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Isolation and Identification of Certain Zoonotic Bacteria from Human, Milk, and Milk Products with study the Pathogenicity of *Salmonella typhimurium* .

A Thesis

Submitted to the Council of the College of Veterinary Medicine, University of Diyala, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Internal and Preventive Veterinary Medicine - Zoonosis

By

Rofeida Mohammad Hussein ALbajaly

High Diploma /University of Baghdad

Supervised by

Prof. Dr. Nazar Jabbar Al-Khafaji

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بسم الله الرحمن الرحيم

وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً ۖ نُّسْقِيكُم مِّمَّا فِي بُطُونِهِ مِن بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَائِغًا لِّلشَّارِبِينَ (66)

صدق الله العظيم

سورة النحل

Supervisor Certification

We certify that the thesis entitled **(Isolation and Identification of Certain Zoonotic Bacteria from Human, Milk and Milk Products with study the Pathogenicity of S*almonella typhimurium*)** has been prepared under my supervision at the Department of Medicine, College of Veterinary Medicine, University of Diyala, as partial fulfillment of the requirements for the Degree of Master of Science in Internal and Preventive Medicine- Zoonosis.

Supervisor

Prof. Dr. Nazar Jabbar Al-Khafaji

College of Veterinary Medicine

University of Diyala

2020

In view of the available recommendation, I forward this thesis for debate by the examining committee

Assist. Prof. Dr. Khalid Ibrahim Abd

Vice Dean of post graduate studies and science affairs

College of Veterinary Medicine

University of Diyala

2020

Examination Committee Certification

We, the examining committee, certify that after reading this thesis entitled **(Isolation and Identification of Certain Zoonotic Bacteria from Human, Milk and Milk Products with study the Pathogenicity of *Salmonella typhimurium*)** was prepared by Rofeida Mohammad Hussein ALbajaly in its contents, we think it is adequate for the award degree of Master of Science in Internal and Preventive Medicine - Zoonosis.

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Assist Prof. Dr.

Mawlood Abbas Ali Al-Grabawi

Member

Assist Prof. Dr.

Raad Mahmood Al-Zubaedi

Member

Prof. Dr. Nazar Jabbar AL-Khafaji

Member and Supervisor

Approved by the council of the College of Veterinary Medicine , University of Diyala

Assist. Prof. Dr. Mohammad Youssif

Head of Department of

Internal and Preventive Medicine

College of Veterinary Medicine

University of Diyala

2020

Prof. Dr. Talib Jawad Kadhim

Dean of College of

Veterinary Medicine

University of Diyala

2020

Dedication

To the spirit of my father who planted in us the love of science from childhood

To my angel in life...

To the meaning of love and the meaning of compassion and dedication ... To the smile of life and the secret of existence my Mother...

To whom I see optimism and happiness in their laughter...

To those who looked forward to my success, my brothers and sister...

I dedicate this humble Work.

Rofeida

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Summary

□o investigate, the bacteria of □oonotic importance, contaminated milk, and milk products, a total of □□□ samples, represent □□ raw milk from cows, buffaloes, sheep, and goats□□□ milk products, cheese, yogurt, and □□ swabs from workers and their equipment in retail shops of milk products, were collected in the period from August 20□□ to April 2020. □he samples were submitted to laboratory investigations, for isolations and identifications of contaminated bacteria, according to their cultural morphology, biochemical properties. Counting of total viable bacteria. Sensitivity to sixteen antibiotics. In addition to investigating the pathogenicity of *Salmonella typhimurium* which was one of the common isolates in the current study. □he results revealed, that from 2□□ isolates that were isolated in the current study□ the highest isolate was *Staphylococcus* spp. □□ □20.□□ □□followed by *Salmonella* spp. □□ □□□.□□ □□ *Lactobacillus* □□ □□2.2□ □□*Pseudomonas* spp. and *E. coli* each □0 □□0.□□ □□ *Klebsiella* spp. 2□ □□0.□□ □□*Enterobacter* 2□ □□.□□ □□*Proteus* spp. and *Citrobacter* each □□ □□.□□ □□*Streptococcus* □ □□.□□ □and *Listeria* □ □□.□□ □. □rom a total of □□ raw milk samples collected from the udder and bulk tank □cows, buffaloes, sheep, and goats□, □□2 isolates were isolated□□rom which the highest isolate was *Salmonella* spp. 2□ □□□.0□ □□followed by *Staphylococcus* spp. 2□ □□□.□□ □□*Lactobacillus* 22 □□□.□□ □□*Enterobacter* □□ □□2.0□ □□*E. coli* □□ □□0.□□ □□*Citrobacter* and *Proteus* each □2 □□.□□ □□ *Klebsiella* □ □□.□□ □and *Pseudomonas* □□□.□□ □. While from a total of □□ samples of milk products □Cheese and □oghurt□, □2 isolates were isolated, the highest isolate was *Lactobacillus* □□ □22.□□ □□*Salmonella* □2 □□□.□□ □□ *E. coli* □0 □□□.□□ □□*Staphylococcus* □ □□□.□□ □□*Pseudomonas* □ □□.□□ □□ *Proteus* □ □□.□□ □□ *Klebsiella* □ □□.□□ □□ *Enterobacter* □ □□.□□ □□ and *Citrobacter* □ □□.□□ □. Meanwhile, from a total of □□ swabs obtained from

workers and their equipment, □□isolates were isolated. □he highest isolate was *Staphylococcus* 2□ □□□.□□ □□followed by *Pseudomonas* □□ □22.□□ □□ *Klebsiella* □□ □20.0□ □□*E. coli*. and *Streptococcus* each □ □□.□□ □□*Listeria*, *Enterobacter*, and *Citrobacter* each □ □□.□□ □.

□rom a total of □□□ samples, 2□ □□□.□□□ □were free from isolates, while from others, □□0 samples □□□.02□ □, 2□□isolates were isolated, from which □□ □2□.0□ □were in a single form, and others in more than one isolate in a sample□two isolates □□ □□2.□□ □□three isolates □□ □□□.□□ □and four isolates 20 □□.□□ □. □he highest isolates were from raw milk □□□□□□□ □□□.□□ □ then, workers □□□□□□ □□□.□□ □, and milk products □2□□□□ □□□.□□ □.

□he highest level of contamination was in cow□s raw milk, as the count was □□.□□□0.0□ $\log_{10}$cfu□ml□□followed by buffalo □□.□0□0.0□□□yogurt □□.2□□0.0□□□cheese □□.□□□0.0□ □□worker□s ear □□.□□□0.0□□and the lowest count was from worker□s hand □□.□□□0.0□ □. While the coliform counts were in cow□s raw milk □□.□0□0.0□□□buffalo□s raw milk □□.0□□0.02□□ yogurt □□.0□□0.0□□□cheese □□.□□□0.0□□□worker□s ear □□.□□□0.0□□and worker□s hand □□.□2□0.0□□.

□he sensitivity of isolates in the current study to sixteen commonly used antibiotics was of high variations. All the isolates were resistant to Clindamycin □DA □0□□Cloxacillin □cx□0□and Piperacillin □prl□00□. □ut all isolates were sensitive to □orfloxacin □□or □0□except *Staphylococcus* and *Listeria*. Also, about 2□□ □□-□□□of antibiotics were resisted by the tested isolates, and the Listeria was the isolate that resists nearly all tested antibiotic

Clinically the animals exposed to *Salmonella typhimurium* exhibit signs of depression, anorexia, isolation from other animals, soft feces.

Heart rates□significantly decreased in those of □roup II, but increased in rabbits of □roup III. Respiratory rates significantly increased in □II and □III. □ody temperature non-significantly increased in □II and III□body weight non-significantly decreased in □II and III.

Hematologically□□otal leucocytes count was significantly increased in both □II and III. While Lymphocytes□ increases in those of □II then decreased, and in □III increased. Meanwhile, □eutrophils □ decreased in □II then increased, but in □III increased then decreased. Monocytes □ significantly increased in both □II and III. □asophils□ and Eosinophils□ showed no significant changes.

□he main gross lesions were in the liver, lung, heart, kidneys, and gastrointestinal tract. Severe congestions, with enlargement of the liver, heart flabby, enlarged, congested, gastrointestinal tracts congested, lungs emphysematous, congested. □idneys are enlarged and congested.

□he main histopathological changes were□ the stomach showed desquamation of mucosal surface with the bleeding, different areas in the mucosal and submucosal layers showed an area of fibrosis and infiltration of mononuclear cells □neutrophils□ hyperplasia of the mucosal gland □hemorrhagic gastritis□. Small intestine showed thread of mucus from the goblet cells, vacuolar degeneration of mucosal glands in the submucosa of the small intestine, and hemorrhage, mucosal erosion, and hyperplasia of peyer□s patchy in the submucosa. Lungs showed edematous fluid with erythrocytes, emphysema which is characteri□ed by highly alveolar wall destruction and thickening of the alveolar wall in some areas □interstitial pneumonia□due to the infiltration of inflammatory cells □neutrophils□with a few mononuclear cells. □he kidney showed focal intertubular mononuclear cells □M□Cs□Infiltration and many vacuoles, infiltration of

multinuclear cells (mesengeal cells), and severe vacuolar degeneration of occluded tubules, hemorrhagic area, the infiltration of mononuclear cells, and vacuolation, hemorrhagic nephritis. Heart showed infiltration of inflammatory cells in the interstitial and presence of vacuole.

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□.□□	*Klebsiella* spp.	□□
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□igure□+□□	□igure □□+□□□□Histopathological section of rabbit Small Intestine for control group showing normal histological structure. □H□E stain □0□□	□□
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List of abbreviations

Aberrations	Key or full name
A□□VA	Analysis of variance
□ cells	□ lymphocyte
C□U	Colony forming unit
EM□	Eosin Methylene blue agar
E□SA	European □ood Safety Authority
E□SA	European Food Safety Authority “
□I□	□astrointestinal tract
gm	□ram
H2S	Hydrogen sulphate
I□D	Indol test
ID	Infective dose
Mac	MacConkey Agar
MSA	Mannitol Salt Agar
ML□S	Mesenteric lymph node
□*m*	Micromole
mg	Milligram
ml	Mole
□SPIs**)**	Salmonella pathogenicity islands
SS	Salmonella Shigella Agar
□SPC□	standard plate count
□ cells	□ lymphocyte

Chapter one
Introduction

1.1. Introduction

Milk and milk products are a source of numerous essential nutrients. Chemically, milk is a complex mixture, being rich in proteins, fats, carbohydrates, vitamins, minerals, essential amino acids, and other constituents (Haug *et al.,* 2007; Marjan *et al*., 2014; van Hooijdonk and Hettinga, 2015). The high nutrient content of milk, all at a near-neutral pH and a high water activity, provides an ideal environment for the growth of many microorganisms. (Haridy, 1992; Frank, 1997; Sangoyomi *et al.,* 2010). Milk may serve as an ideal substrate for the growth and survival of an array of bacteria and fungi, thereby leading to the public health threat (Zucali *et al.*,2011; Aebi *et al*., 2015).

Milk secreted from healthy animal's udder is sterile, becomes contaminated throughout the milking, chilling, and loading (Younan *et al.,* 2005). Bacterial contamination of raw milk can originate from different sources, air, milking equipment, feed, soil, feces, and grass (Coorevits *et al.*, 2008). Microbes on the surface of milking and chilling device, milking workers, the environment of milking animals, and water used in different steps of processing are considered as a common source of contamination (Mbabazi, 2005).

The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed, and animal health (Rogelj and Mleko, 2003). Rinsing water for milking machines and milking equipment washing involves some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley and McKinnon,1990).

Pathogenic bacteria are transmissible to humans through milk and milk products. attention is focused on milk, cheese, and ice cream contaminated

with pathogenic bacteria, viz, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and enteropathogenic *E. coli* (□asavasda, 1988).

1.2. Aims of the study:

1. □solation and □dentification of the most common zoonotic bacteria from milk and milk products of, cows, buffaloes, sheep, and goats.

2. Total viable bacterial counts in milk and milk products

3. □etermining the sensitivity of isolates to the most commonly used antibiotics.

4. Study the pathogenicity of one of the most common isolates in rabbits.