



Cairo University

Faculty of Veterinary Medicine

Department of Veterinary Hygiene and Management

**Study on the impact of cattle production in Egypt on the
environment in different cattle rearing systems**

Thesis presented by

Eslam Mahmoud Mohamed Said Ali

(B.V.Sc., 2007)

Fac. Vet. Med., Cairo University

For the degree of M.V.SC.

(Hygiene of Animal, poultry and Environment)

Under The Supervision Of

Prof Dr. Ehsan Yousef Bashandy

Professor Emeritus of Hygiene of Animal, poultry and Environment.
Faculty of Veterinary Medicine,
Cairo University.

Prof Dr. Wael Anwer Hussein

Professor of Hygiene of Animal, poultry and Environment.
Faculty of Veterinary Medicine,
Cairo University

2018



Cairo University
Faculty of Veterinary Medicine
Department of Veterinary Hygiene and Management

Supervision Sheet

Prof. Dr. Ehsan Yousef Bashandy

Professor Emeritus of Hygiene of Animal, poultry and
Environment.

Faculty of Veterinary Medicine,
Cairo University.

Prof. Dr. Wael Anwer Hussein

Professor of Hygiene of Animal, poultry and Environment.

Faculty of Veterinary Medicine,
Cairo University

2018



Cairo University.
Faculty of Veterinary Medicine.
Department of Veterinary Hygiene and Management

- Name: Eslam Mahmoud Mohamed Said Ali**
- Date of birth: 7/7/1984**
- Nationality: Egyptian.**
- Degree: Master of Veterinary medicine.**
- Specialization: Hygiene of Animal ,Poultry and Environment.**
- Title of thesis: Study on the impact of cattle production in Egypt on the environment in different cattle rearing systems.**

Under supervision of:

Prof Dr. Ehsan Yousef Bashandy

Prof Dr. Wael Anwer Hussein

Abstract

This work was carried out to gain a better understanding of impact of cattle production in Egypt on the environment in different rearing systems by isolation and identification of *E.coli* O157 and Methicillin-Resistant *S.aures* (MRSA) in different Cattle and Buffaloes farms in different samples in Cairo Governorate. A total of 251 samples (129 Rectal swabs from Buffaloes, Cows, 82 milk samples from dairy farms, 7 soil samples , 10 water samples, 10 air samples and 13 human hand swabs) were collected and examined in lab. The occurrence of MRSA and *E.coli* o157 were 32.9%, 49.4% respectively . Application of PCR technique for detection of *mecA* and virulence gene of *E.coli* o157 , for *mec A* 8 out of 21 are positive with percentage 38% . While *E.coli* o157 ,the presence of virulence gene as STX1,HLY,STX2 and EAE with percentage 60%, 60%,10% and 23% respectively .

(Key words: MRSA, *E.coli* o157, *mec A*, STX1,HLY, STX2, EAE, Cattle, Buffaloes, environment)

Acknowledgement

I am extremely grateful to Allah for helping, prosperity and kindness and under whose willing this work was carried out.

*No word can express my deep sincerest gratitude, and deepest thanks to **Prof. Dr. Ehsan Yousef Bashandy**, Professor Emeritus of Hygiene of Animal, Poultry and Environment, Fac. Vet. Med., Cairo University, for her kind encouragement, great interest, valuable advice and unfailing help during the course of this research.*

*All words unable to express my deepest gratitude and high appreciation to **Prof. Dr. Wael Anwer Hussein**, Professor of Hygiene of Animal, Poultry and Environment, Fac. Vet. Med., Cairo University for his advices and help during the practical work of this thesis*

Contents

	Page
1. Introduction.....	1
2. Review of literature.....	6
2.1. Impact of <i>Staphylococcus aureus</i> on the environment.....	6
2.1.1 Epidemiological nature of Methicillin-Resistant <i>S.aureus</i>....	6
2.1.2 Prevalence of MRSA in animal	13
2.1.3 Genotyping of MRSA	16
2.2. Impact of <i>E.coli</i> O157 on the environment.....	20
2.2.1 Epidemiological nature of <i>E. coli</i> O157:H7.....	20
2.2.2 Prevalence of <i>E.coli</i> O157:H7	33
2.2.3 Genotyping of <i>E.coli</i> O157.....	37
3. Materials and Methods.....	41
3.1.Materials.....	41
3.2. Methods.....	49
4.Results.....	59.
5.Discussion.....	68

6. Conclusion.....	79
7. Summary	80.
8. References.....	83

LIST OF TABLES

NO.	Title	Page
1	Types of farms from which the samples have been collected	42
2	Zone diameter interpretation, standard of different antibacterial agents used	45
3	Primers for the genes of <i>S.aureus</i> and <i>E.coli</i> O157	46
4	Components and amplification programs of multiplex PCR used for detection of genes encoding virulence factors..	58
5	Occurrence of <i>Staphylococcus aureus</i> in different samples collected from the examined farms	59
6	Occurrence of MRSA from positive <i>S. aureus</i> samples by applying antibiotic diffusion disc	60
7	Presence of <i>mecA</i> gene from MRSA positive samples	61
8	Occurrence of <i>E.coli</i> O157 from different samples collected from the examined farms	62
9	Genotyping of <i>E.coli</i> O157 by using different primers	63

LIST OF FIGURES

NO.	Title	Page
1	Occurrence of <i>Staphylococcus aureus</i> in different samples collected from the examined farms	59
2	Occurrence of MRSA from positive <i>S. aureus</i> samples by applying antibiotic diffusion disc	60
3	Presence of <i>mecA</i> gene from MRSA positive samples	61
4	Occurrence of <i>E.coli</i> O157 from different samples collected from the examined farms	62
5	Genotyping of <i>E.coli</i> O157 by using different primers	63

LIST OF PHOTOS

NO.	Title	Page
1	<i>S.aureus</i> colony on manitol salt agar media	64
2	Dry spot staphytect plus(oxid)	64
3	Antibacterial sensitivity discs	65
4	<i>E.coli</i> o157 colony on sorbitol macconkey agar media	65
5	<i>E.coli</i> o157 latex test kit	66
6	Agarose (1.5%) gel electrophoresis of <i>mecA</i> <i>Staphylococcus aureus</i> PCR products	66
7	Agarose (1.5%) gel electrophoresis of STX1, HLY and EAE virulence gene of <i>E.coli</i> o157.	67
8	Agarose (1.5%) gel electrophoresis of STX2 virulence gene of <i>E.coli</i> o157	67

Introduction

In Egypt, nearly 70% of the livestock animals are reared by small farmers, where few cattle and buffalo along with small ruminant animals are kept together in their houses to produce milk and other dairy products **Aidaros, 2005**

Livestock have been kept for a variety of purposes, in order that in recent years' communities and researchers are worried about impact of cattle production on the environment.

Livestock activities have significant impact resulting in environmental problems such as land degradation, global warming and climate changes, chemical and bacteriological air and water pollution, water shortage and loss of biodiversity, worldwide. **(Steinfeld, *et al*, 2006).**

In developing countries, the system of raising animals depends on the small mixed farming system while, in the industrial model of livestock production, a lot of animals are housed in confined places.

Livestock in many developing countries are still a critical support to livelihoods of people who live in or near poverty, and it is here that the nonfood uses of livestock such as source of fertilizer (manure). Animals waste generates in massive lagoons waste while many traditional crop use manure as a fertilizer.

In recent years *E.coli* O157 and Methicillin-Resistant *S.aures* (MRSA) emitted from different livestock rearing systems have a serious impact on the environment.

Escherichia coli O157:H7 (STEC) is of a public health problem as it has ability to cause human disease, colonize animal carriers, and survive in the environment. *Methicillin-resistant Staphylococcus aureus* (MRSA) also become one of the most commonly identified antibiotic-resistant pathogens in many parts of the world, so that increased concern about antibiotic resistant strains of *S. aureus* as MRSA infections increase morbidity, mortality, and costs.

S. aureus has a significant pathogenic role as cause of intramammary infections in cattle, buffaloes and small ruminants leading to considerable economic losses in cattle farming. The pathogen plays a role as a frequent causative agent of clinical or subclinical mastitis in cattle. Staphylococci are gram-positive bacteria, non-motile, non-spore forming facultative.

Milk is a good substrate for *S. aureus* growth and dairy products are common sources of staphylococcal food-poisoning may occur due to contamination of milk can also occur from environmental sources as faeces during handling and processing.

In recent years, development of resistance has been related to the extensive therapeutic use of antimicrobials or to their administration as growth promoters in food animal production. Isolates of *S. aureus* are frequently resistant to methicillin and essentially all other β -lactam antibiotics, and called MRSA

There is now increasing concern about the public health impact of MRSA associated with food producing animals, because MRSA and, consequently, their resistance genes can spread from animals to humans by direct contact or through the food chain (**Kluytmans, 2010**)

On the other hand, cattle are believed to be the primary reservoir of *E. coli* O157:H7 (**Garber et al., 1995**), due to the association with human disease, and the organism has been shown to have a specific tropism for adherence to the mucosal epithelium at the terminal rectum of cattle.

Escherichia coli is gram-negative rod-shaped bacteria within the family Enterobacteriaceae, facultative anaerobic, motile, non acid-fast, non spore formers, oxidase negative and catalase positive. It is a large group of germs that are normal inhabitant of animal and human intestinal tract.

Environmental persistence of *E. coli* O157 may play a key role in the epidemiology of this agent on farms. faecal excretion of *E.coli* O157 by cattle is only transient, typically lasting 3 to 4 weeks.

prevalence in cattle are difficult to obtain because shedding is transient and shedding cattle show no clinical signs meaning active faecal sampling is necessary to identify positive farms.

E. coli O157 shedding in cattle populations is spatially and temporally clustered, consistent with point sources of exposure to the organism, such as periodically contaminated feed or water. Water offered to livestock is often of poor microbiological quality, and *E. coli* O157 is present in many of troughs.

Long-term storage of animal wastes reduces, but does not eliminate, the bacterial load therefore opening up the potential to contaminate the environment if any of these are spread on land as fertilizer

This group of bacteria, whose routes of transmission include food and water, are now recognized as an important cause of gastrointestinal disease in humans, particularly since such infections may result in life-threatening consequences such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (**Paton & Paton, 1998**). These for this study was designed for detection of the following:

1. Isolation and identification of *E.coli* O157 and *Methicillin-Resistant S.aurues* (MRSA) in different cattle farms so that, samples including milk, faeces, soil, air, water, and workers'

hands swab were collected randomly from healthy, apparent healthy and diseased animals and human in contact.

2. Determination of the significance of cattle as environmental reservoirs of *E. coli*.
3. Interpretation of the impact of cattle production in Egypt on the environment in different rearing systems to acquire foundational knowledge about the dangers of the two infectious microorganisms on environment and their by on animal and human and their reflection on the surroundings

Review of Literature

There is always need to study the impact of cattle production on the environment in different rearing system to acquire foundational knowledge about the dangerous of *Methicillin-Resistant S.aureus* (MRSA) on cattle and their reflection on the surrounding.

A. Impact of *Staphylococcus aureus* on the environment:

Staphylococcus aureus is highly pathogenic bacteria which play an important role in dairy mastitis and other animal diseases. Resistant *Staphylococcus aureus* to antibiotic agents (MRSA) plays an important role in treatment failure. Increasing concern about impact of MRSA is attributed to the spread from animals to surrounding environment and can survive in the environment for several months.

A.1.Epidemiological nature of Methicillin-Resistant S. aureus (MRSA)

Hartman and Tomasz, 1984 mentioned that the acquired resistance (Methicillin resistance) to the β lactamase stable β -lactam antibiotics is caused by penicillin-binding protein PBP2a and PBP2b.

Foster, 1991 pointed that *Staphylococcus aureus* causes severe animal diseases, such as suppurative disease, mastitis, arthritis, and urinary tract infection, that are associated with numerous virulence factors, such as the production of extracellular toxins and enzymes.