



Faculty of Veterinary Medicine



Cairo University

Development of innate immune stimulants for the control of avian influenza A virus infection

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Preface

The studies illustrated in this thesis were performed at the Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada from December 2015 to November 2017. The thesis contains the materials already published which are listed below:

Ahmed-Hassan, Hanaa, *et al.*, "Potential mediators of *in ovo* delivered double stranded (ds) RNA-induced innate response against low pathogenic avian influenza virus infection." *Virology journal* 15.1 (2018): 43.

Ahmed-Hassan, Hanaa, *et al.*, "Double stranded ribonucleic acid (dsRNA) -mediated antiviral response against low pathogenic avian influenza virus infection." *Viral immunology*.

Abstract

Influenza A viruses (IAVs) are important pathogens of humans and many other species, as, these viruses have been widely circulating in animals are novel to the human immune system; so pose a potential threat to public health. Moreover, recently, low-pathogenic avian influenza viruses (LPAIVs) have caused a global concern to public health therefore attention should be paid to the potential threats to humans posed by LPAIVs. As well as there is an urgent need to more efficient strategies for the prevention and control of such infection. Double stranded ribonucleic acid (dsRNA) has been shown to act as a ligand for Toll like receptor (TLR3) that known to induce type 1 interferons (IFNs) and pro-inflammatory mediators leading to antiviral response against many viral infections. Yet, its effectiveness and involved mechanisms as a mediator against low LPAIV have not been investigated adequately. Therefore, this study was attempted to investigate the effect and mechanism of dsRNA as anti- H₄N₆ LPAIV. Among those, the pre-hatch treatment of specific pathogen free eggs (at 18 days) with dsRNA was performed and evidenced the reduction of H₄N₆ replication in lungs by plaque assay. Further studies revealed that the *in ovo* delivery of dsRNA increases TLR3 expression, type I IFN production and number of macrophages using immunostaining assay in addition to mRNA expression of IL-1 β using RT-PCR in lung 24-hours post-treatment. The same level of induction of innate response was not evident in the spleen. Additionally, the treatment of avian fibroblasts with dsRNA induces antiviral response in avian fibroblasts against H₄N₆ LPAIV through increases the expressions of TLR3, IFN- α , IFN- β and IL-1 β using immunostaining assay. As well as this study emphasized that this antiviral response elicited against H₄N₆ LPAIV infection correlates but is not attributable to type 1 IFNs or IL-1 β . These results imply that the TLR3 ligand, dsRNA has antiviral activity *in ovo* and *in vitro* against LPAIV highlighting potential value of dsRNA as an antiviral agent against LPAIV infections. Although H₄ AIVs are low pathogenic AIVs, it is possible that they will eventually gain the ability to transmit efficiently through the accumulation of mutations and reassortments. Therefore, further investigations are required to determine the potential role of other innate immune mediators or combination of the tested cytokines in the dsRNA-mediated antiviral response against H₄N₆ LPAIV to prepare for potential pandemics.

Key words: *In ovo*, *in vitro*, dsRNA, antiviral agent, type I interferon, macrophage, low pathogenic avian influenza virus, chicken.

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Dedication

I would like to dedicate this thesis work to my beloved family and my future husband

List of abbreviations

Abbreviation	Definition
ADCC	Antibody dependent cell cytotoxicity
AIM2	Absent in melanoma 2
AIV	Avian influenza virus
AP	Activator protein
APCs	Antigen presenting cells
bp	Base pair
CDC	Centers for disease control and prevention
cDNA	Complementary DNA
CDSs	Cytosolic DNA sensors
CEFs	Chicken embryonic fibroblasts
CFIA	Canadian Food Inspection Agency
cGAS	Cyclic GMP-AMP synthetase
CPG	Cytosine--phosphate diester--guanine
DAMPs	Damage associated molecular patterns
DCs	Dendritic cells
DF-1	Douglas Foster
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
dsRNA	Double stranded Ribonucleic acid
ED	Embryo day
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
g	g force

HA	Hemagglutinin
HBSS	Hanks balanced salt solution
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HMM	High molecular mass
HPAIV	Highly pathogenic avian influenza virus
HRIC	Health Research Innovation Center
HSACC	Health Science Animal Care Committee
IFITM	IFN-inducible transmembrane protein
IFN	Interferons
IFN-α	Interferon alpha
IFN-β	Interferon beta
IFNAR1	Interferon alpha receptor 1
IFNAR2	Interferon alpha receptor 2
Ig	Immunoglobulin
IκB	Inhibitor of NF- κ B
IKK	I κ B kinase-related kinase
IL	Interleukin
IL-1R	Interleukin 1 receptor
IL-1Ra	Interleukin 1 receptor antagonist
ILTV	Infectious laryngotracheitis virus
IRAK	IL1 receptor-associated kinases
IRF	Interferon regulatory factor
ISGF	IFN-stimulated gene factor
ISGs	IFN-stimulated genes
ISREs	IFN-stimulated response elements

Iκ B	Inhibitor of NF- κ B
JAK1	Janus kinase 1
JNK	c-Jun N-terminal kinases
L	Liter
LGP2	Laboratory of genetics and physiology 2
LPAIV	Low pathogenic avian influenza virus
LMM	low molecular mass
LRRs	Leucine-rich repeats
LTA	Lipoteichoic acid
M	Matrix protein
MAL	MyD88-adaptor-like
MAPK	Mitogen-activated protein kinase
MD-2	Myeloid Differentiation protein-2
MDA-5	Melanoma differentiation-associated gene 5
MDCK	Madin-Darby canine kidney
MDV	Marek's disease virus
MEM	Minimum Essential Medium
mg	Milligram
mins	Minutes
ml	Milliliter
mM	Milimolar
MOI	Multiplicity of infection
MQ-NCSU	Muquarrab Qureshi-North Carolina State University
mRNA	Messenger RNA
Mx	Myxo-virus resistance
MyD88	Myeloid differentiating factor 88

NA	Neuraminidase
NAK	NF- κ B activating kinase
NAP1	NAK- associated protein 1
NF-κB	Nuclear factor- kappa B
ng	Nanogram
NK	Natural killer
NLRP	NOD-like receptors pyrin domain
NLRs	NOD-like receptors
nm	Nanometer
NOD	nucleotide oligomerization domain-containing protein
NSP	Non structural protein
OAS	oligoadenylate synthetases
OCT	Optimum cutting temperature
OD	Optical density
Opti-MEM	modification of Eagle's Minimum Essential Media
PAMPs	pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFUs	Plaque forming units
PIKA	a stabilized analogue of dsRNA
PKR	protein kinase R
pM	Picomolar
poly (A:U)	Polyadenylic–polyuridylic acid
Poly I:C	Polyinosinic:polycytidylic acid
poly ICLC	polyinosinic–polycytidylic acid-polylysine-carboxymethylcellulose
Poly-G	Poly guanosine

Poly(I:C)	Polyinosinic–polycytidylic acid
PRRs	pattern-recognition receptors
RdRp	RNA dependent RNA polymerase
RIG	retinoic acid-inducible gene
RIP	Receptor interacting protein kinase
RLH	RIG-I-like helicases
RLR	RIG-like receptors
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
rRNA	ribosomal RNA
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction
SNAP	S-nitroso-N-acetyl-DL-penicillamine
SPF	Specific pathogen free
ssRNA	Single stranded ribonucleic acid
STAT	Signal Transducer and Activator of Transcription
TAB	TAK binding protein
TAK	Transforming growth factor beta-activated kinase
TANK	TRAF family member-associated NF- κ B activator
TBK1	TANK-binding kinase 1
TIR	Toll/interleukin -1 (IL-1) receptor
TLRs	Toll like receptors
TNF	Tumor necrosis factor
TPCK	L-tosylamide-2-phenylethyl chloromethyl ketone
TRAF	Tumor necrosis factor receptor associated factor
TRIF	TIR domain-containing adapter inducing IFN- β

TYK2	tyrosine kinase 2
VSV-GFP	vesicular stomatitis virus expressing green fluorescent protein
ZAP	zinc finger antiviral protein
μg	Microgram
μl	Microliter

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