



Cairo University
Faculty of Veterinary Medicine
Department of Medicine and Infectious Diseases

APPLICATION OF BIO RISK REGULATION FOR EPIDEMICSCONTROL IN EQUINE FARMS

A thesis presented by

Hend Ibrahim Mahmoud Abd El-Mahoud

(B.V.Sc., 2005, Faculty of Veterinary Medicine, Cairo University)

For Master Degree in Veterinary Science

(Infectious Diseases)

Under Supervision of

Prof. Dr. Samia Abd-El Hamid Ahmed

Professor of Infectious Diseases Faculty of Veterinary Medicine Cairo University

Dr. Soliman Mohammed Soliman

Dr. Sayed Ahmed Hassan Salem

Lecturer of Infectious Diseases
Faculty of Veterinary Medicine
Cairo University

Chief of Researcher
Virology Research Dept.
Animal Health Research Institute

Cairo University
Faculty of Veterinary Medicine
Department of Medicine and Infectious Diseases

Name: Hend Ibrahim Mahmoud Abd El Maaboud

Date of Birth: 25/6/1982

Specialization: Infectious diseases

Title of thesis:

Application of Bio Risk Regulation for Epidemics Control in Equine Farms

Supervisors:

Prof. Dr. Samia Abd-El Hamid Ahmed

Professor of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University

Dr. Soliman Mohammed Soliman

Lecturer of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University

Dr. Sayed, Ahmed Hasan Salem

Chief Researcher, Virology Research, Animal Health Research institute

Abstract

Equine influenza virus (EIV) is considered the most important cause of respiratory affection of horses because it is highly contagious disease that has potential to disrupt major equestrian events. Equine influenza (EI) can be controlled by vaccination, but without Biosecurity measures there is no control on the respiratory problems as well as antigenic drift impacts on vaccine efficacy so the bio risk regulations is urgently needed, Appropriate biorisk management strategies should be developed, established and implemented in the control programs to deal with this problem.

In this study a total number of 357 of different equidae species (horse-donkey-mule) were examined for the presence of clinical signs of respiratory problem including fever, harsh sound, dry cough and mucopurulent discharge. 357 serum samples and 74 nasal swabs were collected from the examined equidae from 10 Egyptians governorates classified into three regions , Upper Egypt(Beni-suef-Fayoum-Luxor-Qena) , Central region (Cairo –Giza) and Delta region (Alexandria-Gharbia-Beheira –Kafr ElShiekh) . ELISA and HI was conducted on serum samples to detect antibodies against EIV, where (58%) were tested positive for antibody against EIV by ELISA and (51.24%) by HI.

Real Time PCR was carried out directly on nasal swabs using specific primers where all samples tested were negative.

Biosecurity measures were applied on farm against another one with no biosecurity measures for a period from 2014 till 2016. The noticed was decreasing the percentage of the clinically suspected cases(from 33.33% till 12.5%) in the farm which apply the biosecurity measures and the other one still show increasing in the new clinically suspected cases (from 40%, 45% to 50%).

DEDICATION

I dedicate this work to ALLAH almighty my creator, my strong pillar, my source of inspiration, strength, wisdom, knowledge and understanding.

A special thanks to my family. Words cannot express how grateful I am to my mother and my father for all of the sacrifices and being a constant source of support, their pray for me was what sustained me thus for. I am truly thankful and proud for having you in my life.

ACKNOWLEDGEMENT

First of all, my deep prayerful gratitude should be submitted to merciful ALLAH, whose blessings enabled me to complete this study and without his willing I will achieve nothing.

No words can adequately express my deepest gratitude and faithful thanks to **Prof. Dr. Samia Abd- El hamid Ahmed**, Professor of Infectious diseases, faculty of veterinary medicine, Cairo University for her supervision, continuous aid and fruitful advice, great help and encouragement throughout this study.

I would like to express my deep appreciation and gratitude to **Dr. Sayed Ahmed Hassann Salem**, chief researcher of virology, Animal Health Research

Institute (AHRI), Dokki and **Dr. Soliman Mohammed Soliman**, lecturer of Infectious diseases, faculty of veterinary medicine, Cairo University for kind encouragement, help, great support, guidance and supervision.

I would like to offer my grateful thanks to all medicine infectious diseases department staff, faculty of veterinary medicine, Cairo University and virology department, Animal Health Research Institute (AHRI), Dokki ,for their help and support.

Finally, I would like to express my special thanks to my **family** for the encouragement and support given to me to fulfill this work, and for help throughout my life.

CONTENTS

Item	Page
INTRODUCTION	1
REVIEW OF LITERATURE	5
1- History of equine influenza viruses	5
2- Etiology	6
3- The Economic impact of EIV	6
4- Epidemiology of EIV	7
5- Prevalence of EIV	9
6- Diagnosis of Equine influenza	9
6.1-Field diagnosis (clinical signs)	9
6.2- Hemagglutination Inhibition test	10
6.3- Enzyme Linked Immune Sorbent Assay (ELISA)	11
6.4- Real time-polymerase chain reaction (rt-PCR)	13
7-Importance of bio security	15
MATERIAL AND METHODS	18
1.Material	18
1.1. Animals	18
1.2. Samples	21
1.3. Biosecurity measures SOPs	21
1.4. Kits used for detection of antibodies against EIV in equidae serum	22
1.5 Material used for extraction of RNA	22
1.6.Material used for real time PCR	23
1.7. Material used for Heamagglutination Inhibition test	24
2. Methods	25
2.1. Clinical examination of (equidea) Animals	25
2.2. Sample collection	25
2.3. Indirect ELISA for detection of EI antibodies in serum samples	25
2.4. Method of extraction of Influenza virus RNA according to QIAamp Vira	al 28
RNA Mini handbook	
EXPERIMENTS AND RESULTS	34
DISCUSSION	51
SUMMARY	60
REFERENCES	62
الملخص العربي	١

LIST OF ABBREEVATIONS

EIV	Equine Influenza Virus
PCR	Polymerase Chain Reaction
HI	Heamagglutination Inhibition
HA	Heamagglutination
FAO	Food and Agriculture Organization
OIE	Office of International Epizootics
ELISA	Enzyme Linked Immune Sorbent Assay
rt-PCR	Real Time Polymerase Chain Reaction
rpm	Revolutions per minute
lab	Laboratory
min	Minute
sec	second
SOPs	Standard operator procedure
PPE	Personal protective equipments
Ab	antibodies
qRT-PCR	Qualitative reverse transcription PCR
PBS	Phosphate buffer saline
RBCs	Red Blood Cells

LIST OF TABLES

Table no.	Item	Page
1	Distribution of equidae which examined and from	19
	which serum samples collected for Ab detection in	
	some Egyptians governorates	
2	Distribution of equidae by region in relation to	20
	biosecurity measures application (2014-2016)	
3	Distribution of equidae serum samples in relation to	20
	Age	
4	Distribution of equidae serum samples in relation to	20
	species	
5	Distribution of equidae serum samples in relation to	20
_	vaccine status (2014-2016)	
6	The Oligonucleotide Primers and probes	24
7	Preparation of PCR Master Mix according to	29
	Quantities probe RT-PCR kit handbook	
8	Cycling conditions of Primers and probes	30
9	Results of indirect ELISA of the total collected sera	34
10	of clinically diseased Equidae	
10	Results of indirect ELISA of each governorate	35
11	Results of indirect ELISA of the total sera in relation	36
	to region	
12	Results of indirect ELISA of the total collected	38
	equidae sera inrelation to sex	
13	Results of indirect ELISA of the total collected	39
4.4	equidae sera in relation to age	40
14	Results of indirect ELISA of the total collected	40
1.7	equidae sera samples in to Species	40
15	the results of total serum samples for Ab detection	42
16	the results of HI test in different Egyption	43
15	governorates	
17	Matching between results of ELISA& HI for specific	45
10	Ab detection in Equidae sera	4.5
18	the result of the tested 74 nasal swabs samples by PCR	46
19	Modeling schedule for application of bio risk	50
	measures in two farms under experiment 2014-2016	

LIST OF FIGURES

Fig. no.	Item	Page
1	bio security measures application	32
2	Showing results of indirect ELISA of the total collected	36
	sera of clinically diseased equidae	
3	Prevalence of equine influenza in different governorates	39
4	Results of indirect ELISA of total sera in relation to	37
	localities	
5	Distribution Results of indirect ELISA of the total	38
	collected sera in relation to sex	
6	Distribution of the results of indirect ELISA of the total	39
	collected equidea sera in relation to age	
7	Distribution of results of indirect ELISA of the total	40
	collected sera in relation to Species	
8	Result of total positive samples against negative by HI	42
9	Result of positive samples by HI test in each	43
	governorate	
10	Matching between the positive results of ELISA& HI	45
	for specific Ab detection in Equidae sera	
11	The result of the tested 74 nasal swabs samples by PCR	47
12	The difference between the farm (A) which applied the	50
	biosecurity measures and the farm (B) which didn't	
	applied biosecurity measure	