



Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

**Factors Affecting Capability of Toxin Production of
Lyophilized *Clostridium tetani* (Harvard Strain)**

A thesis presented by

Shereen Said Ahmed AbdElwahed
B.V.Sc., Cairo University, 2008

**For the Master Degree in Veterinary Medical sciences,
Microbiology**

Under the supervision of

Prof. Dr. Mona Ibrahim Hassan El-Enbaawy
Prof. and Head of Department Microbiology
Faculty of Veterinary Medicine
Cairo University

Dr. Wafaa Ragab Abd El-Aziz El-Sayed

Senior Researcher
Department of Bacterial Sera and Antigens Research
Veterinary Serum and Vaccine Research Institute,
Abbasia, Cairo
2018

Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

APPROVAL SHEET

This is to approve that dissertation presented by
Shereen Said Ahmed AbdElwahed

To Cairo University
Entitled

Factors Affecting Capability of Toxin Production of Lyophilized Clostridium Tetani
(Harvard Strain)

For the Master Degree

has been approved by the examining committee:

Prof. Dr. Ashraf Awaad Abd Eltwab

Professor and Head of Bacteriology, Immunology and Mycology
department
Faculty of Veterinary Medicine
Benha University

Ashraf Awaad

Dr. Sherif Abdelmonem Omar Marouf

Assistant Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Sherif

Prof. Dr. Mona Ibrahim Hassan El-Enbaawy

Professor and Head of Microbiology department
Faculty of Veterinary Medicine
Cairo University
(Supervisor)

Mona EL-Enbaawy

Dr. Wafaa Ragab Abd El-Aziz El-Sayed

Senior Researcher
Department of Bacterial Sera and Antigens Research
Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo
(Supervisor)

Wafaa Ragab

Date: 21 / 6 / 2018

بسم الله الرحمن الرحيم

"إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ إِنَّ اللَّهَ
عَزِيزٌ غَفُورٌ"

صدق الله العظيم

(سورة فاطر – الآية ٢٨)

Supervision sheet:

This thesis is under supervision of:

Prof. Dr. Mona Ibrahim Hassan El-Enbaawy

Prof. and Head of Microbiology Department
Faculty of Veterinary Medicine
Cairo University

Dr. Wafaa Ragab Abd El-Aziz El-Sayed

Senior Researcher
Department of Bacterial Sera and Antigens Research
Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo



Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Name: **Shereen Said Ahmed Abdelwahed**

Date of Birth: **5/5/1986**

Nationality: **Egyptian**

Degree: **Master degree of Veterinary Medical Sciences.**

Specialization: **Microbiology.**

Title of the Thesis: **Factors Affecting Capability of Toxin Production of Lyophilized *Clostridium tetani* (Harvard Strain)**

Supervisors:

1. **Prof. Dr. Mona Ibrahim Hassan El-Enbaawy.**
2. **Dr. Wafaa Ragab Abd El-Aziz El-Sayed**

Abstract

In order to preserve the characters of the highly toxigenic *C. tetani* (Harvard strain) as long as possible, a combined approach of pre-lyophilization treatment of microorganisms and subsequent storage was developed in order to improve cell survival and toxin production capability. Different stabilizers have been tried in combination with different storage temperatures. The study revealed that -20 °C is the best temperature for storing *C. tetani* (Harvard strain) and that stabilizer III (modified **Pivnick**) is the best stabilizer for lyophilization of *C. tetani* (Harvard strain) as it preserves the strain's toxigenic capability till the 19th month post lyophilization with minimum reduction in toxin titer. By PCR assay, the samples were found to be positive for *tetX* gene, thus confirming their potential to produce the tetanus toxin.

In conclusion: preserving *C. tetani* by lyophilization using stabilizer III (modified Pivnick) retains its toxigenic power more than other stabilizers for longer periods. Also, the optimum temperature for storing lyophilized *C. tetani* (Harvard strain) is at -20 °C.

Key words: *C. tetani* - Harvard strain – Lyophilization – Stabilizer.

Dedicated to:

My father

my mother

my Husband

my daughter

and

my son

Acknowledgment

I would like to start by thanking **Lord Allah** for helping me during all the stages of this work.

Then ,I would like to express my sincere gratitude for the kindness and encouragement of **Prof. Dr. Mona Ibrahim Hassan El-Enbaawy, Prof. and Head of Department of Microbiology , Faculty of Veterinary Medicine , Cairo University** , *for her valuable advices , constructive criticism , cooperation and support during the course of this study* under whose stimulating supervision, guideness , this work was carried out .

I wish to express my appreciation and deep thanks to **Dr. Wafaa Ragab AbdelAziz, Senior Researcher, Veterinary Serum and Vaccine Research Institute, Abbasia.Cairo** *for his valuable advices , constructive criticism , cooperation and intense kind efforts during the course of this study.*

Also many thanks to **all members in the Department of Microbiology, Faculty of Vet. Med. Cairo University and Bacterial Sera and Antigens Research Department, Vet. Serum and Vaccine Research Institute, Abbasia, Cairo**, for their support.

Contents

Subject	Page
List of Tables	IV
List of Figures	V
List of Photos	VI
1. INTRODUCTION.....	1-4
2. REVIEW of LITERATURE.....	5-28
2.1. Importance of <i>C. tetani</i> (toxin and disease).....	5-9
2.2. Factors affecting cell survival and toxin production.....	9-16
2.3. Preservation of bacterial culture.....	16-19
2.4. Stabilizers.....	19-23
2.5. Clostridium tetani toxigenic gene.....	24-27
3. Material and Methods.....	28-49
3.1. Materials.....	28-38
3.1.1. Bacterial strains.....	28
3.1.2. Media.....	28
3.1.3. Stabilizer used for lyophilization of bacteria.....	32
3.1.4. Tetanus antitoxin for flocculation test	33
3.1.5. Experimental animals	33
3.1.6. Buffers and solutions.....	33
3.1.7. Preservative	34
3.1.8. Miniprep plasmid extraction of plasmid DNA.....	34
3.1.9. Chemicals used for detection of <i>Clostridium tetani</i> (Harvard strain) toxin gene by PCR	35
3.1.10. Buffers and solutions used for agarose gel electrophoresis.....	36
3.1.11. Equipment.....	38
3.2. Methods.....	40-49
3.2.1. Preparation of seed culture for lyophilization	40
3.2.2. Stabilizers used for lyophilization of cultures.....	40
3.2.3. Lyophilization of cultures.....	41
3.2.4. Evaluation of Lyophilized cultures.....	41
3.2.5. Detection of plasmid encoded toxin gene of <i>C. tetani</i> (Harvard strain) lyophilized with different stabilizers and stored at different temperatures	43
4. RESULTS.....	50-77
5. DISCUSSION.....	78-87