

An-Najah National University

11/1/2000

***Prevalence and Serovar Distribution of  
Some Species of Enterobacteriaceae in  
Fresh Meat of Cattle, with Special  
Emphasis on Salmonella, and  
Escherichia coli O157: H7***

***By***

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**2000**

An-Najah National University  
Faculty of Graduate Studies

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Submitted in Partial Fulfillment of the Requirements for the Degree of  
Master of Environmental Science, Faculty of Graduate Studies, at An-  
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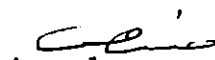
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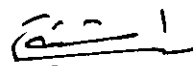
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TO

*EVERYONE SUPPORTS ME IN MY  
STUDYING AND ALWAYS, TO MY  
FAMILY, WIFE AND DAUGHTER.*

*TO MY DEAR FATHER, GREAT  
MOTHER, AND BROTHERS. FOR  
THEIR ENCOURAGEMENT AND  
SUPPORT WITH LOVE.*

## *Acknowledgment*

*It is a great pleasure to have the opportunity to record a measure of thanks to those who have directly or indirectly helped in the completion of this thesis.*

*I am grateful to my supervisor Dr. Yahya Faydi, for his guidance, encouragement and support through the master period.*

*Special thanks are due to my father, mother, brothers and wife, for all they have done for me.*

*Thanks also are due to my colleagues Lubna Al Kharraz, Majdi Dwekat, and Suheil Abu Ghdeib for their help and encouragement.*

*Finally it would be wrong to fail to mention the people who had a great influence on my work and life, namely Professor Dr. Ali Al-Darraji, of Jordan University of Science and Technology, Jordan.*

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## Abstract

A Total of 150 fresh samples were taken from Nablus Municipality slaughterhouse, including 37 liver samples of young calves, 38 liver samples of old cows, 37 meat samples of young calves, and 38 meat samples of old cows. These samples were cultured for total bacterial count, total coliform count (by pour plate count), *Salmonella*, and *E.coli* O157: H7, in Nablus area. This was done by standard methods of enrichment, plating, biochemical and serological techniques. Total bacterial count and total coliform count indicate the overall microbial quantity, and hence the quality of meat, and the degree of contamination of these samples either from endogenous sources or exogenous ones. Ten percent of the total samples were unacceptable either due to high total bacterial count (4%) or due to the presence of *Salmonella* (6%). All the samples were negative for *E.coli* O157: H7. The average total bacterial count for the samples was  $2.8 \times 10^5$  CFU/gm, whereas the average total coliform count was 26 CFU/gm. Young calves showed 5.3% unacceptable samples which was higher than that of old cows that appeared to be 4.7%. For the presence of *Salmonella*, old cows showed an incidence of 3.3%, which was higher than that of calves that appeared to be 2.7%. The average total bacterial count for calves samples was  $3.1 \times 10^5$  CFU/gm, while that of cows samples was  $2.5 \times 10^5$  CFU.

No significant correlation was found between total bacterial count and total coliform count on one hand, and the presence or absence of *Salmonella* on the other. The study also showed that there was no correlation between the age of the animal and the acceptance or rejection of the sample. There was also no significant correlation between the age of the animal and the presence or absence of *Salmonella*, or the total bacterial count.

# **Chapter 1**

## ***Introduction***

# CHAPTER 1

## 1.INTRODUCTION

Meat is normally regarded as the edible parts (muscle and offal) of the food animals which consume mainly grass and other arable crops [1]. Meat is a fine culture medium for many organisms because it is high in moisture, rich in nitrogen, minerals and other growth factors. It usually has some fermentable carbohydrate (glycogen) and is at a favorable pH for most microorganisms [2].

Nablus Municipality slaughterhouse serves the whole city of Nablus and all related refugee camps, with a slaughtering capacity of 100 calves, and 500 sheep per day.

Food-borne infections have been the focus of much public health attention over the past century. The most common food infections are related to bacteria, viruses, and parasites. The symptoms caused by these infections varied, usually related to a particular organisms' method of attacking the gastrointestinal tract [3]. There are no obvious signs of spoilage to indicate the presence of pathogens or toxins [4]. For most food-borne pathogens, no vaccines are available [22].

Illnesses caused by food-borne microorganisms are serious health problems throughout the world. Verotoxigenic *Escherichia coli*

(VTEC) and *Salmonella* are two examples of food-borne pathogens capable of causing disease [5].

*E.coli* usually remains harmlessly confined to the intestinal lumen, however in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal “nonpathogenic” strains of *E.coli* can cause infection [42].

*E.coli* is the predominant non-pathogenic facultative flora of the human intestine. Some *E.coli* strains, however, have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system. Several distinct clinical syndromes accompany infection with diarrheagenic *E. coli* categories, including traveler’s diarrhea (enterotoxigenic *E. coli*), Hemorrhagic colitis and hemolytic uremic-syndrome (enterohemorrhagic *E. coli*), persistent diarrhea (enteroaggregative *E.coli*), and watery diarrhea of infants (enteropathogenic *E. coli*) [6].

Enteropathogenic *E.coli* (EPEC) strains were first recognized as pathogens >50 years ago. Their involvement as a major cause of infantile diarrheal outbreaks throughout the world has been well-documented [8]. Infections due to pathogenic *E.coli* may be limited to the mucosal surfaces or can disseminate through out the body. Three general clinical syndromes result from infection with

inherently pathogenic *E.coli* strains: (1) urinary tract infection, (2) sepsis/meningitis, and (3) enteric/diarrheal disease [42].

The recognition of enterohemorrhagic *E.coli* (EHEC) as a distinct class of pathogenic *E. coli* resulted from two key epidemiological observations. The first was the 1983 report by Riley et al [9], who investigated two outbreaks of a distinctive gastrointestinal illness characterized by severe crampy abdominal pain, watery diarrhea followed by grossly bloody diarrhea, and little or no fever. This illness, designated hemorrhagic colitis (HC), was associated with the ingestion of undercooked hamburgers at a fast food restaurant chain. Stool cultures from these patients yielded a previously rarely isolated *E.coli* Serotype *O157: H7*. The second key observation was by Karmali et al. [10], also in 1983 who reported the association of sporadic cases of hemolytic uremic syndrome (HUS) with fecal cytotoxin and cytotoxin-producing *E.coli* in stools.

*E.coli O157: H7* is one of hundreds of strains of bacterium *Escherichia coli*. Although most strains of *E.coli* are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness [7].

Most outbreaks caused by *E.coli O157: H7* have been food or water related. Likely vehicles of infection have been undercooked ground beef according to a report by the Centers of Disease Control



(CDC) in 1993. Additionally raw milk, cold sandwiches, vegetables and water have been implicated as sources of some outbreaks [11].

Cattle are found consistently to be a reservoir for this organism in the environment [12].

*Salmonella* species are some of the major causes of food poisoning in the developed world [13]. Gastroenteritis is the most common clinical manifestation of *Salmonella* infection [14]. *Salmonella* enteritis is an acute gastrointestinal disease frequently associated with ingestion of contaminated food. There are more than 1600 types of *Salmonella*; many of them are capable of causing enteric illness. The usual forms of this illness consists of vomiting, diarrhea, and fever alone; however, spread of the disease to almost every other system by way of the bloodstream has been documented [3]. Human *Salmonella* infection can lead to several clinical conditions, including enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by non-typhoid microorganisms [24].

The role of animal and poultry excretors of Salmonellae and the spread of the organism to the carcass meat of both animals and poultry and so to the human population is illustrated in many outbreaks [15].

## **1.1 Enterobacteriaceae (enteric gram-negative rods):**

The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (e.g., *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, and others) [16]. Members of this family have six major features: [17]

- 1- They ferment glucose.
- 2- Reduce nitrates to nitrites.
- 3- None produce cytochrome oxidase.
- 4- Except *Klebsiella*, *Shigella*, and *Yersinia* are motile; with rare exceptions, the flagellar arrangement is peritrichous if the organism is motile.
- 5- Do not require NaCl for growth.
- 6- Catalase positive.

The antigenic structures: capsule (K), cell wall (O), and flagella (H), are significant in the identification and epidemiological studies of this group of organisms. Capsular antigen (K) are heat labile polysaccharides that can mask the heat stable somatic (O) antigens. In some species, such as *Salmonella typhi*, this capsular antigen functions as a virulence factor. Somatic (O) cell wall antigen is the LPS component of the cell wall. It is useful as a biological marker, & also

works as a virulence factor. Flagellar (H) antigens are proteins; these antigens are particularly helpful in the classification and serologic specification of members of the genus *Salmonella* [18].

## 1.2 Coliform Group:

This group of bacteria is an indicator organisms and their presence indicates fecal contamination [19], since this organism was universally present at high numbers in both human and animal feces [20]. Coliforms are specific groups of bacteria or individual species are commonly used to provide evidence of poor sanitary practices, inadequate processing or post process contamination of food [20]. The coliform group contains all aerobic and facultatively anaerobic, gram negative, non-spore forming rods able to ferment lactose with the production of acid and gas at 32 °C or 35°C within 48-h [21]. The genera that satisfy the definition include: [20]

1-*Klebsiella*.

2-*Escherichia*.

3-*Enterobacter*.

4-*Citrobacter and Serratia*.

*E. coli* comprises nearly 95% of the coliforms in feces and is present in the feces of warm-blooded animals at densities of  $10^8$ – $10^9$  per gram [20].

### 1.3 Genus *Salmonella*:

*Salmonella* are members of the family Enterobacteriaceae, they are gram negative bacilli growing aerobically and anaerobically at an optimum temperature of 37°C, readily killed by temperatures above 55°C, they may be isolated from the intestines of man and animals and from foods of animal origin [15].

The genus *Salmonella* was named in 1900 after the American veterinarian Dr. Salmon who was the first to describe a member of the group *Salmonella choleraesuis* [2]. Gastroenteritis is the most common clinical manifestation of *Salmonella* infection [14]. By no means all the types of organisms included in the genus *Salmonella* are related to food poisoning, many *Salmonellae*, however have a wide host range and it is these which commonly cause food poisoning [1].

Salmonellosis is a disease caused by many species of *Salmonellae* and is characterized clinically by one or more of the three major syndromes: septicemia, acute enteritis, and chronic enteritis [23]. *Salmonella* can withstand drying for years, especially in dried feces, dust, and other dry materials such as feeds and certain food [25]. Nomenclature of the *Salmonella* group has progressed through a succession of taxonomical schemes based on biochemical and serological characteristics and on principles of numerical taxonomy [24]. In the early development of taxonomic schemes, determinant

biochemical reactions were used to separate *Salmonella* into subgroups. The Kauffmann-White scheme stands prominently as the first attempt to systemically classify Salmonellae by using these scientific parameters [24].

At present, more than 2300 serovars are known to exist based on the 67 O-antigen groups and the numerous H-antigens [25, 26]. One classification system had three primary species: *Salmonella typhi* (one serotype), *Salmonella choleraesuis* (one serotype) and *Salmonella enteritidis* (over 1500 serotype)[27].

In 1984, Canada experienced its largest outbreaks of foodborne salmonellosis, which was attributed to the consumption of cheddar cheese manufactured from heat treated and pasteurized milk; the episode resulted in no fewer than 2700 confirmed cases of *S.typhimurium* infection [28]. In the United States, during the 1977, 27850 cases of Salmonellosis excluding typhoid fever were reported [29]. In England and Wales, *Salmonella* infection account for more than 80% of cases reported for foodborne diseases in 1981-1983 [25]. Recently national epidemiological registries continue to highlight the importance of *Salmonella* species as the leading cause of food-borne bacterial diseases in humans, in who reported incidents of food-borne salmonellosis tend to dwarf those associated with other food-borne pathogens [24].

In 1985 low fat and whole milk in one Chicago dairy caused a salmonellosis outbreak involving 16000 cases in 6 states. This was the largest outbreak of food salmonellosis in the U.S. [33].

### 1.3.1 Microbiology of *Salmonella*:

*Salmonella* organisms are gram negative facultatively anaerobic rods that morphologically resemble other enteric bacteria [17]. Although members of this genus are motile by peritrichous flagella, nonflagellated variants, such as *S.pullorum* and *S.gallinarum*, and nonmotile strains resulting from dysfunctional flagella do occur [24]. Salmonellae grow readily on simple media, but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce H<sub>2</sub>S. They survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (e.g., brilliant green, sodium tetrathionate, and sodium deoxycholate) that inhibit other enteric bacteria [27].

### 1.3.2 Pathogenicity of *Salmonella*:

Many Salmonellae have a wide host range, and it is these which commonly cause food poisoning [1]. Detailed investigations from foodborne outbreaks have indicated that the ingestion of just a few *Salmonella* cells can be infectious, more recent evidence suggests that

1 to 10 cells can constitute a human infectious dose, depending upon the health of the host, and strain differences among the members of the genus. [30,31,33]. Clinical diseases induced by Salmonellae are shown in Table 1.1.

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Table 1.1 Clinical diseases induced by Salmonellae \*:

	Enteric Fevers	Septicemia	Enterocolitis
Incubation period	7-20 days	Variable	8-48 hours
Onset	Insidious	Abrupt	Abrupt
Fever	Gradual, then high plateau.	Rapid rise then spiking (septic) temperature	Usually low
Duration of disease	Several weeks	Variable	2-5 days
Gastrointestinal symptoms	Often early constipation, later bloody diarrhea.	Often none	Nausea, vomiting, and diarrhea at onset
Blood cultures	Positive in 1 <sup>st</sup> -2 <sup>nd</sup> weeks of disease.	Positive duration high fever	Negative
Stool cultures	Positive from 2 <sup>nd</sup> week on; negative earlier in disease	Infrequently positive	Positive soon after onset

\* After: Brooks [27]



### 1.3.3 Role of animal in disease transmission:

Most types of *Salmonella* live in the intestinal tracts of animals and birds and are transmitted to humans by contaminated foods of animal origin [32].

The chains of infection are maintained by a large number of mammals, birds, and reptiles. Insects and rodents can be considered as important vectors particularly when they are in contact with domestic animals or wastes that are not kept hygienically and disseminate them directly to other prepared or raw foods [21,25,34]. Of the many sectors within the meat industry, poultry products remain the principal reservoirs of *Salmonellae* in many countries, dominating other meat products such as pork, beef, and mutton as potential vehicle of infection [35,36,37]. A further significant observation was made that samples of ruminal fluid from cattle slaughtered at a number of abattoirs in Queensland shows that an average of 45% of samples contained *Salmonella* organisms [1]. The slaughtering of a healthy animal carrier may lead to gross contamination of carcasses with *Salmonella* [21].

## **1.4 *Escherichia coli* O157: H7:**

Among the many strains of *E.coli* found in stools of humans, a few can cause diarrhea [38]. *E.coli* is a bacterium that is a common inhabitant of the gut of warm-blooded animals, including man. Most strains of *E.coli* are harmless, some strains, such as *E.coli* O157: H7 can cause severe foodborne disease and are referred to as enterohemorrhagic *E.coli* [39]. It is an emerging cause of foodborne illness. An estimated of 10000 to 20000 cases of infection occurs in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure [40]. EHEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure [16]. Symptoms of hemorrhagic colitis include crampy abdominal pain followed within 1 to 2 days by a nonbloody diarrhea which progress within 1 to 2 days to bloody diarrhea that lasts for 4 to 10 days [24].

### **1.4.1 Microbiology of *E.coli* O157: H7:**

Most biochemical reactions of *E.coli* O157: H7 are typical of *E.coli*, with the exception of sorbitol fermentation and  $\beta$ -glucuronidase activity [41]. *E.coli* is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the family

*Enterobacteriaceae* [42]. *E.coli* ferment lactose rapidly. It produces large, usually  $\beta$  -hemolytic gray colonies on blood agar [18]. *E.coli* typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose, typical colonial morphology with an iridescent “sheen” on differential media as EMB agar. Over 90% of *E.coli* isolates are positive for  $\beta$ -glucuronidase using the substrate 4-methylumbelliferyl-  $\beta$ -glucuronide (MUG) [16]. Sorbitol MacConkey agar culture (SMAC) has been the most commonly used method for the isolation of *E.coli* O157: H7, which is unable to ferment sorbitol, which distinguishes them from the majority of fecal *E.coli* [43].

#### 1.4.2 Pathogenicity of *E.coli* O157: H7:

The most highly conserved feature of diarrheagenic *E.coli* strains is their ability to colonize the intestinal mucosal surface due to the presence of surface adherent fimbriae, despite peristalsis and competition for nutrients by the indigenous flora of the gut [42].

The pathogenicity of EHEC appears to be associated with a number of several cytotoxins referred to as Shiga-like toxins [41], because of their similarity to the toxins produced by *Shigella Dysentriae* [39]. Shiga toxin-producing *E.coli* (STEC) strains have emerged as an important cause of serious human gastrointestinal

disease, which may result in life threatening complications such as hemolytic uremic syndrome [43]. *E.coli* O157: H7 produces one or two cytotoxins that are cytotoxic to Vero cells, an African green monkey kidney cell line, and thus were originally named VT1 and VT2 [44]. Shiga toxins are the major virulence factor, and defining characteristics of EHEC. This potent cytotoxin is the factor that leads to death and many other symptoms in patient infected with EHEC [42].

#### **1.4.3 Role of animal in disease transmission:**

Shiga toxin-producing *E.coli* can be found in the fecal flora of a wide range of animals including cattle, sheep, goats, pigs, cats, dogs, chicken, and gulls. The most important animal species in terms of human infection is cattle [45,46].

Cattle have long been regarded as the principal reservoir of STEC strains, including those belonging to serotype O157: H7 [43]. While many domestic animals carrying STEC are asymptomatic, certain STEC strains are capable of causing diarrhea in cattle, particularly calves [47,48].

Most confirmed human *E.coli* O157:H7 outbreaks have been associated with the consumption of undercooked ground beef and less

frequently, unpasteurized milk, hence cattle have been the focus of many studies to determine their involvement in transmitting the pathogen [49,50]. Dirty hides, hoofs, and hair of the animals harbor large number of bacteria from soil, feed, manure, and water which are important sources of contamination of the surface of the carcass during skinning [25]. Consequently, the potential exist for the spread of VTEC from healthy animals to land grazed by these animals and then cause infection in people who consumed poorly washed vegetables [20].

## **1.5 Microbiological guidelines:**

Microbiological standards for meat and other food products vary from area to another. Table 1.2 shows microbiological standards for meat in different areas.

Table 1.2 Microbiological standards for meat in different areas:

Item	Aerobic Plate Count (APC)	Coliforms	<i>Salmonella</i>	Reference
Chilled fresh meat	$1 \times 10^6/\text{gm}$		0 in 25 gm	Palestinian Standards Inst.(PSI)
Frozen meat	$1 \times 10^6/\text{gm}$		0 in 25 gm	Palestinian Standards Inst.(PSI)
Carcass meat	$10^6\text{-}10^7/\text{gm}$			FAO
Beef			0/gm	*
Raw beef products	$<5 \times 10^5/\text{gm}$	$<1000/\text{gm}$	Absent in 100 gm	**
Crowd meat	$1 \times 10^7/\text{gm}$	5000/gm	0/20gm	***

\* After: Doyle [24].

\*\* After: Government of Abu-Dhabi [57]

\*\*\*After: The Palestinian National Authority [61].

## **1.6 The hazard analysis critical control point (HACCP) system:**

It is a systems' approach to assuring product safety. It is based on identifying and monitoring the most critical points in the production process rather than relying on testing the final product [1].

Hazard analysis evaluates the risks associated with various operations of food production, distribution and use of raw materials and food products to ensure food safety and quality [51]. The systematic approach to food safety embodied by HACCP is based on seven principles [24]:

- 1-Conduct a hazard analysis. Prepare a list of steps in the process where significant hazards occur, and describe the preventive measures.
- 2-Identify the critical control points (CCPs) in the process.
- 3-Establish critical limits for preventive measures associated with each identified CCP.
- 4-Establish CCP monitoring requirements. Establish procedure for using the results of monitoring to adjust the process and maintain control.
- 5-Establish corrective action to be taken when monitoring indicates that there is a deviation from an established critical limit.
- 6-Establish effective record-keeping procedures that document the HACCP system.
- 7-Establish procedures for verification that the HACCP system is working correctly.

## 1.7 Objectives:

The objectives are aimed to determine:

- 1- The microbial quality of meat at Nablus slaughterhouse.
- 2- The degree of contamination of meat with *Salmonella* and *E.coli O157:H7* microorganisms at the slaughterhouse and before being transferred to the butchers shops, with the comparison between young and old cattle, taking samples from the inner and the outer tissues of the animal.
- 3- Decrease the contamination of meat prior distribution.
- 4- The relationship between total bacterial counts, total coliform counts, and the presence or absence of *Salmonella* and *E.coli O157:H7* in fresh meat in the slaughterhouse.



# **Chapter 2**

## ***Materials & Methods***

## CHAPTER 2

### **2. MATERIALS and METHODS:**

#### **2.1 Sample collection:**

A total of 150 representative 35 g portions of fresh meat and liver of cattle including old cows and young calves were collected randomly from Nablus Municipal slaughterhouse, during the period from 14 February 2000 to 5 April 2000.

Young calves were less than 1 year old, usually males, while old cows were more than one year, usually females.

The samples include 74 samples from calves (37 samples from external meat and 37 samples from livers of the same calves). Also 76 samples from cows (38 samples from external meat and 38 samples from livers of the same cows). Samples were collected using a sterile blade and a rat tooth forceps. The meat samples were taken from the diaphragm area as it is, after entering the carcasses to the refrigeration units in the slaughterhouse. The internal samples were taken immediately during evisceration and before being contaminated from the ruminal fluid or the intestinal contents of the abdomen, so as to keep it as sterile as possible to get a clear picture for the microbial situation of the internal parts of the animal. Samples were taken to the laboratory in a chilled container after keeping it in the refrigerator for about 12 hours.

## **2.2 Total bacterial count test and total coliform count test [52]:**

Figure 2.1 shows the procedure for total bacterial count and total coliform count tests. As the samples reached the laboratory the following steps were done:

- 1-Each sample was assigned an individual unit number, using odd numbers for the internal samples and even numbers for the external samples.
- 2-After sterilizing a blender by washing with hot water, rinsing with 95% alcohol and then allowing the remaining alcohol to burn, about 25 grams of each sample were aseptically transferred to the blender and blended with 225 ml of sterile nutrient broth (Oxoid, CM1) for two minutes to get a homogenate mixture, and a concentration of 0.1.
- 3-The homogenate mixture was aseptically transferred to a sterile 500-ml bottle having the sample number, and mixed well by swirling the bottle.
- 4- Using a sterile pipette, 1 ml of the homogenate mixture was aseptically transferred to a sterile 9ml normal saline tube, to give a concentration of 0.01.
- 5-Step 4 was repeated using 9ml sterile saline tubes to prepare 10 fold dilutions of the sample. After loosing the bottle cap the homogenate mixture was then incubated at 35°C for 24 hours, for the isolation of *Salmonella*.

- 6- 1ml from each serial dilution was then transferred aseptically each to one of 2 sterile plates having the sample number and the decimal dilution of the sample.
- 7- About 10 to 15ml of plate count agar (Oxoid CM 325), tempered to 44-46°C, were poured each into one of the two plates, to determine total bacterial count.
- 8- Step 6 and 7 were repeated using 10-15 ml violet red bile agar (VRBA) (Oxoid, CM 107) tempered to 44-46°C to determine the total coliform count.
- 9- Duplicate plates and agar control plates were run for each series of samples.
- 10- The number of samples to be plated in any one series were selected so there was no more than a 20 minutes time lapse between diluting the first sample and pouring the last plate in the series.
- 11- The contents of the plates were mixed thoroughly by using conventional mixing procedures, and allow to solidify on a level surface.
- 12- The plates were then inverted and incubated for 24 hours at 35°C.

## **2.3 Identification of coliform bacteria (lactose fermenter):**

Four well-separated red colonies, which were suspected to be Coliform from the suitable violet red bile agar plate, were randomly chosen for biochemical identification. Including gram stain, oxidase, catalase, triple-sugar iron, urea, sulfide, indole, motility (SIM), Methyl Red-Voges Proskauer (MR-VP) test, and citrate utilization tests as follows [17,18,53,54,55,56]:

### **2.3.1 Gram stain:**

Gram stain was done as follows:

- 1-A thin smear was done using a sterile loop. One drop of normal saline is emulsified with part of a colony, and spread into a slide and left to dry, then fixation by passing through the flame three times, and left to cool.
- 2- The smear was flooded with crystal violet stain for 10 seconds then poured off, and washed with gram iodine solution.
- 3-Decolorization with absolute ethanol acetone solution (1:1) until no further color flew from the slide.

- 4- The smear was then counterstained by safranin for 30 seconds, followed by washing with water, and air-dried.
- 5- After that the smear was examined under the microscope using the oil immersion lens.

### **2.3.2 Oxidase test:**

- 1- A piece of filter paper is moistened by oxidase reagent (0.1% tetramethyl paraphenyline diamine dihydrochloride).
- 2- A loop from the colony to be tested is smeared on the moistened filter paper.  
(Coliforms are oxidase negative, and show no change in color of the filter paper).

### **2.3.3 Catalase test:**

Portion of the colony to be tested is emulsified aseptically with 3% hydrogen peroxide ( $H_2O_2$ ) on a glass slide. Positive reaction is recognized by elaboration of bubbles of oxygen immediately.

(Coliforms are catalase positive).

### **2.3.4 Triple sugar iron (TSI) test (Oxoid, CM227):**

1 –Using a sterile inoculating needle, the center of the colony is picked up and inoculated into TSI agar slant tubes by streaking the slant and stabbing the butt.

2- The tubes were then incubated at 35°C for 24-48 hours.

Table 2.1 shows typical reactions on TSI [53].

Table 2.1 Typical reactions of different types of bacteria on TSI:

Organism	Butt	Slope	H <sub>2</sub> S
<i>Escherichia coli</i>	AG	A	Negative
<i>Salmonella sp.</i>	A or AG	NC or ALK	Positive except for <i>Salmonella paratyphi</i>
<i>Enterobacter sp.</i>	AG	A	Negative
<i>Proteus vulgaris</i>	AG	NC or ALK	Positive
<i>Morganella morganii</i>	A or AG	NC or ALK	Negative
<i>Shigella sp.</i>	A	NC or ALK	Negative

AG = acid (yellow) and gas formation.

A = acid (yellow).

NC = no change.

Alk = alkaline (red).

Positive = hydrogen sulfide (black).

Negative = no hydrogen sulfide (no black).

### 2.3.5 Urease test:

1-Part of a colony is picked up with a sterile inoculating needle, and streaked into the slant of urea agar (Oxoid, CM53).

2-The urea agar slant was then incubated at 35°C for 24 hours.

Urease producing organisms hydrolyze the urea to form ammonia, and the medium changes to purple red.



### **2.3.6 Sulfide, Indole and Motility (SIM) test:**

- 1- Inoculation of part of the colony into a sterile tube of SIM agar dip (Oxoid, CM435) by inserting a straight wire to about one third of the depth of the medium.
- 2-Incubation at 35°C for 18 hours or longer.

### **2.3.7 Colonial appearance on SIM:**

- 1-Using a sterile inoculating needle, part of the colony was stabbed into the upper 2/3 of SIM tube.
- 2-The SIM tube was then incubated at 37°C for 24 hours.
- 3-Non-motile organisms grow only along the line of inoculation, whereas motile species show either a diffuse even growth spreading from the inoculum, or turbidity of the whole medium.
- 4-Blackening of the medium, indicates hydrogen sulfide production.
- 5-For testing indole: addition of 0.2ml of kovac's reagent to the tube and allow to stand for 10 minutes. A dark red color in the reagent constitutes a positive indole test. No change in the original color of the reagent constitutes a negative test.

### 2.3.8 Methyl Red-Voges Proskauer (MR-VP) test:

- 1-Portion of the bacterial colony is inoculated into a sterile tube of 2ml MR-VP broth (Oxoid, CM43) using a sterile loop.
- 2-Incubation for 18 hours at 35°C.
- 3-For methyl red test, 0.5 ml of MR-VP culture was transferred aseptically to another sterile tube, and the remainder was incubated for another 30 hours at 35°C.
- 4-One drop of methyl red reagent was added aseptically to the tube (0.1gm methyl red dissolved in 300ml ethyl alcohol and then completed to 500ml by distilled water).

A positive reaction was indicated by a distinct red color, while negative reaction is indicated by a yellow color.

- 5-About 0.6ml of 5% 1-naphthol solution (5gm of 1-naphthol in 100ml of ethyl alcohol) and 0.2ml of 40% potassium hydroxide with creatine (40gm KOH in 100 ml distilled water) were added to the remainder incubated culture of MR-VP tube and mixed.
- 6-The tube was shaken and left to stand for 10 to 20 minutes.

A positive reaction was indicated by a bright orange-red color in the medium. Negative reaction was indicated by colorless or yellow color.

## **2.4 Isolation of *Salmonella*:**

Figure 2.1 shows the steps of isolation of *Salmonella*, which was done as follows:

### **2.4.1 Pre-enrichment:**

About 25 grams of the sample was aseptically blended with 225 ml of sterile nutrient broth for two minutes to obtain a homogenate mixture, then the mixture was aseptically transferred to 500 ml sterile bottles, the cap was loosen and the mixture was incubated at 35°C for 24 hours.

### **2.4.2 Selective enrichment:**

After mixing the incubated homogenate, 1ml was transferred aseptically by a micropipet to 10ml tetrathionate broth (Oxoid, CM 29) which is a selective enrichment medium for the isolation of *Salmonellae*, that can reduce tetrathionate. Bile salts in conjugation with thiosulfate and added iodine-iodine solution inhibits the growth of gram-positive organisms and most gram negative-rods except *Salmonellae* [53,18]. The medium was then incubated at 35°C for 24 hours.

### 2.4.3 Selective growth:

- 1-About 3mm loop-full of the incubated medium after mixing was taken aseptically from each culture and streaked on xylose lysine desoxycholate agar (XLD) (Oxoid, CM 469), and brilliant green (Oxoid, CM 263). XLD is a selective differential primary plating medium used to isolate *Salmonellae* and *Shigellae* from clinical specimens and foods [53,18]. Brilliant green agar is a selective medium for the isolation of *Salmonellae* other than *Salmonella typhi* [53]. It should be used in parallel with other selective plating media such as XLD agar [53]. The use of enrichment\selective broths prior to subculture on brilliant green agar will improve the probability of isolating *Salmonellae* [53].
- 2-The plates were incubated at 35°C for 24 hours.

### 2.4.4 Biochemical conformation:

#### Triple Sugar Iron test (TSI)

- 1-All colonies typical or suspected to be *Salmonella* were selected from each selective agar and inoculated into TSI agar slant by streaking the slant and stabbing the butt. Then incubation for 24-48 hours at 35°C.

2- Cultures that produce alkaline (red) slant and acid (yellow) butt with or without blackening of the medium (production of  $H_2S$ ) were retained as potential *Salmonella* isolates.

Urease test, Lysine iron agar test, Sulfide, Indole and Motility (SIM) [54,56]:

With a sterile needle small amount of growth from TSI agar suspected to be *Salmonella* was aseptically streaked into the slant of urea agar (Oxoid, CM53), and lysine iron agar (Oxoid, CM 381), and stabbed into SIM media (Oxoid, CM 435).

All cultures that gave negative urease test (no change in the color of the medium), alkaline reaction at lysine iron agar (purple color through out the medium), and indole negative at SIM (absence of a dark red ring in the reagent layer after the addition of kovac's reagent), were kept for serological identification.

Most *Salmonellae* are motile as indicated by the cloudiness in the medium or by growing in brush-like patterns around the line of inoculation, and some *Salmonellae* produce hydrogen sulfide as indicated by blackening of the line of inoculation or the whole tube.

#### 2.4.5 Serological test:

Serological test was carried out by *Salmonella* rapid test (Oxoid, FT 201). This test was demonstrated by slide agglutination of *Salmonella* species [53], and was done according to the instruction manual:

- 1-The latex reagent is brought to room temperature, then vigorous shaking to mix the latex suspension.
- 2-One free-falling drop of the test latex is dispensed onto one of the reaction circles of the test card.
- 3-One free-falling drop of the control latex is dispensed onto an adjacent reaction circle on the test card.
- 4-Using a sterile loop, a loopful of the suspected colony is removed and mixed with the test latex drop. Mixing with the loop for 10-15 seconds while spreading the drop to cover most of the reaction circle.
- 5- A second loopful of the colony is removed, and mixed into the control latex on the test card for 10-15 seconds, while spreading the drop to cover most of the reaction area.
- 6- The card is gently rocked in a circular motion for up to 2 minutes and agglutination is observed.

A result is positive if agglutination of the blue latex particles occurs within 2 minutes, this indicates the presence of *Salmonella*.

## **2.5 Isolation of *E.coli* O157: H7**

Most biochemical reactions of *E.coli* O157: H7 are typical of *E.coli*, with the exception of sorbitol fermentation and  $\beta$ -glucuronidase activity [41]. Figure 2.2 shows the isolation of *E.coli* O157: H7.

Steps for the isolation of *E.coli* O157: H7 were done as follows:

### **2.5.1 Selective enrichment:**

The recovery rate of *E.coli* O157: H7 on sorbitol macConkey agar (SMAC) (Oxoid, CM813) can be improved by prior enrichment in selective broth for four hours to overnight [42], this was done as followed:

- 1- About 10gm of meat was aseptically blended for 2 minutes with 90 ml of sterile lauryl tryptose broth (Oxoid, Cm 451) to get a 1:10 homogenate.
- 2-The mixture was aseptically transferred to sterile 250-ml bottles, which were incubated at 35°C for 24 hours.

### **2.5.2 Differential growth:**

- 1- Using a sterile loop, 3mm loop-full were aseptically taken from the incubated lauryl tryptose broth, and streaked into eosin methylene

blue agar (EMB) (Oxoid, CM 69), and incubated at 35°C for 24 hours [53].

- 2-Isolated colonies of *E.coli* exhibit a green metallic sheen by reflected light, and dark purple centers by transmitted light [53].
- 3-Almost all the colonies exhibiting the *E.coli* characteristics were subcultured on (SMAC), which is a selective and differential medium for the isolation of *E.coli* O157: H7 [53], the agar medium most commonly used for the isolation of *E.coli* O157: H7 [41,42,43,53,58,59].
- 4- Incubation at 35°C for 24 hours.
- 5-*E.coli* O157: H7 will form colorless colonies [53], but otherwise typical *E.coli* colonies [53].
- 6-The inability of *E.coli* O157: H7 to produce glucuronidase can be tested by using 4-methyl umbellifery 1-B-D-4glucuronide (MUG) [42].
- 7- Multiple sorbitol-nonfermenting colonies (at least 3 and up to 10) [42] were selected, and each colony was streaked onto SMAC containing (MUG).
- 8-The plates were incubated overnight at 35°C.

The isolates that were MUG-negative (no fluorescence under UV light), were suspected to be *E.coli* O157: H7, and subjected for further identification by serological test.



### 2.5.3 Serological test:

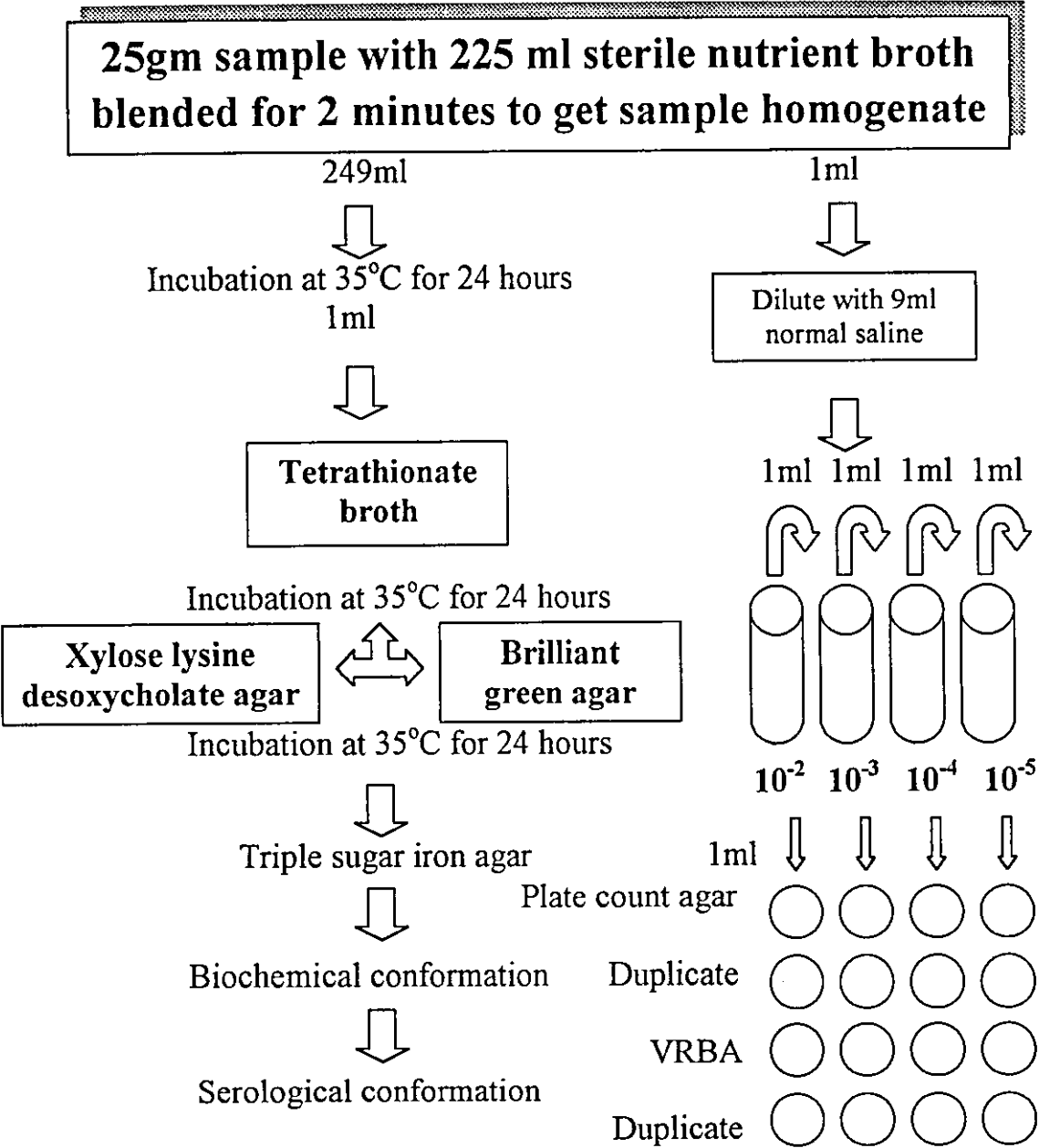
Presumptive identification of *E.coli* O157: H7 can be reported for confirmed *E.coli* strains that are sorbitol negative on SMAC agar and agglutinate in O157 antiserum [60].

*E.coli* O157 latex test (Oxoid, DR 620M) had been used for the identification of *E.coli* serogroup O157; this latex test was demonstrated by slide agglutination *E.coli* strains possessing the O157 antigen [53].

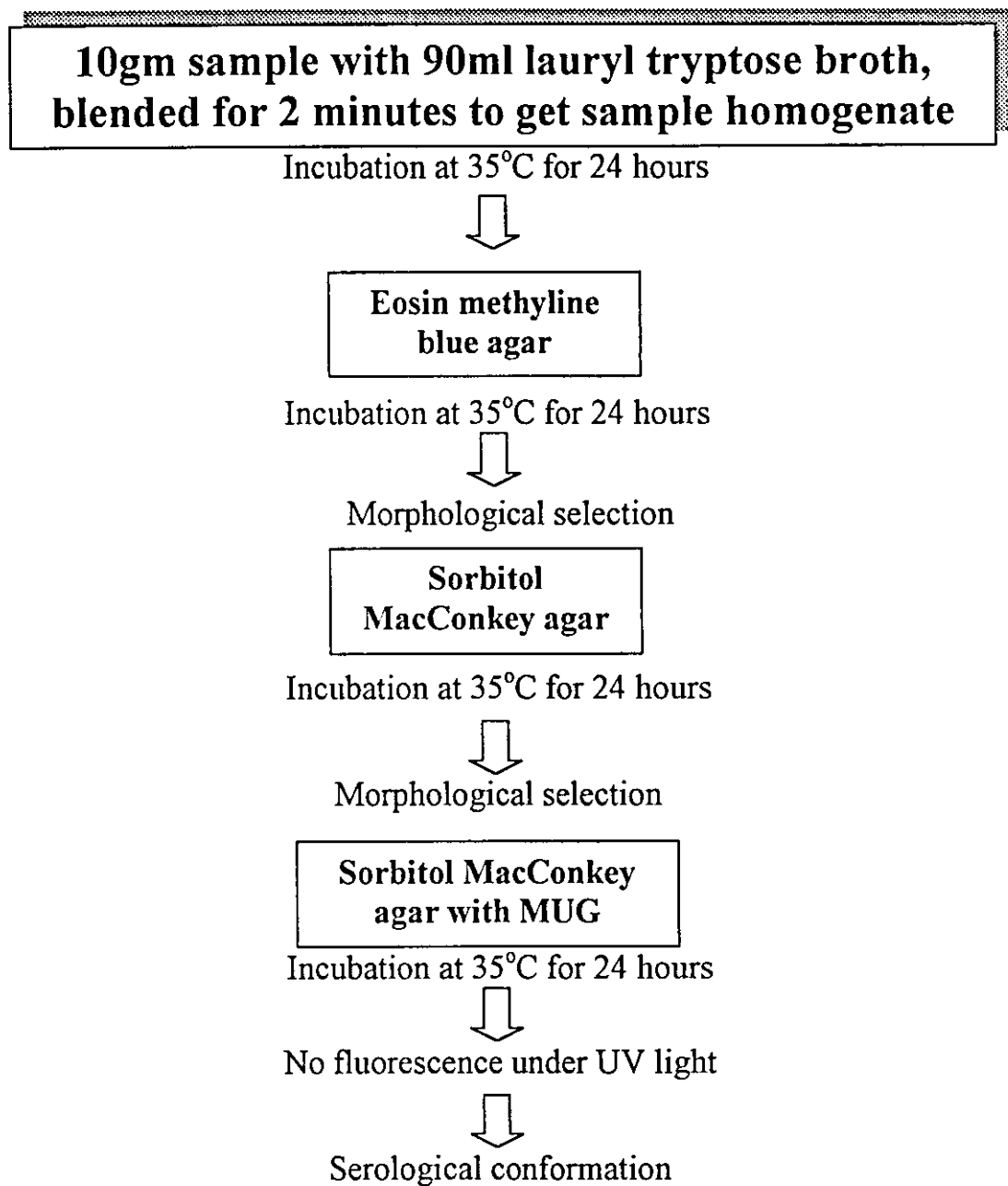
- 1-After getting the reagent to room temperature, vigorous shaking to mix the latex suspensions.
- 2-One drop of the test latex was dispensed onto a circle on the reaction card close to the edge of the circle.
- 3-A Pasteur pipette drop of saline was added to the circle, but not mixed to the latex drop.
- 4-A portion of the bacterial growth was picked off aseptically, by using a loop, from fresh nutrient agar slant culture and then emulsified carefully in the saline drop, then mixing the test latex and the suspension together to cover the reaction area.
- 5-The loop was flamed, and the card was rocked in a circular motion for only one minute.

Positive reaction was indicated by agglutination with the test reagent within one minute and in this case a further portion of the colony was tested with the control latex reagent to ensure that the isolate was not an auto-agglutinating strain.

**Figure 2.1** Isolation of *Salmonella* and preparation of 10-fold dilution of the sample for total bacterial count, and total coliform count.



**Figure. 2.2** Isolation of *E.coli* O157: H7:



# **Chapter 3**

## ***Results***

## CHAPTER 3

### 3.RESULTS

During the period from 14 February 2000 to 5 April 2000, a total of 150 fresh meat and liver specimens were obtained from Nablus Municipality slaughterhouse. The representative samples include 74 samples from calves (37 meat samples and 37 liver samples), and 76 samples from cows (38 meat samples and 38 liver samples).

The samples were cultured for total bacterial count, total coliform count, *Salmonella*, and *E.coli* O157: H7.

#### **3.1 Total bacterial count:**

Plate count agar plates that contained between 15 and 150 colonies were considered for calculating the total bacterial count. The average number of the bacterial colonies of two plates was multiplied by the reciprocal of the dilution to determine the total bacterial count as the average bacterial count per gram.

As shown in Table 3.1, the average total bacterial counts for calves meat and liver were  $2.4 \times 10^5$  CFU/g, and  $3.8 \times 10^5$  CFU/g, respectively, and for cows meat and liver  $3.8 \times 10^5$  CFU/g, and  $1.2 \times 10^5$  CFU/g, respectively.

### 3.2 Total coliform count:

Violet red agar plates that contained between 15 and 150 round purple-red colonies [52,53] were considered for determining the total coliform count. The average number of the bacterial colonies of two plates was multiplied by the reciprocal of the dilution to calculate the total coliform count as the average bacterial count per gram, which is equal to number of colonies X reciprocal of the dilution.

As shown in Table 3.1 the average total coliform count (CFU/g) for calves meat and liver was 31 and 11, respectively. While the average total coliform count (CFU/g) for cows meat and liver was 49.8 and 12.5, respectively.

Tables 3.2-6 show the different biochemical reactions for the identification of the different types of coliforms in calves and cows liver and meat. *Citrobacter* was found to be the predominant coliform either in liver of calves or cows, whereas *E.coli* was the predominant coliform in meat of either calves or cows.

### 3.3 Salmonella:

Morphological appearance of *Salmonella* appears as follows: [53]

On XLD: red colonies with or without black centers.

On brilliant green agar: red pink white opaque colored colonies surrounded by brilliant red zone.

The isolates that gave alkaline/acid on TSI media (red/yellow), urea negative, lysine decarboxylase positive, and indole negative, were suspected to be *Salmonella*, and were subjected to further identification by serological test.

As shown in Table 3.7-8, three samples of liver of calves were positive (8.1% of the total samples of calves' livers), 1 sample of meat of calves (2.7% of the total samples of calves' meat), 3 samples of liver of cows (7.9% of the total samples of cows' livers) and 2 samples of meat of cows (5.3 of the total samples of cows' meat). As for the total samples 9 were *Salmonella* positive (6% of the total samples).

### **3.4 *E.coli* O157: H7:**

The isolates that were MUG negative (no fluorescence under UV light) from SMAC containing MUG were suspected to be *E.coli* O157: H7. These colonies were subjected for further identification by serological test.

All samples, calves and cows, meat and liver were negative for *E.coli* O157: H7.

### **3.5 Acceptable and unacceptable liver and meat samples:**

As it is evident from Table 3.7, the total number of calves liver and meat acceptable samples was 32 (86.5%) and 34 (91.9%) respectively. While that for unacceptable samples was 5 (13.5%) for liver, and 3 (8.1%) for meat. In case of cows liver and meat, the total number of acceptable samples was 34 (89.5%), and 35 (92.1%) respectively. However, the total number of unacceptable samples was 4 (10.5%) for cows liver and 3 (7.9%) for cows' meat.

Thus the total number of acceptable samples, for both calves and cows liver and meat was 135 (90%), while that of unacceptable samples was 15 (10%) (Table 3.7).

Table 3.9 shows the correlation between the average total bacterial count (TBC) and *Salmonella* positive and negative samples. The total bacterial count (CFU/g) for calf liver and meat, *Salmonella* positive samples were  $3 \times 10^5$ , and  $1 \times 10^4$ , respectively, while that for calves liver and meat, *Salmonella* negative samples were  $3.9 \times 10^5$ , and  $2.5 \times 10^5$ , respectively. As for cows liver and meat *Salmonella* positive samples, it was  $1.3 \times 10^5$ , and  $5.3 \times 10^5$ , respectively, while that for cows liver and meat *Salmonella* negative samples was  $1.2 \times 10^5$ , and  $3.7 \times 10^5$ , respectively



Concerning the correlation between the average total coliform count (TCC) (CFU/g), and *Salmonella* positive and negative samples, Table 3.10 showed that *Salmonella* positive calf liver and meat samples have 12, and 50 TCC, respectively, while *Salmonella* negative calf liver and meat samples have 11.4, and 30.5 TCC, respectively. However the average TCC for *Salmonella* positive cows liver and meat was 83, and 40, respectively, while the average TCC for *Salmonella* negative samples for cows liver was 6.5, and 50.4, respectively.

**Table 3.1** The average total bacterial count, and the average total coliform count for different samples:

Sample	Average total bacterial count (CFU/g)	Average total coliform count (CFU/g)
Liver of calf	$3.8 \times 10^5$	11.4
Meat of calf	$2.4 \times 10^5$	31
Liver of cow	$1.2 \times 10^5$	12.5
Meat of cow	$3.8 \times 10^5$	49.8

**Table 3.2** Biochemical reactions for identification of coliform in liver of calf:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H <sub>2</sub> S	Butt	Slant	
1	5	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	<i>Enterobacter</i>
2	20	-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	<i>Citrobacter</i>
3	42	-	-	+	+	-	-	+	-	+	+	+	+	A	A	<i>Proteus</i>
4	9	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	<i>E.coli</i>
5	1	-	-	+	-	+	-	+	+	-	-	+	-	A	A	N.I.
6	1	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	N.I.
7	3	-	-	+	-	-	-	+	-	+	-	+	-	A	A	N.I.

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskauer.

Alk: Alkaline reaction, which gives red color.

Alk/A: Some strains give alkaline results, others give acidic results.

A: Acid reaction, which gives yellow color.

+: Positive result.

-: Negative result.

d: Different strains give different results.

N.I. : Not identified, need other biochemical reactions.

**Table 3.3** Biochemical reactions for identification of coliform in meat of calf:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H <sub>2</sub> S	Butt	Slant	
1	35	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	<i>Enterobacter</i>
2	27	-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	<i>Citrobacter</i>
3	17	-	-	+	-	+	+	-	d	-	d	+	-	A	Alk/A	<i>Klebsiella</i>
4	25	-	-	+	+	-	-	+	-	+	+	+	+	A	A	<i>Proteus</i>
5	46	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	<i>E.coli</i>
6	12	-	-	+	-	+	-	+	-	-	-	d	-	A	Alk/A	<i>Hafina alvei</i>
7	18	-	-	+	-	+	-	+	+	-	-	+	-	A	A	N.I.
8	3	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	N.I.
9	7	-	-	+	-	-	-	-	-	-	+	+	-	A	A	N.I.
10	5	-	-	+	-	-	-	-	-	-	-	+	-	A	A	N.I.
11	1	-	-	+	-	+	+	+	+	+	+	+	+	A	Alk	N.I.

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaur.

Alk: Alkaline reaction, which gives red color.

Alk/A: Some strains give alkaline results, others give acidic results.

A: Acid reaction, which gives yellow color.

+: Positive result.

- : Negative result.

d : Different strains give different results.

N.I. : Not identified, need other biochemical reactions.

**Table 3.4** Biochemical reactions for identification of coliform in liver of cow:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H <sub>2</sub> S	Butt	Slant	
1	15	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	<i>Enterobacter</i>
2	28	-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	<i>Citrobacter</i>
3	17	-	-	+	-	+	+	-	d	-	d	+	-	A	Alk/A	<i>Klebsiella</i>
4	22	-	-	+	+	-	-	+	-	+	+	+	+	A	A	<i>Proteus</i>
5	14	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	<i>E.coli</i>
6	8	-	-	+	-	+	-	+	-	-	-	d	-	A	Alk/A	<i>Hafina alvei</i>
7	2	-	-	+	-	+	-	+	+	-	-	+	-	A	A	N.I.
8	13	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	N.I.

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskauer.

Alk: Alkaline reaction, which gives red color.

Alk/A: Some strains give alkaline results, others give acidic results.

A: Acid reaction, which gives yellow color.

+: Positive result.

- : Negative result.

d : Different strains give different results.

N.I. : Not identified, need other biochemical reactions.

**Table 3.5** Biochemical reactions for identification of coliform in meat of cow:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H <sub>2</sub> S	Butt	Slant	
1	34	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	<i>Enterobacter</i>
2	22	-	-	+	+	-	+	+	d	d	d	d	D	A	Alk/A	<i>Citrobacter</i>
3	25	-	-	+	-	+	+	-	d	-	d	+	-	A	Alk/A	<i>Klebsiella</i>
4	34	-	-	+	+	-	-	+	-	+	+	+	+	A	A	<i>Proteus</i>
5	52	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	<i>E.coli</i>
6	13	-	-	+	-	+	-	+	-	-	-	d	-	A	Alk/A	<i>Hafina alvei</i>
7	12	-	-	+	-	+	-	+	+	-	-	+	-	A	A	N.I.
8	7	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	N.I.
9	9	-	-	+	-	-	-	-	-	-	+	+	-	A	A	N.I.
10	1	-	-	+	-	-	-	-	-	-	-	+	-	A	A	N.I.
11	1	-	-	+	-	+	+	+	+	+	+	+	+	A	Alk	N.I.
12	2	-	-	-	-	+	-	+	+	-	-	-	-	A	Alk	N.I.

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaur.

Alk: Alkaline reaction, which gives red color.

Alk/A: Some strains give alkaline results, others give acidic results.

A: Acid reaction, which gives yellow color.

+: Positive result.

- : Negative result.

d : Different strains give different results.

N.I. : Not identified, need other biochemical reactions.

**Table 3.6** Incidence of coliform organisms (percentage occurrence) in different samples:

Sample	Number of colonies	<i>E.coli</i> %	<i>Klebsiella</i> %	<i>Enterobacter</i> %	<i>Citrobacter</i> %	<i>Hafnia alvei</i> %	<i>Proteus</i> %	N.I %
Liver of calf	81	24.7	0.0	6.2	51.9	0	11.1	6.2
Meat of calf	196	23.5	8.7	17.9	13.8	6.1	12.8	17.3
Liver of cow	119	11.8	14.3	12.6	23.5	6.7	18.5	12.6
Meat of cow	212	24.5	11.8	16	10.4	6.1	16	15.1

N.I. : Not identified, need other biochemical reactions.

**Table 3.7** Number and percentages of acceptable and unacceptable samples and the reason for rejection:

Sample	Acceptable		Unacceptable									
	No.	%	High TBC		High TCC		<i>Salmonella</i>		<i>E.coli</i> O157:H7		Total	%
			No.	%	No.	%	No.	%	No.	%		
Liver of calf	32	86.5	2	5.4	0	0	3	8.1	0	0	5	13.5
Meat of calf	34	91.9	2	5.4	0	0	1	2.7	0	0	3	8.1
Liver of cow	34	89.5	1	2.6	0	0	3	7.9	0	0	4	10.5
Meat of cow	35	92.1	1	2.6	0	0	2	5.3	0	0	3	7.9
Total	135	90	6	4	0	0	9	6	0	0	15	10

Note: For total bacterial count the Palestinian Ministry of Health standards were the reference, while for total bacterial count and *Salmonella*, the Palestinian standard institution were the reference.

**Table 3.8** Number and percentage of *Salmonella* and *E.coli* O157:H7 in different samples of meat:

Sample	Number of Samples	<i>Salmonella</i> positive		<i>E.coli</i> O157:H7 Positive	
		Number	Percent	Number	percent
Liver of calf	37	3	8.1%	0	0%
Meat of calf	37	1	2.7%	0	0%
Liver of cow	38	3	7.9%	0	0%
Meat of cow	38	2	5.3%	0	0%
Total	150	9	6%	0	0%

**Table 3.9** Average total bacterial count (TBC) in positive and negative samples of *Salmonella* and *E.coli* O157:H7:

Sample	Average TBC(CFU/g) in <i>Salmonella</i>		Average TBC(CFU/g) in <i>E.coli</i> O157:H7	
	+ve samples	-ve samples	+ve samples	-ve samples
Liver of calf	$3 \times 10^5$	$3.9 \times 10^5$	-----	$3.8 \times 10^5$
Meat of calf	$1 \times 10^4$	$2.5 \times 10^5$	-----	$2.4 \times 10^5$
Liver of cow	$1.3 \times 10^5$	$1.2 \times 10^5$	-----	$1.2 \times 10^5$
Meat of cow	$5.3 \times 10^5$	$3.7 \times 10^5$	-----	$3.8 \times 10^5$

**Table 3.10** Average total coliform count (TCC) in positive and negative samples of *Salmonella* and *E.coli* O157:H7:

Sample	Average TCC(CFU/g)in <i>Salmonella</i>		Average TCC(CFU/g) in <i>E.coli</i> O157:H7	
	+ve samples	-ve samples	+ve samples	-ve samples
Liver of calf	12	11.4	-----	11.4
Meat of calf	50	30.5	-----	31
Liver of cow	83	6.5	-----	12.5
Meat of cow	40	50.4	-----	49.8



# **Chapter 4**

## ***Discussion***

## CHAPTER 4

### 4. DISCUSSION

According to the Palestinian Standard Institution (PSI) for the standards of total bacterial count and *Salmonella*, and based on the Palestinian Ministry of Health for total coliform count, and based on internationally acceptable microbial standards for fresh meat, the samples that were *Salmonella* negative (0/25g), and had a total bacterial count  $< 1 \times 10^6$  CFU/g, and a total coliform count  $< 1 \times 10^3$  CFU/g were considered as acceptable samples, otherwise were rejected and considered as unacceptable samples.

This study showed that the liver of calves have the highest unacceptable samples, as 8.1% of the samples are positive for *Salmonella* and 5.4% of the samples have high TBC. Liver of cows in which 7.9% of the samples are positive for *Salmonella* follows this, and 2.6% of the samples have high TBC. All samples had low total coliform counts that appear higher in meat samples. This may be due to previous infection of the animal before slaughtering, or regurgitation of the ruminal fluid after slaughter, reaching the liver through blood circulation.

Statistical analysis using EPI2000 into statistical system was used.  $\chi^2$  test was used to indicate the p-value that shows if there is a relation or not between two parameters, if this value was less than 0.05

this indicates a significant relation, and a value more than 0.05 indicates no significant relation.

The results showed no significant relation ( $P=0.797$ ) between the age of the animal and numbers of accepted or rejected samples (Table 4.1). There was also no significant relation between the inner and the outer surfaces of the same animal, this was clear in all samples positive for *Salmonella* or had high total bacterial count that appeared either inside or outside the animal but not in the other part from the same animal. The age of the animal had no significant relation ( $P=0.735$ ) with the presence or the absence of *Salmonella* (Table 4.2). Table 4.3 showed that no significant relation ( $P=0.865$ ) was found comparing the age of the animal and the total bacterial count. On the other hand there was no significant relation between the TBC and TCC, as the samples with high TBC appeared completely normal for TCC. Also no significant relation between TCC and TBC on one hand and the presence or absence of *Salmonella* on the other hand, this was clear that all samples positive for *Salmonella* were low in TBC, and TCC, and all samples high in TBC were *Salmonella* negative. This may be due to previous infection of the animal, or contamination of meat with the gastrointestinal tract contents during skinning, evisceration, and handling of meat.

It is generally agreed that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughter [62].

One of the sources of carcass contamination in the meat plant is the animal itself [1]. Bacteria play an important role in the spoilage and decomposition of meat and also in food poisoning [1]. Spoilage defects in meat become evident when the number of bacteria at the surface reaches  $10^7$ CFU/cm<sup>2</sup>, and off odors are first detected. When numbers reach  $10^8$ CFU/cm<sup>2</sup>, the muscle tissue surface will begin to feel tacky, representing the first stage in slime formation which is attributed to the growth of bacteria and synthesis of polysaccharides which gradually form a confluent, sticky layer on the surface of the tissue. Since spoilage characteristics do not become evident until amino acids are degraded, the concentration of glucose present in the tissue is a primary factor governing the time necessary for the onset of aerobic spoilage [24].

The percentage occurrence of coliform organisms in different kinds of samples showed that *E.coli*, and *Citrobacter* were the most frequently isolated coliform. This indicates exposure of meat to fecal contamination during slaughtering, evisceration, skinning, and handling. *Citrobacter* is mainly found in environmental source.[20]. These results are in agreement with those of Kay and Fricker[20], which indicated that *E.coli* would be always present in fecal contamination event, as it comprises 95% of the coliform group, unlike *Klebsiella*, *Enterobacter*, and *Citrobacter*, which may be present, but at much lower densities.

These results are in agreement also with those of Al-Kharraz[63], which indicates that *E.coli* and *Citrobacter* were the most frequently isolated coliforms from different samples of meat in the butcher shops.

Comparing ground beef microbial results of Al-Kharraz showed that 8.1% of the ground beef samples were *Salmonella* positive, and 4.8% were *E.coli* O157:H7 positive, which appears higher compared with the microbial results of this work. This may be attributed to several reasons[63]. First, ground meat consists of trimming from various cuts, providing greater surface area for contamination. Second, the greater surface area of ground meat favors the growth of aerobic bacteria, the low temperature spoilage bacteriobiota. Third, in some butchers shops, the meat grinders, cutting knives, and storage utensils are rarely cleaned as often and as thoroughly as is necessary to prevent the successive build up of microbial numbers. Fourth, one heavily contaminated piece of meat is sufficient to contaminate others, as well as the entire lot as they pass through the grinder.

Bacterial contamination of meat occurs in several ways [1,24,62]:

- 1-The knives: either during the act of sticking where the bacteria can enter the jugular vein or anterior vena cava and travel in the blood to the muscles, lungs and bone marrow, or through skinning of the animal.
- 2-Animal hide: It is generally agreed that the majority of bacteria on a dressed red meat carcass originate from the hide.

3-Gastrointestinal tract: under ordinary conditions the heaviest and potentially the most dangerous load of bacteria is in the animals' digestive tract. It is estimated that 28 g of fresh bovine feces contain 1500 million bacteria. Microorganisms may also be introduced to the carcass surface during the evisceration process, where careful evisceration will reduce the potential for contamination. Contamination from the digestive tract also appears clear in the regurgitation of the animal when bled, so it is usually advisable to keep the animals for about 18 hours drinking only water to decrease the contamination expected during bleeding or evisceration [1]. A further significant observation was made that samples of ruminal fluid from cattle slaughtered at a number of abattoirs in Queensland showed that an average of 45% of samples contained *Salmonella* organism [1].

4- Lymph nodes: these that are usually imbedded in fat often contain large numbers of organisms especially bacteria. If they are cut through or added to portion that are ground, one may expect this biota to become prominent.

5- Muscles may be infected before slaughter by specific organisms responsible for illness of the animal.

In addition to being present in the hide and viscera, bacteria may originate from sources in the processing environment, such as floors, walls, contact surfaces, knives, and workers' hands.

In addition to these hazard points, persons with occupational exposure to animals, and clinical stool specimens, handlers of unprocessed meat may also be at increased risk of illness particularly if personal hygiene is poor or preventive measures are inadequate [58].

Table 4.1 The relation between the age of the animal and the acceptance or rejection of the sample:

Sample	Acceptable	Unacceptable	Total
Liver of calf	32	5	37
Meat of calf	34	3	37
Liver of cows	35	3	38
Meat of cows	35	3	38
<b>Total</b>	<b>135</b>	<b>15</b>	<b>150</b>

Chi square = 1.02

Degrees of freedom = 3

P-value = 0.797

Table 4.2 The relation between the age of the animal and the presence or absence of *Salmonella*:

Sample	+ve	-ve	Total
Liver of calf	3	32	35
Meat of calf	1	34	35
Liver of cows	3	34	37
Meat of cows	2	35	37
<b>Total</b>	<b>9</b>	<b>135</b>	<b>144</b>

Chi square = 1.27

Degrees of freedom = 3

P-value = 0.735

Table 4.3 The relation between the age of the animal and the total bacterial count:

Sample	+ve	-ve	Total
Liver of calf	2	32	34
Meat of calf	2	34	34
Liver of cows	1	34	35
Meat of cows	1	35	35
<b>Total</b>	<b>6</b>	<b>135</b>	<b>141</b>

Chi square = 0.73

Degrees of freedom = 3

P-value = 0.865

## **Recommendations:**

On January 12, 1998, the British medical association warned in a report to members of the Parliament in London that “The current state of food safety in Britain is such that all raw meat should be assumed to be contaminated with pathogenic organisms” and “The only safe approach for the food industry and general public is to treat all raw meat as infected and adopt universal precautions in handling and cooking raw meat”.

- 1-Make sure that meat is thoroughly cooked and still hot when served, and cold storage (chilling) of food which is not consumed immediately after preparation.
- 2-Wash hands, counters, and utensils thoroughly before and after handling raw meat.

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3-Maintenance of all meat processing premises in a clean state before and after dealing with meat.

For the slaughterhouse:

- 1- All animals should be kept for 18 hours before slaughter without feeding, drinking only water, to decrease the bulk of intestinal and ruminal material before being slaughtered, in order to decrease the contamination expected during bleeding and evisceration.
- 2- Keeping the meat after slaughtered in the refrigerator for at least 24 hours in the slaughterhouse. Change of the pH of meat and chilling will limit the bacterial growth. And maintenance of the low temperature either in the butchers shops or before and during being processed and cooked.
- 4- Intensive meat inspection at the slaughterhouse either organoleptic examination or laboratory one, in order to decrease the bacterial transfer to other places disseminating diseases.
- 5-The Ministry of Health, Ministry of Agriculture and Veterinary Services should make more work and further research on farm level, animal level, slaughterhouse, in food processing plant and butcher shops, because controlling all the hazard points and the source of infection can reduce the possibility of producing products with the ability of transferring diseases.

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بسم الله الرحمن الرحيم

توزيع ووجود بعض أنواع مجموعة البكتيريا المعوية في اللحوم الطازجة للماشية، مع تأكيد خاص على بكتيريا السالمونيلا وبكتيريا اشريشيا القولون صنف (O157: H7).

إعداد الطالب

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إشراف

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الملخص العربي

تم جمع مائة وخمسين عينة طازجة من مسلخ بلدية نابلس، تشمل هذه العينات ٣٧ عينة من كبـد عجول صغيرة و ٣٧ عينة لحوم من نفس العجول، ٣٨ عينة من كبـد أبقار كبيرة، و ٣٨ عينة لحوم من نفس الأبقار.

تم فحص العينات لمعرفة عدد البكتيريا الكلي، والعدد الكلي لبكتيريا القولون، اثناهما بطريقة الأطباق المصبوبة. وكذلك البحث عن وجود بكتيريا إشريشيا القولون صنف (*E.coli* O157: H7) ووجود بكتيريا السالمونيلا وذلك لتقييم دور اللحوم الطازجة كمصدر للتسمم الغذائي في منطقة مدينة نابلس. وذلك بعدة طرق مرجعية للإكتار، الزراعة، والاختبارات البيوكيميائية والسيرولوجية.

عدد البكتيريا الكلي والعدد الكلي لبكتيريا القولون يدلان على الجودة الميكروبية للحوم، وكذلك درجة تلوث العينات سواء داخليا من نفس الحيوان، أو خارجيا من العوامل المحيطة.

تبين أن ما نسبته ١٠% من العدد الكلي للعينات كان غير مقبول سواء بسبب الزيادة في العدد الكلي للبكتيريا (٤%)، أو بسبب وجود السالمونيلا (٦%). لم تسجل من العينات أي حالة ملوثة ببكتيريا اشريشيا القولون نوع (*E.coli* O157:H7).

المتوسط لعدد البكتيريا الكلي لجميع العينات كان ( $2.8 \times 10^5$  CFU/gm) في حين أن المتوسط للعدد الكلي لبكتيريا اشريشيا القولون كان (26 CFU/gm). العجول الصغير أظهرت ما نسبته ٥,٣% من العينات غير المقبولة، والتي كانت أكثر من العينات المأخوذة من البقر كبير السن، الذي أظهر ما نسبته ٤,٧% من العينات المرفوضة.

بالنسبة لوجود السالمونيلا، الأبقار الكبيرة أظهرت نسبة ٣,٣% والتي كانت أعلى من مثيلاتها في العجول حيث كانت ٢,٧%.

لم تظهر الدراسة أي علاقة بين العدد الكلي للبكتيريا والعدد الكلي لبكتيريا القولون من جهة، ووجود بكتيريا السالمونيلا من جهة أخرى. وكذلك لم تظهر الدراسة علاقة بين عمر الحيوان، وبين قبول أو رفض العينة، والإصابة سواء ببكتيريا السالمونيلا أو العدد الكلي للبكتيريا.