ISOLATION AND IDENTIFICATION OF SOME STRAINS OF AVIAN PARAMYXOVIRUS -1 FROM POULTRY BIRDS

By

SAMAR SAYED AHMED EBRAHIM

B.SC. Agric. SC. (Agricultural Microbiology), Ain Shams Univ., 2008

M.SC. Agric. SC. (Agricultural viruses), Ain shams Univ., 2012

A thesis submitted in partial fulfillment

of

The requirements for the degree of

DOCTOR OF PHILOSOPHY
in
Agriculture Science
(Agricultural Viruses)

Department of Agricultural Microbiology

Faculty of Agriculture

Ain shams University

2016

Approval Sheet

ISOLATION AND IDENTIFICATION OF SOME STRAINS OF AVIAN PARAMYXOVIRUS -1 FROM POULTRY BIRDS

By

SAMAR SAYED AHMED EBRAHIM

B.SC. Agric. SC. (Agricultural Microbiology), Ain Shams Univ., 2008

M.SC. Agric. SC. (Agricultural viruses), Ain shams Univ., 2012

This thesis for Ph.D. degree has been approved by:

Dr. Sayed Ahmed Hassan Salem ..……..……

Chief of Research, Virology Department, Animal Health Research Institute.

Dr. Sohair Ibrahim El-Afifi ..……

Prof. Emeritus of Agric. Viruses, Fac. of. Agriculture, Ain Shams University .

Dr. Badawi Abd El-Salam Othman ..……

Prof. Emeritus of Agric. Viruses, Fac. of. Agriculture, Ain Shams University

Dr. Khalid Abd El-Fattah El-Dougdoug ..……

Prof. Emeritus of Agric. Viruses, Fac. of. Agriculture, Ain Shams University .

Date of Examination: / / 2016

ISOLATION AND IDENTIFICATION OF SOME STRAINS OF AVIAN PARAMYXOVIRUS -1 FROM POULTRY BIRDS

By

SAMAR SAYED AHMED EBRAHIM

B.SC. Agric. SC. (Agricultural Microbiology), Ain Shams Univ., 2008
M.SC. Agric. SC. (Agricultural viruses), Ain shams Univ., 2012

Under the Supervision of:

Dr. Khalid Abd El-Fattah El-Dougdoug

Prof. Emeritus of Agric.Virology, Agric. Microbiol. Dept., Fac. of Agriculture, Ain Shams University (Principal Supervisor).

Dr. Badawi Abd El-Salam Othman

Prof. Emeritus of Agric.Virology, Agric. Microbiol. Dept., Fac. of Agriculture, Ain Shams University.

ABSTRACT

Samar Sayed Ahmed: Isolation and identification of some strains of avian paramyxovirus -1 from poultry birds. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2016.

Newcastle disease (ND) is a contagious and damaging viral infection of fowl and orderly by immunization. Despite immunization, prevalence of ND was described in marketable chicken farms. Our study was carrying out to isolate some Newcastle virus isolates and using them for vaccine production. 13 Samples which collected from dead birds based on postmortem investigation from poultry farms from different areas were exposed to isolation of NDV in specific pathogenic free embryonated chicken eggs. Detection of this isolates was done by serological tests and electron microscope examination. Identification of viral isolates was done by biological properties, virion properties, antigenic properties and genome organization. From biological properties, the pathogenicity tests, host range and tropisms of Newcastle virus were studied. From virion properties, the effects of some physical and chemical agents on stability of viral isolates were determined. The serological relationships between viral isolates were studied using agar gel precipitation test after injection the velogenic strain in rabbits and antiserum was obtained. Among the properties that have been reliable in the definition of viral isolates it was viral genome properties. The fusion gene was chosen as it is responsible for identifying pathological capacity of Newcastle disease virus strains and it amplified by the polymerase chain reaction and the sequence of nucleotides was studied and the bioinformatics used to analyze the results of the sequence of nucleotides and build the phylogenetic tree. It is the principle that prevention is better than cure, two application experiments were done to raise the immune status of the flock and protect it against infection with Newcastle disease

virus, the first experiment is aims to produce a vaccine from lentogenic strain after inhibited by formalin then infection with the velogenic strain was done. It appeared from the results that , despite all the birds were infected with velogenic strain but the strong importance of the vaccine appeared to prevent mortality among chickens and minimizing clinical and postmortem signs compared with untreated chickens. Our study also was planned to study the effect of some nutrition additives (Yeast, Ginger and Cinnamon) on the chicken challenged with NDV. One hundred and fifty six chicks by one-day old were weighted and casually separated into equivalent three main groups, group 1 (ginger, 1 kg/tons), group 2 (cinnamon, 2kg/tons) and group 3 (Yeast, 8 kg/tons), each group was categorized into four sub group A (control without virus or improver), B (additive only) C (chickens infected + flavor) and D (chickens infected without additive), each one contains thirteen chicks. The chicks were kept in cages, in a room with controlled temperature and air conversation and were providing with light and water was delivered *ad libitum*. All chicks were reserved on basal diet for two weeks before impurity with NDV. The preservative was added to chicken on basal diet for extra two weeks after injection with NDV (velogenic strain / 8 HAU) in drinking water (3 ml / 100ml). Birds were reserved under daily surveillance. Weight (every week), signs, % mortality were detailed. Blood collected randomly from two birds to describe protein profile. The result presented that all flavors reducing % mortality and aggregate the weight of birds. The cytological investigation of the chickens displayed that there were hemorrhages in trachea, liver, proventriculus and intestine in all sub groups excepting sub group A (Control). All values for globulin were sig. dissimilar ($P<0.05$) in the three main group. This exposed the capacity of this food additive to influence protection in chickens infected with (NDV).

Key words: Newcastle Virus, viral isolation, Veleogenic, ICPI, IVPI, MDT, haemagglutination inhibition test (HI),

haemagglutination test (HA), food additive, ginger, PCR , vaccine production.

AKNOWLEDGMENT

″ **Praise and thanks be to ALLAH the most merciful for assisting and directing me to the right way** ″

My sincere appreciation to the soul of **Prof. Dr. Esmat. Kh. Allam**, Prof. Emeritus of Agric. Virology, Department of Agric. Microbiology, Fac. of Agric., Ain shams University, for his efforts to open new branches in virology school, before his death.

My heartily thanks are due to **Prof. Dr. Khalid A. El-Dougdoug**, Prof. Emeritus of Agric. Virology, Department of Agric. Microbiology, Fac. of Agric., Ain shams University, for supervision, suggesting the problem and valuable advice , unlimited support encouragement during this work and progressive criticism during the study.

I feel most indebted and grateful to **Prof. Dr. Badawi A. Othman**, Prof. Emeritus of Agric. Virology, Department of Agric. Microbiology, Fac. of Agric., Ain shams University, for supervision, suggesting the problem , his interest , continuous help and issuing this work in the best possible scientific form , and great thank to him for long days of his time that he gave me to complete this work.

Deepest gratitude to **Prof. Dr. Suzan Sayed El Mahdy**, Prof. of virus, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt, for her previous supervision, valuable advice and providing me with polyclonal antiserum for NDV and AIV.

Special thanks are due to **Eng/ Ahmed Mohamed Abd-Elhady** , Head of Department of Poultry Projects , Ministry of Agriculture , for helping me in collection of samples during the study.

My heartily thanks are due to **Prof. Dr. Safwat Hassan Ali**, professor of Agric. chemistry, Fac. of Agric., Ain Shams Univ. For his sincere, encouragement, continuous kindly and support during the study, his truly scientific nature was a continuous source of inspiration for me during the work and his important accurate notices in the part of blood biochemistry.

Special thanks are due to **Dr. Mohamed Abd-El basir**, researcher of virology, faculty of medicine, El Azhar University, for training me on tissue culture technique.

I´m greatly indebted to express my special thank and great appreciation to **Dr. Allam Arafat Megahed**, researcher in National Research Center for unlimited help and constant support and training me on electrophoresis technique.

We also acknowledge the technical support of **Khadra Foad Abdallah** for her efforts for rearing the experimental birds and chickens.

My thanks have to be extended to all my professors' colleagues and all members of virology Laboratory and Agricultural Microbial., Fac. of Agric. Ain Shams Univ. for their Continuous assistance and supplying all Facilities throughout this thesis.

CONTENTES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

APMV : Avian Paramyxovirus
Dpi : Day post infection
et al., : And others
F : Fusion
Fig. : Figure
gm : Gram
HA : Hemagglutination
HI : Hemagglutination Inhibition
ICPI : Intracerebral Pathogenicity Index
IVPI : Intravenous Pathogenicity Index
kbp : Kilobase pair
MDT : Mean Death Time
min : Minute
ml : Milliliter
ND : Newcastle disease
NDV : Newcastle disease virus
nm : Nanometer
nt : Nucleotide
OIE : Office des International Epizootic
PBS : Phosphate Buffer Saline
pH : Potential for hydrogen ion concentration
pmol : Picomole
RBC : Red blood cell

RNA	:	Ribonucleic acid
Rpm	:	Revolutions per minutes
RT-PCR	:	Reverse transcription-polymerase chain reaction
sec	:	Second
SPF	:	Specific pathogen free
TAE	:	Tris – acetate –EDTA- buffer
TEM	:	Transmission electron microscope
UV	:	Ultra violet
%	:	Percent
Mg	:	Microgram
µl	:	Microliter
µM	:	Micromole
^{o}C	:	Degree Celsius