An-Najah National University

Isolation of *Salmonella* and *Escherichia coli 0157:H7* from Fresh Meat of Turkey and Imported Frozen Cattle Meat with Emphasis on Isolation of *Salmonella* from Poultry Eggs

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Faculty of Graduate Studies

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DEDICATION

TO EVERYONE THAT ENCOURAGED

AND

SUPPORTED ME IN MY STUDY

TO MY DEAR FATHER, GREAT MOTHER,

BROTHERS AND SISTERS,

FOR THEIR ENCOURAGEMENT AND SUPPORT

WITH LOVE.

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Issam M. Al-Karablieh

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LIST OF ABBREVIATIONS

$CCP_{(s)}$	Critical Control Point
CDC	Centers for Disease Central
CFU	Colony Forming Unit
EAEC	Entroaggregative *E.coli*
EHEC	Entrohemorrhagic *E.coli*
EIEC	Entroinvasive *E.coli*
EMB	Eosin Methline Blue Agar
EPEC	Entropathogenic *E.coli*
ETEC	Entrotoxigenic *E.coli*
FDA	Food & Drug Administration
H_2O_2	Hydrogen Peroxide
HACCP	Hazard Analysis Critical Control Point
HUS	Hemolytic Uremic Syndrome
IMViC	Indole-Methyl Red Proskauer Citrate
KOH	Potassium Hydroxide
LPS	Lipopolysaccharide
MR	Methyl Red
MUG	4-Methylumbelliferyl β-D-Glucuronide
NASA	National Aeronautics and Space Administration
PCA	Plate Count Agar
PSI	Palestine Standards Institution
SIM	Sulfide Indole Motility
SLT	Shiga-Like Toxin
SMC	Sorbitol MacConkey
SS	*Salmonella Shigella*
Stxs	Shiga Toxins
TBC	Total Bacterial Count
TCC	Total Coliform Count
TSI	Triple Sugar Iron
TTP	Thrombotic Thrombocytopenic Purpura
USDA	U.S. Department of Agriculture
UV	Ultra Violet
VP	Voges-Proskauer
VRBA	Violet Red Bile Agar
VTEC	Verotoxigenic *Escherichia Coli*
XLD	Xylose Lysine Desoxycholate

ABSTRACT

A total of 290 samples, 150 meat samples and 140 fresh chicken eggs samples were collected randomly from butchers shops in Nablus area in the period from September 2000 to April 2001. The meat samples include 76 fresh turkey meat, 68 frozen cattle meat and 6 frozen sheep meat. The meat samples were assayed and studied for total bacterial count, total coliform count using pour plate technique, and the incidence of *Salmonella* and *E.coli O157:H7* in order to assess the role of animal meat as a source of these pathogens. The egg samples were studied for incidence of *Salmonella* & other enteric bacteria in order to assess the role of chicken egg as a source of *Salmonella*. Both types of samples were studied using standard enrichment, plating, biochemical and serological techniques.

Total bacterial count and total coliform count indicate the overall microbial quantity, and hence the quality of meat.

This work showed that fresh turkey meat had the highest percentage (57.9%) of unacceptable samples depending on Palestine acceptable microbial standards, followed by frozen cattle meat (29.4%) and frozen sheep meat (16.7%). All the samples of meat were negative for *E.coli O157:H7*.

For the presence of *salmonella*, fresh turkey meat showed an incidence of (5.3%), which was higher than that of frozen cattle meat (1.5%), frozen sheep meat (0.0%), and fresh chicken eggs (0.0%). The average total bacterial count for the meat samples was 4.12×10^6 CFU/g, whereas the average total coliform count was 2.4×10^5 CFU/g. The average total bacterial count for turkey meat samples was 4.1×10^6 CFU/g, cattle meat samples was 6.46×10^6 CFU/g, and sheep meat samples was 2.03×10^6 CFU/g. The average total coliform count for turkey samples meat was 4.24×10^5 CFU/g, cattle meat samples was 2.55×10^5 CFU/g, and sheep meat samples was 0.42×10^5 CFU/g.

CHAPTER ONE

1. INTRODUCTION

Some foods particularly favor the growth of pathogenic microorganisms, poisoning bacteria, and food spoilage bacteria. Foods which encourage the growth of bacteria are usually high in protein, moisture and minerals. Examples being meat, poultry, turkey, eggs, milk and "made-up" products where any of these foods is an ingredient (4). Many such bacteria can grow rapidly in food that is not properly refrigerated or cooked, and they may spoil the food (6).

Meats contain an abundance of all nutrients required for the growth of bacteria (11). Meat is a fine culture medium for many organisms (5), because it is high in moisture, rich in nitrogenous foods of various degrees of complexity, well supplied with minerals and accessory growth factors (8, 12). It usually has some form of fermentable carbohydrate (glycogen) present (5, 8, 12). Fresh meats such as beef, as well as fresh poultry and processed meats, have pH values within the growth range of most of the organisms (11). The pH of raw meat may vary from about 5.7 to over 7.2 depending upon the amount of glycogen present at slaughter and subsequent changes in the meat (8), so meat is

more subjective to microbial attack than any other food products which may result in spoilage of meat and in extreme cases may cause food-borne infection or food-borne intoxication (51). Factors associated with spoilage may include color defects or changes in texture, the development of off-flavors, off-odors and slime, or any other characteristics that indicate the growth of pathogenic microorganisms which renders food undesirable for consumption (1, 3). If pathogenic bacteria are present, they may cause food poisoning or food infection or both (6).

In fact meat is the most common vehicle of reportable cases of food-born illness (52). Every year the majority of food poisoning outbreakes are caused by meat and meat products (4).

Raw egg is ideal for bacterial growth. Hens' eggs are not infected with bacteria (4). Although the majority of freshly laid eggs are sterile inside, the shells soon become contaminated; by fecal matter from the hen, by the lining of the nest, by wash water if the eggs are washed, by handling, and, perhaps, by the material in which the eggs are packed (8). However, in a relativity short period of time after laying, numerous microorganisms may be found on the outside and, under the proper conditions, may enter eggs, grow, and cause spoilage. The speed at which microbes enter eggs is related to temperature of storage, age of

eggs, and level of contamination (11). The entry of microorganisms into whole eggs is favored by high humidity. Under such conditions, growth of microorganisms on the surface of eggs is favored, followed by penetration through the shell and inner membrane. The latter structure is the most important barrier to the penetration of bacteria into eggs, followed by the shell and the outer membrane (14). In general, more spoilage of eggs is caused by bacteria then by molds (8). So care must be taken to prevent transfer of bacteria from the shell to the raw egg or any other food in the kitchen (4). Certain pathogenic microorganisms in or on food of animal origin still constitute a particular hygienic risk (53).

Because even small numbers of infectious organisms may cause disease, they should be absent from ready – to eat foods (1). For toxin – forming organisms, however, much higher numbers (> 10^5 to 10^6 organisms per ml) are usually required to produce food-borne illness (1). For most food-borne pathogens, no vaccines are available (60).

Illnesses caused by food-borne micooganisms are serious health problems through out world. Verotoxigenic *Escherichia coli* (VTEC) and *Salmonella* are two examples of food-born pathogens capable of causing disease (54).

There are a variety of *E.coli* bacteria present in nature. They are usually found in the intestines of healthy humans and healthy animals

(19). *E.coli O157:H7* is one of hundreds of strains of bacterium *E.coli* (55). The entrohemorrhagic *E.coli* (EHEC) strain serotype *O157:H7* has since been associated with hemorrhagic diarrhea, colitis, and hemolytic uremic syndrome (HUS). HUS is characterized by low platlet count, hemolytic anemia, and kidney failure (2). Also an increasing number of disease outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS) in human have been linked to the ingestion of beef and dairy products (9, 57) contaminated with verocytotoxin (Shiga-like toxin) producing *E.coli* VTEC (56). The most frequently known outbreaks of *E.coli O157:H7* are associated with fecal contaminated meats in slaughter houses (20). Investigative reports have shown that all *E.coli O157:H7* strains have so far been found to produce high levels of verotoxin (2).

The *Salmonella* infection that are called "food poisoning" may be caused by any of a large number of species of that genus (8). Some species of these enteric pathogens (e.g., *Salmonella enteritidis*) can grow in foods and cause food infections, and others are commonly only transported by foods, e.g., *S. typhosa*, the cause of typhoid fever, and *S. paratyphi*, causing the B type of paratyphoid fever (8). *Salmonella* species are some of the major causes of food poisoning in the developed world (58), which is caused by consumption of raw products of animal

origin (53). *Salmonella* food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers (11).

Because it is not feasible to test food for all pathogens, the detection of indicator organisms such as coliform group is used as an indication of fecal contamination and possible presence of other entropathogens (2, 11, 59).

1.1 Family Enterobacteriaceae:

The Enterobacteriaceae family are a large, heterogeneous group of gram-negative bacilli, nonspore-forming short rods, aerobic and facultative anaerobes ranging from 1 to 2 μm in diameter and from 3 to 10 μm in length, either non-motile or motile by peritrichous flagella (6, 22, 41), whose natural habitat is the intestinal tract of humans and animals (41).

Members of this family have many features (2, 8, 41):

1- All ferment glucose with the production of acid or acid and gas.

2- Some species ferment lactose with gas formation.

3- All grow well on and in artificial media.

4- All reduce nitrates to nitrites.

5- None produce cytochrome oxidase (oxidase negative).

6- All except *Klebsiella, Shigella,* and *Yersinia* are motile. With rare exceptions, motile organism have peritrichous flagella.

7- Do not require NaCl for their growth.

8- All are catalase positive.

Enterobacteriaceae are present as normal human fecal flora, and in natural habitats worldwide, including soil, water, plants of all types, fish, insects, and other animals. Their importance in human disease is at least equaled by their importance as causes of disease in poultry, domestic livestock, fish, and several vegetable and plant crops, with major economic consequences (22).

The family Enterobacteriaceae, often referred to as "enterics" consists of a large number of diverse organisms (2). Several Enterobacteriaceae have been associated with gastroenteritis and food-borne disease (22).

Within the last several years the number of recognized species of Enterobacteriaceae has grown from 26 to more than 100 (approximately 90 species have been named), and more species are being validated monthly (23).

The family includes several genera and species (2, 5, 6, 8, 41):

1- *E.coli:*

The common inhabitant of the lower part of the intestine of warm-blooded animals.

2- *Aerobacter:*

Aerobacter aerogenes, although occasinally from the intestines.

3- *Salmonella:*

To which the typhoid fever organism *S. typhi* belongs.

4- *Shigella dysenteria:*

Causing bacillary dysenteries.

5- *Klebsiella:*

Commonly associated with the respiratory and intesinal tracts of man. *K. pneumoniae* is the cause pneumonia in man.

6- *Proteus vulgaris:*

Bacteria of this genus have been involved in the spoilage of meats, seafood, and eggs.

7- *Erwinia:*

The species of this genus are plant pathogens.

8- *Serratia*:

Many species produce a pink or magenta pigment and may cause red discolorations on the surface of foods. *S. marcescens* is the most common species.

9- *Yersinia*:

Can be found in the soil. *Y. pestis* is the causative organism of plague in man.

The first two genera are important saprophytes in foods, and the other genera consist chiefly of pathogens (8). The first two genera together are called the coliform, or colon-aerogenes, group and the organisms are called coliform bacteria (8). Many of the relatively harmless organisms used as models in the laboratory belong to the Enterobacteriaceae. They provide good examples of pathogens (6).

1.1.1 Antigenic Structure:

Members of family Enterobacteriaceae have the following antigens:

Capsular (K), cell wall (O), and flagella (H). These are important in the identification and epidemiological studies of this group of organisms. (K) antigen is heat labile polysaccharides that can mask the heat stable somatic (O) antigens. In some species, such as *S. typhi*, (K)

antigen functions as a virulance factor. (O) antigen is the LPS component of the cell wall. It is useful as a biological marker, and also works as a virulance factor. (H) antigens are proteins; that are particularly helpful in the classification and serologic specification of members of the genus *Salmonella* (125).

Gram-negative bacteria are characterized by their production of endotoxins, which consist of a lipopolysaccharide (LPS) layer outside the outer membrane of the cell envelope. The LPS is pyrogenic and responsible for some of the symptoms that accompany infections caused by gram-negative bacteria (11).

1.2 Coliform Group:

Detection of pathogenic organisms in foods further – more requires the use of selective techniques to separate the target organisms from others that may be present in much greater numbers (1). Therefore, testing for indicator organisms has been introduced as a simpler means of controlling the hygienic status of foods and helps to ensure production of safe food (1). Thus, the indicator organisms used should be selected carefully, and the results should be interpreted with caution (1). The coliform bacteria are commonly referred to as indicator organism (61).

While attempting to isolate the etiologic agent of cholera in 1885, Escherich (16) isolated and studied the organisms that is now *E.coli*. It was originally named Bacterium coli commune because it was present in the stools of each patient he examined.

Schardinger (15) was the first to suggest the use of this organism as an index of fecal pollution because it could be isolated and identified more readily than individual waterborne pathogens. A test for this organism as a measure of drinking water portability was suggested in 1895 by T. Smith (17). This marked the beginning of the use of coliforms as indicators of pathogens in water, a practice that has been extended to foods (11).

The coliform bacteria are short rods, Gram-negative, non-spore-forming that are defined in standard methods for the examination of both water and milk as "all aerobic and facultative anaerobic bacteria which ferment lactose with the production of acid and gas at 32°C or 35°C with in 48h (8, 18, 63), and produce dark colonies with a metallic sheen on Endo-type agar (18). By and large, coliforms are represented by four genera of the family Entrobacteriaceaes (11). The genera that satisfy the definition (11, 61) include:

1- *Citrobacter* and *Serratia*: found in environmental sources.

2- *Enterobacter*: may be found in feces and ubiquitous in the environment.

3- *Escherichia*: always found in human and other animal feces.

4- *Klebsiella*: may be found in feces and ubiquitous in the environment.

For their presence in some foods and water, is considered to be indicative of sewage contamination (8).

Consequently, coliform – group can be classified into those of fecal and non-fecal origin (63). The fecal coliform group is referred to as organisms that grow in the gastrointestinal tract of humans and other warm-blooded animals and includes members of 3 genera: *Escherichia*, *Klebsiella*, and *Enterobacter* (63).

Thereafter, the use of indicator organisms came into vogue for evaluating hygiene in food processing (1).

The presence of any of these groups in food particularly meats, meat products and fresh vegetables is used as indication of fecal contamination (62), and as a marker of un-sanitary conditions or practices during production, processing or storage (59).

The coliform organisms are well established as fecal indicators for water. Their use as indicators of food sanitary quality derives from their successful use for water (64).

Fecal coliforms are defined by the production of acid and gas in EC broth between 44 °C and 46 °C, usually 44.5 °C or 45.5 °C. EC broth, for *E.coli*, was developed in 1942 by Parry and Hajna (21). This concept was important because it recognized feces as a significant source of human pathogens (1).

E.coli is more indicative of fecal pollution than the other coliform genera and species especially *E. aerogenes.* It is often desirable to determine its incidence in a coliform population (11). *E.coli* comprises nearly 95% of the coliforms in feces and are universally present in the feces of warm-blooded animals at densities of 10^8-10^9 per gram (61).

In addition to its role as indicator of fecal contamination, *E.coli* is one of the most commonly used index organisms for *Salmonella* in food (61).

Because *E.coli* and other coliform species are considered to be indicative of sewage contamination and hence of the possible presence of enteric pathogens; and growth in foods results in their spoilage, the presence of coliform with large numbers in foods, water and oysters is highly undesirable (8, 11) .

Some of the characteristics that make the coliform bacteria important in food spoilage are (8):

1- Their ability to grow well in a variety of substrates and to utilize a number of carbohydrates and some other organic compounds as food for energy and a number of fairly simple nitrogenous compounds as a source of nitrogen.

2- Their ability to synthesize most of the necessary vitamins.

3- The ability of the group to grow well over a fairly wide range of temperature, from below 10 °C to about 46 °C (8). In foods, growth is poor or very slow at 5 °C, although several authors have reported the growth of coliforms at 3-6 °C (11).

4- Coliforms have been reported to grow over a pH range 4.4-9.0 (11).

5- Their ability to produce considerable amounts of acid and gas from sugars.

6- Their ability to cause off-flavors, often described as "unclean" or "barny".

In examination of food the presence of intestinal inhabitants should be taken to indicate a lack of cleanliness, not of safety. The safety of food can be assessed only by examining for the presence of pathogens (64). Fecal coliform validity as indicators for the presence of *Salmonella* in irrigation water has been found to be high. When the fecal coliform

density/100 ml was above 1000 CFU, occurrence exceeded 96% frequency, while a frequency of only about 54% was associated with fecal coliform level of less than 1000/100 ml (64). From about 1920 on awards, coliform group (coli-aerogens) has been used as indicator or index organisms in the examination of bottled milk, ice cream and shellfish and, more recently, of animal feeds (65).

1.3 Genus *Salmonella*:

Several Enterobacteriaceae species have been associated with gastroenteritis and food borne disease. One member of the family, *Salmonella sp.*, is the etiological agent of most food-borne gastroenteritis in the United States.

Genus *Salmonella* was named in 1900 after a U.S. Department of Agriculture (USDA) bacteriologist, Dr. Salmon, who was the first to describe a member of the group, *S. choleraé-suis* (1, 5), which he thought caused hog cholera. It was later discovered that a virus caused hog cholera and Salmon's bacterium was an incidental isolate (1).

1.3.1 Microbiology of *Salmonella*:

Salmonella species are facultatively anaerobic gram-negative bacteria (1). Salmonellae are short, thin, rod-shaped bacteria, non-spore-

forming bacteria (4, 8). Salmonellae ferment glucose, usually with gas production, but not lactose or sucrose (1, 8, 41).

Biochemical features for the genus include the following (2, 41):

- They don't ferment lactose.
- They are negative for indole, the Voges-Proskauer test, phenylalanine, and urease.
- Catabolize D-glucose and other carbohydrates with the production of acid and gas.
- Most species except *S.pullorum-gallinarum* are motile with peritrichous flagella.
- Most produce hydrogen sulfide on triple sugar iron agar.
- They don't grow in potassium cyanide.

Salmonella is able to multiply in an environment with a low level or no oxygen (28). They grow best in nonacid foods; thus salad with a pH between 5.5 and 5.7 has been found unfavorable for growth. They have an optimum temperature of about 37 °C, but grow well at ordinary room temperatures, but not at refrigerator temperatures (2). Readily killed by temperature above 55 °C (15).

Salmonellae are widespread in nature, occurring in animals, poultry, insects and swine, and environment including water, soil,

factory surfaces, kitchen surfaces, and raw sea foods, to name only a few (7, 10).

1.3.2 Sources of *Salmonella*:

Human beings and animals are directly or indirectly the source of the contamination of foods with salmonellae. The organisms may come from actual cases of the disease or from carriers. Most frequently *S. typhimurium*, *S. montevideo*, *S. oranienburg*, and *S. newport* cause human gastroenteritis, but any of many other types may be responsible. The organisms also may come from cats, dogs, swine, and cattle, but more important sources for foods are poultry and their eggs and rodents. Chickens, turkeys, ducks and geese may be infected with any of a large number of types of *Salmonella*, which are then found in the fecal matter, in eggs from the hens, and in the flesh of the dresses fowl (5, 8). Various *Salmonella* species have long been isolated from the outside of eggs shells. The present situation with *S. enteritidis* is complicated by the presence of the organism inside the egg, in the yolk (7).

1.3.3 Pathogenicity & Clinical Finding:

Salmonella is often pathogenic to humans or animals when aquired by the oral route (41, 46, 47). *Salmonella* species are able to invade the intestinal mucosa. Some strains produce an entrotoxin (32). The *Salmonella* food-poisoning syndrome is caused by the ingestion of foods that contain significant numbers of non-host-specific species or serotypes of the genus *Salmonella*. From the time of ingestion of food, symptoms usually develop in 12-14h, although shorter and longer times have been reported. The symptoms consist of nausea, vomiting, abdominal pain (not as severe as with staphylococcal food poisoning), headache, fever, chills, and diarrhea. These symptoms are usually accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness, and drowsiness. Symptoms usually presist for 2-3 days (11). *Salmonella* infection is responsible for 10 to 15% of acute gastroenteritis cases (46). Detailed investigations from food-borne 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 (7, 48, 49).

It is estimated that from 2 to 6 million cases of salmonellosis occur in the U.S. annually. The incidence of salmonellosis appears to be

rising both in the U.S. and in other industrialized nations (7). The symptoms of salmonellosis include the sudden onset of headache, chills, vomiting, and diarrhea, followed by a fever that lasts a few days (4, 9). All age groups are susceptible, out symptoms are most severe in the elderly, infants, and the infirm. AIDS patients suffer salmonellosis frequently (estimated 20-fold more than general population) and suffer from recurrent episodes (7).

In a study of 61 outbreaks of human salmonellosis from the period 1963 to 1965, eggs and egg products accounted for 23, chicken and turkey for 16, beef and pork for 8, icecream for 3, potato salad for 2, and other miscellaneous foods for 9 (24). In 1967, the most common food vehicles involved in 12836 cases of salmonellosis from 37 states were beef, turkey, eggs and egg products, and milk (11). Of 7907 salmonellae isolates made by the Centers for Disease Control (CDC) in Atlanta in 1966, 70% were from raw and processed food sources with turkey and chicken sources accounting for 42% (11).

In September 1973, in Mid-Western America, 125 of 173 people who had eaten a meal in a certain restaurant developed diarrhea, severe abdominal pains and other symptoms, on average 23 hours after the meal. Eleven of the people had to be taken to hospital for treatment.

However, a study of the food eaten by the victims suggested that potato salad and chicken dressing were the vehicles of transmission (12).

In 1974, temperature abuse of egg containing potato salad served at an outdoor barbecue led to an estimated 3400 human cases of *S. Newport* infection; cross – contamination of the salad by an infected food handler was suspected (25).

In October 1977, newspapers carried reports of a massive outbreak of *Salmonella* food poisoning in Stockholm: more than 1000 school children and teachers became ill after eating ham salad served in school cafeterias. The food was prepared in a central kitchen and distributed to several schools in Western Stockholm (12).

The large Swedish outbreak of *S. enteritidis* in 1977 was attributed to the consumption of a mayonnaise dressing in a school cafeteria (26). In the United States, during the 1977, 27850 cases of Salmonellosis excluding typhoid fever were reported (27).

A *Salmonella* food poisoning outbreak occurred in Riyadh, Saudi Arabia in 1980 where twelve persons out of 21 who attended a home dinner were affected and 6 of these required the care of a physician (33).

In England and Wales, *Salmonella* infection account for more than 80% of cases reported for food-borne diseases in 1981-1983 (28).

In 1984, Canada experienced its largest outbreaks of food-borne 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 (29).

In Scotland, 224 outbreaks of salmonellosis associated with poultry meat were reported between 1981-1985. In total 2245 persons were affected, 12 of whom died (34).

The following year witnessed the largest outbreak of food-borne salmonellosis in the United States, involving 16284 confirmed cases of illness (30, 31), it is one of the worst food poisoning incidents in the history of United States occurred in Chicago in the spring of 1985 when 16284 cases and 7 deaths were documented when pasteurized milk somehow became decontaminated with a potent strain of *S. typhimurium* (1). More than 50000 cases were reported to the Centers for Disease Central (CDC) in 1987 (22).

Until 1988, poultry meat was considered the major source of *Salmonella* food poisoning in Britain (35). In 1989, *S. enteritids* was responsible for at least 49 food-borne outbreaks and 13 deaths in United States, in which raw shell eggs and unpasturized liquid egg products were the food vehicles in the majority of outbreaks (36).

The incidence of typhoid fever in the United States is quite low, about 400 cases reported each year between 1980 and 1990 and in approximately half of these the disease was acquired during foreign travel (22). By 1990 the number had dropped somewhat, with 20 cases per 100000 individuals in the U.S. population becoming infected (22).

A large outbreak of salmonellosis affecting more than 10000 Japanese consumers was attributed to a cooked egg dish (37).

In 1993, paprika imported from South America was incriminated as the contaminated ingredient in the manufacture of potato chips distributed in Germany (38).

In 1994, the latest major outbreak of food-borne salmonellosis occurred in the United States and involved icecream contaminated with *S. enteritidis*. This was exceeded by a national outbreak of *S. enteritidis* affecting 225000 people who consumed contaminated ice-cream products. The transportation of ice-cream mix in an unsanitized truck that previously carried raw eggs was identified as the source of contamination (39, 40).

1.3.4 Classification of the Genus *Salmonella*:

Food microbiologist / scientists and epidemiologists treat the 2324 *Salmonella* serovars as though each was a species. All salmonellae have been placed in two species, *S. enterica* and *S. bongori*, with the 2324 serovars being divided into 5 subspecies or groups, most of which are classified under the species *S. enterica*, as the number of base differences between different *Salmonella* strains is minimal (42, 127). The major groups correspond to the following subspecies: Group II (*S. enterica* subspecies salamae); Group IIIa (*S. enterica* subspecies arizonae); Group IIIb (*S. enterica* subspecies diarizonae); Group IV (*S. enterica* subspecies houtenae); and Group VI (*S. enterica* subspecies indica). The former Group V organisms have been elevated to species status as *S. bongori* (43).

If and when this taxonomic scheme is fully adopted, the long-standing practice of treating salmonellae serovars as species is no longer valid. For example, *S. typhimurium* should be *S. enterica* serovar typhimurium, or *S. typhimorium* (note that "*typhimurium*" is capitalized and not italicized) (11).

For epidemiologic purposes, the salmonellae can be placed in three groups (11):

1- Those that infect humans only: These include *S. typhi, S. paratyphi A, S. paratyphi C.* This group includes the agents of typhoid and the paratyphoid fevers, which are the most severe of all diseases caused by *Salmonella.*

2- The host-adapted serovars (some of which are human pathogens and may be contracted from foods): Included are *S. gallinarum* (poultry), *S. dublin* (cattle), *S. abortus-equi* (horses), *S. abortus-ovis* (sheep) and *S. choleraesuis* (swine).

3- Unadapted serovars (no host preference). These are pathogenic for humans and other animals, and they include most food-borne serovars.

1.3.5 Serotyping of *Salmonella*:

The majority of outbreaks in livestock are caused by a select number of serotypes, serotyping is not an adequate method for determination of the source of contamination during an outbreak (13).

Species and serovars are placed in groups designated A, B, C, and so on, according to similarities in content of one or more (O) antigens. Thus, *S. hirschfeldii, S. cholerae-suis, S. oranienberg,* and *S. montevideo*

are placed in group C, because they all have (O) antigens 6 and 7 in common. *S. newport* is placed in Group C_2 due to it is possession of antigens (K) and 8 (Table 1.1) (11). For further classification, the (H) antigens are employed. These antigens are of two types: specific phase or phase 1, and group phase or phase 2. Phase 1 antigens are shared with only a few other species or varieties of *Salmonella*; phase 2 may be more widely distributed among several species. Any given culture of *Salmonella* may consist of organisms in only one phase or of organisms in both flagellar phases. The (H) antigens of phase 1 are designated with small letters, and those of phase 2 are designated by Arabic numerals. Thus, the complete antigenic analysis of *S. cholerae-suis* is as follows: 6, 7, c, 1, 5, where 6 and 7 refer to (O) antigens, c to phase-1 flagellar antigens, and 1 and 5 to phase-2 flagellar antigens (Table 1.1). *Salmonella* subgroups of this type are referred to as serovars (11).

The (O) antigens are lipopolysacharides (LPs) located on the external surface of the bacterial outer membrane, (H) antigens are associated with the peritrichous flagella. The capsular (Vi) antigen occurs only in *S. typhi, S. paratyphi C*; and *S. dublin* (28, 44).

The heat-stable (O) antigens are classified as major or major antigens (5), (H) antigen are heat-labile proteins; individual *Salmonella*

strains may produce one (monophasic) or two (diphasic) (H) antigens (5).

Table (1.1) Antigenic structure of some common salmonellae: (11, 45):

Group	Type	Somatic antigens	Flagellar Antigens	
			Phase 1	Phase 2
A	*S. paratyphi A*	1, 2, 12	A	-
B	*S. paratyphi B*	1, 4, 5, 12	B	1, 2
	S. typhinurium	1, 4, 5, 12	I	1, 2
	S. stanley	4, 5, 12	D	1, 2
	S. heidelberg	1, 4, 5, 12	R	1, 2
	S. abortus-equi	4, 12	-	e, n, x
	S. abortus-ovis	4, 12	C	1, 6
C_1	*S. paratyphi C*	6, 7, Vi	C	1, 5
	S. cholerae-suis	6, 7	C	1, 5
	S. thompson	6, 7	K	1, 5
	S. bareilly	6, 8	Y	1, 5
C_2	*S. newport*	6, 8	E, h	1, 2
	S. bovis-morbificans	6, 8	R	1, 5
D	*S. typhi*	9, 12, Vi	D	-
	S. dublin	1, 9, 12	G, p	-
	S. enteritidis	1, 9, 12	G, m	-
	S. gallinarum	1, 9, 12	Non Flagellate	Non Flagellate
E_1	*S. anatum*	3, 10	E, h	1, 6
	S. meleagridis	3, 10	E, h	1, w
	S. london	3, 10	l, v	1, 6
E_4	*S. senftenberg*	1, 3, 19	G, s, t	-
F	*S. aberdeen*	11	I	1,2
G	*S. poona*	13, 22	Z	1, 6

1.4 Genus *E.coli: O157:H7:*

E.coli is the predominant, facultative anaerobe of the intestinal tract of healthy humans and warm-blooded animals.

Beneficial strains of *E.coli* typically colonize the infant gastrointestinal tract within a few hours after birth. The presence of this bacterial population in the intestine suppresses the growth of harmful bacteria and is important for synthesizing appreciable amounts of B vitamins. *E.coli* usually remains harmless when confined to the intestinal lumen. However, in debilitated or immuno-suppressed humans, or when gastrointestinal barriers are violated, even normal, "non-pathogenic" strains of *E.coli* can cause infection (1, 70, 72, 76, 77).

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

Characteristically, *E.coli* have the following (2, 41):

- Mannitol fermentation and produce gas from glucose.
- Ferment glucose, lactose, trehalose, and xylose.
- Indole and methyl red tests.
- Dose not produce H_2S, DNase, urease, or phenylalanine deaminase.
- Dose not grow in the presence of potassium cyanide.
- Cannot utilize citrate as a sole source of carbon.
- May be motile or nonmotile.

- Produce a negative result with Voges-Proskauer test.

Most *E.coli* strains are harmless commensals, however, several strains of *E.coli* have recently emerged as potent food-borne pathogens (9). Some *E.coli* strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even very healthy people (70, 72, 75, 77). They are characterized by their ability to produce potent enterotoxins (9).

E.coli may cause several types of diarrheal illnesses. Strains of *E.coli* that cause diarrhea include five major categoris of diarrheogenic *E.coli*, based on definite virulence factors, clinical manifestation produced, epidemiology, and different O:H serotypes.

These include the following: (2, 70, 72, 75, 77):

- Entropathogenic *E.coli* (EPEC): watery diarrhea of infants.
- Entrotoxigenic *E.coli* (ETEC): traveler's diarrhea.
- Entroaggregative *E.coli* (EAEC): persistent diarrhea.
- Entroinvasive *E.coli* (EIEC).
- Entrohemorrhagic *E.coli* (EHEC): hemorrhagic colitis (bloody diarrhea) and hemolytic urimic syndrome / serotype *O157:H7*.

E.coli serotypes associated with bloody diarrhea or HUS are listed in (Table 1.2).

Infections due to pathogenic *E.coli* may be limited to mucosal surface or can disseminate through out the body. Three general clinical syndromes result from infection with inherently pathogenic *E.coli* strains (75):

1- Enteric / diarrheal disease.

2- Urinary tract infection.

3- Sepsis / meningitis.

Table (1.2): *E.coli* serotypes associated with bloody diarrhea or Hemolytic Uremic Syndrome (HUS) (73):

O2:H5; H6	O48:H21	O112:H2	O145:H25; H-
O4:H10; H-	O50:H7	O113:H2; H21	O146:H8
O5: H-	O55:H6, H7; H10; H-	O114:H4	O153:H25
O6:H2; H-	O86:H40	O115:H10	*O157:H7*; H-
O18:H70	O91:H10; H21; H-	O117:H4	O163:H19
O22:H8	O98:H-	O118:H12	O165:H25; H-
O26:H11; H-	O103:H2	O119:H6	O168:H-
O38:H21	O104:H; H21	O121:H19	O?:H2; H7; H19; H21
O452:H2	O105:H18	O125:H-	
O46:H31	O111:H2; H8; H-	O128:H2; H25; H-	

1.4.2 Infective Dose:

According to the FDA, the infectious dose for *E.coli O157:H7* is unknown, since the infectious dose of *E.coli O157:H7* is low, ingestion of even a small number of surviving *E.coli O157:H7* could cause an illness. However, a compilation of outbreak data indicates that it may be as low as 10 organisms (1, 72). In a review, Paton and Paton (77) cite references for infectious dose as ranging from 1 to 100 CFU. These data show that it takes a very low number of microorganisms to cause illness in young children, the elderly and immune – compromised people.

1.4.3 Pathogenicity of *E.coli O157:H7*:

Most *E.coli* strains are harmless commensals in the human gut (92). There are those variations, or strains, that are pathogenic (have the ability to cause disease). However, *E.coli O157:H7* is one particular strain that is a rapid emerging food-borne illness (19, 92). The spectrum of human illness of *E.coli O157:H7* infection includes non-bloody diarrhea, hemorrhagic colitis, hymolytic urimic syndrome (HUS) (78), and thrombotic thrombocytopenic purpura (TTP) (70). Some persons are infected but asymptomatic (1). Symptoms of hemorrhagic colitis include bloody diarrhea and abdominal cramps (19, 101). The elderly and children under five years old are highly susceptible to HUS, a disease in which red blood cells are destroyed and kidneys fail (19).

The mechanism of *E.coli O157:H7* pathogenesis is not fully documented, but a likely scenario occurs as follows: after *E.coli O157:H7* cells are ingested, the bacteria colonize the large intestine and adhere to and possible invade colonic mucosal epithelial cells, replicate and destroy colonic cells, and damage the underlying tissue and vasculature, possibly by both exotoxin-related and endotoxin-related mechanisms, thereby producing bloody diarrhea (107).

1.4.4 Entrohemorrhagic (EHEC):

were first identified as a human pathogen in 1982, when *E.coli* of serotype *O157:H7* was associated with two food-borne outbreaks of hemorrhagic colitis (80, 82).

The main pathogenic property of EHEC is belived to be associated with the formation of toxins. *E.coli O157:H7* produces a large quantity of toxin(s) that cause severe damage to the lining of the intestine and other organs of the body. These toxins [referred to as verotoxin (VT) or shiga-like toxin (SLT)] are very similar, if not identical, to the toxin produced by *Shigella dystenteriae* (72). *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 (96, 108).

Recent studies support the concept that shiga toxins (Stxs) contribute to pathogenesis by directly damaging vascular endothelial

cells in certain organs, thereby disrupting the homeostatic properties of these cells (109).

The incubation period for EHEC diarrhea is usually 3 to 4 days, although incubation times can be as long as 5 to 8 days or as short as 1 to 2 days. Usually the first complaint of illness is non-bloody diarrhea, which is preceded by crampy abdominal pain and fever of short duration in many patients. Vomiting occurs in about half of the patients during the period of non-bloody diarrhea and/or other times in the illness. Within 1 or 2 days, the diarrhea becomes bloody, and the patient experiences increased abdominal pain. This state usually lasts between 4 to 10 days. In severe cases, fecal specimens are described as "all blood and no stool". In most patients, the bloody diarrhea resolves with no long-term impairment. However, in about 10% of patients younger than 10 years and in many elderly patients, the illness will progress to hemolytic uremic syndrome (HUS) and other complications (75, 85).

The syndrome is characterized by a traid of features: acute renal insufficiency, microangiopathic hemolytic anemia, and thrombocyto-penia. Thrombocytopenia is a typical feature but is transient and is therefore occasionally missed. HUS is a leading cause of kidney failure in children, which often requires dialysis and may ultimately be fatal (2, 85).

Other systemic manifestations of illness due to *E.coli O157:H7* include a central nervous system involvement, hypertension, myocarditis, and other cardiovascular complications that may result in death or severe disability. In some cases, the illness is indicative of some forms of heart disease and has been responsible for strokes in small children. These complications are attributed to direct or indirect actions of verotoxins absorbed from the intestinal tract (73, 75). (TTP) largely affects adults and resembles HUS histologically. It is accompanied by distinct neurological abnormalities resulting from blood clots in the brain. Fortunately, TTP is rare syndrome of *E.coli O157:H7* infection (1).

There is evidence of transmission of this pathogen through direct contact between people. *E.coli O157:H7* can be shed in feces for a median period of 21 days with a range of 5 to 124 days, according to studies reported by Nataro and Kaper (75).

Most strains of *E.coli O157:H7* posses several characteristics uncommon to most other *E.coli*: inability to grow well, if at all, at temperatures of ≥ 44.5 °C, inability to ferment sorbitol within 24 hours, inability to produce B-glucuronidase (i.e., inability to hydrolyze 4-methyl-umbelliferyl- D-glucuronide), possession of an attaching and effacing (eae) gene (84).

Outbreaks and clusters of *E.coli O157:H7* peak during the warmest months of the year (83). Of breaks and clusters reported in the United States, 83.4% (57 of 68) occurred from May to October (1). The reasons for this seasonal pattern are unknown, but they could hypothetically include: (i) an increased prevalence of the pathogen in cattle or other livestock or vehicles of transmission during the summer, (ii) greater human exposure to ground beef or other *E.coli O157:H7* contaminated foods during the "cook-out" months, and / or (iii) greater improper handling (temperature abuse) or incomplete cooking of products such as ground beef during warm months than other months (1).

Geographically, the focus of attention for addressing *E.coli O157:H7* infection has been largely on the North America continent. Most occurrences of *E.coli O157:H7* infection outside North America have involved sporadic diarrheal disease cases. Although some outbreaks have been reported in Europe and Japan (83).

A large epidemic involving several thousand patients was reported from Swaziland and South Africa, following consumption of surface water contaminated with *E.coli O157:NM* (86).

Data from the United Kingdom indicate that reported isolations of *E.coli O157:H7* from patients increased from 178 in 1989 to 532 in 1991 (87).

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 (88, 89, 90, 91).

Investigators at the centers for disease control and prevention estimate that *E.coli O157:H7* infection accounts for approximately 20000 cases of illness and 250 deaths annually (9, 104).

The prototype strain for the syndrome below is *E.coli O157:H7*. The H7 type was initially isolated in 1944 from a human diarrheal specimen, whereas the O157 type was first isolated and named in 1972 from diarrheal swine feces (93). However, the first *O157:H7* strain was recovered in 1975 from a patient with bloody diarrhea. Stx-producing strains of *E.coli* were identified in 1977 in the United States (94, 95) and Canada (96). Following its original isolation in 1975, the next recorded isolation of *E.coli O157:H7* was in 1978 when it was recovered from diarrheal stools in Canada (11).

The first notable incident of *E.coli O157:H7* in the United States was reported in 1982, when two outbreaks of hemorrhagic colitis occurred, one in Oregon and one in Michigan. The outbreak occurred in Oregen in 1982 documented with 26 cases and 19 persons hospitalized. All patients had bloody diarrhea and sever abdominal pain. The median

age was 28 years, with a range of 8 to 76 years. This outbreak was associated with eating undercooked hamburgers (that were contaminated) from fast-food restaurants of a specific chain (1, 71, 76, 82).

Since 1982, a continuing number of outbreaks of infection due to *E.coli O157:H7* have been reported in the U.S., Canada, and the U.K. These incidents have occurred in homes, child-day care centers, swimming pools, schools, nursing homes, institutions for the mentally retarded, and in fast-food and other restaurant operations (75, 76, 77).

In the fall of 1988, an incident occurred at a junior high school in Minnesota that led to illness in 30 students. Four were hospitalized. There were no fatalities. Frozen, partially cooked beef patties were incriminated in this incident. The patties had not been heated sufficiently by the processor to inactivate *E.coli* in the center. The beef patties were reheated before they were served to students, but some, again, were not reheated adequately to inactivate *E.coli O157:H7* (74).

Recently more than 8000 children (including seven fatalities), in 43 of the 47 total Japanese prefectures, were shoe to excrete *E.coli O157:H7* after consuming the midday school meat (92).

A large outbreak of *E.coli O157:H7* infection associated with contamination of municipal water occurred in Cabool, Mo., between December 16, 1989, and January 15, 1990 (97). Among the 243 affected

people identified, 86 had bloody diarrhea, 32 were hospitalized, 2 had HUS, and 4 died. The mean age was 38 years, and all four deaths were women 79 years of age or older. The city had an unchlorinated water supply (97).

In the summer of 1991, an outbreak of *E.coli O157:H7* was traced to a lakeside park near Portland, Ore (98). Twenty-one cases of park-associated *E.coli O157:H7* infection were identified from 19 households. All of the patients were children (median age 6 years; range, 1 to 16 years); seven, including three with HUS, were hospitalized. The vehicle was fecally contaminated lake water ingested by bathers, among whom were many toddlers not yet toilet trained. All 21 patients reported swimming (98).

Among the unusual food-borne *E.coli O157:H7* outbreaks in the United States was an occurrence in southeastern Massachusetts in the fall of 1991 that was traced to apple cider (99). Twenty-three cases of *E.coli O157:H7* infection involving 13 families were identified, with one to five cases per family. Four children had a diagnosis of HUS, and six patients were hospitalized (99). although *E.coli O157:H7* was not isolated from apple cider made by the implicated processor, a case-control study identified the apple cider as the vehicle of transmission. The implicated cider processor may have pressed apples that were contaminated by soil containing *E.coli O157:H7*, because more than

90% of the apples used in the cider were collected from the ground, or contamination may have occurred during processing (1). *E.coli O157:H7* is acid tolerant and able to survive in apple cider for 20 days at 8 °C (100).

The largest documented outbreak of *E.coli O157:H7* infection in the United States occurred from November 15, 1992 through February 28, 1993 in 4 states (Washington, Idaho, California, and Nevada). More than 500 laboratory-confirmed infections with *E.coli O157:H7* and 4 associated deaths. Many young children were involved. The outbreaks were traced to a fast-food chain and restaurants in these states serving regular hamburgers and jumbo hamburgers. In total 731 cases were identified: 629 in Washington, 13 in Idaho, 57 in Las Vegas, Nevada, and 34 in southern California (69, 76, 101, 102, 103).

Another unusual outbreak was associated with consumption of dry fermented salami in 1994 in Washington and California and involved 19 cases (104). Dry-cured salami is not cooked but is usually fermented and dried. The implicated product contained 85% pork, 15% beef, and spices and was fermented with a lactic acid bacterial culture to a final pH of (4.9). Inoculation studies have revealed that *E.coli O157:H7* can survive the fermentation, drying, and storage of fermented sausage (105).

Through 1994, 68 outbreaks of clusters of *E.coli O157:H7* infection have been documented (102). They have increase from an

average of 2 per year between 1982 and 1992 to 17 in 1993 and 30 in 1994 (1).

In the fall of 1996, unpasteurized apple juice from a processing facility in California was determined to be responsible for an outbreak of *E.coli O157:H7*. In August 1997, Hudson foods recalled 25 million pounds of ground beef after an *E.coli* outbreak was traced to it is plant in Columbus, Nebraska (76).

Most *E.coli O157:H7* infections are single cases and not associated with outbreaks. The numbers of reported cases in Washington of *E.coli O157:H7* peaked in 1993, and have mostly been dropping since then. Case counts for 1991-1997 are listed below (20):

1991 – 164 cases

1992 – 300 cases

1993 – 741 cases

1994 – 174 cases

1995 – 140 cases

1996 – 187 cases

1997 – 149 cases

1998 – 144 cases

1999 – 186 cases

1.5 Microbiological Guidelines:

Microbiological standards for red meat, poultry, eggs, and other food products vary from area to another. Microbiological standards are very important to determine the degree of safety and quality of foods. Currently, microbiological standards are used to assess (i) the safety of food, (ii) adherence to good manufacturing practices (GMPs), (iii) keeping quality (shelf-life) of certain perishable foods, and (iv) the utility (suitability) of a food or ingredient for a particular purpose when appropriately applied, microbiological standards can be useful means for ensuring the safety and quality of foods, which inturns elevates consumer confidence.

Table 1.3: Palestinian microbiological standards for red meat, poultry, and chicken eggs:

Item	Aerobic Plate Count (APC) (CFU / g)	*Salmonella* (CFU / g)	Coliforms (CFU / g)	Standards use
Fresh chilled meat	1×10^{6}-1×10^{7}	0 in 25	—	(PSI)
Frozen meat	1×10^{6}	0 in 25	—	(PSI)
Fresh chilled chicken	2.5×10^{5}	0 in 25	—	(PSI)
Frozen chicken	5×10^{5}/ in skin layer	0 in 25	—	(PSI)
Fresh chicken eggs	Zero	Zero	Zero	(PSI)

PSI: Palestine Standards Institution (50, 81, 106, 122, 128).

Table 1.4: Microbiological standards for meat in different areas:

Item	Aerobic Plate Count (APC) (CFU / g)	*Salmonella* (CFU / g)	Coliforms (CFU / g)	Standards use
Carcass meat, chilled	1×10^{6}-1×10^{7}	---	---	FAO
Raw chicken (fresh or frozen) during processing	5×10^{5}-5×10^{7}	---	---	FAO
Beef	---	0.00	---	*
Raw beef products	$< 5\times10^{5}$	Absent in 100g	< 1000	**
Poultry Spin chilled breast. Skin.	$< 25\times10^{4}$ /16cm^2 at 22 °C	---	<1000 /16cm^2	**
Crowed meat	1×10^{7}	0 in 20	5000	***

FAO- After: Andrews, 1992 (63).

* After: Doyle, 1997 (1).

** After: Government of Abu-Dhabi, 1979 (67).

*** After: The Palestinian National Authority, 2000 (68).

1.6 The Hazard Analysis Critical Control Point (HACCP) System:

Among the desirable qualities that should be associated with foods is freedom from infectious organisms (11). The production of foods with the lowest possible numbers is the desirable goal (11). The Hazard Analysis Critical Control Point (HACCP) System is presented as the method of choice for ensuring the safety of food from farm to home (11). The HACCP concept was advanced in 1971 by H.E. Bauman and other scientists at the Pillsburg Company in collaboration with the National Aeronautics and Space Administration (NASA) and the U.S. Army Research Laboratories. It was presented at the first national Conference on Food Protection (110, 111).

First applied to low-acid canned foods, the HACCP concept the since been applied throughout the food industry to a large variety of products and to the food service industry (112). The HACCP use in meat and poultry inspection (113), production of meat and poultry products (114). Its use in Canada and control of salmonellosis (115, 116, 117). HACCP is a system that leads to the production of microbiologically safe foods by analyzing for the hazard of raw materials-those that may appear throughout processing and those that may occur from consumer abuse. Although some classical approaches to food safety rely heavily on product testing, the HACCP system places emphasis on the quality of all ingredients and all process steps on the premise that safe products will

result if these are properly controlled. The system is thus designed to control organisms at the point of production and preparation (11). Mishandling of foods in food service establishments in Canada in 1984 was involved in about 39% of food borne incidents (118). Proper implementation of HACCP in food service establishments and the home will lead to a decrease in food-borne illness (11).

The systematic approach to food safety embodied by HACCP is based on seven principles (11):

1- Assess the hazards and risks associated with the growing, harvesting, raw materials, ingredients, processing, manufacturing, distribution, marketing, preparation, and consumption of the food in question.

2- Determine the $CCP_{(s)}$ required to control the identified hazards.

3- Established the critical limits that must be met at each identified CCP.

4- Establish procedures to monitor the CCP(s).

5- Establish corrective actions to be taken when there is a deviation identified by monitoring a given CCP.

6- Establish effective record keeping systems that document the HACCP plan.

7- Establish procedures for verification that the HACCP system is working correctly.

1.7 Objectives:

Meat and eggs pass through various steps during processing. Meat steps beginning from farming, then transporting, slaughtering, evisceration, chilling or freezing, selling in butchers shops, and finally handling in the kitchen.

The present work was therefore aimed to determine:

1- The sanitary quality of fresh turkey meat and imported frozen red meat (beef and sheep) in butchers shop in Nablus city by determining the total bacterial count and the total Coliform count.

2- The degree of contamination of fresh turkey with *Salmonella* and *E.coli O157:H7* microorganisms at the butchers shop after being transferred from the slaughterhouse.

3- The degree of contamination of imported frozen red meat with *Salmonella* and *E.coli O157:H7* microorganisms at the butchers shop.

4- The transovarian transmission of *Salmonella* in poultry eggs.

5- Comparison between the degree of contamination with *Salmonella* and *E.coli O157:H7* between fresh turkey meat and imported frozen red meat.

CHAPTER TWO

2. MATERIALS & METHODS

2.1 Sample Collection:

A total of 150 samples 100g portions each of fresh turkey meat and imported frozen red meat, in addition to 140 samples of chicken eggs (one egg/sample) were collected randomly according to standard protocols from butchers shops, in between 8:00-10:00 a.m, from Nablus city, during the period from September 2000 to April 2001.

The meat samples include 76 turkey, 68 cattle, & 6 sheep. They were collected at weekly intervals. The meat samples were immediately sent refrigerated in an icebox within 1 hr of collection to the microbiology laboratory at An-Najah National University.

Frozen red meat imported from many countries, such as Belgium, Brazil, China, Germany, Holland, and Netherlands. The source of fresh turkey meat is Israel, whereas the source of chicken eggs is many villages in Nablus area.

2.2 Processing and Culturing of Meat Specimens:

As the sample arrived at the laboratory, each sample was assigned an individual unit number and was analyzed as discrete unit as follows:

1- About 25 grams of each meat sample were aseptically transferred to sterile blending container, previously sterilized by washing with hot water & rinsing with 95% ethanol alcohol and then allowing the remaining alcohol to burn. The sample was then blended with 225 ml of sterile nutrient broth for 2 minutes to obtain homogenate mixture with 0.1 ml concentrations.

2- The homogenate mixture was aseptically transferred to a sterile 500 ml bottle having the sample number, and mixed well by swirling the bottle. Then the bottle cap was loosen, and the homogenate mixture was incubated at 35 °C for 24 hr, for the isolation of *Salmonella*.

2.3 Total Bacterial Count Test:

Figure 2.1 shows the steps for the determination of the total bacterial count.

These steps are:

1- Using a suitable micropipet and sterile disposable tips, 1 ml of the homogenate mixture (0.1 ml concentration) was aseptically

transferred to a sterile 9 ml normal saline tube, to give a concentration of 0.01.

2- Step 1 was repeated to give the appropriate decimal dilution for each specimen.

3- 1 ml from each decimal dilution was then transferred aseptically each to one of the two sterile plates having the sample number and the decimal dilution of the sample.

4- About 10 to 15 ml of Plate Count Agar (PCA), tempered to 44-46 °C, were poured each into one of the two plates.

5- Duplicate plates and agar control plates were run for each series of samples.

6- 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.

7- The contents of the plates were mixed thoroughly by using conventional mixing procedures, and allow to solidify (5 to 10 minutes) on a level surface.

8- The plates were then inverted and incubated for 24 hourse at 35°C.

9- After incubation, the number of colonies (CFU/g) was counted, using plates with 25-250 colonies (119).

2.4 Total Coliform Count Test:

Figure 2.2 shows the procedure for total coliform count.

The procedure outline in following steps:

1- Using a suitable micropipet and sterile disposable tips, 1 ml of the homogenate mixture (0.1 ml concentration) was aseptically transferred to a sterile 9 ml normal saline tube, to give a concentration of 0.01.

2- Step 1 was repeated to give the appropriate decimal dilution for each specimen.

3- 1 ml from each decimal dilution was then transferred aseptically each to one of the two sterile plates having the sample number and the decimal dilution of the sample.

4- About 10 to 15 ml of violet red bile agar (VRBA), tempered to 44-46 °C, were poured each into one of the two plates.

5- Duplicate plates and agar control plates were run for each series of samples.

6- 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.

7- The contents of the plates were mixed through by using conventional mixing procedures, and allow to solidify (5 to 10 minutes) on a level surface.

8- The plates were then inverted and incubated for 24 hours at 35 °C.

Coliforms in VRBA appear as typical dark red colonies normally measuring at least 0.5 mm in diameter on uncrowded plate (59).

After incubation, the number of coliform colonies (CFU/g) was counted, using plates with 15-150 coliform colonies (59).

2.5 Identification of Coliform Bacteria (Lactose Fermenter):

Four well-separated dark red colonies, which were suspected to be coliform from uncrowded violet red bile agar were randomly chosen for biochemical identification (22, 59, 119, 120).

Gram stain, oxidase, catalase, triple-sugar iron, urea, and IMViC tests were carried out for each colony.

2.5.1 Gram Stain:

The Gram stain is a very useful stain for identifying and classifying bacteria. The Gram stain is a differential stain that allows you to classify bacteria as either gram-positive or gram-negative. The Gram staining technique was discovered by Hans Christian Gram in 1884,

when he attempted to stain cells and found that some lost their color when excess stain was washed off (119).

Gram stain was done as follows:

1- Using a sterile loop, part of isolated colony was emulsified in one drop of sterile saline & spread on a slide to make a thin film. The slide was left to dry, then fixation by passing through the flame three times, and left to cool.

2- The smear was covered with crystal violet and left for 30 seconds.

3- The slide was washed carefully with water and covered with gram iodine for 30 seconds.

4- The smear was then decolorized with acetone alcohol until no large amounts of purple wash out (usually a few seconds).

5- Then the smear was washed gently with distilled water & countered stained with safranin for 30 seconds, and wash with distilled water and was blot with a paper towel or absorbent paper.

6- This is followed by microscopic examination of the smear using the oil immersion objectives at 1000X.

2.5.2 Spot Oxidase Test (Kovac's Method):

The cytochrome oxidase enzyme is able to oxidize the substrate tetramethyl-p-parapheny-line diamine dihydrochloride, forming a colored end product, indophenol. The dark-purple end product will be

visible if a small amount of growth from a strain that produces the enzyme is rubbed on substrate-impregnated filter paper (22).

The test is outlined in the following procedure:

1- With a sterile platinum loop or wooden stick, a small portion of the bacterial colony to be tested, is smeared on a filter paper moistened with oxidase reagent (0.1% tetramethyl-p-paraphenyline diamine dihydrochloride).

A positive reaction (oxidase positive) was recognized by dark purple color, developed in 5 to 10 seconds followed by black color due to death of bacteria.

Coliform is oxidase negative. Colonies of *E.coli* (oxidase negative) and *Neisseria gonorrhoeae* (oxidase positive) were used as controls.

2.5.3 Catalase Test [3% H_2O_2 Solution]:

The enzyme catalase catalyzes the libration of water and oxygen from hydrogen peroxide, a metabolic end product toxic to bacteria. When a small amount of an organism that produces catalase is introduced into hydrogen peroxide, rapid elaboration of bubbles of oxygen occurred (22).

The test is outlined in the following procedures:

1- With a loop or sterile wooden stick, a small amount of pure bacterial growth is transferred from the agar culture onto the surface of a clean, dry glass slide.

2- Immediately, a drop of 3% hydrogen peroxide (H_2O_2) is placed onto the bacterial growth on the slide.

Bubbles of gas are released with catalase positive organisms.

Colonies of *Staphylococcus aureus* and *Streptococcus pyogenes* were used as control.

2.5.4 Triple Sugar Iron (TSI) Test:

TSI agar is very useful in the presumptive identification of enetrics, particularly in secreening for intestinal pathogens. The formulas for TSI agar is it contains sucrose in addition to glucose and lactose. The contents of TSI agar are outlined in the followings (2, 120):

a- Lactose is present in a concentration 10 times that of glucose (10:1) ratio.

b- Sucrose is present in a concentration 10 times that of glucose (10:1) ratio.

c- Ferrous sulfate (0.02%) and thiosulfate are added to detect the production of hydrogen sulfide.

d- Phenol red is used as the pH indicator, which is yellow below the pH of (6.8). Uninoculated medium is red, since the pH is buffered at (7.4).

TSI agar is useful in detecting the ability of the microorganism to produce gas from the fermentation of sugars.

The test is outlined in the following procedure:

1- With sterile inoculating needle, the center of the bacterial colony is picked up (the colony was lightly touched), and inoculated into TSI agar slant tubes by streaking the slant and stabbing the butt.

2- The tubes are incubated at 35°C for 24 hr.

Table 2.1: Shows typical reactions on TSI (120):

Organism	Butt	slope	H_2S
Escherichia coli	AG	A	Negative
Salmonella sp.	A or AG	NC or ALK	Some Salmonellae are positive
Enterobacter sp.	AG	A	Negative
Proteus vulgaris	AG	NC or ALK	Positive
Morganella morgianii	A orAG	NC or ALK	Negative
Shigella sp.	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).

2.5.5 Urease Test:

The alkaline end products cause the indicator phenol red to change from yellow to pink or red. This test can be used as a component of screening tests for lactose-negative colonies on differential media plated with material from stool specimens, helping to differentiate *Salmonella* and *Shigella sp.* which are urease negative, from the urease-positive non-pathogens (22).

The test is outlined in the following procedure:

1- With a sterile inoculating needle, a small portion of the bacterial colony was streaked on the surface of urea agar slant.

2- The tubes were incubated at 35 °C for 24 hr.

Positive test is indicated by development of pink color, where as in negative test no change in the color of the media can be detected.

Colonies of *Proteus* (urease positive) and *E.coli* (urease negative) were used as control

2.5.6 Indole-Methyl Red-Voges Proskauer Citrate (IMViC) Test:

The clinical microbiology laboratory must identify bacteria quickly and accurately. Accuracy is improved by using standardized tests. The IMViC tests were developed as means of separating enterics, particularly the coliforms,* using a standard combination of four tests (2, 119, 120).

Table 2.2: IMViC reactions for selected species of enterics:

Species	indole	Methyl red	Voges-Pros-kauer	Citrate
Escherichia coli	+ (v)	+	-	-
Citrobacter freundii	-	+	-	+
Enterobacter aerogenes	-	-	+	+
Enterobacter cloacae	-	-	+	+
Serratia marcescens	-	+ or - *	+	+
Proteus vulgaris	+	-	+	- (v)
Proteus mirabilis	-		- or + **	+ (v)

V = variable

* majority of strains give + results

** majority of strains give – results

2.5.6.1 Sulfide, Indole and Motility (SIM) Test:

The test is outlined in the following procedure:

1- With a sterile inoculating needle, a tube of SIM media was aseptically inoculated with a part of bacterial colony by stabbing the media once to depth of ½ to ¼ inch.

2- The tubes were incubated at 35 °C for 18 hr or longer.

SIM Reaction:

a- Sulfide: Blacking the medium, indicates H_2S production sulfide positive. No change in the color of medium, indicates sulfide negative.

b- Indole: Indole is one of the degradation products of the amino acid tryptophan. For testing indole production, addition of 0.2 ml (1 to 2 drops) Kovac's reagent (p-dimethyl aminobenzaldehyde) to the SIM tube will result in cherry red color in the top layer of the tube within 10 seconds, which indicates positive test.

c- Motility: Motile organisms show either a diffuse even growth spreading from the inoculum, or turbidity of the whole medium, whereas non motile organisms grow only along the line of inoculation.

2.5.6.2 Methyl Red-Voges Proskauer (MR-VP) Test:

The MR-VP test is used to distinguish between organisms that produce large amounts of acid from glucose and those that produce the natural product acetone. MR-VP medium is a glucose-supplemented nutrient broth used for the methyl red (MR) test and the Voges-Proskauer (V-P) test.

Rapid Methyl Red Test (121):

The test is outlined in following procedure:

1- Using a sterile inoculating loop, a portion of bacterial colony was aseptically inoculated into a sterile tube of 2 ml MR-VP broth.

2- The tube was incubated at 35 °C for 18 hr.

3- Using automatic pipette, 0.5 ml of MR-VP culture was transferred under a sterile condition to another sterile tube for methyl red test.

4- One drop of methyl red reagent was added especially to the tube. The reagent prepared by (0.1 g) methyl red dissolved in 300 ml ethyl alcohol and then completed to 500 ml by distilled water.

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

Voges-Proskauer Test:

1- The remainder of the MR-VP culture was incubated at 35°C for additional 30 hr.

2- Using automatic pipette, 0.6 ml of 5% 1-naphthol solution (5 g of 1-naphthol in 100 ml of ethyl alcohol) and 0.2 ml of 40% potassium hydroxide with creatine (40 g KOH in 100 ml distilled water) was added to the remainder incubated culture of MR-VP tube and mixed.

3- 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 where as a negative reaction was indicated by colorless or yellow color.

Colonies of *Escherichia coli* (MR-positive, VP-negative) and *Klebsiella pneumonia* (MR-negative, VP-positive) were used as control (22, 121).

2.5.6.3 Citrate utilization:

The citrate utilization test determines whether the organism can utilize sodium citrate as the sole source of carbon for metabolism. The alkaline pH that results from the use of citrate turns the indicator in the medium from green to blue. It is important to keep the inoculum light, since dead organisms can be a source of carbon, producing a false-positive reaction (2, 22).

The test is outlined in the following procedure:

1- With a sterile inoculating needle, a portion of bacterial colony, was picked and streaked on Simmon citrate agar slant surface.

2- The tube was incubated at 35 °C for 24 hr with a loose cap.

A positive reaction was indicated by growth of the organism on the slant, with change of the color indicator from green to blue, where as, a negative reaction was indicated by no growth or very little growth and no color change. *Klebsiella pneumonia* (citrate positive) and *E.coli* (citrate negative) were used as control.

2.6 Isolation of *E.coli O157:H7*:

The *E.coli O157:H7* culture may be performed using MacConkey agar containing sorbital instead of lactose. *E.coli O157:H7* does not ferment sorbitol in 48 hours, a characteristic that differentiates it from most *E.coli*. The use of this differential medium facilities the primary screening of *E.coli O157:H7*, which ordinarily would not be distinguished from other *E.coli* on lactose-containing MacConkey or other routine enteric agar. *E.coli O157:H7* appears colorless on sorbitol-MacConkey agar (2). The commercially available MUG assay (4-methylumbelliferyl β-D-glucuronide) is a biochemical test that may

be used to screen for *O157:H7*, in addition to testing for sorbitol fermentation (75, 120, 123, 124).

E. coli O157:H7 rarely produces the enzyme β-D-glucuronidase, whereas 92% of the other strains do (2). Figure 2.3 shows the isolation of *E.coli O157:H7*.

Steps for the isolation of *E.coli O157:H7* were outlined in the following procedure:

2.6.1 Selective Enrichment:

Lauryl tryptose broth was used as enrichment media. Enrichment media contain chemicals that enhance the growth of desired bacteria. Other bacteria will grow, but the growth of the desired bacteria will be increased (119).

1- Approximately 10 gm of meat was aseptically blended for 2 minutes with 90 ml of sterile lauryl treptose broth to get 1:10 homogenate of the meat.

2- The mixture was aseptically transferred to a sterile 250 ml bottles, which was incubated at 35 °C for 24 hr.

2.6.2 Differential Growth:

1- With a sterile 3 mm loop, a loop-full of lauryl treptose broth culture was aseptically taken from the incubated lauryl treptose broth, and streaked onto eosin methyline blue agar (EMB).

2- The plates were incubated at 35 °C for 24 hr.

3- Wet a sterile loop, at least 3 colonies of *E.coli* (exhibit a green metalic sheen by reflected light, and dark purple centers by transmitted light) were aseptically taken from the incubated EMB agar and streaked onto MacConkey sorbitol agar (MSA) for each colony.

4- The plates were incubated at 35 °C for 24 hr.

5- Four sorbitol negative colonies (white) from the incubated MacConkey sorbitol agar were selected, and each colony was streaked onto MacConkey sorbitol agar containing 4-methylumbelliferyl-β-D-glucuronide (MUG).

6- The plates were incubated at 35 °C for 24 hr.

The isolated colonies 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.

E.coli O157:H7 show the same biochemical reactions like other *E.coli* strain. So all cultures which produce acid (yellow) slant and acid

(yellow) butt in TSI agar, urease negative and ++— IMViC tests (22, 120).

2.6.3 Serological Test:

For the identification of *E.coli* serogroup O157, a latex agglutination test [Unipath Limited, Basingstoke, Hampshire, England] had been used.

Serological test outlined in following procedure:

1- After the reagent had been brought 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 the latex and saline were not mixed at this stage.

4- With sterile loop, a portion of the bacterial growth was picked off aseptically from fresh nutrient agar slant culture and then emulsified carefully in the saline drop.

5- With sterile loop, the test latex and suspension were mixed together and spreaded to cover the reaction area.

6- The loop was flamed. 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, whereas negative reaction was indicated by absence of agglutination.

In positive reaction case, to ensure that the isolate was not an auto-agglutination strain, a further portion of the colony was tested with the control latex reagent.

2.7 Isolation of *Salmonella*:

Figure 2.4 shows the isolation of *Salmonella* from meat, and figure 2.5 shows the isolation of *Salmonella* from fresh eggs.

Steps for the isolation of *Salmonella* from meat were outlined in the following procedure (22, 63):

2.7.1 Pre-enrichment:

1- About 25 g of meat sample were blended with 225 ml of sterile nutrient broth in a mixer for 2 minutes to get a homogenate mixture. The mixture was then aseptically transferred to 500 ml sterile bottles.

2- The cap was loosen and the mixture was incubated at 35 °C for 24 hr.

2.7.2 Selective Enrichment:

1- After mixing the incubated homogenate mixture, 1 ml was transferred aseptically by a micropipet to 10 ml tetrathionate broth (120,125).

2- The tube was incubated at 35 °C for 24 hr.

2.7.3 Selective Growth:

1- After incubation , the tubes were mixed by a vortex.

2- With a sterile loop, 3 mm loop-full were aseptically taken from each tube and streaked on xylose lysine desoxycholate (XLD) agar and *Salmonella-Shigella* (SS) agar.

3- The plates were incubated at 35 °C for 24 hr.

4- In the following day, the plates were examined for the presence of *Salmonella* suspected colonies.

On XLD agar, *Salmonella* appeared as pink-red colonies with or without black centers, while on SS agar, *Salmonella* appeared as colorless colonies with or without black centers (2).

For the isolation of *Salmonella* from eggs the same procedure for isolation of *Salmonella* from meat was used.

However, one egg contents were added to 50 ml of sterile nutrient broth, after disinfecting the egg shell with tincture of iodine & 70% ethanol.

2.7.4 Biochemical Conformation:

2.7.4.1 Triple Sugar Iron Test (TSI):

1- With a sterile inoculating needle, at least three colonies typical or suspected to be *Salmonella* were selected from selective agar (XLD and SS), (the very center of the colony) to be picked was lightly touched and inoculated into TSI agar slant by streaking the slant and stabbing the butt.

2- The TSI tube was incubated at 35 °C for 24 hr with loose cap to maintain aerobic condition.

Cultures which produced alkaline (red) slant and acid (yellow) butt with or without production of H_2S (blacking) in TSI agar, were retained as potential *Salmonella* isolates and were submitted for other biochemical and serological tests (63).

2.7.4.2 Urease Test:

1- With a sterile inoculating needle, small amount of growth from TSI agar suspected to be *Salmonella* was aseptically taken and inoculated into urea agar slant by streaking the slant.

2- The urea agar tube was incubated at 35 °C for 24 hr.

Culture that give negative urease test (no change in color of the medium) were retained for further identification, whereas cultures cause

positive urease test (change in color of medium to pink color) were discarded (63).

2.7.4.3 Lysine Iron Agar Test:

1- With a sterile inoculating needle, small amount of growth from TSI agar suspected to be *Salmonella* was aseptically taken and inoculated into lysine iron agar slant by streaking the slant and stabbing the butt.

2- The lysine iron agar tube was incubated at 35 °C for 24 hr.

Cultures that gave alkaline reaction at lysine iron agar (purple color through out the medium) were considered as suspected *Salmonella* and were retained for further identification, whereas cultures caused development of yellow color throughout medium were discarded (63).

2.7.4.4 SIM Test:

All *Salmonella sp.* are indole negative as indicated by absence of a dark red ring in the reagent layer after the addition of Kovac's reagent.

Most *Salmonella sp.* are motile organisms as indicated by producing cloudiness in the medium or by growing in brush – like patterns around the line of inoculation. Some *Salmonella sp.* Produce

hydrogen sulfide (H_2S) as indicated by blackening of the line of inoculation or the whole tube, whereas others can not (63).

1- With a sterile inoculating needle, growth on the TSI agar slant, biochemicaly identified as *Salmonella* was aseptically inoculated into sterile nutrient agar slant by streaking the slant.

2- The slant was incubated at 35 °C for 24 hr.

From fresh nutrient agar slant culture, gram stain, oxidase, catalase and serological tests were done.

2.7.5 Serological Test:

Serological test was carried out by *Salmonella* polyvalent somatic (O) antiserum (Murex ZCO2).

Serological test outlined in the following procedure:

1- After the reagent had been brought to room temperature, vigorous shaking to mix the latex suspensions.

2- On a clean slide, with a sterile loop, portion of the fresh, pure isolates of suspected colony which had been cultured on nutrient agar was emulsified in sterile saline to obtain smooth, fairly dense suspensions.

3- One drop of undiluted antiserum was added to the suspension followed by mixing.

4- The slide was then rocked gently for one minute and observed for agglutination using indirect light over a dark background.

Positive reaction was indicated by agglutinaion with the test reagent within one minute.

As control, one drop of saline was added to another bacterial suspension.

2.8 Statistical Package

Statistical analysis: EPI2000 statistical system was used. χ^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 < 0.05 it indicates significant relation, and a value > 0.05 indicates no significant relation.

Figure 2.1 Preparation of 10-fold dilutions of the sample for total bacterial count.

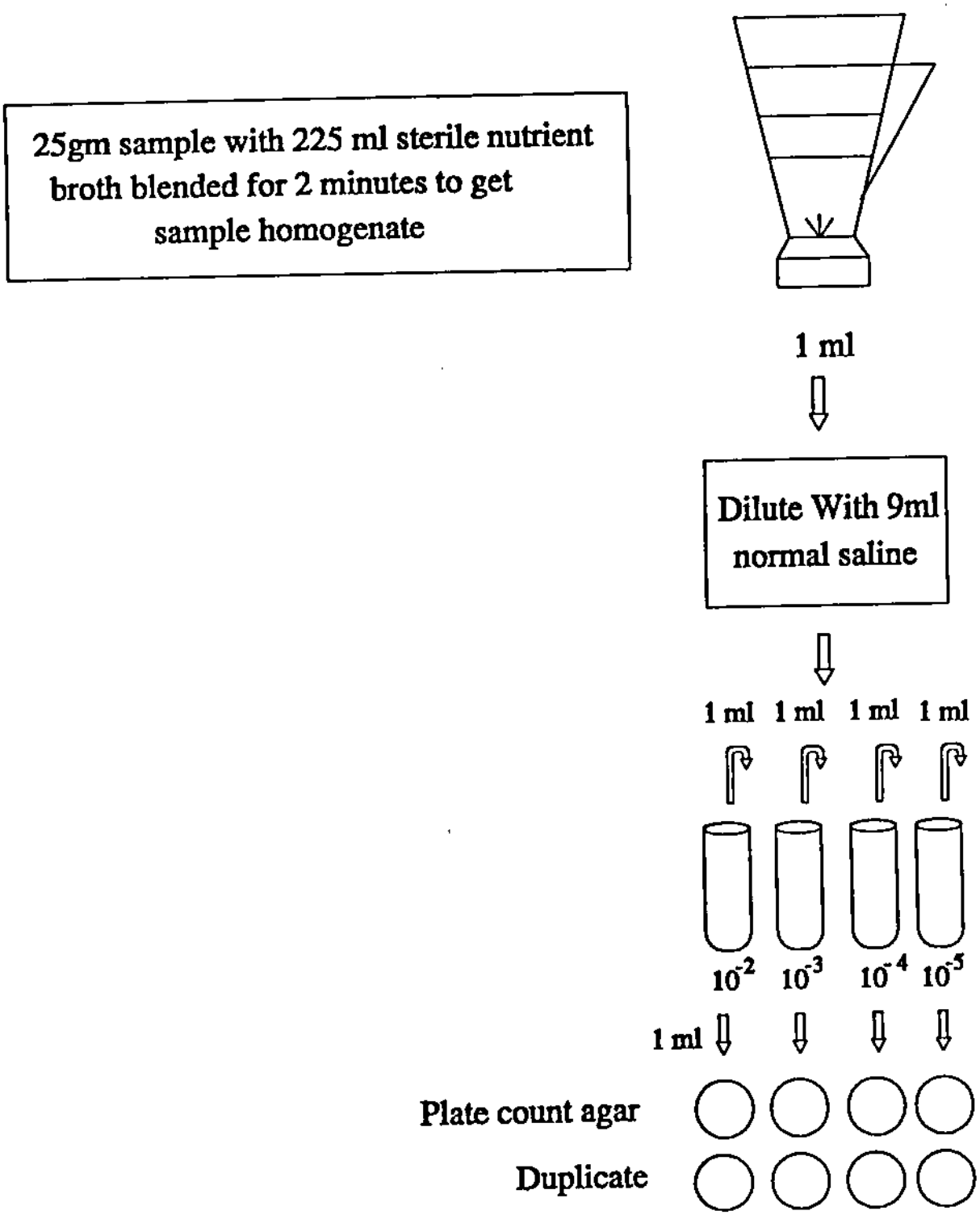

Figure 2.2 Preparation of 10-fold dilutions of the sample for total coliform count.

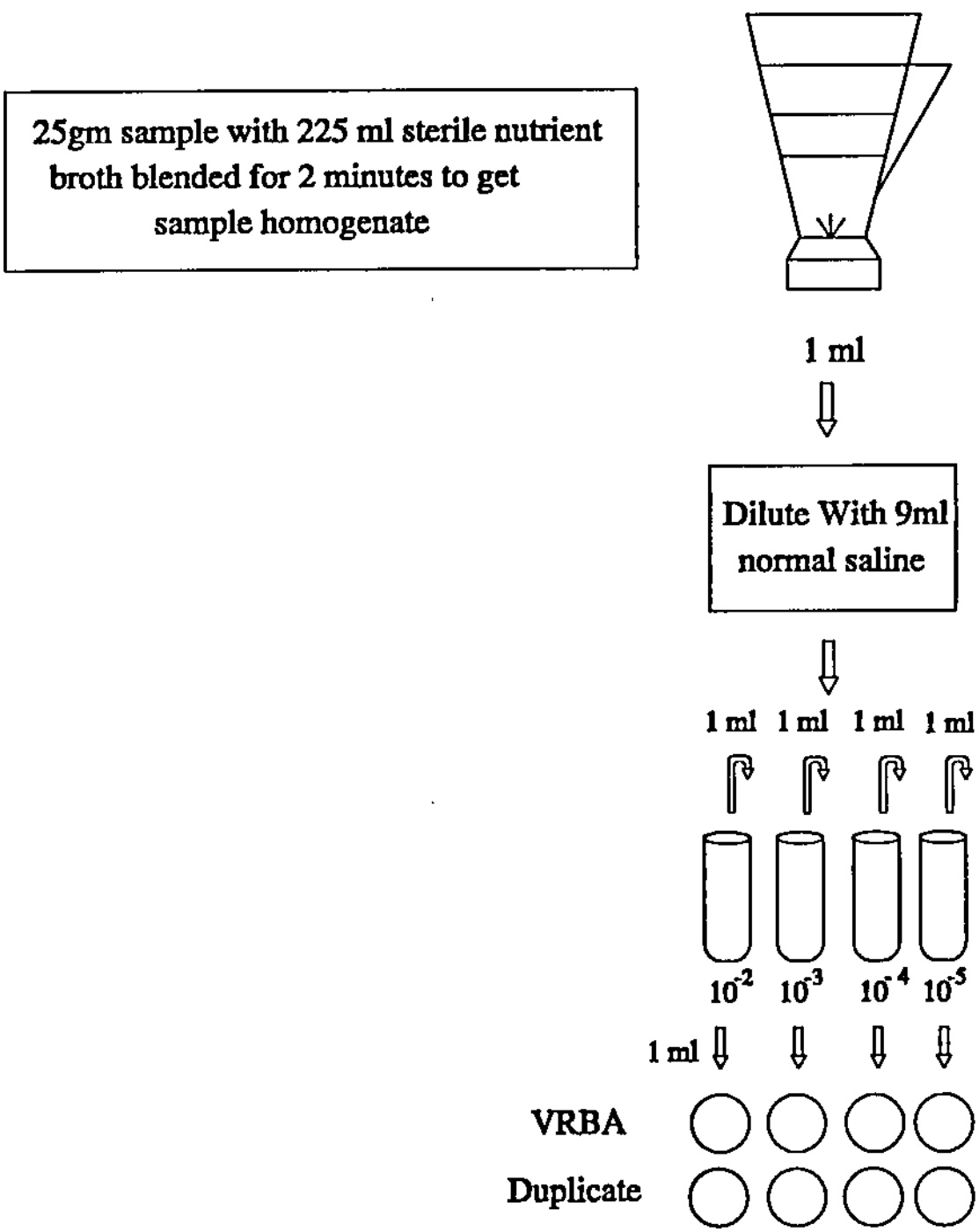

Figure 2.3 Isolation of E.coli O157:H7

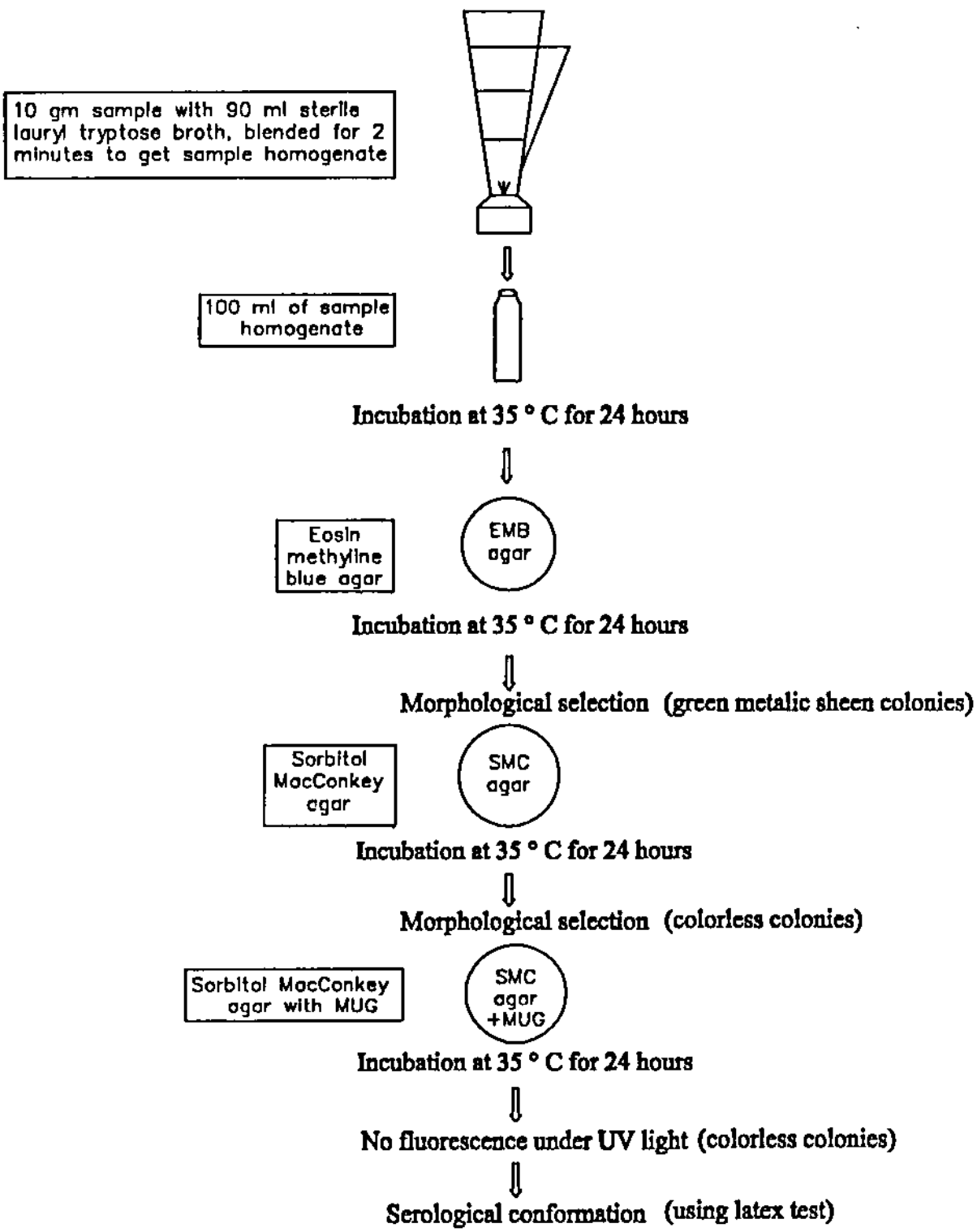

Figure 2.4 Isolation of Salmonella from meat

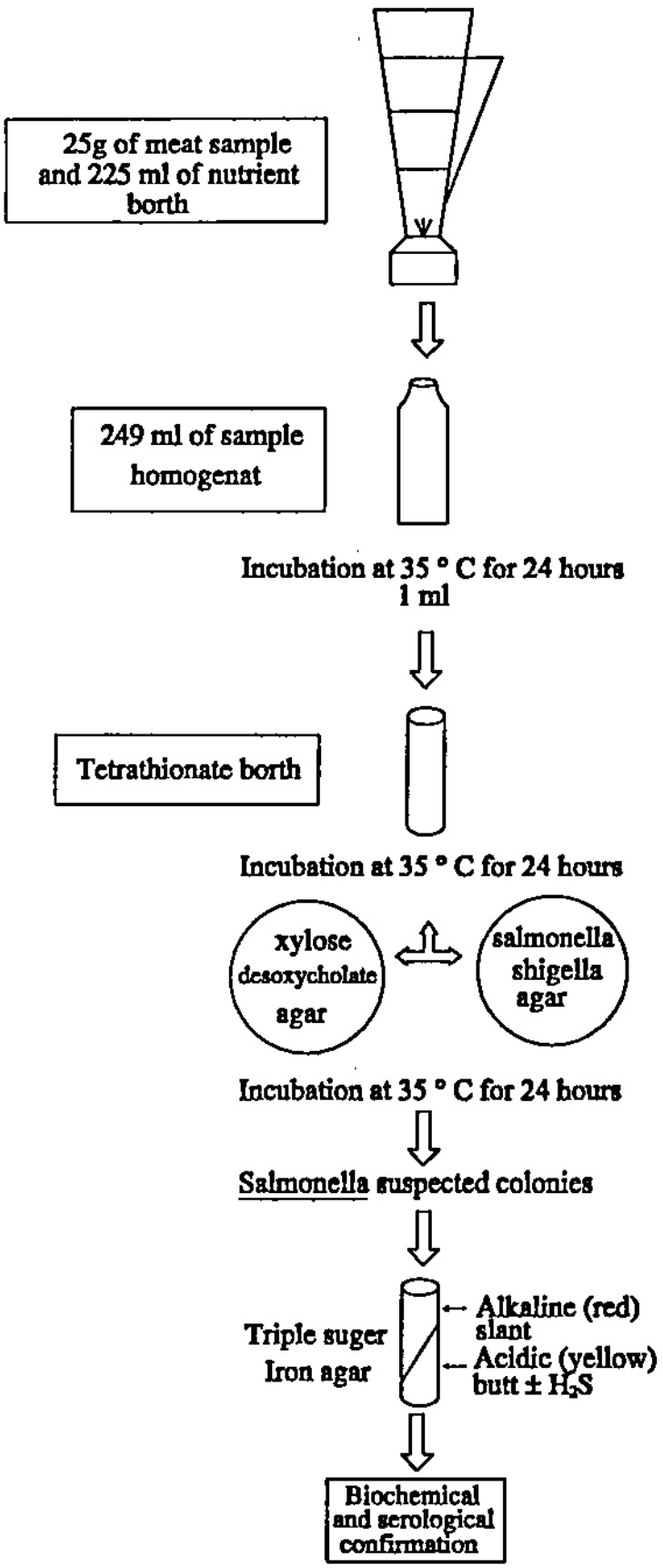

Figure 2.5 Isolation of Salmonella from fresh chicken eggs

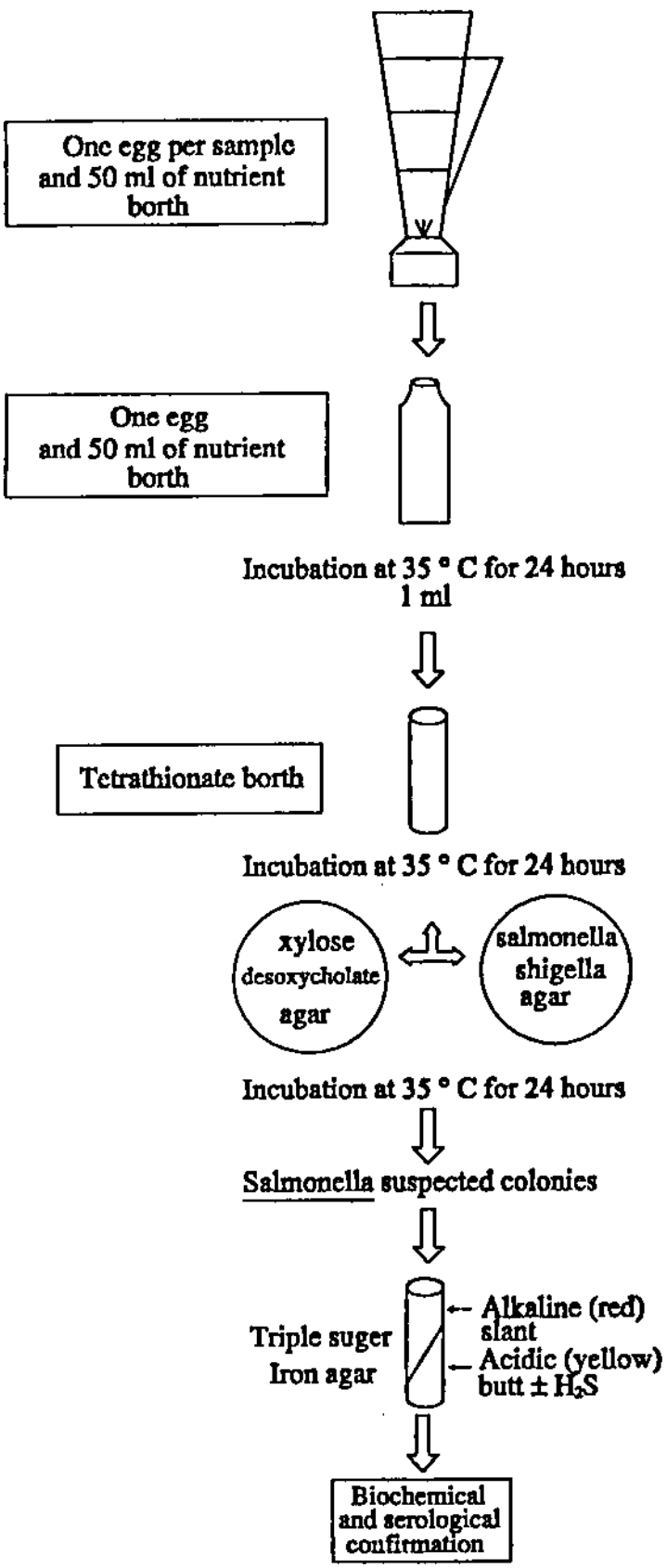

Table 2.3: Biochemical reactions for identification of enteric organisms:

Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
						Motility	Indole	Sulfide		Gas	H2S	Butt	Slant	
-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	*Citrobacter*
-	-	+	+	-	-	+	-	-	-	d	-	A	A	*E.coli*
-	-	+	-	+	+	-	d	-	d	+	-	A	A	*Klebsiella*
-	-	+	+	-	-	+	+	-	+	+	-	A	Alk	*Morgenella*
-	-	+	+	-	-	+	-	+	+	+	+	A	Alk	*Proteus*
-	+	+	-	-	+	+	-	-	-	-	-	Alk	Alk	*Pseudomonas*
-	-	+	+	-	V	+	-	V	-	V	V	A	Alk	*Salmonella*
-	-	+	+	-	+	+	-	-	-	V	-	A	Alk/A	*N.I.*

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaeur

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.

V: Variable (-ve or +ve).

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

CHAPTER THREE

3. RESULTS

During the period from September 2000 to April 2001, a total of 290 samples, 150 samples of meat including 76 samples of fresh turkey meat, 74 samples of imported frozen meat (68 cattle, 6 sheep), and 140 samples of fresh chicken eggs, were studied for the total bacterial count, total coliform count, and incidence of *Salmonella* and *E.coli O157:H7*.

3.1 Total Bacterial Count:

For determining the total bacterial count, plate count agar (PCA) plates that contained between 25-250 colonies were considered. The total bacterial count (TBC) was calculated by multiplying the reciprocal of the dilution used by the average number of the bacterial colonies of two plates. It is reported as CFU/g.

Total bacterial count = number of colonies × reciprocal of the dilution

As shown in table 3.1, the average total bacterial count for fresh turkey meat, frozen cattle meat, and frozen sheep meat was 4.1×10^6 CFU/g, 6.5×10^6 CFU/g, and 2.03×10^6 CFU/g respectively.

3.2 Total Coliform Count:

For determining the total coliform count (TCC), violet red bile agar (VRBA) plates that contained between 15-150 round purple-red colonies were considered. The total coliform count was calculated by multiplying the reciprocal of the dilution used by the average number of the bacterial colonies of two plates. It is reported as CFU/g.

Total coliform count = number of round purple-red colonies × reciprocal of the dilution.

As shown in table 3.1, the average total coliform count for fresh turkey meat, frozen cattle meat and frozen sheep meat was 4.24×10^5 CFU/g, 2.55×10^5 CFU/g, and 0.42×10^5 CFU/g, respectively.

Tables 3.2, 3.3, 3.4, 3.5 and 3.6 show the different biochemical reactions for the identification of the different types of enteric organisms in fresh turkey meat, frozen cattle meat, frozen sheep meat and fresh chicken eggs. *Proteus* was found to be the predominant enteric organisms in fresh turkey meat, whereas *E.coli* was the predominant enteric organisms in imported frozen red meat (cattle and sheep meat).

3.3 *Salmonella:*

Morphological appearance of *Salmonella* was as follows (2, 120):

- On (SS) agar: colorless colonies with or without black centers.
- On (XLD): red colonies with or without black centers.

The bacterial isolates that gave alkaline/acid (red/yellow) on TSI media, urea negative (yellow), lysine decarboxylase positive (violet/violet), and indole negative (absence of a red color in the top layer of SIM tube), were suspected to be *Salmonella*, and were subjected to further identification by serological test.

As shown in Table 3.7, 3.8, four (5.3%) samples of fresh turkey meat & one (1.5%) sample of frozen cattle meat (imported beef meat from China) were positive for *Salmonella*. Thus a total of five (3.3%) samples were *Salmonella* positive of the total meat samples and (1.7%) of the total samples (meat and egg samples). However, samples of frozen sheep meat & all fresh chicken eggs were negative for *Salmonella*.

3.4 *E.coli O157:H7*:

The bacterial isolates that were MUG negative (no flourescence 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 studied samples (frozen red meat, and fresh turkey meat) were negative for *E.coli O157:H7.*

3.5 Acceptable and Unacceptable Fresh Turkey Meat and Frozen Red Meat Samples:

As it is evident from (Table 3.7), the total number of fresh turkey meat, frozen cattle meat, and frozen sheep meat acceptable samples were 32 (42.1%), 48 (70.6%) and 5 (83.3%) respectively. While that for unacceptable samples was 44 (57.9%) for fresh turkey meat, 20 (29.4%) for frozen cattle meat and 1 (16.7%) for frozen sheep meat.

Thus the total number of acceptable samples, for both fresh turkey meat and frozen red meat was 85 (56.7%), while that of unacceptable samples was 65 (43.3%) (Table 3.7). The total number of fresh chicken egg acceptable samples was 140 (100%) (Table 3.7).

Table 3.9 shows the correlation between the average (TBC) and *Salmonella* positive and negative samples. The average (TBC) for fresh turkey meat and frozen cattle meat, *Salmonella* positive samples were 5.83×10^{4} and 5×10^{3} respectively, while that for fresh turkey meat, frozen

cattle meat, and frozen sheep meat, *Salmonella* negative samples were 4.33×10^6, 6.56×10^6, and 2.03×10^6, respectively.

The average (TBC) for negative *E.coli O157:H7* for fresh turkey meat, frozen cattle meat, and frozen sheep meat were 4.1×10^6, 6.5×10^6, and 2.03×10^6, respectively.

Table 3.10 showed that *Salmonella* positive fresh turkey meat and frozen cattle meat samples have 1.2×10^5 & 0.00 TCC, respectively, while *Salmonella* negative fresh turkey meat, frozen cattle meat, and frozen sheep meat have 4.414×10^5, 2.59×10^5, and 4.2×10^4 TCC/g, respectively.

The average (TCC) for negative *E.coli O157:H7* for fresh turkey meat, frozen cattle meat and frozen sheep meat were 4.24×10^5, 2.55×10^5, and 4.2×10^4, 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)
Fresh turkey meat	4.1×10^6	4.24×10^5
Frozen cattle meat	6.5×10^6	2.55×10^5
Frozen sheep meat	2.03×10^6	0.42×10^5

Table 3.2: Biochemical reactions for identification of enteric organisms in **fresh turkey meat**:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H2S	Butt	Slant	
1	16	-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	*Citrobacter*
2	38	-	-	+	+	-	-	+	-	-	-	d	-	A	A	*E.coli*
3	19	-	-	+	-	+	+	-	d	-	d	+	-	A	A	*Klebsiella*
4	40	-	-	+	+	-	-	+	+	-	+	+	-	A	Alk	*Morgenella*
5	89	-	-	+	+	-	-	+	-	+	+	+	+	A	Alk	*Proteus*
6	5	-	+	+	-	-	+	+	-	-	-	-	-	Alk	Alk	*Pseudomonas*
7	4	-	-	+	+	-	V	+	-	V	-	V	V	A	Alk	*Salmonella*
8	25	-	-	+	+	-	+	+	-	-	-	V	-	A	Alk/A	*N.I.*

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaeur

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.

V: Variable (-ve or +ve).

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

Table 3.3: Biochemical reactions for identification of enteric organisms in **frozen cattle meat**:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H2S	Butt	Slant	
1	13	-	-	+	+	-	+	+	d	d	d	d	d	A	Alk/A	*Citrobacter*
2	55	-	-	+	+	-	-	+	-	-	-	d	-	A	A	*E.coli*
3	9	-	-	+	-	+	+	-	d	-	d	+	-	A	A	*Klebsiella*
4	21	-	-	+	+	-	-	+	+	-	+	+	-	A	Alk	*Morgenella*
5	34	-	-	+	+	-	-	+	-	+	+	+	+	A	Alk	*Proteus*
6	4	-	+	+	-	-	+	+	-	-	-	-	-	Alk	Alk	*Pseudomonas*
7	1	-	-	+	+	-	V	+	-	V	-	V	V	A	Alk	*Salmonella*
8	25	-	-	+	+	-	-	+	+	-	-	+	-	A	Alk/A	*N.I.*

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaeur

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.

V: Variable (-ve or +ve).

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

Table 3.4: Biochemical reactions for identification of enteric organisms in **frozen sheep meat**:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H2S	Butt	Slant	
1	4	-	-	+	+	-	-	+	-	-	-	d	-	A	A	*E.coli*
2	3	-	-	+	+	-	-	+	+	-	+	+	-	A	Alk	*Morgenella*
3	2	-	-	+	+	-	-	+	-	+	+	+	+	A	A	*Proteus*
4	3	-	-	+	+	-	+	+	-	-	-	V	-	A	Alk/A	*N.I.*

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaeur

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.

V: Variable (-ve or +ve).

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

Table 3.5: Biochemical reactions for identification of enteric organisms in **fresh chicken eggs**:

Colony Number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H2S	Butt	Slant	
1	3	-	-	+	+	-	-	+	-	-	-	d	-	A	A	*E.coli*
2	8	-	-	+	-	+	+	-	d	-	d	+	-	A	A	*Klebsiella*
3	1	-	-	+	+	-	-	+	+	-	+	+	-	A	Alk	*Morgenella*
4	3	-	-	+	+	-	+	+	-	-	-	V	-	A	Alk/A	*N.I.*
5	82	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	*N.I.*

TSI: Triple sugar iron agar

MR: Methyl red

VP: Vogas Proskaeur

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.

V: Variable (-ve or +ve).

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

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

Sample	Number of colonies	Citrobacter %		E.coli %		Klebsiella %		N.I. %		Morgenella %		Proteus %		Pseudomonas	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh turkey meat	232	16	6.9	38	16.4	19	8.2	25	10.8	40	17.2	89	38.4	5	2.2
Frozen cattle meat	161	13	8.1	55	34.2	9	5.6	25	15.5	21	13.0	34	21.1	4	2.5
Frozen sheep meat	12	0	0.0	4	33.3	0	0.0	3	25	3	25	2	16.7	0	0.0
Fresh chicken eggs	97	0	0.0	3	3.09	8	8.25	85	87.63	1	1.03	0.0	0.0	0.0	0.0

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

Sample	Acceptable		Unacceptable															
	No	%	High TBC		High TCC		TBC∩TCC		*Salmonella*		*Salmonella* ∩TBC		*Salmonella* ∩TCC		*E.coli O157:H7*		Total	%
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Fresh turkey meat	32	42.1	24	31.6	38	50	19	12.7	4	5.3	0	0	3	2	0	0	44	57.9
Frozen cattle meat	48	70.6	16	23.53	15	22.06	12	8	1	1.5	0	0	0	0	0	0	20	29.4
Frozen sheep meat	5	83.3	1	16.7	1	16.7	1	0.7	0	0	0	0	0	0	0	0	1	16.7
Total	85	56.7	41	27.3	54	36.1	32	21.4	5	3.3	0	0	3	2	0	0	65	43.3
Fresh chicken egg	140	100	—	—	—	—	—	—	0	0	—	—	—	—	—	—	0	0

Note: for total coliform 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 percentages of *Salmonella* and *E.coli O157:H7* in different samples:

Sample	Number of Samples	*Salmonella* Positive		*E.coli O157:H7* Positive	
		No.	%	No.	%
Fresh turkey meat	76	4	5.3	0	0
Frozen cattle meat	68	1	1.5	0	0
Frozen sheep meat	6	0	0	0	0
Fresh chicken eggs	140	0	0	0	0
Total	290	5	1.7	0	0

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

Sample	Average TBC (CFU/g) in *Salmonella*		Average TBC (CFU/g) in *E.coli O157:H7*	
	+ve samples	-ve samples	+ve samples	-ve samples
Fresh turkey meat	5.83×10^{4}	4.33×10^{6}	---	4.1×10^{6}
Frozen cattle meat	5×10^{3}	6.56×10^{6}	---	6.5×10^{6}
Frozen sheep meat	---	2.03×10^{6}	---	2.03×10^{6}
Fresh chicken eggs	---	---	---	---

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

Sample	Average TCC (CFU/g) in *Salmonella*		Average TCC (CFU/g) in *E.coli O157:H7*	
	+ve samples	-ve samples	+ve samples	-ve samples
Fresh turkey meat	1.2×10^5	4.414×10^5	---	4.24×10^5
Frozen cattle meat	0.0	2.59×10^5	---	2.55×10^5
Frozen sheep meat	---	4.2×10^4	---	4.2×10^4
Fresh chicken eggs	---	---	---	---

CHAPTER FOUR

4. DISCUSSION

4.1 Discussion:

Based on the Palestinian Standards Institution (PSI) for the standards of total bacterial count and *Salmonella* (Table 1.3), and according to the Palestinian Ministry of Health for total coliform count, the samples had a total bacterial count (< 1×10^6 CFU/g), were *Salmonella* negative (0/25 g), and a total coliform count (< 1×10^3 CFU/g) were considered as acceptable samples, otherwise were rejected and considered as unacceptable samples.

This study showed that the fresh turkey meat have the highest unacceptable samples, as 5.3% of the samples are positive for *Salmonella*, 31.6% of the samples have high TBC, and 50% of the samples have high TCC. The low TCC of some meat samples, specially frozen imported red meat may be due to the followings:

1- The separation between meat and animal intestine to prevent contamination.

2- The temperature of frozen meat is very low which lead to inhibition of bacterial activity and multiplication.

Statistical analysis using EPI2000 into statistical system was used. χ^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 < 0.05 it indicates significant relation, and a value > 0.05 indicates no significant relation.

The results showed significant relation (P=0.000) between the type of meat samples and numbers of acceptable or unacceptable samples (Table 4.2). There was also significant relation (P=0.000) between the type of meat and egg samples and numbers of acceptable or unacceptable samples (Table 4.3). The type of meat samples had no significant relation (P=0.182) with the presence or absence of *Salmonella* (Table 4.4).

Table (4.5) showed that significant relation (P=0.029) was found comparing the type of samples and the presence or the absence of *Salmonella*. Also no significant relation between TBC and TCC on one hand and the presence or absence of *Salmonella* on the other hand. According to Palestinian standards, it was clear that samples positive for *Salmonella* have low TBC. Some samples positive for *Salmonella* have high TCC and other samples positive for *Salmonella* have 0.0 CFU/g as shown in (Table 4.1). This may be due to previous infection of the animal or contamination of meat with the gastrointestinal tract content during skinning and evisceration in slaughterhouse or contamination of meat due to handling of meat in butchers' shops (use contaminated

cutting knives or contaminated cutting board). Thus our results proved that low or absence of coliforms is not an indication of absence of pathogens from meat.

Bacteria play an important rule in spoilage and decomposition of meat and also in food poisoning. Spoilage defects in meat become evident when the number of bacteria at the surface reaches 10^7 CFU/cm^2, and off odors are first detected. When numbers reach 10^8 CFU/cm^2, 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 (1).

Our results showed that the percentage of occurrence of coliform organisms in different kinds of meat samples showed that *E.coli*, *Proteus*, and *Morgenella* were the most frequently isolated coliforms (Table 3.6). This indicates exposure of meat to fecal contamination during slaughtering, evisceration, skinning, and handling.

Kay & Fricker (61), found that *E.coli* compromised 95% of the coliform group, unlike *Klebsiella*, *Enterobacter* and *Citrobacter*, which may be present at a much lower densities in fecal contamination.

Our results which showed that *E.coli*, *Proteus*, and *Morgenella*, are the most frequently isolated coliforms from meat samples, differ from that of Al-Kharraz (1999) and Abu-Seir (2000), which indicates that *E.coli* and *Citrobacter* were the most frequently isolated coliforms from different samples of meat in the butcher shops and slaughterhouse (129, 130).

Comparing ground beef microbial results of Al-Kharraz (1999) showed that 8.1% of the ground beef samples were *Salmonella* positive, and 4.8% were *E.coli O157:H7* positive.

Comparing liver of calf microbial results of Abu-Seir (2000) showed that 8.1% of liver of calf were *Salmonella* positive, and 0.0% were *E.coli O157:H7* positive, which appears higher compared with the microbial results of this work (130).

The presence of different types of bacteria especially pathogenic bacteria (ex. *Salmonella* and *E.coli O157:H7*) in meat is due to many factors. These are (5, 28, 51, 64, 123, 127):

1. Infection of the animal with bacteria (*Salmonella* and *E.coli O157:H7*).
2. The slaughtering of a healthy animal *Salmonella* carrier may lead to gross contamination of the carcasses with *Salmonella*. Dirty hide, 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.

3. The knives used to bleed and cut animals serve as intermediate sources of contaminants as any bacteria on the knife will be found in various parts of the carcass carried there by blood and lymph.
4. Another possible source of contamination may be found in butchers shops or in open air markets where hygiene standards are inadequate especially when wooden cutting surfaces are used which absorb and hold moisture, thus allowing penetration of bacteria through cracks where it can multiply and transmit to the next item by way of wooden surfaces.
5. Keeping meat together with animal intestine and using contaminated scales, slicers, and grinders will increase the possibility of contamination.
6. 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 measure are inadequate.

The different types of bacteria isolated from fresh chicken eggs may enter the egg by penetration the egg shell and inner membrane, where they grow and cause spoilage. This is related to the temperature

and humidity of storage, age of eggs and level of contamination (11, 14). In general, more spoilage of eggs is caused by bacteria then by molds (8).

All types of bacteria isolated from chicken eggs in this work are non-pathogenic enteric bacteria. All tested egg samples are free of *Salmonella*. Accordingly, all egg samples are acceptable. It is known that *Salmonella pullorum gallinarium* group are transmitted through the ovary to the eggs.

Table 4.1: TBC and TCC for samples positive for *Salmonella*:

Type of sample	TBC	TCC
Fresh turkey meat	130000	2000
Fresh turkey meat	0.00	0.00
Fresh turkey meat	0.00	440000
Fresh turkey meat	103000	20000
Frozen cattle meat	5000	0.00

Table 4.2: The relation between the type of meat samples and numbers of acceptable or unacceptable samples:

Type of meat	Acceptable	Unacceptable	Total
Fresh turkey meat	32	44	76
Imported frozen red meat (cattle & cheep)	53	21	74
Total	85	65	150

Chi square = 13.302
Degree of freedom = 1
P-value = 0.000

Table 4.3: The relation between the type of meat and egg samples and numbers of acceptable or unacceptable samples:

Type of samples	Acceptable	Unacceptable	Total
Meat (fresh turkey & frozen red)	85	65	150
Fresh chicken egg	140	0	140
Total	225	65	290

Chi square = 78.193
Degree of freedom = 1
P-value = 0.000

Table 4.4: The relation between the type of meat samples and the presence or absence of *Salmonella*:

Type of meats	+ve	-ve	Total
Fresh turkey meat	4	72	76
Imported frozen red meat (cattle & cheep)	1	73	74
Total	5	145	150

Chi square = 1.781
Degree of freedom = 1
P-value = 0.182

Table 4.5: The relation between the type of meat and egg samples and the presence or absence of *Salmonella*:

Type of samples	**+ve**	**-ve**	**Total**
Meat (fresh turkey & frozen red)	5	145	150
Fresh chicken egg	0	140	140
Total	5	285	290

Chi square = 4.749
Degree of freedom = 1
P-value = 0.029

4.2 Recommendation

To prevent food-borne disease, especially these caused by *Salmonella* and *E.coli O157:H7*, the following prophylactic measures may be taken into account:

4.2.1 For Consumers:

Always handle raw beef, poultry, turkey and eggs as if they are contaminated:

1. Refrigerate foods promptly, don't let them sit at room temperature.
2. Wash cutting boards and counters used for preparation immediately after use to avoid spreading the bacteria to other foods.
3. Don't eat raw or undercooked meats. Beef meat, ground beef or hamburger must be cooked thoroughly. Make certain that cooked meat is gray or brown color throughout (not pink) and the meat juice run clear, the inside should be hot.
4. Keeping eggs adequately refrigerated prevents any *Salmonella* present in the eggs from growing to higher numbers, so eggs should be held refrigerated until they are needed.
5. Discard cracked or dirty eggs.
6. Wash hands and cooking utensils with soap and water after contact with raw eggs.

7. Eat eggs promptly after cooking. Do not keep eggs warm for more than 2 hours.
8. Don't eat raw or undercooked foods containing raw eggs (as in homemade ice-cream or eggnog) Commercially manufactured ice cream and eggnog are made with pasteurized eggs and have not been linked with *S. enteritidis* infections.
9. Avoid restaurant dishes made with raw or undercooked, un pasteurized eggs. Restaurants should use pasteurized eggs in any recipe (such as Hollandaise sauce or Caesar salad dressing) that calls for pooling of raw eggs.
10. Eat and drink only pasteurized milk and milk products (for example, cheese). Don't drink un pasteurized apple cider and untreated water.
11. Wash hands thoroughly with soap and warm water before and after preparing food, eating and feeding children.
12. Make sure children, particularly those who handle pets, wash their hands carefully after handling these animals.
13. Carefully wash hands after changing diapers or using the toilet.
14. Anyone who has diarrhea, should not use a pool or swim in a pond.

4.2.2 For Butchers' Shops:

1. Wrap fresh meats in plastic bags at the market to prevent bloody liquid from dripping on other foods.
2. Keeping fresh meat in refrigerator and frozen meat in freezer until being sold.
3. In the refrigerator, separate between meat and animal intestine.
4. Maintenance of all meat handling in a clean state combined with rodent-proofing and anti-fly measures.
5. All equipment which are used in butchers' shops (such as refrigerator, knives, cutting surfaces) should be washed between carcasses with easy access for regular cleaning and disinfecting.
6. Supervision of the health of food handlers and their education in methods of preventing contamination of foodstuff is very important.

4.2.3 For Future Research:

1. Laboratory examination of meat samples at varying seasons and time periods.
2. Laboratory examination of meat samples from rural areas.
3. Laboratory examination of meat samples for other types of bacteria such as *Listeria monocytogenes*.

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بسم الله الرحمن الرحيم

عزل بكتيريا السالمونيلا وبكتيريا إشريشيا القولون صنف O157:H7 من لحوم الديك الرومي الطازجة ولحوم الماشية المجمدة المستوردة مع التأكيد على عزل السالمونيلا من بيض الدجاج الطازج.

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الملخص العربي

تم جمع 150 عينة لحوم و 140 عينة بيض دجاج طازج بطريقة عشوائية من سوق مدينة نابلس، تشتمل عينات اللحوم على 76 عينة لحوم حبش طازج و74 عينة لحوم حمراء مجمدة والمستوردة من عدة دول في القارة الأوروبية (ألمانيا، هولندا، نيوزلندا، بلجيكا) ودول أخرى مثل البرازيل والصين منها 68 عينة لحوم أبقار وعجول مجمدة و6 عينات من لحوم الخراف المجمدة.

تم فحص العينات لمعرفة عدد البكتيريا الكلي، والعدد الكلي لبكتيريا القولون، كلاهما بطريقة الأطباق المصبوبة. وكذلك البحث عن وجود بكتيريا السالمونيلا *Salmonella* في اللحوم بمختلف أنواعها إضافة إلى بيض الدجاج الطازج، ووجود بكتيريا إشريشيا القولون صنف *E.coli O157:H7*، وذلك لتقييم دور لحوم الحبش الطازجة واللحوم الحمراء المجمدة والمستوردة وبيض الدجاج الطازج كمصدر للإصابة ببعض الأمراض الخطيرة والتسمم الغذائي في منطقة مدينة نابلس وذلك بعدة طرق مرجعية للإكثار، الزراعة، والاختبارات البيوكيميائية والسيرولوجية.

والهدف من معرفة عدد البكتيريا الكلي والعدد الكلي لبكتيريا القولون هو الدلالة على الجودة الميكروبية للحوم بمختلف أنواعها سواء اللحوم الطازجة أو المجمدة والتي شملتها هذه الدراسة، وكذلك الدلالة على درجة تلوث العينات سواء داخلياً من نفس الحيوان أو خارجياً من العوامل المحيطة.

بالاعتماد على المواصفات والمقاييس الفلسطينية الصادرة عن مؤسسة المواصفات الفلسطينية ووزارة الصحة الفلسطينية، تبين أن ما نسبته (43.3%) من العدد الكلي لعينات اللحوم بمختلف أنواعها كان غير مقبول سواء بسبب الزيادة في العدد الكلي للبكتيريا (27.3%) أو بسبب الزيادة في العدد الكلي لبكتيريا القولون (36.1%) أو بسبب وجود بكتيريا السالمونيلا (3.3%) حيث أن ما نسبته (12.7%) من عينات لحم الحبش الطازج غير مقبول بسبب زيادة العدد الكلي للبكتيريا وزيادة العدد الكلي لبكتيريا القولون لنفس العينات و (2%) من عينات لحم الحبش غير مقبول بسبب زيادة العدد الكلي لبكتيريا القولون وبكتيريا السالمونيلا لنفس العينات حيث كان العدد الكلي للبكتيريا لهذه العينات منخفض، إضافة إلى ما نسبته (8%) من عينات اللحم البقري غير مقبول بسبب زيادة العدد الكلي للبكتيريا وزيادة العدد الكلي لبكتيريا القولون لنفس العينات، وما نسبته (0.7%) من لحم الخراف المجمدة غير مقبول لنفس السبب لنفس العينات.

أما بالنسبة لبكتيريا إشريشيا القولون *E.coli O157:H7* فلم يتم تسجيل أي حالة ملوثة بهذه البكتيريا في جميع عينات اللحوم باختلاف أنواعها.

لوحظ من هذه الدراسة أن العينات غير المقبولة كانت أعلى نسبة لها من لحم الحبش الطازج (57.9%)، وكانت قيمة المتوسط للعدد الكلي للبكتيريا في اللحم البقري أعلى قيمة (4.24×10^{6})، أما المتوسط للعدد الكلي لبكتيريا القولون فكانت أعلى قيمة له في لحم الحبش الطازج (4.24×10^{5}).

بالنسبة لوجود السالمونيلا فكانت لحوم الحبش الطازجة لها أعلى نسبة مقارنة مع باقي العينات حيث تم عزل بكتيريا السالمونيلا من أربع عينات (5.3%) من مجموع عينات لحم الحبش الطازج، وعينة واحدة (1.5%) من مجموع اللحم البقري، حيث لم يتم عزل بكتيريا السالمونيلا من لحم الخراف المجمد وبيض الدجاج الطازج.

أما عينات البيض الطازج فكانت جميعها مقبولة وذلك لخلوها من البكتيريا المرضية خاصة لبكتيريا السالمونيلا.

من خلال عملية التحليل الإحصائي للنتائج، لم تظهر الدراسة أي علاقة بين العدد الكلي للبكتيريا والعدد الكلي لبكتيريا القولون من جهة، ووجود بكتيريا السالمونيلا من جهة أخرى حيث أن جميع العينات التي وجد فيها بكتيريا السالمونيلا كان العدد الكلي للبكتيريا فيها منخفض، كما أن العدد الكلي لبكتيريا القولون كان مرتفعاً في بعض العينات وفي البعض الآخر كانت تساوي صفر.

كما أظهرت الدراسة أن هناك علاقة بين نوع العينة ووجود بكتيريا السالمونيلا، كما لم تظهر الدراسة علاقة بين نوع العينة وبين قبول أو رفض العينة.