Cairo University
Faculty of Veterinary Medicine
Department of Microbiology



Bacteriological and Molecular Studies on Salmonella species Isolated from Beef Meat Products in El-Gharbia Governorate

A Thesis Presented By

Ahmed Abo El yazeed Fawaz Ahmed Fawaz

(B.V.Sc., 2010, Cairo University) For The Degree of M.V.Sc. (Microbiology)

Under Supervision of

Prof. Dr. Khaled Farouk El Amry

Professor of Microbiology Faculty of Veterinary Medicine Cairo University

Prof .Dr. Heidy Mohamed Shawky

Prof. Dr. Nesreen Zakaria Helmy

Professor and Head of

Chief Researcher of Meat Hygiene

Faculty of Veterinary Medicine

Microbiology Dep.

Animal Health Research Institute
Tanta lab

Cairo University

2015

Cairo University

Faculty of Veterinary Medicine

Department of Microbiology



Approval Sheet

This is to certify that the dissertation submitted by **Vet./ Ahmed Abo Elyazeed Fawaz Ahmed Fawaz** to Cairo University, for the master degree of Veterinary Medical Sciences, Microbiology (Bacteriology, Immunology and Mycology) has been approved by the examining committee:

• Prof. Dr./ Ashraf Awad Abd-El-Twab

Ashruf Amed

Professor and head of Bacteriology, Immunology and Mycology Department Faculty of Veterinary Medicine Benha University

• Prof. Dr./ Jakeen Kamal Abdel Haleem Eljakee

Professor of Microbiology

Faculty of Veterinary Medicine

Cairo University

• Prof. Dr./ Khaled Farouk Mohamed Abdel Hamid El-Amry (Supervisor)

Professor of Microbiology

Faculty of Veterinary Medicine

Cairo University

• Prof. Dr./ Heidy Mohamed Shawky (Supervisor)

Professor and head of of Microbiology Department

Faculty of Veterinary Medicine

Cairo University

• Prof. Dr./ Nesreen Zakaria (Supervisor)

Chief researcher of meat hygiene

Animal Health Research Institute

Tanta Lab.

Wesreen

Heidy shanky



سورة البقرة الآية: ٣٢

Acknowledgment

Firstly, thanks to ALLAH for helping me in completing and direction of this work.

I would like to express my sincere thanks and gratitude to Prof. Dr. Khaled Farouk El-Amry, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

My special thanks are expressed to Dr. Heidy Mohamed shawky, Professor and Head of Microbiology Dep. Faculty of Veterinary Medicine, Cairo University.

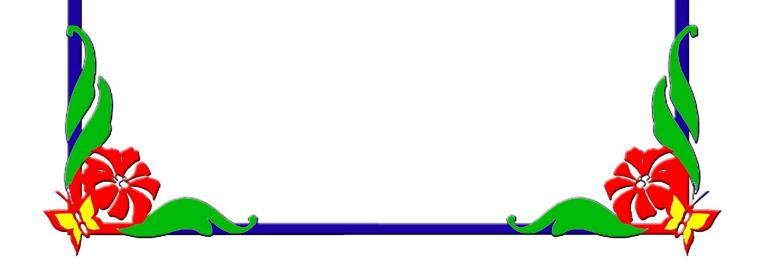
Words are not enough to express my gratitude to Dr.

Nesreen Zakaria Helmy, Chief researcher of meat hygiene,

Animal Health Research Institute, Tanta lab.

My deepest thanks to All staff members of Animal Health Research Institute for their continuous help and encouragement.

Ahmed Abo El yazeed Fawaz Ahmed Fawaz



Dedication

- This work is dedicated to those who gave meaning to my life.
- To my Father and Mother who gave me every thing and took nothing.
- To my brothers (Hany- Ibrahim -Mostafa).
- To my future wife soad who gave a smile to my life.

Ahmed Abo El yazeed Fawaz Ahmed Fawaz

Vita

- Name: Ahmed Abo-Elyazeed Fawaz Ahmed
- **Date of birth:** 17 / 08/ 1988
- Place of birth: Santa, Gharbia, Egypt
- His primary and preparatory education was completed in Erfan and Gaafaria School – Santa.
- His secondary education was completed in Gaafaria secondary
 School Santa.
- His undergraduate and professional education was completed in Faculty of Veterinary Medicine, Cairo University from which received a B.V.Sc in 2010.
- He began the master degree in Faculty of Veterinary Medicine,
 Cairo University in September 2012.
- He works in Animal Health Research Institute, Tanta branch, Agriculture Researches Center.

Contents

Title	Page
1. Introduction	1
2. Review of Literature	6
- 2.1. Prevalence of Salmonella species in meat products:	6
- 2.2. Salmonella species associated with human infection	13
- 2.3. General characteristics of Salmonella species	18
- 2.4. Disease and pathogenesis	23
- 2.5. Isolation technique and biochemical identification of Salmonella species	26
- 2.6. Molecular characteristics of Salmonella genome	31
3. Materials and Methods	40
4. Results	58
5. Discussion	86
6. Summary	94
7. Recommendations	95
8. References	99
9. Arabic Summary	

List of Abbreviations

bp	:	base pair.
CDC	:	Center of Disease Control and Prevention
CIDRAP	:	Center for Disease Research and Policy
		Academic Health Center.
DW	:	Distilled Water.
E. coli	:	Escherichia coli.
ELISA	:	Enzyme-Linkad Immunosorbant assay.
FAO	:	Food and Agriculture Organization.
FSIS	:	Food Safety and Inspection Service.
НАССР	:	Hazard Analysis and Critical Control Point.
ICMSF	:	International Commission on Micropiological
		Specification for Foods.
MR	:	Methyl Red.
PCR	:	Polymerase Chain Reaction.
S.Arizonae	:	Salmonella Arizonae.
S.enterica	:	Salmonella Enterica.
S.Typhimurium	:	Salmonella Typhimurium.
SS	:	Salmonella-Shigella agar.

List of Abbreviations

TSI	•	Triple Sugar Iron Agar.
VP	:	Voges Proskauer.
WHO	:	World Health Organization.
XLD	:	Xylose Lysine Desoxycholate.

List of Tables

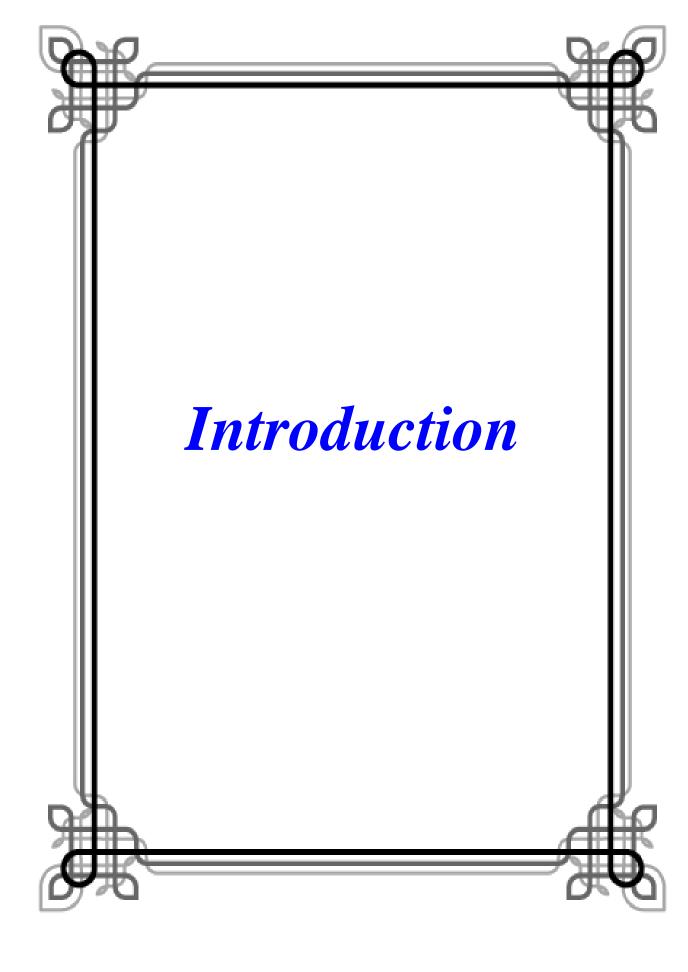
Title	Page
Table (1): primers used in PCR reactions for the detection of invA virulent gene of Salmonella	46
Table (2): PCR protocol for amplification conditions of PCR	40
products	55
Table (3): Results of biochemical identification of the isolated salmonellae using standard laboratory tests	59
Table (4): Result of biochemical identification of the isolated	
salmonellae using confirmatory test (API 20 E)	59
Table (5): Prevalence of isolated Salmonella from collected samples	64
Table (6): The prevalence of salmonellae recovered from each	0.
product	65
Table (7): Results of Salmonella serotyping	66

List of Photos

Title	Page
Photo.(1): Salmonella organism on XLD agar (smooth colonies with black center)	60
Photo.(2): Salmonella on Brilliant Green agar (pink colonies)	60
Photo.(3): Salmonella on S.S. agar appeared as pale coloured colonies with or without black center	
Photo.(4): Salmonella organism on Hektone enteric agar	61
(appeared as deep blue colonies)	61
Photo.(5): Results of Salmonella organism (biochemical identification). Tube (1) positive result on urea agar – tube (2) negative result on urea agar – tube (3) positive result on lysine iron agar– tube (4) negative on lysine iron agar– tube (5) positive on TSI agar – tube (6) negative on TSI	
	62
Photo.(6): Results of Salmonella organism on Simmon's Citrate (biochemical identification). Positive result (left tube), negative result (right tube)	
	63
Photo.(7): API 20 technique reaction of Salmonella (rapid biochemical test)	
	63
Photo.(8): Agarose gel electrophoresis of Salmonella	67

List of Figures

Title	Page
Fig. (1): Percentages of isolated Salmonella from samples	64
Fig. (2): Percentages of Salmonellae recovered from meat	
products	65



Introduction

Bacteria of the genus Salmonella are members of the family Enterobacteriaceae. They are rod-shaped gram-negative, facultative anaerobes and inhabit the intestinal tract of animals and may be thus recovered from a wide variety of hosts, specially poultry, swine, humans, foods and environment. Besides, these bacteria may be pathogenic to wild and domestic animals, and humans (Holt et al., 1994). Salmonella bacteria are between 2 and 5 µm long and 0.7 to 1.5 µm in diameter. They have flagella, which are tail-like projections made of proteins that help the bacteria to move. There is not a single method that can assure that Salmonella is found if it is present. Finding Salmonella is in many cases like finding a needle in a hay stack. Therefore, if a detection method does not find Salmonella, it does not mean that the bacteria are not there (Hendriksen, 2003).

Salmonellosis is a zoonotic bacterial disease of national and international importance. The worldwide distribution of salmonellosis often parallels the patterns of trade of animal products and food and the migration patterns of human and animals (Gilbert et al., 2010). Consumption of raw or undercooked contaminated poultry products can induce acute gastroenteritis in humans. Faced with the public health concerns associated with salmonellosis, the European Union has established a European regulation forcing member states to implement control programs aimed at reducing Salmonella prevalence in poultry production especially at the primary production level (Fica et al., 2012).

More than 2,500 different serovars of *S.enterica* have been identified and most of them have been described as the cause of human infections,

but only a limited number of serovars are of public health importance. Most reports have mentioned *S.enterica* serovar Typhimurium and *S.enterica* serovar Enteritidis as the most common causes of human salmonellosis worldwide (**Tavechio et al., 1996**).

In recent years, problems related to Salmonella have increased significally, both in terms of incidence and severity of cases of human and animal salmonellosis, new concerns have been identified. Since the beginning of the 1990s, strains of Salmonella which are resistant to a range of antimicrobials, including first-choice agents or the treatment of human and animals, have emerged and are threatening to become a serious public health problem. This resistance results from the use of antimicrobials both in human and animal husbandry (WHO, 2006).

Differences in virulence among Salmonella serovars and in the course of Salmonella infections in various host species have been attributed to the variable acquisition and evolvement of virulence genes (Falkow, 1996).

For Salmonella to be virulent, the expression of numerous genes is necessary, which encode some factors with the ability to be located in transmissible genetic elements such as plasmids, bacteriophages and transposons and may be part of specific regions in the chromosome of the bacterium (Hacker et al., 1997).

A wide range of food has been implicated in food borne Salmonellosis. However, as the disease is primarly zoonotic, food of animal origin has been consistently implicated as the main source of human salmonellosis (FAO/WHO, 2002). Salmonellosis is considered to be one