Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Studies on *Riemerella anatipestifer* isolated from ducks

A thesis presented by

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Supervision sheet

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Abstract

Ducks have an important role in the poultry industry especially in developing countries like Egypt. However, duck diseases received a little attention from the veterinary research as well as veterinarians. *Riemerella anatipestifer* is considered one of the most important threats for ducks and ducklings worldwide. In the present study, isolation and molecular characterization for *R. anatipestifer* based on PCR for full length outer membrane protein A (*omp*A), sequencing and phylogeny followed by prediction for the ligand binding sites on the surface of the immunogenic protein (OmpA). BLAST result for the obtained nucleotide sequences revealed the circulation of different serotypes among the Egyptian duck flocks indicating progressive adaptation and evolution of *R. anatipestifer* among ducks and ducklings. Phylogeny was conducted based on full length *omp*A gene that classifies the diversity of *R. anatipestifer* worldwide into two main lineages (lineage 1 and lineage 2); each lineage diversified into three main clusters based on the evolution studies and confirmed by 3D prediction. Till now, at least 21 *R. anatipestifer* serotypes had been identified. In spite of the molecular techniques regarding its pathogenicity and antigenicity, they are not well understood. This study reports the first genotyping of *R. anatipestifer* based on an immunogenic protein (OmpA) and confirm the co-circulation of different *R. anatipestifer* clusters in the Egyptian duck flocks. The isolates were belonging to lineage 1 (cluster I and II) and linage 2 (cluster I and III). These findings represent a start to what should be an expanded investigation of the genetic diversity of *R. anatipestifer* at national and regional levels to better understand evolution dynamics, distribution and the genetic relatedness among the duck industry. In conclusion, identifying the disease cause, source of infection, mode of transmission, and the immunogenic factors of *R. anatipestifer* is essential for its molecular characterization and analysis of virulence mechanisms as well as its prevention and control.

Key words: Ducks, *Riemerella anatipestifer*, *omp*A gene, OmpA protein, Phylogeny, 2D, 3D structure of protein and Evolution.

List of abbreviations

2D structure: two dimensional structure

3D structure: three-dimensional structure

AGP Agar Gel Precipitin

AGPT: Agar Gel Precipitation Test

ATCC: American Type Culture Collection

bp: base pair

CASP: Caspase, Apoptosis-Related Cysteine Peptidase

CFU/ml: colony forming units/ml

DDW: Double Distilled Water

DNA: deoxy-ribonucleic acid

EDTA: Ethylene Diamine Tetra Acetic acid

ELISA: Enzyme Linked Immunosorbent Assay

ERIC: Enterobacterial Repetitive Intergenic Consensus sequence

FAME: Fatty Acid Methyl Ester

G+C: Guanine + Cytosine

I-TASSER: Iterative Threading ASSEmbly Refinement

i/v: intra venous

kDa: kilodalton

LOMETS: Local Meta-Threading-Server

LPS: Lipopolysaccharide

MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization-Time Of Flight

NCBI: National Center for Biotechnology Information

OIE: World Organization for Animal Health

***omp*A:** outer membrane protein A gene

OmpA: Outer membrane protein A

OMPs: Outer membrane proteins

***P. multocida*:** *Pasteurella multocida*

PCR: polymerase chain reaction

PCR-RFLP: PCR-restriction fragment length polymorphism

PDB: Protein Data Bank

PFGE: pulsed-field gel electrophoresis

PH: power of hydrogen

PM: postmortem

***R. anatipestifer*:** *Riemerella anatipestifer*

***rpo*B:** Rifampin resistance gene

rRNA: ribosomal RNA

TAE: Tris Acetic acid EDTA

TE: Tris EDTA

VapD: virulence associated protein D

DEDICATION

TO PROPHET

MOHAMMAD

SALLA ALLAH ALAIHE

WA SALM

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