	Kirkos
54	Pastry shop	Pelican	Ice cream and cake	4	Bole
55	Pastry shop	Pizzeria	Ice cream and cake	3	Kirkos
56	Pastry shop	Purple café	Ice cream and cake	3	Kirkos
57	Pastry shop	Rainbow	Cake	2	Arada
58	Pastry shop	Ras shell	Ice cream and cake	3	Kirkos

59	Pastry shop	Rea cafe	Ice cream & cake	2	Kirkos
60	Pastry shop	Riche cafe	Ice cream and cake	3	Kirkos
61	Pastry shop	Romina	Ice cream and cake	3	Arada
61	Pastry shop	Saay	Ice cream and cake	3	Bole
63	Pastry shop	Saay-22	Ice cream and cake	3	Bole
64	Pastry shop	Sara	Cake	2	Nifs Silk Lafto
65	Pastry shop	Seasons cafe	Ice cream and cake	4	Nifs Silk Lafto
66	Pastry shop	Simret	Cake	2	Kirkos
67	Pastry shop	Sofnia	Ice cream and cake	3	Arada
68	Pastry shop	Soul kid	Ice cream and cake	3	Arada
69	Pastry shop	Spot cafe	Ice cream and cake	2	Kirkos
70	Pastry shop	Temesgen	Cake	2	Kirkos
71	Pastry shop	Top view	Ice cream and cake	3	Arada
72	Pastry shop	TS cafe	Cake	1	Kirkos
73	Pastry shop	T-Top	Cake	2	Arada
74	Pastry shop	Unique	Cake	2	Arada
75	Pastry shop	Veronica	Ice cream and cake	3	Yeca
76	Pastry shop	Wubet beisu	Ice cream and cake	3	Kirkos
77	Pastry shop	Youth cafe	Cake	2	Arada
78	Pastry shop	Z cafe	Ice cream and cake	3	Arada

TNS = total number of samples taken in each pastry shop

Annex omposition and preparation of culture media used for the study

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

Comp on	(g/l)
- Meat one (peptic digest of animal tissue)	5.0
- Tryp (peptic digest of casein)	5.0
- Beef ct	5.0
- Yeas ract	5.0
- Sodi hloride	20.0
- Diso hydrogen phosphate dehydrate	9.6
- Potas dihydrogen phosphate	1.35
- Aesc	1.0
- Lithi hloride	3.0
- Acrif hydrochloride	0.0125
- Nalid acid	0.01

Direct

- 1 55.0 g of the powder into one litter of distilled water
- carefully until complete dissolution
- ilize at 121° C for 15 minutes (N.B: Do not overheat)
- ptically add 0.5 g of ferric ammonium citrate after filter sterilization

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

Comp n	(g/l)
- Meat one (peptic digest of animal tissue)	5.0
- Trypt (peptic digest of casein)	5.0
- Beef act	5.0
- Yeast ract	5.0
-Sodiu loride	20.0

- Disodium hydroge osphate dehydrate 9
- Potassium dihydro phosphate 1
- Aesculin 1
- Lithium chloride 3
- Acrifalvin hydroch de 0 5
- Nalidixic acid 0

Directions:

- Add 55.0 g o e powder into one litter of distilled ater
- Mix carefull til complete dissolution
- Sterilize at 1 C for 15 minutes (N.B: Do not ov eat)
- Aseptically a 0.1 g of ferric ammonium c trate a filter ste zatio
- Dispense int erile test tubes

c) Polymixin Acrifl n Lithium chlori Cefta dime culin M nitol (LCAM): *Listeria* Selective ating- ut mediu

i) **Agar base** (Lab, Combourg, France)

Composition

- Peptones
- Starch
- Sodium chloride
- Yeast extract
- Glucose
- Mannitol
- Aesculin
- Ferric ammonium c ate
- Phenol red 08
- Litium chloride
- Agar

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

Composition	(g/l)
- Polymixin B sulfate (100,000IU)	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 ampules of the reconstituted PALCAM supplement
- Mix carefully and dispense into sterile petri-dishes

d) Oxford *Listeria* Selective Plating-out Medium

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

Composition	(g/l)
- Proteose peptone	23
- Starch	1
- Sodium chloride	5
- Agar (depending on the gel strength of the agar)	9-18
- Aesculin	1

- Ammonium iron (III) citrate 0.5
- Lithium chloride 15

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

Composition	(g/l)
- Cyclohexamide	400
- Colistin sulfate	20
- Acriflavin hydrochloride	5
- Cefotetan	2
- Fosfomycin	10

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 till the powder dissolves completely
- Sterilize at 121° C for 15 minutes
- Cool it to about 45°-50° C and add the 2 ampules 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
- Soya peptone	3
- Sodium chloride	5

- Dipotassium phosphate	2.5
- Glucose	2.5
- Agar	15
- Yeast extract	6

Directions:

- Suspend 40 g of the powder in 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 (TSYEB) (Merck, Darmstadt, Germany)

Composition	(g/l)
- Tryptone	17
- Soya peptone	3
- Sodium chloride	5
- Dipotassium phosphate	2.5
- Glucose	2.5
- Yeast extract	6

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

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 to 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 1 litter of purified water
- Mix thoroughly
- Heat with frequent agitation and boil for one minute
- Autoclave at 121° C for 15 minutes

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

i) Phenol red broth base (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 15g of powder in 1 litter of purified water
- Autoclave at 121° C for 15 minutes and cool to about 60 ° C

ii) Carbohydrate solutions

a) Rhamnose (AES Lab., Combourg, France)
b) Xylose (AES Lab., Combourg, France)
c) 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

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

Known β-hemolytic *Staphylococcus aureus* (CIP, Collection of Institute of Pasteur, 5710), *Rhodococcus equi* (CIP, 5869), *L. ivanovii* (CIP, 7842) and *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 them 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 research period.

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)

Annex 6: Record sheet for laboratory analysis of *Listeria* species in foods (using PALCAM agar)

SN	Date	Sample number	Sample type	Source S/market	Half Fraser broth	Fraser Broth	Growth on PALCAM	Colony number	Growth on TSYEA	Gram stain	Catalase	Motility	Hemolysis
								1					
								2					
								3					
								4					
1								5					
								1					
								2					
								3					
								4					
2								5					
								1					
								2					
								3					
								4					
3								5					
								1					
								2					
								3					
								4					
4								5					
								1					
								2					
								3					
								4					
5								5					

Annex 7: Record sheet for laboratory analysis of *Listeria* species in foods (using Oxford agar)

SN	Date	Sample number	Sample type	Source S/market	Half Fraser broth	Fraser Broth	Growth on Oxford	Colony number	Growth on TSYEA	Gram stain	Catalase	Motility	Hemolysis
								1					
								2					
								3					
								4					
1								5					
								1					
								2					
								3					
								4					
2								5					
								1					
								2					
								3					
								4					
3								5					
								1					
								2					
								3					
								4					
4								5					
								1					
								2					
								3					
								4					
5								5					

Annex 8: Record sheet for biochemical reactions of *Listeria* species

Colony from	Sample No	Colony No	Carbohydrate utilization test			CAMP test with	
			Rhamnose	Xylose	Mannitol	*S. aureus*	*R. equi*
PALCAM	1	1					
		2					
		3					
		4					
		5					
Oxford	1	1					
		2					
		3					
		4					
		5					
PALCAM	2	1					
		2					
		3					
		4					
		5					
Oxford	2	1					
		2					
		3					
		4					
		5					
PALCAM	3	1					
		2					
		3					
		4					
		5					
Oxford	3	1					
		2					
		3					
		4					
		5					
PALCAM	4	1					
		2					
		3					
		4					
		5					
Oxford	4	1					
		2					
		3					
		4					

ULUM VITAE

AL DATA

– Desalegne Mengesha Degefaw

Male

f birth – 28 August 1977 G.C

of birth – Gondar

ality– Ethiopian

l status – Single

sion – Veterinarian

age – Amharic – speaking and writing

- English – speaking and writing

address – Estie District Office of Agriculture, South Gondar

- Tel (08) 115705, (08) 114886, (09) 763203

- E-mail – desmen96@yahoo.com

IONAL BACKGROUND

Education – Key Amba Primary School, Gondar

ry Education – Atse Bekafa Junior Secondary School, Gondar

Education – Atse Fasiledes Comprehensive Senior Secondary School, Gondar – 1991-1994

ity Education - Addis Ababa University, Faculty of Science - 1994-1995

- Addis Ababa University, Faculty of Veterinary Medicine - 1995-2000

Degree award – DVM, Degree of Doctor of Veterinary Medicine

duate Education - AAU, Faculty of Veterinary Medicine, 2003-2005

Degree award – Master of Science (MSc) in Tropical Veterinary Medicine

3. EMPLOYMENT RECORD

3.1. Date of employment – 15 Sep., 2000 G.C.

3.2. Position – Veterinary services team leader and field veterinarian

3.3. Employer – Amhara Bureau of Agr., Estie District Office of Agriculture, South Gondar

3.4. Major tasks – Coordinating veterinary services in Estie District

– Training of animal health assistants and technicians

4. RESEARCH ACTIVITIES

4.1. Study on *L. monocytogenes* and other *Listeria* species in milk and meat products in retail markets of Addis Ababa, Ethiopia (MSc thesis, 2005)

4.2. Study on major infertility problems of crossbred dairy herds in Adaa district of central Ethiopia (DVM thesis, 2000)

4.3. Status of wildlife management in Ethiopia (Seminar paper, 1999)

5. OTHER EXPERIENCES

5.1. Computer Literacy - Microsoft word, Microsoft excel, Microsoft Power Point, data entry and analysis.

6. REFERENCES

6.1. Dr. Bayleyegn Molla (DVM, MSc, PhD), Associate Professor of Microbiology and Veterinary Public Health, Addis Ababa University, Faculty of Veterinary Medicine, Department of Microbiology and Veterinary Public Health. P.O Box #34, Debre Zeit, Ethiopia.

6.2. Dr Ademe Zerihun (DVM, MSc, PhD), Assistant Professor, Addis Ababa University, Faculty of Veterinary Medicine, Department of Microbiology and Veterinary Public Health. P.O Box #34, Debre Zeit, Ethiopia.

9. CURRICULUM VITAE

1. PERSONAL DATA

1.1. Name – Desalegne Mengesha Degefaw

1.2. Sex - Male

1.3. Date of birth – 28 August 1977 G.C

1.4. Place of birth – Gondar

1.5. Nationality– Ethiopian

1.6. Marital status – Single

1.7. Profession – Veterinarian

1.8. Language – Amharic – speaking and writing

- English – speaking and writing

1.9.Home address – Estie District Office of Agriculture, South Gondar

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2. EDUCATIONAL BACKGROUND

2.1. Primary Education – Key Amba Primary School, Gondar

2.2. Secondary Education – Atse Bekafa Junior Secondary School, Gondar

2.3. Tertiary Education – Atse Fasiledes Comprehensive Senior Secondary School, Gondar – 1991-1994

2.4. University Education - Addis Ababa University, Faculty of Science - 1994-1995

- Addis Ababa University, Faculty of Veterinary Medicine - 1995-2000

Degree award – DVM, Degree of Doctor of Veterinary Medicine

2.5. Postgraduate Education - AAU, Faculty of Veterinary Medicine, 2003-2005

Degree award – Master of Science (MSc) in Tropical Veterinary Medicine

6.3. Dr. Tamiru Negash (DVM, MSc), Assistant Professor, Addis Ababa University, Faculty of Veterinary Medicine, Department of Pathology and Parasitology. P.O Box #34, Debre Zeit, Ethiopia.

10. DECLARATION

"I declare that the information presented here in my thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged".

Name: Desalegne Mengesha Degefaw

Signature: ____________________

Date of Submission: ____________________

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

Dr. Bayleyegn Molla (DVM, MSc, PhD, Associate Professor)

Signature: ____________________

1088/DES/2005 C-1

AUTHOR Desalegne Mengesha

TITLE Study on Listeria Monocytogenes & other Listeria ....

DATE DUE	BORROWER'S NAME

1088
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2005

Study on Listeria Monocytoogenes & other listeria species in milk & Meat Products in

Desalegne Mengesha

C-1