

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Studies on the Role of Virulence Factors of *S. enterica* and its Pathogenicity in Dairy Farms

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(Infectious Diseases)

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ABSTRACT

Bovine Salmonellosis is a worldwide bacterial disease causing great zoonotic impact, animal losses and economic problems. The present study was conducted on a total number of 313 samples divided into, 188 milk samples (124 from bulk tank milk and 64 from individual mastitic milk) and 125 fecal samples (100 diarrheic fecal samples and 25 from apparently normal calves in contact with the diseased ones) which collected from different localities of governorates in Egypt. Samples were examined bacteriologically and serologically, revealed that *Salmonella* was isolated in young diarrheic calves and apparent normal calves in 11% and 4% respectively, while mastitic caws exhibited 3.1% positive results and from bulk tank milk 3.2%. Serological examination 18 of *Salmonella* isolates were typed as 15 *S. typhimurium*, two as *S. sekondi* and only one as *S. chester* in percentage of 83.3%, 11.1% and 5.6% respectively. Epidemiological studies showed that some risk factors as (age, sex, housing, and hygiene) have significant effect on *Salmonella* prevalence. Statistical analysis showed that there were significant results. The highest rate of salmonellosis was recorded in small animals and in bad hygienic farms specially containing rodents and steer animals. Conventional PCR was performed on 18 *Salmonella* isolates for the presence of seven virulence genes (*invA*, *bcfC*, *stn*, *pefA*, *mgtC*, *csgD*, and *fimH*). These genes were detected in 18 (100%), 18(100%), 16(88.9%), 14(77.7%), 3(16.7%), 1(5.5%) and 0(0%) respectively. In conclusion, *Salmonella* is one of the most important causes of diarrhea in calves and in adult cattle causing mastitis. The application of PCR assay is important for rapid detection of virulence profile of *Salmonella*.

Key words: *Salmonella enterica*, cattle, virulence genes, risk factors & PCR.

ACKNOWLEDGMENTS

First of all I would like to thank Allah .who gave me the ability to finish this work, I wish to express my cordial thanks and deep gratitude to my PROF. Mahmoud Amin, professor of Infectious diseases, Faculty of veterinary medicine ,Cairo University, for his deeply kind encouragement, valuable guidance, constructive criticism and supervision.

My grateful thanks and deep gratitude are particular to Dr. Wagdy El ashmawy, Lecturer of Infectious diseases Faculty of Veterinary Medicine, Cairo University, for his deeply kind encouragement

My grateful thanks and deep gratitude are particular to Dr. Ebtsam El sayed “senior Researcher in animal Health Research” Institute, Al-Haram, Giza for her continuous help, kind advice, scrupulous guidance, encouragement and supervision.

My grateful thanks are of fered to all members and workers of the medicine & infectious Diseases Department .Faculty of veterinary medicine, Cairo University for their help and encouragement.

My grateful thanks are of fered to workers of the udder health and neonatal diseases Department .Animals reproduction Research Institute, Al-Haram, Giza.

My grateful thanks to my friends who help me to collecting of samples.

Special thanks go to my family. I could not have managed to finish my M.V.Sc. without their love and encouragement.

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List of Abbreviations

bcfC	Colonization Factor-Fimbrial Gene
BTM	Bulk Tank Milk
CFSPH	Center Of Food Security And Public Health
DNA	Deoxy Ribonucleic Acid
fimA	Fimbriae Virulence Gene
invA	Invasion gene
ISO	International Standard Organization
LIA	Lysine Iron Agar
mgtC	Mg ²⁺ -Transporting Protein
OIE	International Office Of Epizootics
PCR	Polymerase Chain Reaction
pefA	Plasmid Encoding Fimbriae
RV	Rappaport-Vassiliadis
S.S	Salmonella-Shigella
SPIs	Salmonella Pathogenicity Islands
spp.	Species
stn	Enterotoxin Gene
TSI	Triple Sugar Iron
VI	Virulence
VP	Voges-Proskauer
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate Agar

Introduction

Bovine salmonellosis is a worldwide bacterial disease causing economic losses in cattle and has zoonotic importance in man. Salmonella infection had many impacts on animal and human health that are considered a major wide problem (**Youssef and El_Hiag 2012**).

Salmonella enterica is a wide bacterial species that involved in field problems. Ninety-nine percent of human and animal infections are caused by the subspecies *enterica*. Within this subspecies more than 2600 serotypes have been classified, based on serological reactions to cell wall lipopolysaccharide (O), flagellar (H) and capsular (Vi) antigens (**Sanderson & Nair, 2012, Agren 2017**).

Clinical signs of bovine salmonellosis include diarrhea, fever, anorexia, dehydration, abortion and evidence of endotoxaemia, although many infection remain a symptomatic (**Nielsen et al., 2012b and OIE 2016**).

Epidemiological studies as measurements of disease occurrence and risk factor analysis, dynamics of transmission of salmonellosis in cattle herds are of great importance to be studied. The detection and identification of salmonella required not only isolation but also biochemical, serological and molecular assay that detect salmonella species or even strain level for accurate epidemiological survey (**Azooz 2017**).

The use of DNA based method is becoming increasingly important in epidemiological studies and differentiation of salmonella species is being used as a rapid and reliable tool for epidemiological survey. PCR provides a specific method and superior ability to detect *Salmonella enterica* serovars in the presence of other bacteria simultaneously (**Moussa et al., 2012**).

Virulence of *Salmonella* is linked to a combination of chromosomal and plasmid factors. *Salmonella* pathogenicity islands (SPIs) are large gene cassettes within the *Salmonella* chromosome that encode determinants responsible for establishing specific interaction with the host, and are required for bacterial virulence in a given animal. There are more than 20 SPIs have been described (**Sabbagh et al., 2010 and Aleslamboly 2011**).

The most common detected virulence genes (invA and bcfC) using specific primer for each .invA and bcfC gene were expressed in all examined salmonella serotypes from examined samples (**Mohammed 2014**).

Diarrhea induced by salmonella infection is a complex phenomenon involving several pathogenic mechanisms including production of enterotoxin .this enterotoxin production is mediated by salmonella enterotoxin virulence gene stn (**Murugkar et al .,2003**).

The objective of the present work is to investigate the risk factors associated with isolation of *Salmonella enterica* microorganisms and some of its virulence genes (invA, stn, pefA, csgD, bcfC, fimH and mgtC) by PCR and In-vitro antibiotic sensitivity testing of the isolated local strains of different to detect the best antibiotic to be used.