Cairo University Faculty of Veterlaary Medicine Bepartment of Virology

## **Approval Sheet**

This is to approve that the dissertation presented by:

### AHMED MOHAMED AHMED ABDUL-ATY ELHARIRY

to Cairo University-Faculty of Veterinary Medicine, entitled:

# "Studies on Adenoviruses in animal and human"

For the degree of **Ph.D.** (**VIROLOGY**) has been approved by the examining committee:

Prof. Dr. Gabr Fekry El-Bajoury

Professor and Head of Virology Department Faculty of Veterinary Medicine Banha University

Prof. Dr. Mohamed Samy Saber

Professor of Virology Faculty of veterinary medicine Cairo University

Prof. Dr. Mohamed Abd El-Hamid Shalaby

Professor of Virology
Faculty of veterinary medicine
Cairo University
(Supervisor)

Dated on 25th of August 2005.

G.F. El Barry



# بسبم اللسه الرحمين الرحيم للما علم لنا إلا ما علم لنا علم لنا علم الما علم الما علم النا إلا ما علم الما علم النا إنك أنت العليم العليم المحكيم

صدق الله العظيم

### **ACKNOWLEDGMENT**

"Praise be to Allah, who has guided us to this, never could we have found guidance, had it not been for the guidance of Allah; Indeed it was the truth."

I am greatly indebted to Prof. Dr. M.A. Shalaby, The former Chairman of Virology Department, Faculty of Veterinary Medicine- Cairo University, who kindly devoted a great deal of his valuable time in planning, and entire development of this work. It is a great honour to work under his supervision.

Sincere thanks for Prof. Dr. A.M. Samy, Professor of virology, Faculty of Veterinary Medicine- Cairo University, for his persistent help, valuable advice, stimulating suggestions and constructive criticism.

It is a pleasure to record my grateful acknowledgement and appreciation to Prof. Dr. R.M. El-Karamany, the former Head of the Research Department at VACSERA, for his actual and great supports, generous advice and scientific guidance, in addition to the supplies, that facilitate carrying out of the present work.

Special thanks, to Prof. Dr. M. S. Saber and Prof. Dr. A. Elsonosy, Fac. of Vet. Med.-Cairo Univ., for their great scientific and technical support all over the study, and for dedicating a lot of their valuable time in monitoring and follow up of all the study procedures and results.

I must introduce my deep gratitude and appreciation to Chairman of VACSERA, Dr. M. El-Abbadi, for the continuous support and empowering enhancements to apply the practical experiments at various VACSERA laboratories.

My deep thanks for Prof. Dr. M. Salama, Fac. of Science-Ain Shams Univ., and Dr. Ali Fahmy, *Vacsera*; for their continuous support and encouragement during the whole study, by them the work was obviously promoted and improved.

Particular gratitude should be paid for Prof. Dr. Annika Allard, Virology Department, Ume University, Sweden, for her continuous beneficial technical support and supply of references and materials.

I should not forget to acknowledge all of my colleague staff members at VACSERA laboratories [Virology Research Dept., Cell Culture Dept. Influenza and Respiratory Inf. Dept., Molecular Biology Dept.], for their great help and supporting efforts.

\*\*\*\*

### **LIST OF CONTENTS**

Acknowledgement	
List of contents	í
List of tables	]]iii
List of figures	iv
List of Photos	V
List of Charts	vi
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	7
2.1. Description and Nature of adenovirus	7
2.1.1. Virion Properties	7
2.1.1.1. Morophology	7
2.1.1.2. Viral Structure	9
2.1.1.3. Biophysical Properties	12
2.1.1.4. Sensitivity to physicochemical agents	13
2.1.1.5. Biological Properties	13
2.1.1.6. Viral Replication	15
2.1.1.7. Antigenic Properties.	18
2.1.1.8. Phylogony and Genetic Organization	19
2.1.1.9. Growth in Cell Culture	23
2.1.1.10. Adenovirus as Vectors for delivery of foreign DNA	25
2.1.2. Classification of Family: Adenoviridae	26
2.1.3. Taxonomic Structure of Adenoviridae	32
2.2. Clinical Aspects and Epidemiology of Adenoviruses	35
2.2.1. Adenovirus in Human	35
2.2.2. Adenovirus in Bovines	48
2.2.3. Adenovirus in Equines	54
2.2.4. Adenovirus in Canines	55
2.2.5. Adenovirus in Avians	58
2.3. Immunologic Aspects, Prevention and Treatment of Adenovirus	61

LIST OF CONTENTS Studies on Adenoviruse	es in Animal and Humo
2.4 Laboratory Diagnosis of Adenoviruses	69
2.4.1. Direct Detection in Clinical Samples	69
2.4.2. Virus Isolation	78
2.4.3. Identification of Isolates	B0
2.4.4. Serologic Diagnosis	83
3. MATERIALS AND METHODS	85
3.1. Materials	85
3.2. Methods	93
4. RESULTS	115
5. DISCUSSION	142
6. CONCLUSION AND RECOMMENDATIONS	157
7. SUMMARY ,	162
8. REFERENCES	167
9. ARABIC SUMMARY	
<u> </u>	

### LIST OF TABLES

Table N°. 1 : Classification of Family Adenoviridae	27
Table N°. 2 : Adenovirus serotypes infecting Animals	28
Table N°. 3 : Subdivision of Human Adenoviruses	[31
Table №. 4 : Age distribution of human study population	85
Table No. 5: Nucleotide ambiguity codes	113
Table N°. 6 : Results of Direct IFA (in human samples)	116
Table No. 7 : Results of Direct IFA (in bovine samples)	118
Table N°. 8 : Results of Adenovirus isolation from human samples	122
Table N <sup>6</sup> . 9 : Results of Adenovirus isolation from bovine samples	[123]
Table No. 10 : Statistical analysis of IFA (in human samples)	127
Table N°. 11 : Statistical analysis of IFA (in bovine samples)	128
Table N°. 12 : Results of ELISA test	130
Table N°. 13 : Results of single-step and Nested PCR techniques in	136
human and bovine samples	
Table N°. 14 : Statistical analysis of single-step and Nested PCR	141
techniques	
•	
	•

### **LIST OF FIGURES**

Fig No. 1: Structure of Adenovirus	9
Fig N°. 2: Structure of Adenovirus	9
Fig N°. 3: Adenoviral Replication	15
Fig N°. 4: Phylogenetic relationship among Adenoviridae Family	20
Fig No. 5: Genus-Common and Genus-Specific genes in Adenoviridae	22
· · · · · · · · · · · · · · · · · · ·	
Θ.	
	<b>,</b>
·	· ,
·	

### **LIST OF PICTURES**

Picture N°. 1: Electron micrograph o Adenovirus particle	7
Picture No. 2: Electron microscope picture of human Adenovirus	8
Picture No. 3: Electron microscope picture of porcine Adenovirus	8
Picture Nº. 4: Electron microscope picture of avian Adenovirus	8
Picture N°. 5: Example for positive reacting sample for direct IFA (slide)	119
Picture N°. 6: Example for negative reacting sample for direct IFA (slide)	119
Picture N°. 7: Example for complete CPE by Adenovirus in Hep-2 cells	121
Picture N°. 8: Example for partial CPE by Adenovirus in Hep-2 cells	121
Picture N°. 9: Example for absence of CPE in Hep-2 cells	121
Picture Nº.10: Positive samples by single-step PCR (100 bp ladder scale)	137
Picture N°.11: Positive samples by single-step PCR (50 bp ladder scale)	137
Picture N°.12: Positive samples by single-step PCR (100 bp tadder scale)	138
Picture Nº.13: Positive samples by Nested PCR (100 bp ladder scale)	139
	<u> </u>
<u></u>	•
<u> </u>	
<u> </u>	
<u> </u>	
· · · · · · · · · · · · · · · · · · ·	
<u> </u>	
<del></del>	
<u> </u>	h

### **LIST OF CHARTS**

Chart No. 1: ELISA results using human antiAdeno-antibodies (2726 SE)	131
Chart N°. 2: ELISA results using human antiAdeno-antibodies (947 SD)	132
Chart No. 3: ELISA results using human antiAdeno-antibodies (1504 SD)	133
Chart N°. 4: ELISA results using human antiAdeno-antibodies (1517 SD)	134
	<u> </u>
	L:
f <del></del>	<del></del>
·	
<u> </u>	
[	
	<del></del>
	<u> </u>
	_
	<del></del>
	<del></del> -1
<u> </u>	ь

### **LIST OF ABBREVIATIONS**

Ads	Adenoviruses
AHC	Acute Haemorrhagic Conjunctivitis
AIDS	Acquired Immuno Deficiency Syndrome
ARD	Acute Respiratory Disease
BAdV	Bovine Adenovirus
bp	Base Pair
BRSV	Bovine Respiratory Syncytial Virus
BSA	Bovine Serum Albumin
CAdV	Canine Adenovirus
CCIDso	Cell Culture Infective Dose 50
CE	Counter Electrophoresis
CF	Complement Fixation
CNS	Central Nervous System
CPE	Cyto-Pathic Effect
DNA	Deoxyribo-Nucleic Acid
EAdV	Equine Adenovirus
EIA	Enzyme Immuno-Assay
ELISA	Enzyme Linked Immuno Sorbent Assay
EKC	Epidemic Kerato-Conjunctivitis
EM	Electron Microscopy
EV70	Enterovirus type 70
FAdV	Fowl Adenovirus
FBS	Foetal Bovine Serum
FCS	Foetal Calf Serum
FITC	Fluorescein IsoThioCyanate
FrAdV	Frog Adenovirus
НА	Haemagglutination
VbAH	Human Adenovirus
HEK	Human Embryonic Kidney
HEV/TAdV	Haemorrhagic enteritis virus/Turkey Adenovirus

## LIST OF ABBREVIATIONS (cont.)

н	Haem-agglutination Inhibition
ICTV	International Committee on Taxonomy of Viruses
IEM	Immuno Electron Microscopy
IFA	Immuno-Fluorescent Assay
IIF	Indirect Immuno-Fluorescent
ILD	Influenza Like Disease
ITR	Inverted Terminal Repetitions
kDa	Kilo Dalton
LA	Latex Agglutination
LRI	Lower Respiratory Illness
Mab	Monoclonal antibodies
WAdV	Murine Adenovirus
MEM	Minimal Essential Medium
МоН	Ministry of Health
NCBI	National Centrer for Biotech Information
	Nanometer
nm	Malioniere
OAdV	Ovine Adenovirus
VbAO	Ovine Adenovirus
OAdV OD	Ovine Adenovirus Optical Density
OAdV OD PAdV	Ovine Adenovirus  Optical Density  Porcine adenovirus
OAdV OD PAdV PBS	Ovine Adenovirus  Optical Density  Porcine adenovirus  Phosphate buffered saline
OAdV OD PAdV PBS PCF	Ovine Adenovirus  Optical Density  Porcine adenovirus  Phosphate buffered saline  Pharyngeo-Conjunctival Fever
OAdV OD PAdV PBS PCF PCR	Ovine Adenovirus  Optical Density  Porcine adenovirus  Phosphate buffered saline  Pharyngeo-Conjunctival Fever  Polymerase Chain Reaction
OAdV OD PAdV PBS PCF PCR	Ovine Adenovirus Optical Density Porcine adenovirus Phosphate buffered saline Pharyngeo-Conjunctival Fever Polymerase Chain Reaction Proportionate Distance
OAdV OD PAdV PBS PCF PCR PD	Ovine Adenovirus Optical Density Porcine adenovirus Phosphate buffered saline Pharyngeo-Conjunctival Fever Polymerase Chain Reaction Proportionate Distance Possum Adenovirus
OAdV OD PAdV PBS PCF PCR PD PoAdV RBCs	Ovine Adenovirus Optical Density Porcine adenovirus Phosphate buffered saline Pharyngeo-Conjunctival Fever Polymerase Chain Reaction Proportionate Distance Possum Adenovirus Red Blood Cells
OAdV OD PAdV PBS PCF PCR PD PoAdV RBCs RE	Ovine Adenovirus Optical Density Porcine adenovirus Phosphate buffered saline Pharyngeo-Conjunctival Fever Polymerase Chain Reaction Proportionate Distance Possum Adenovirus Red Blood Cells Restriction Enzyme
OAdV OD PAdV PBS PCF PCR PD PoAdV RBCs RE	Ovine Adenovirus Optical Density  Porcine adenovirus Phosphate buffered saline Pharyngeo-Conjunctival Fever Polymerase Chain Reaction  Proportionate Distance Possum Adenovirus Red Blood Cells Restriction Enzyme Radio Immuno Assay

### LIST OF ABBREVIATIONS (cont.)

TNF	Tumour Necrosis Factor
TP	Terminal Protein
TR-IFA	Time resolved Immunofluorescent Assay
URI	Upper Respiratory Illness
USA	United States of America
WBCs	White Blood Cells
WHO	World Health Organization
μg	Micro gram
μl	Micro litter
	<u> </u>
	,
<del>,</del>	
	<u>,, , , , , , , , , , , , , , , , , , ,</u>
	<del>                                      </del>
	, , , , , , , , , , , , , , , , , , ,

# INTRODUCTION

ð,

### 1. INTRODUCTION

Adenovirus is one of a group of small double-stranded DNA viruses of animal cells capable of causing various disorders in human and animals, and of transforming cells into tumour cells.

Adenoviruses (Ads) were discovered in the early 1950s during the period of active searching for agents of the common cold and other respiratory infections of children. The first Adenoviruses were found during investigations of the cause of spontaneous degeneration of cell cultures of adenoidal tissue obtained by routine adenoidectomies in children. This was discovered in 1953 by Wallace Rowe and colleagues (*Rowe et al 1953*).

Others were found at the same time in HeLa cell cultures inoculated with respiratory specimens from patients with acute respiratory disease (ARD) or primary atypical pneumonia. Of particular significance during this period was the prominent role that adenoviruses played in non-influenzal acute respiratory disease (ARD) in new military recruits (Hillman and Werner 1954).

By the end of the 1950s, 24 antigenically distinct adenoviruses had been described, including some found during a search for the cause of poliomyelitis and others associated with eye disease (Bell et al 1960).

In 1954 Cabbasso and colleagues demonstrated that the etiological agent of infectious canine hepatitis was an adenovirus. Subsequently, many adenoviruses, each appearing to be highly host specific, were isolated from humans and many other mammals and birds, usually from the upper respiratory tract, but some-times from feces. Most of these viruses produce