

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



# **Virucidal Ability of Some Disinfectants Against Locally Isolated Avian Viruses in Egypt**

Thesis

Presented by:

**Abd Elrahman Mohammed Gamal**

(B.V.Sc., Faculty of Vet. Med, Cairo Uni., 2010)

(M.V.Sc., Faculty of Vet. Med, Cairo Uni., 2014)

For the degree of

(Ph.D. in Veterinary Medicine)

Animal, Poultry and Environment Hygiene

Under the Supervision of:

**Prof. Dr. Osama Zahran**

Emeritus Professor of Animal, Poultry and  
Environmental Hygiene.

Department of Veterinary Hygiene  
Faculty of Veterinary Medicine  
Cairo University.

**Prof. Dr. Mohamed Mohamed Ali**

Professor of Animal, Poultry and  
Environmental Hygiene.

Department of Veterinary Hygiene  
Faculty of Veterinary Medicine  
Cairo University.

**Prof. Dr. Sherif Tawfik Moubarak**

Professor of Animal, Poultry and  
Environmental Hygiene

Department of Veterinary Hygiene  
Faculty of Veterinary Medicine  
Cairo University.

**Dr. Elshaimaa Ismael Sayed Solayman**

Lecture of Animal, Poultry and  
Environmental Hygiene.

Department of Veterinary Hygiene  
Faculty of Veterinary Medicine  
Cairo University.

**2018**

This is to approve that the dissertation presented by Abdelrhman Mohamed gamal to Cairo university for the degree of Ph.D. (Animal, Poultry and Environmental Hygiene) has approved by the examining committee.

<b>1. Prof. Dr. Ahmed Mohamed Byomi</b> Professor of Animal, Poultry and Environmental Hygiene Faculty of Veterinary Medicine University of Sadat City and the Head of Sadat City University.	Ahmed M. Byomi
<b>2. Prof. Dr. Hassan Moustafa El-Agrab</b> Professor of Animal, Poultry and Environmental Hygiene Faculty of Veterinary Medicine Cairo University.	Hassan El-Agrab
<b>3. Prof. Dr. Osama Mohamed Kamel Zahran</b> Professor of Animal, Poultry and Environmental Hygiene Faculty of Veterinary Medicine Cairo University.	Osama Zahran
<b>4. Prof. Dr. Mohamed Mohamed Ali</b> Professor of Animal, Poultry and Environmental Hygiene Faculty of Veterinary Medicine Cairo University.	Dr. Mohamed Ali
<b>5. Prof. Dr. Sherif Tawfik Moubarak</b> Professor of Animal, Poultry and Environmental Hygiene Faculty of Veterinary Medicine Cairo University.	S.T. Moubarak

(2018)

## **Supervision sheet**

This thesis is under supervision of:

### **Prof. Dr. Osama Mohamed Kamel Zahran**

Emeritus Professor of Animal, Poultry and Environmental Hygiene-  
Faculty of Veterinary Medicine- Cairo University

### **Prof. Dr. Mohamed Mohamed Ali**

Professor of Animal, Poultry and Environmental Hygiene- Faculty  
of Veterinary Medicine- Cairo University

### **Prof. Dr. Sherif Tawfik Moubarak**

Professor of Animal, Poultry and Environmental Hygiene- Faculty  
of Veterinary Medicine- Cairo University

### **Dr. Elshaimaa Ismael Sayed Solayman**

lecturer of Animal, Poultry and Environmental Hygiene- Faculty of  
Veterinary Medicine- Cairo University.

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



- **Name:** Abd Elrahman Mohammed Gamal
- **Nationality:** Egyptian
- **Date of birth:** 15 / 7 / 1988
- **Specialization:** Animal, poultry and environmental hygiene
- **Title of thesis** Virucidal Ability of Some Disinfectants Against Locally Isolated Avian Viruses in Egypt

**Supervisors:**

**Prof. Dr. Osama Mohamed Kamel Zahran**

Emeritus Professor of Animal, Poultry and Environmental Hygiene - Faculty of Veterinary Medicine - Cairo University.

**Prof. Dr. Mohamed Mohamed Ali**

Professor of Animal, Poultry and Environmental Hygiene-Faculty of Veterinary Medicine - Cairo University.

**Prof. Dr. Sherif Tawfik Moubarak**

Professor of Animal, Poultry and Environmental Hygiene - Faculty of Veterinary Medicine - Cairo University.

**Dr. Elshaimaa Ismael Sayed Solayman**

lecturer of Animal, Poultry and Environmental Hygiene- Faculty of Veterinary Medicine - Cairo University.

**Abstract:**

This study was carried out to help improving two of the most important pillars of any biosecurity programs, Disinfection and Footbath. The aim of this study was to evaluate the viricidal ability of fourteen commonly used and commercially available disinfectants on poultry farms against velogenic Newcastle disease virus (NDV), Low Pathogenic Avian

Influenza Virus (LPAI) H9N2 that were isolated locally in Egypt. To simulate the Egyptian field conditions, we used two different surfaces (cement and rubber) contaminated experimentally with the previously mentioned viruses, we also added two interfering materials together, organic matter and hard water. Against NDV our results revealed that three disinfectants out of fourteen were able to pass the test criteria (achieving at least 2.8 log reductions, and no recoverable viruses were isolated after the treatment) these disinfectants were calcium hypochlorite, Halamid® and ZixVirox® while Virkon S®, PIQuat 20®, Aquazix E52®, Fumagri Effisafe® GroundZero®, Synergize®, Formic, and Citric acid, all failed to pass the test criteria (either not achieving the required log reduction or stopping the viral propagation). Against (LPAI) H9N2 our results revealed that most of the used disinfectants were effective in destroying the virus after the treatment as disinfectants (VirkonS®, Halamid®, Ca.Hypochlorite, PIQuat20®, Aquazix E52®, Zixvirox®, Dyne-O-Might® and Fumagri Effisafe®) were all able to achieve the previously mentioned test criteria. While disinfectants (Synergize®, Groundzero®, Iodis® formic and citric acid) all failed to pass the test criteria. The study was also done to evaluate disinfectants suitability to the footbath task, for this purpose we used disinfectants that commonly used in the Egyptian poultry farms in footbath. Different forms of disinfectants were used (liquid, solid and semisolid). We had concluded after this experiment that aldehydes, iodophors, phenols and powder form disinfectants (VirkonS®+ salt, Halamid®+ calcium carbonate, paraformaldehyde+ calcium carbonate, and Ca.Hypochlorite + calcium hydroxide) were the most effective in the footbath, on the other hand the semisolid form of the tested disinfectants gave variable results in the footbath.

**Key words:** Viricidal – Chemical disinfectant – Avian influenza virus – New castle disease virus - Inactivation – Footbath- Biosecurity – Egypt.

## *Dedication*

*I would like to dedicate this work to  
My Teacher, My Wife Asma, Daughter  
Aliyah, and My family, I hope I made  
you happy.*

## **ACKNOWLEDGMENT**

*Thanks for Allah. The Master of Universe, Most Gracious,*

*Most Merciful.*

*Without the generous aid of many people the successful completion of this research would not have been possible; therefore, I would like to express my greatest appreciation to the following personnel.*

*I would like to express my thanks for **Prof. Dr. Osama Zahran**, professor of veterinary hygiene and management, Faculty of Veterinary Medicine, Cairo University who assisted me every step and under his supervision this work was planned and carried out with the support of his kindness and encouragement, he was like my father.*

*I am very thankful to **Prof. Dr. Mohamed Mohamed Ali**, professor of veterinary hygiene and management, Faculty of Veterinary Medicine, Cairo University for the honest unselfish and unlimited help he gave me during the application and preparation of this work under his supervision this work was planned and carried out.*

*My deep greeting appreciation to **Prof. Dr. Sherif Moubarak**, professor of veterinary hygiene and management, Faculty of Veterinary Medicine, Cairo University, for his unselfish help he gave me during this work,*

*I am very thankful to **Dr. El-Shaimaa Ismael** lecturer of veterinary hygiene and management, Faculty of Veterinary Medicine, Cairo University for her continuous supportive cooperation during the application of this work,*

*I would like also to thank **Prof. Dr. Manal zaki** professor of veterinary hygiene and management Faculty of Veterinary Medicine, Cairo University for her precious advices and help offered through this work,*

*My deep thanks and gratefulness are due also to all the staff members of Department of veterinary hygiene and department, Faculty of Veterinary Medicine, Cairo University especially **Ass. Prof. Shimaa Nasr, Ass. Prof. Tamer fawzey, and Dr. Samah El-saied** for their kind support and brotherhood feelings they expressed to me during this work.*

*I would like also to thank **Ass. Prof. Mohamed Mamdouh Hamoud** Assistant Prof. of poultry and rabbit diseases, Faculty of Veterinary Medicine, Cairo University for his precious advices and help offered through this work,*

*I would like also to thank my colleague **Mohammed abdelmohsen** for his precious advices and help offered through this work,*

*Finally, no acknowledgment is complete without thanking **my wife Asma, my daughter Aliyah and my family** for their immense spiritual support during my research work,*



## **Table of Contents:**

<b>Introduction:</b> .....	1
<b>1. Study 1: In vitro Evaluation of the Viricidal Efficacy of Commercially used Disinfectant against Velogenic Newcastle Disease Virus</b> .....	6
1.1. <b>Review of Literature:</b> .....	6
1.1.1. Epidemiology of Newcastle Disease Virus: .....	6
1.1.2. Principles of Viricidal Testing: .....	8
1.1.2.1.Suspension Test and Carrier Test: .....	10
1.1.3. Approved Test Used for Evaluation of Viricidal Effect of Disinfectants: .....	13
1.1.4. Chemical Inactivation of Newcastle Disease Virus:.....	14
1.1.4.1.Chlorine and Chlorine Releasing Compounds.....	15
1.1.4.2. Oxidizing Agents: .....	16
1.1.4.3.Aldehydes .....	19
1.1.4.4.Quaternary Ammonium Compounds:.....	20
1.1.4.5.Phenolic Compounds: .....	22
1.1.4.6.Iodine Related Compounds:.....	23
1.2. <b>Materials and Methods</b> .....	22
1.3. <b>Results:</b> .....	31
1.4. <b>Discussion:</b> .....	37
<b>2. Study 2: In vitro Evaluation of the Viricidal Efficacy of Commercially used Disinfectant against Low Pathogenic Avian Influenza Virus (LPAI) H9N2</b> .....	46
2.1. <b>Review of Literature:</b> .....	46

2.1.1. Epidemiology of Low Pathogenic Avian Influenza Virus (LPAI) H9N2:	46
2.1.2. Chemical inactivation of Low Pathogenic Avian Influenza Virus (LPAI):	48
2.1.2.1. Chlorine releasing agents:	48
2.1.2.2. Aldehydes and Quaternary Ammonium Compounds (QACS):	51
2.1.2.3. Oxygen Releasing Agents:	54
2.1.2.4. Iodine Related Compounds:	54
2.1.2.5. Phenol and Phenolic Compounds:	55
2.1.2.6. Organic Acids:	57
2.2. <b>Materials and Methods</b>	58
2.3. <b>Results:</b>	62
2.4. <b>Discussion:</b>	68
<b>3.1. Study 3: Evaluation of The Viricidal Ability of Some Footbath Disinfectants Against Locally Isolated Velogenic Newcastle Disease Virus:</b>	77
3.2. <b>Review of Literature:</b>	77
3.2.1. Foot Bath Definition:	77
3.2.2. Efficacy of disinfectant in footbath:	78
3.3. <b>Materials and Methods:</b>	81
3.4. <b>Results:</b>	85
3.5. <b>Discussion:</b>	88
<b>Conclusion:</b>	99
<b>Summary:</b>	104
<b>References:</b>	111

## LIST OF TABELS

<b>Table</b>	<b>Title</b>	<b>Page</b>
<b>(1-1)</b>	Chemical Disinfectants.	27
<b>(1-2)</b>	chemical neutralizers.	28
<b>(1-3)</b>	Viricidal effect of Chlorine releasing Disinfectants on cement coupons.	31
<b>(1-4)</b>	Viricidal effect of Chlorine releasing Disinfectants on rubber coupons.	31
<b>(1-5)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on cement coupons.	32
<b>(1-6)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on rubber coupons.	32
<b>(1-7)</b>	Viricidal effect of iodine Based Disinfectants on cement coupons.	33
<b>(1-8)</b>	Viricidal effect of iodine Based Disinfectants on rubber coupons.	33
<b>(1-9)</b>	Viricidal effect of Oxidizing agents on cement coupons.	34
<b>(1-10)</b>	Viricidal effect of Oxidizing agents on rubber coupons.	34
<b>(1-11)</b>	Viricidal effect of Organic acids on cement coupons.	35
<b>(1-12)</b>	Viricidal effect of Organic acids on rubber coupons.	35
<b>(1-13)</b>	Viricidal effect of Phenolic compounds on cement coupons.	36
<b>(1-14)</b>	Viricidal effect of Phenolic compounds on rubber coupons.	36
<b>(2-1)</b>	Viricidal effect of Chlorine releasing Disinfectants on cement coupons.	62
<b>(2-2)</b>	Viricidal effect of Chlorine releasing Disinfectants on rubber coupons.	62
<b>(2-3)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on cement coupons.	63
<b>(2-4)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on rubber coupons.	63
<b>(2-5)</b>	Viricidal effect of iodine Based Disinfectants on cement coupons.	64
<b>(2-6)</b>	Viricidal effect of iodine Based Disinfectants on rubber coupons.	64

(2-7)	Viricidal effect of Oxidizing agents on cement coupons.	65
(2-8)	Viricidal effect of Oxidizing agents on rubber coupons.	65
(2-9)	Viricidal effect of Organic acids on cement coupons.	66
(2-10)	Viricidal effect of Organic acids on rubber coupons.	66
(2-11)	Viricidal effect of Phenolic compounds on cement coupons.	67
(2-12)	Viricidal effect of Phenolic compounds on rubber coupons.	67
(3-1)	Liquid footbath.	81
(3-2)	Dry foot bath disinfectants.	82
(3-3)	Re-isolation of the virus from liquid footbath.	85
(3-4)	Results of Liquid footbath.	85
(3-5)	Results of semi-solid footbath (PRIL).	86
(3-6)	Results of semi-solid footbath (Silica Gel)	86
(3-7)	Solid Footbath.	87

## LIST OF FIGUERS

<b>Figure</b>	<b>Title</b>	<b>Page</b>
<b>(1-1)</b>	Building materials	25
<b>(1-2)</b>	Viricidal effect of Chlorine releasing Disinfectants on cement and rubber coupons	31
<b>(1-3)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on cement and rubber coupons	32
<b>(1-4)</b>	Viricidal effect of iodine Based Disinfectants on cement and rubber coupons	33
<b>(1-5)</b>	Viricidal effect of oxidizing agents on cement and rubber coupons	34
<b>(1-6)</b>	Viricidal effect of organic acids on cement and rubber coupons	35
<b>(1-7)</b>	Viricidal effect of Phenolic compounds on cement and rubber coupons	36
<b>(2-1)</b>	Viricidal effect of Chlorine releasing Disinfectants on cement and rubber coupons	62
<b>(2-2)</b>	Viricidal effect of Aldehyde and Quaternary ammonium compound Disinfectants on cement and rubber coupons	63
<b>(2-3)</b>	Viricidal effect of iodine Based Disinfectants on cement and rubber coupons	64
<b>(2-4)</b>	Viricidal effect of oxidizing agents on cement and rubber coupons	65
<b>(2-5)</b>	Viricidal effect of organic acids on cement and rubber coupons	66
<b>(2-6)</b>	Viricidal effect of Phenolic compounds on cement and rubber coupons	67

## **LIST OF ABBREVIATIONS:**

AI	Avian Influenza
AusVetPlan	Australian Veterinary Emergency Plan
DVG	Deutsche Veterinärmedizinische Gesellschaft
DVV	German Association for the Control of Virus Disease
ECEs	Embryonated Chicken Eggs
EID50	Mean Embryo Infective Dose
EMA	European-Middle Eastern-African
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
HA	Haemagglutination
HA	Haemagglutinin
HI	Haemagglutination Inhibition
HPAIV	Highly Pathogenic Avian Influenza Virus
LPAIV	Low Pathogenic Avian Influenza Virus
NDV	Newcastle disease virus
OIE	World Organisation for Animal Health
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SPF	Specific Pathogen Free
WHO	World Health Organization
TCID	Tissue Culture Infective Dose
ASTM	American Society for Testing and Materials
AFNOR	Association Francaise de Normalisation

## **Introduction:**

In Egypt the rapidly growing sector of poultry farming, plays an important role in the Egyptian food security chain. The sustainability of this sector is continuously threatened by emergence of different viral pathogens e.g. Newcastle disease virus and avian influenza virus. These viruses can hypothetically be transmitted to chickens via contaminated inanimate surfaces. Many of these viruses exhibit long-lasting stability on surfaces, especially when organic matter is present. Newcastle disease virus (NDV) was first identified in Egypt in 1948 (**Daubney and Mansy 1948**). The disease spread widely throughout the country and has been recorded as an “endemic” at the beginning of 1960s. (**El-Nassary and Eskarous, 1960**). Trials for controlling the disease were practised by mass vaccination programs using combined live attenuated and inactivated vaccines to develop solid immunity against the virus. (**Alexander, 2000, Ewies et al., 2017**). Since the early months of 2011, many NDV outbreaks with high mortality rates have been recorded regardless the adopted intensive vaccination programs, these outbreaks were attributed to the circulation of the new genotype VIIId that wasn't reported before in Egypt. (**Radwan et al., 2013, Ewies et al., 2017**). The first report of Low pathogenic avian influenza H9N2 infection in Egypt was in November 2011, when the virus was