



Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Comparative study on different types of inactivated Pasteurella vaccine for rabbits

A Thesis Presented By

Mahmoud Tawfik Ahmed Ismail

B.V.Sc., Cairo University, 2007

M.V.Sc., Cairo University, 2015

For the Ph.D. Degree in Veterinary Medical sciences

(Microbiology)

Under the supervision of

Prof. Dr. Mona Ibrahim El-Enbaawy

Prof. and head of Microbiology Department

Faculty of Veterinary Medicine

Cairo University

Prof. Dr. Eman Mohamed El Rawy

Chief researcher and head of Aerobic bacteria vaccine research Department

Veterinary Serum and Vaccine

Research Institute, Abbasia, Cairo

2019



Cairo University



Supervision sheet:

This thesis is under supervision of:

Prof. Dr. Mona Ibrahim Hassan El-Enbaawy

Professor and head of Microbiology Department

Faculty of Veterinary Medicine

Cairo University

Prof. Dr. Eman Mohamed El Rawy

Chief researcher and head of Aerobic bacteria vaccine research
Department

Veterinary Serum and Vaccine Research Institute

Abbasia, Cairo



Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Name: **Mahmoud Tawfik Ahmed Ismail**

Date of birth: **30/06/1985**

Nationality: **Egyptian**

Degree: **PhD of Veterinary Medical Sciences.**

Specialization: **Microbiology (Bacteriology, Immunology, Mycology)**

Title of thesis: **Comparative study on different types of inactivated Pasteurella vaccine for rabbits**

Supervisors:

1. Prof. Dr. Mona Ibrahim. H. El-Enbaawy

2. Prof. Dr. Eman Mohamed El Rawy

Abstract

Snuffle disease is one of the most important health problems in rabbits. It is caused by *P. multocida*. A total of 116 New-Zealand rabbits were used in evaluation of four prepared polyvalent *P. multocida* (serotypes A: 1, A: 3, A: 12 and D: 2) vaccines. First vaccine was formalized non adjuvanted Pasteurella vaccine (FV). Second one was Lipid A adjuvanted Pasteurella vaccine (AV) in which the Lipid A was self-prepared, extracted from *E. coli* O: 157 and evaluated by High Performance Liquid Chromatography (HPLC). Third one was Montanide™ ISA 70 VG adjuvanted Pasteurella vaccine (MV). Fourth one was Montanide™ IMS1313 VG N PR adjuvanted Pasteurella vaccine (NV) and finally control groups. Comparisons between the prepared vaccines were done by challenge test, Lysozyme activity test, IHA test and ELISA. Statistical analysis was done. Montanide™ ISA 70 VG adjuvanted Pasteurella vaccine and Lipid A adjuvanted Pasteurella vaccines were the best, followed by Formalized non adjuvanted Pasteurella vaccine then Montanide™ IMS1313 VG N PR adjuvanted Pasteurella vaccine.

In conclusion, the 1st dose vaccination may need bootstring for better results. Montanide™ ISA 70 VG and Lipid A adjuvanted Pasteurella vaccines were the best two in the prepared vaccines. Vaccination and bootstring by Montanide™ ISA 70 or Lipid A adjuvanted Pasteurella vaccines give 100 % protection.

Key words: *P. multocida*, Lipid A, Montanide, Vaccine, one-shot, HPLC.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ﴾ إِنَّ اللَّهَ

عَزِيزٌ غَفُورٌ ﴿

طَبَرُوا (اللَّهُ الْعَظِيمُ)

الآية (٢٨) من سورة فاطر

Dedicated

To:

My Mother,

My Father,

My wife,

My sister,

My Brothers

And

My daughters.

Acknowledgement

First and foremost extremely grateful and thankful to Allah

Who gave me the ability and power to finish 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 Microbiology Dept., 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, guidance, this work was carried out.*

*Very special thanks all my life is extending to **Prof. Dr. Eman Mohamed El Rawy**, Chief researcher and head of Aerobic bacteria vaccine research Department, Veterinary Serum and Vaccine Research Institute, for her guidance and advices through the practical work,*

*And a lot of thanks for **Dr. Mai AbdElmoném** the researcher in Animal health research institute for her help in extraction and validation of Lipid A.*

Finally, I would like to thank all persons who aid me in this work with great special thanks for ever to all members of Aerobic bacteria vaccine research Department, Veterinary Serum and Vaccine Research Institute.

Contents

Subject	Page
List of Tables	IV
List of Figures	V
List of Photos	V
List of Abbreviations.....	VI
Introduction.....	1
Review of literature.....	4
2.1. Important of Pasteurella in rabbits	4
2.2. Formalized Inactivated Pasteurella vaccine	17
2.3. Lipid A as an adjuvant.....	21
2.4. Montanide adjuvants.....	31
2.5. Evaluation of Pasteurella vaccines in rabbits.....	39
Materials and Methods.....	48
3.1. Materials.....	48
3.1.1. Bacterial strains.....	48
3.1.2. Media used for isolation.....	48
3.1.3. Media used for biochemical identification.....	50
3.1.4. Media used for testing vaccine sterility.....	50
3.1.5. Reagents.....	51
3.1.6. Stains used for confirmation of <i>P. multocida</i>	52
3.1.7. Lipid A extraction and evaluation by HPLC.....	53
3.1.8. Adjuvants.....	54
3.1.9. Materials used for evaluation of Montanide™ IMS1313 VG N PR (containing nano particles) adjuvanted vaccine	54
3.1.10. Inactivators.....	54
3.1.11. Preservative.....	55
3.1.12. Materials for immunity evaluation.....	55
3.1.12.1. Materials for evaluation of innate immunity estimation (serum lysozyme activity).....	55
3.1.12.2. Materials used for evaluation of humoral immunity...	56
3.1.13. Buffers and solutions.....	58
3.1.14. Experimental animals.....	59
3.1.15. Other materials and equipment.....	60

3.2. Methods.....	61
3.2.1. <i>P. multocida</i> strains subculture.....	61
3.2.2. Biochemical confirmation of <i>P. multocida</i> strains.....	61
3.2.3. Preparation of <i>E. coli</i> for Lipid A extraction	63
3.2.4. Evaluation of Lipid A extract by HPLC.....	64
3.2.5. Preparation of the <i>Pasteurella</i> vaccines.....	66
3.2.6. Addition of preservative (thiomersal).....	69
3.2.7. Quality control on the prepared <i>P. multocida</i> vaccines.....	70
3.2.8. Evaluation of the immunizing potency of the prepared Vaccines.....	74
3.2.9. Statistical analysis.....	81
Results.....	82
4.1. Subculture and confirmatory tests for <i>P. multocida</i> field Strains.....	82
4.2. High pressure liquid chromatography (HPLC) for confirmation of Lipid A extraction purity.....	83
4.3. Formalin inactivation of the <i>P. multocida</i> bulk culture.....	88
4.4. Results of determination of chemical residues in the prepared vaccine.....	88
4.5. Quality control on the prepared <i>P. multocida</i> vaccines.....	88
4.6. Bio assay (challenge) test results.....	90
4.7. Lysozyme activity results.....	93
4.8. Indirect Hemagglutination (IHA) test results.....	100
4.9. Enzyme Linked Immuno Sorbent Assay (ELISA) test Results.....	106
Discussion.....	114
Summary.....	136
References.....	139
Arabic summary.....

List of Tables

No.	Title	Page
1	The specific characteristic feature for <i>P. multocida</i>	63
2	The concentrations of Lipid A standard ($\mu\text{g/ml}$) and their corresponding peak response	83
3	The precision results of Lipid A	85
4	The recovery studies Lipid A	86
5	The accuracy and recovery studies of Lipid A	86
6	The mortality and protection rates 3 weeks of first dose of vaccination	91
7	The mortality and protection rates after 3 weeks of booster dose of vaccination	92
8	The results of lysozyme activity after 1 st dose of vaccination for each group on days 1, 3, 5, and 7 in $\mu\text{g/ml}$ concentration	95
9	The results of lysozyme activity after 2 nd dose of vaccination for each group on days 1, 3, 5, and 7 in $\mu\text{g/ml}$ concentration	98
10	The results of IHA test measured by GMT after the 1 st dose of Pasteurella vaccination and 3 weeks after the challenge test	101
11	The IHA results by GMT after the booster dose and 3 weeks after the challenge test	104
12	The results of ELISA test after 1 st dose of Pasteurella vaccination and 3 weeks after the challenge test	108
13	The results of ELISA test after the booster dose of Pasteurella vaccination and 3 weeks after challenge test	112

List of Figures

No.	Title	Page
1	The liquid chromatogram of 1 µg/ml Lipid A standard	84
2	The standard curve of Lipid A	85
3	The liquid chromatogram of Lipid A extract of <i>E. coli</i> O:157 bacteria cells with a concentration 1 µg/ml	87
4	The comparisons between all groups results of Lysozyme activity after the 1 st dose of vaccination	96
5	The lysozyme activity after the booster dose of vaccination	99
6	The results of IHA test measured by GMT after the 1 st dose of Pasteurella vaccination and 3 weeks after the challenge test	102
7	The IHA results by GMT after the booster dose and 3 weeks after the challenge test	105
8	The results of ELISA test after 1 st dose of Pasteurella vaccination and 3 weeks after the challenge test	109
9	The ELISA test results after the booster dose and 3 weeks after the challenge test	113

List of Photographs

No.	Title	Page
1	The nano particle size: 48.71 nm	88
2	the nano particle size: 24.68 nm	88

List of abbreviations

AEs	Adverse events
AF	Acriflavine test
Ag	Antigen
AI	Avian influenza
ALV	Aluminum hydroxide gel vaccine
AMPT	Active mouse protection test
APCs	Antigen presenting cells
Bb	<i>Bordetella bronchiseptica</i>
CD	Cluster of differentiation
CFU	Colony forming unit
CIE	Counter immunoelectrophoresis
D.W.	Distilled water
ELISA	Enzyme linked immunosorbent assay
EMB	Eosin Methylene Blue Agar
FI-RSV	Formalin-inactivated respiratory syncytial virus
GA-SRBC	Gluteraldehyde fixed sheep red blood cells
GDPT	Gel-diffusion precipitin test
GMT	Geometric Mean Titer
HA	Hemagglutination test
HI	Hemagglutination inhibition test
HPLC	High performance liquid chromatography
ICH	International conference harmonization
I/D	Intradermal
IM	Intra muscular
IFN- γ	Interferon gamma
IHA, IHAT	Indirect haemagglutination test
IL-2	Interleukin 2
ISA	Incomplete Seppic Adjuvant
LBP	Lipopolysaccharide binding protein
LD ₅₀	Lethal dose 50
LOD	Limit of detection
LOQ	Limit of quantification

LPS	lipopolysaccharides
MAbs	Monoclonal antibodies
MAT	Micro agglutination test
MD	Myeloid differentiation
ME	Multiple emulsion adjuvant vaccine
MHCII	Major histocompatibility class II
Mins	Minutes
MN	Microneedle
MPL A	Monophosphoryl Lipid A
MyD88	Myeloid differentiation factor 88
No.	Number
OAV	Oil adjuvant vaccine
OPD	Ortho-phenylene-diamine
OV	Oily adjuvant vaccine
PBS	Phosphate buffer saline
PM	Post mortem
PMT	<i>Pasteurella multocida</i> toxin
PMPT	Passive mouse protection test
RSD	Relative standard deviation
RSV	Respiratory syncytial virus
S/C	Subcutaneous
SAEs	Serious adverse events
SPF	Specific pathogen free
TAAAs	Tumor-associated antigens
Th2	T helper type 2
TIR	Toll-interleukin 1 receptor
TLR	Toll like receptor
TNF	Tumor necrosis factor
TRIF	Toll-interleukin 1 receptor domain-containing adapter inducing interferon- β
TSA	Tryptone soya agar medium