

Epidemiological studies on avian influenza and other  
respiratory viruses in backyard poultry in Oman

*Thesis submitted in accordance with the requirements of the University of Liverpool  
for the degree of Doctor of Philosophy*

By

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Epidemiological studies on avian influenza and other respiratory viruses in backyard poultry in Oman

This thesis is based on research carried out in the Department of Epidemiology and Population Health and Department of Infection Biology at the University of Liverpool. Except where indicated, the content of this thesis is my own work.

Thunai Obaid Al Shekaili

## TABLE OF CONTENTS

<b>ABSTRACT .....</b>	<b>VII</b>
<b>DECLARATION.....</b>	<b>XI</b>
<b>DEDICATION.....</b>	<b>XII</b>
<b>ACKNOWLEDGMENTS .....</b>	<b>XIII</b>
<b>LIST OF FIGURES .....</b>	<b>XV</b>
<b>LIST OF TABLES .....</b>	<b>XVIII</b>
<b>LIST OF ABBREVIATIONS.....</b>	<b>XXII</b>
<b>PUBLICATIONS AND PRESENTATIONS .....</b>	<b>XXV</b>
<b>CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW .....</b>	<b>1</b>
1.1    THE GLOBAL POULTRY PRODUCTION .....	3
1.2    THE SULTANATE OF OMAN .....	6
1.3    POULTRY PRODUCTION IN OMAN.....	11
1.4    POULTRY VIRAL RESPIRATORY DISEASES.....	15
1.5    AVIAN INFLUENZA VIRUSES (AIV).....	17
1.5.1    High pathogenic versus low pathogenic viruses.....	18
1.5.2    General epidemiology .....	19
1.5.3    Prevention, control and eradication .....	21
1.5.4    Epidemiology in Oman.....	22
1.5.5    Zoonosis .....	24
1.6    NEWCASTLE DISEASE VIRUS (NDV): .....	26
1.6.1    General epidemiology .....	27
1.6.2    Epidemiology of Newcastle disease in Oman .....	28
1.7    AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV).....	29
1.7.1    General epidemiology .....	30
1.8    AVIAN METAPNEUMOVIRUS (AMPV).....	33
1.8.1    General epidemiology .....	33

1.9	WEST NILE VIRUS (WNV) .....	36
1.9.1	General epidemiology .....	37
1.9.2	Vector preference .....	38
1.9.3	West Nile virus in Oman .....	39
1.10	AIM OF THE THESIS .....	40
<b>CHAPTER TWO: MATERIALS AND METHODS .....</b>		<b>41</b>
2.1	SAMPLE SIZE CALCULATION.....	42
2.1.1	Sampling Criteria .....	46
2.1.1.1	Criteria for inclusion.....	46
2.1.1.2	Criteria for exclusion:.....	46
2.2	SAMPLING.....	48
2.2.1	Blood.....	48
2.2.2	Oropharyngeal swabs .....	48
2.2.3	Data .....	54
2.3	SEROLOGY .....	55
2.3.1	Detection of AIV and NDV antibodies .....	55
2.3.2	WNV antibody detection.....	56
2.3.2.1	Sample preparation.....	57
2.3.2.2	ELISA.....	57
2.4	MOLECULAR TESTING.....	58
2.4.1	RNA extraction from FTA cards.....	58
2.4.1.1	Elution of RNA from FTA cards .....	58
2.4.1.2	RNA Extraction.....	58
2.4.2	Reverse transcriptase polymerase chain reaction (RT-PCR).....	59
2.4.2.1	Reverse transcription (RT) reaction.....	61
2.4.2.2	Nested PCR 1 .....	61
<b>CHAPTER THREE: SYSTEMATIC RESEARCH ON THE EPIDEMIOLOGY OF AVIAN INFLUENZA AND OTHER VIRAL RESPIRATORY DISEASES OF POULTRY IN THE MIDDLE EAST.....</b>		<b>62</b>
3.1	ABSTRACT .....	63

3.3	INTRODUCTION: .....	65
3.3	RESEARCH JUSTIFICATIONS .....	67
3.4	LITERATURE SEARCHES .....	68
3.4.1	Results for the literature searches .....	69
3.5	AVIAN INFLUENZA (AI) IN THE MIDDLE EAST .....	76
3.5.1	Epidemiology of HPAI H5N1 .....	76
3.5.2	Control of H5N1 .....	78
3.5.3	Other avian influenza viruses .....	80
3.5.4	Future of HPAI H5N1 and LPAI .....	82
3.6	EPIDEMIOLOGY OF OTHER POULTRY RESPIRATORY VIRUSES IN THE MIDDLE EAST .....	84
3.6.1	Newcastle disease .....	84
3.6.1.1	Epidemiology in the Middle East countries .....	84
3.6.2	Avian infectious bronchitis (IB) .....	86
3.6.3	Epidemiology in the Middle East .....	86
3.6.3	AVIAN METAPNEUMOVIRUS (AMPV) .....	88
3.6.4	INFECTIOUS LARYNGOTRACHEITIS (ILT) .....	89
3.6.5	FUTURE OF NDV, IBV, AMPV AND ILTV IN THE MIDDLE EAST .....	90
3.7	CONCLUSION .....	92

**CHAPTER FOUR: SERO-SURVEILLANCE FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES IN BACKYARD POULTRY IN OMAN..... 93**

4.1	ABSTRACT .....	95
4.2	INTRODUCTION .....	96
4.3	RESEARCH JUSTIFICATION .....	97
4.4	MATERIALS AND METHODS .....	98
4.4.1	Sampling method .....	98
4.4.2	Detection of AIV and NDV antibodies .....	98
4.4.3	RNA extraction from FTA cards .....	99
4.4.4	Reverse transcriptase polymerase chain reaction (RT-PCR) .....	99
4.5	RESULTS .....	100
4.5.1	Flocks and ELISA results .....	100

4.5.2	AIV and NDV RT-PCR.....	112
4.6	DISCUSSION .....	113
<b>CHAPTER FIVE: RISK FACTORS AFFECTING THE EPIDEMIOLOGY OF AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES IN BACKYARD POULTRY IN OMAN..... 118</b>		
5.1	ABSTRACT .....	119
5.2	INTRODUCTION .....	120
5.3	RESEARCH JUSTIFICATIONS .....	122
5.4	MATERIALS AND METHODS .....	123
5.4.1	Data sources .....	123
5.4.1.1	Infection intensity .....	123
5.4.1.2	Explanatory variables .....	123
5.4.2	Statistical methods.....	131
5.5	RESULTS .....	133
5.5.1	Risk factors for NDV seropositivity .....	133
5.5.2	Risk factors for AIV seropositivity .....	141
5.6	DISCUSSION .....	148
<b>CHAPTER SIX: MOLECULAR DETECTION OF INFECTIOUS BRONCHITIS AND AVIAN METAPNEUMOVIRUSES IN OMANI BACKYARD POULTRY ..... 152</b>		
6.1	ABSTRACT .....	154
6.2	INTRODUCTION .....	155
6.3	RESEARCH JUSTIFICATIONS.....	156
6.4	MATERIALS AND METHODS .....	157
6.4.1	Sampling method.....	157
6.4.2	RNA extraction from FTA cards .....	159
6.4.3	aMPV RT-PCR.....	159
6.4.4	IBV RT-PCR and amplicon sequencing.....	159
6.4.5	Reverse transcription (RT) reaction .....	160
6.4.6	Nested PCR 1 .....	160
6.4.7	Nested PCR 2 .....	161

6.4.8	Gel Electrophoresis .....	161
6.4.9	DNA sequencing of IBV .....	162
6.4.10	Phylogenetic analysis and nucleotide comparison.....	162
6.5	RESULTS .....	164
6.5.1	Detection of aMPV .....	164
6.5.2	Detection and genotyping of IBV.....	164
6.5.3	Phylogenetic analysis of IBV isolates .....	165
6.6	DISCUSSION .....	169
<b>CHAPTER SEVEN: SEROPREVALENCE OF WEST NILE VIRUS SEROPREVALENCE IN BACKYARD POULTRY IN OMAN .....</b>		<b>174</b>
7.1	ABSTRACT .....	175
7.2	INTRODUCTION: .....	176
7.3	RESEARCH JUSTIFICATIONS .....	177
7.4	MATERIALS AND METHODS .....	178
7.4.1	Sampling of poultry.....	178
7.4.2	Detection of West Nile virus antibodies.....	178
7.4.3	Mosquito Sampling .....	179
7.4.3.1	Selection of sampling farms .....	179
7.4.3.2	Criteria for farm selection.....	179
7.4.3.3	Random jittering of the mosquito sampled farms .....	180
7.4.3.4	Mosquito Trapping .....	182
7.4.3.5	Mosquito Identification .....	183
	Keys for Culex quinquefasciatus mosquito identification according to (Harbach, 1985) .....	183
7.5	RESULTS: .....	184
7.5.1	Serological results .....	184
7.5.2	Mosquito trapping results .....	189
7.6	DISCUSSION .....	192
<b>CHAPTER EIGHT: BIOSECURITY AND FLOCK HEALTH PRACTICES IN COMMERCIAL BROILERS POULTRY FARMS IN OMAN.....</b>		<b>196</b>
8.1	ABSTRACT .....	197

8.2	INTRODUCTION .....	198
8.3	RESEARCH JUSTIFICATIONS .....	202
8.4	METHODOLOGY .....	203
8.4.1	Study area .....	203
8.4.2	Data collection.....	203
8.4.3	Statistical Analysis .....	206
8.5	RESULTS .....	207
8.6	DISCUSSION .....	216
<b>CHAPTER NINE: GENERAL DISCUSSION AND FUTURE WORK .....</b>		<b>221</b>
<b>CHAPTER TEN: REFERENCES .....</b>		<b>233</b>
<b>APPENDICE .....</b>		<b>270</b>
	APPENDIX 1 DATA COLLECTION FORMS .....	271
	APPENDIX 2 PROTOCOLS AND REAGENTS .....	286
	APPENDIX 3 SAMPLED FLOCKS DATA .....	291
	APPENDIX 4: R CODES FOR RISK FACTOR ANALYSIS .....	323
	APPENDIX 5: PUBLICATIONS .....	330

## Abstract

This thesis describes studies on the epidemiology of the avian influenza virus (AIV) and other respiratory viruses such as Newcastle disease (NDV), infectious bronchitis (IBV) and avian metapneumovirus (aMPV) in backyard poultry in Oman. Also, I utilized backyard birds as sentinels to detect the presence of West Nile virus (WNV) in Oman backyard poultry. Additionally, I tried to investigate the risk factors contributing to the spatial distribution of AIV and NDV diseases in backyard poultry farms. Management biosecurity and health programmes in commercial broiler poultry farms were also examined.

Chapter 3 reviews the epidemiology of the viral respiratory diseases affecting poultry in the Middle East (ME) in relation to diseases reported in Oman. The review was undertaken to identify knowledge gaps. The review focused more on the highly pathogenic avian influenza H5N1 outbreak in the ME since most of the published poultry articles were on this virus. There was a clear gap in the knowledge on the epidemiology of respiratory viral pathogens except for H5N1.

Chapter 4 describe a sero-surveillance study on backyard poultry flocks in Oman. A snapshot two-stage cluster sampling was done during the summer of 2012 on 2350 backyard poultry birds from 238 farms from all of the Oman's regions and governorates. The dominant poultry species in the visited farms was the chickens; however, ducks, turkeys, geese and guinea fowls were present and sampled. The overall seroprevalence of the avian influenza and Newcastle disease viruses were 37.5% and 42.1%, respectively, and the flock's positive level was 84% and 90.2%, respectively. The mean within-flock seroprevalences were 37.6% and 43.4%. All

the PCR results were negative for NDV and AIV. In conclusion, both disease viruses are endemic in the backyard poultry in Oman.

Chapter 5 studies the risk factors associated with the intensity of the infection of both avian influenza and Newcastle disease in Omani backyard farms (serological results from chapter 4). A number of previous studies have investigated and identified a number of risk factors for both diseases, especially the highly pathogenic avian influenza. I obtained the risk factors that are present in Oman from those previously identified and modelled their association with the intensity of the AIV and NDV infection in Omani backyard flocks using general linear models (GLM). There was a regional effect on the level of exposure to both viruses; however there was no North-South pattern. Also, there was a highly significant association between the presence of AIV and NDV infection which may be attributed to the level of biosecurity applied in the farms. Furthermore; there was a negative association between the farm altitude and the AIV intensity of infection. The flock size was marginally negatively associated with the NDV infection rate.

Chapter 6 describes a study on the prevalence of IBV and aMPV using molecular methods including RT-PCR, sequencing and phylogenetic analysis from the same sampled backyard flocks. Five flocks from the northern regions were positive for aMPV subtype B and 39 flocks were positive for 5 different genotypes of the IBV virus. The 793B like was the most prominent genotype. However, genotypes M41, IS885, IS94 and D274 were also identified. The presence of the IBV viral genome in the FTA card collected from ducks and turkeys raises the question of their role in IBV epidemiology. The study concluded that both viruses are endemic in Oman backyard poultry.

Chapter 7 describes a study of the serological prevalence of the West Nile virus using backyard birds as sentinels. The sera from backyard birds were used for the detection of antibodies against WNV. The total flock prevalence was 45% and the total bird prevalence was 21%. All the tested bird species showed positive ELISA results. Later a snapshot mosquito sampling was done in 16 of the previously identified WNV positive farms. The mosquito species recorded on WN positive farms were *Culex quinquefasciatus*, a known vector of WNV, and *Anopheles stephensii*, a malaria vector. The study concluded that WNV is endemic in backyard poultry in Oman.

Management, biosecurity and health practices are the core of poultry farming success and the main defence against the introduction of diseases to the farm, as well as dissemination between farms. Chapter 8 describes the management and health practices applied in commercial poultry production farms in Oman. A questionnaire designed to investigate the management, biosecurity and health practices in broiler production poultry farms was filled in by Ministry of Agriculture and Fisheries animal production engineers for 69 broiler poultry farms. These 69 broiler farms produce around 95% of the poultry meat produced in Oman. The Southern region Dhofar governorate, has the highest reported number of birds, nearly 20 million, which makes up almost (82%) of the responding broiler production farms. The majority of the farms use closed houses with evaporating/cooling fans and cooling beds (46/69, 66.7%). All farms vaccinate against Newcastle disease. infectious bursal disease (IBD), avian influenza virus H9N2, avian infectious bronchitis and aMPV were also been reported in vaccination programs with different percentages. The bigger farms apply better biosecurity measures and their managers have better

knowledge. Medium and small farms vary greatly in their application of biosecurity measures.

## Declaration

All techniques and studies performed and described in this thesis were undertaken by myself as a PhD student at the University of Liverpool between January 2012 and August 2015, unless otherwise acknowledged.

Neither this thesis nor any part of it has been submitted in support of an application of another degree or qualification of this or any other University or other institute of learning.

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Thunai Al Shekaili

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## **Dedication**

This thesis is dedicated to my parents, my brothers and sisters, my wife, my children and for my friend Munir Omar and Faiza Salem for their endless love and encouragement.

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“Who followed a way seeking knowledge, Allah (the God) will pave way for him to the paradise” Prophet Mohammad

Primarily all thanks goes to Allah who gave me the energy and patience to finish the PhD work.

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## LIST OF FIGURES

**Page 7:** Figure 1.1 Sultanate of Oman’s different geographical regions and governorates

**Page 8:** Figure 1.2 Different topographical environments of Oman (A) rocky valleys environment in Northern regions, (B) lush tropical environment in the monsoon-affected region of Dhofar in southern Oman (C) desert environment.

**Page 10:** Figure 1.3 Location map of Oman

**Page 45:** Figure 2.1 Estimated flock numbers to be sampled from each region

**Page 50:** Figure 2.2 Distribution of sampled farms from each region

**Page 53:** Figure 2.3 (A) Blood samples (1-2ml) were collected from the wing (brachial) vein, (B) oropharyngeal swab samples were taken using sterile wooden swabs

**Page 53:** Figure 2.4 (A) The swabs of each flock/species were pooled in 1.5 ml distilled water, (B) then 80-100 µl was inoculated onto each of the circles on the FTA cards

**Page 71:** Figure 3.1 (A) Middle Eastern countries coloured according to the total number of poultry viral diseases articles published from each country, (B) Middle Eastern countries coloured according to the total number of poultry viral diseases genomic sequencings uploaded to the Genbank from each country

**Page 72:** Figure 3.1 (B) Middle Eastern countries coloured according to the total number of poultry viral disease genomic sequencings uploaded to Genbank from each country

**Page 75:** Figure 3.2: The square root of number of genomic sequences vs the number published articles from Middle Eastern countries

**Page 108:** Figure 4.1 (A) percentages of positive avian influenza virus (AIV) in the backyard birds in each region indicated by colour zones

**Page 109:** Figure 4.1 (B) percentages of positive avian influenza virus (AIV) in the backyard flocks in each region indicated by colour zones

**Page 110:** Figure 4.2 (A) Percentages of positive Newcastle disease virus (NDV) in the backyard birds, in each region indicated by colour zones

**Page 111:** Figure 4.2 (B) Percentages of positive Newcastle disease virus (NDV) in the backyard flocks in each region indicated by colour zone

**Page 128-30:** Figure 5.1 Plotting of location of sampled backyard farms with other risk factors such as production farms (C), water areas (temporary lakes) (D), coast line in Oman map

**Page 135-38:** Figure 5.2 The GAM plot of the NDV seroprevalence with the continues explanatory variables showing liner or closer to linear relations (A-J), the solid line represent the fitted line (the smooth) and the dashed lines represent 95% CI and the rugged plot on the x axis represent the data points

**Page 143-45:** Figure 5.3 The GAM plot of the AIV seroprevalence with the continues explanatory variables showing liner or closer to linear relations (A-F), the solid line represent the fitted line (the smooth) and the dashed lines represent 95% CI and the rugged plot on the x axis represent the data points

**Page 166:** Figure 6.1: (A) Locations aMPV positive flocks (n=5). (B) Locations of IBV positive flocks (n=39)

**Page 168:** Figure 6.2 Maximum likelihood analysis of 32 isolates (accession numbers included in brackets) obtained from the regions of Oman, and 11 IBV reference strains from GenBank, the isolates obtained from the turkey and duck

flocks are highlighted (Oman-Mosanah-612 and Oman-Alhamra-3-12) and arrows indicating the vaccine strains

**Page 181:** Figure 7.1 Map of the mosquitos sampled backyard farms in Oman

**Page 182:** Figure 7.2 CDC light trap hanging around 1.5 m from the backyard poultry house (A & B)

**Page 188:** Figure 7.3 Distribution of backyard farms positive for West Nile virus antibodies and the percentage of seropositivity in each regions in Oman

**Page 189:** Figure 7.4 Photo of a *Culex quinquefasciatus* mosquito

**Page 190:** Figure 7.5 Randomly jittered (11.1 km) location of the mosquito sampled backyard farms which were previously found positive for West Nile Virus (WNV).

**Page 205:** Figure 8.1 locations of 303 production poultry farms reported in the 2012/13 census produced by Ministry of Agriculture and Fisheries, Oman

**Page 209:** Figure 8.2 Falajs are small rivers used mainly for palm tree irrigation in the North of Oman

## **LIST OF TABLES**

**Page 5:** Table 1.1. Poultry meat production by region and country 2013-14 in 1000s tonnes (FAO)

**Page 14:** Table 1.2 The number of farms and the biosecurity levels of different broiler poultry systems in Oman

**Page 16:** Table 1.3 The distribution of viral poultry disease worldwide and its presence in Oman

**Page 25:** Table 1.4 Influenza pandemics since the 16<sup>th</sup> Century

**Page 44:** Table 2.1 Stratification of the total required 269 backyard farms calculated from Department of Rural Women Development estimation (10000 flocks) for each Oman region

**Page 51:** Table 2.2 The number of flocks sampled, the number and type of each poultry species sampled in the eight regions of Oman

**Page 52:** Table 2.3 The number of birds sampled from each bird species from Oman backyard poultry

**Page 60:** Table 2.4 Reverse transcription polymerase chain reaction (RT-PCR) oligonucleotides

**Page 73:** Table 3.1 Published papers on poultry viral diseases from Middle Eastern countries until January 2015

**Page 74:** Table 3.2 Number of gene sequencings from each ME countries found in EID2 database

**Page 102:** Table 4.1 Number of birds of each poultry species present in farms visited in different regions of Oman in 2012

**Page 103:** Table 4.2 Number of flocks of each poultry species present in farms visited in different regions of Oman in 2012

**Page 104:** Table 4.3 Bird level avian influenza virus (AIV) and Newcastle disease virus (NDV) serological results in different regions of Oman

**Page 105:** Table 4.4 Flock level avian influenza virus (AIV) and Newcastle disease virus (NDV) serological results in different regions of Oman

**Page 106:** Table 4.5 Number of avian influenza virus (AIV) and Newcastle disease virus (NDV) positive birds from each bird type

**Page 107:** Table 4.6 Number of avian influenza virus (AIV) and Newcastle disease virus (NDV) positive flocks from each bird type

**Page 124:** Table 5.1 A list of risk factors previously identified for avian influenza and Newcastle disease viruses found in the PubMed database search

**Page 127:** Table 5.2 The source of data for the studied risk factors in Oman

**Page 139:** Table 5.3 Analysis of deviance for the reduced model for Newcastle disease virus (NDV) prevalence in Omani backyard chicken flocks fitted using quasi-likelihood methods

**Page 140:** Table 5.4 Coefficients, standard errors and 95% confidence intervals on parameter estimates from a reduced model for Newcastle disease virus (NDV) prevalence in backyard chicken flocks in Oman; the Ad Dakhliyah region was the reference for the statistical comparison of different regions; flock size and avian

influenza virus (AIV) seropositivity (number of positive birds/ number of sampled birds) are continuous variables

**Page 146:** Table 5.5 Analysis of deviance for the reduced model for avian influenza virus (AIV) prevalence in Omani backyard chicken flocks fitted using quasi-likelihood methods

**Page 147:** Table 5.6 Coefficients, standard errors and 95% confidence intervals on parameter estimates from the reduced model for avian influenza virus (AIV) seroprevalence in backyard chicken flocks in Oman; The Dhofar governorate was the reference for the statistical comparison of different regions; altitude (m) is a continuous variable

**Page 158:** Table 6.1 The estimated total number of flocks in each region, the number of flocks sampled, the number and type of each poultry species sampled in the eight regions of Oman

**Page 167:** Table 6.2 Prevalence of identified genotypes within each sampled region from 39 IBV positive isolates

**Page 185:** Table 7.1 Percentage WNV seropositivity of flocks and birds in different regions of Oman

**Page 186:** Table 7.2 Percentage of birds positive for West Nile virus antibodies in backyard poultry in Oman

**Page 187:** Table 7.3 Percentage of flocks positive for West Nile virus antibodies in backyard poultry in Oman

**Page 191:** Table 7.4 Number and species of the trapped mosquitos in the three sampled regions

**Page 204:** Table 8.1 The scores for both general farm biosecurity and the managers' knowledge of biosecurity concepts

**Page 212:** Table 8.2 Number of responding commercial broiler farms from each Omani region and their total numbers of birds produced annually

**Pages 213-15:** Table 8.3 The characteristics of broiler poultry farms in Oman

## LIST OF ABBREVIATIONS

Common abbreviations are defined below, whilst specialist abbreviations are defined below and on their first use within the text.

Common abbreviations are defined below, whilst specialist abbreviations are defined below and on their first use within the text.

AI	Avian Influenza
AIV	Avian influenza virus
aMPV	Avian metapneumovirus
APV	Avian pneumovirus
Ark	Arkansas
AVL	A viral lysis buffer
Bp	Base pair
°C	Degrees Celsius
C	Culex
Conn	Connecticut
Edf	Estimated degrees of freedom
E	East
EID2	the Emerging Infectious diseases database
ELISA	Enzyme-linked immunosorbent assay
F	Fusion
FAO	Food and Agriculture Organization
FTA	the Flinders Technology Associates
GAMs	Generalised Additive Models
GLM	General linear model

GIS	Geographical Information Systems
HA	Haemagglutination
HI	Haemagglutination inhibition
HPAI	Highly pathogenic avian influenza
HPNAI	high pathogenic notifiable avian influenza
IB	Infectious bronchitis
IBV	Infectious bronchitis virus
IgG	Immunoglobulin G
JE	Japanese encephalitis
ILT	Infectious laryngotracheitis
ILTV	Infectious laryngotracheitis virus
IVPI	intravenous pathogenicity index
LPAI	Low pathogenic avian influenza
LPNAI	low pathogenic notifiable avian influenza
Kg	Kilogram
Km	Kilometre
ME	Middle East
ml	Millilitre
Mass	Massachusetts
M41	Massachusetts 41
µl	Microlitre
N	North
NA	Neuraminidase
NAIV	Notifiable avian influenza viruses
ND	Newcastle disease

NDV	Newcastle disease virus
NCSI	National Centre for Statistics
NP	Nucleoprotein
ODs	Optical density
OIE	World Organisation for Animal Health
RPM	Revolutions Per Minute
RNA	Ribonucleic acid
RT	Reverse transcription reaction
RT-PCR	Reverse transcriptase polymerase chain reaction
SAP	Shrimp alkaline phosphatase
S/N	the sample to negative ratios
S/P	the sample to positive ratios
SE	Standard Error
SHS	Swollen head syndrome
SLE	St. Louis encephalitis
TBE	Tris-borate-EDTA
UK	United Kingdom
USA	The United states of America
UV	Ultraviolet
WHO	World Health Organization
WN	West Nile
WNV	West Nile virus

## **Publications and presentations**

**Al Shekaili T.**, Baylis, M. & Ganapathy, K. (2015) Molecular detection of infectious bronchitis and avian metapneumoviruses in Oman backyard poultry. *Research in Veterinary Science*, 99, 46-52.

**Al Shekaili T.**, Helen Clough, K. Ganapathy & Baylis, M. (2015). Sero-surveillance and Risk factors for Avian Influenza and Newcastle Disease virus in backyard poultry in Oman. *Preventive Veterinary Medicine*

**Al-Shekaili, T.**, Baylis, M. and Ganapathy, K. (2013). Sero-surveillance for Avian Influenza and Newcastle Disease virus in backyard poultry in Oman.. *British Veterinary Poultry Association (BVPA) meeting. Harrogate, UK, 14<sup>th</sup>-15<sup>th</sup> March 2013* (Oral presentation).

**Al-Shekaili, T.**, Baylis, M. and Ganapathy, K. (2014). Molecular detection of infectious bronchitis and avian metapneumoviruses in Oman backyard poultry. 8<sup>th</sup> International symposium on avian corona-and pneumovirus infections/2nd cost action, Rauschholzhausen, Germany, 20<sup>th</sup>-23<sup>rd</sup>, June 2014 (Oral presentation).

**Al Shekaili T.**, K. Ganapathy & Baylis, M (2013). Surveillance and risk factors for Avian Influenza and Newcastle Disease virus in backyard poultry in Oman. *Presentation in Departmental Research seminar.*

**Al Shekaili T.**, K. Ganapathy & Baylis, M (2015). A study on the prevalence of West Nile Virus in backyard poultry in Oman. *Presentation in Ministry of agriculture and Fisheries, Oman.*

**Al Shekaili T., K. Ganapathy & Baylis, M (2014).** Serological and molecular results of the Oman backyard sampling. Presentation in Ministry of agriculture and Fisheries, Oman.

**Al Shekaili T., K. Ganapathy & Baylis, M (2013).** Surveillance of Avian Influenza and Newcastle Disease virus in backyard poultry in Oman. Liverpool University Poster day 2014.

## **1- Chapter one: Introduction and literature review**

In this chapter I will give an overview on the global poultry production, Oman location, environments, and poultry production in Oman. Then I will elaborate some of the viral respiratory pathogens that affect poultry production world-wide such as avian influenza viruses (AIVs), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and avian metapneumovirus (aMPV) and their epidemiology. In addition, I will give insight into the epidemiology of West Nile virus (WNV) which causes neurological disease in both human and equines, which also circulates in local birds, including poultry.

## **1.1 The global poultry production**

Poultry meat is one of the fastest growing food sectors. According to the Food and Agriculture Organization (FAO), poultry meat is the second most common source of meat reaching more than 35% of the total global meat production (Anon, 2015b). There was an increase in global poultry production between 1990 and 2012 of more than 104%.

Although the single biggest poultry meat producer is Brazil, followed by the United States of America, Asia has been leading the global poultry growth rate by around 4.3% in the last 15 years. Table 1.1 shows the total poultry meat production in different regions of the world in 2013 and 2014 (Anon, 2015b).

FAO experts expect that by 2020 the projected trends in poultry meat consumption will be more than three times what they were in 1993. The expected increase will be greater in countries such as China, India and Brazil than the rest of the world.

Modernization and expansion of poultry production is derived by the integrated, intensive poultry enterprises in most of developed countries, for example in the US, over 97% of broiler poultry products come from this system (Leibler et al., 2008).

This increase was despite the presence of the biggest ever avian influenza outbreak, which started in 2003 (H5N1) and affected more than 60 countries, with its heaviest toll on South-East Asia and Egypt (Brown, 2010). Hundreds of millions of poultry birds died due to the highly pathogenic effects of the virus or because they were culled to control and/or eradicate the disease. This had profound consequences for the global poultry industry. The cost of controlling and monitoring the virus included trade bans in the afflicted areas, which had an effect on the entire production and marketing cycle. Moreover, people's fear of eating poultry meat and other poultry by-products aggravated the situation (Lee and Saif, 2009). Similarly,

other viral respiratory viruses such as Newcastle disease virus, infectious bronchitis and avian metapneumovirus have a high impact on the growth of poultry farming in many countries world-wide.

In this thesis, I will focus on the epidemiology of the viral respiratory diseases with more emphasis on avian influenza in Oman poultry. I chose to do epidemiological studies on the Oman backyard poultry since it is the only sector not practicing any type of vaccination.

**Table 1.1.** Poultry meat production by region and country 2013-14 in 1000s tonnes  
(FAO) (Anon, 2015b)

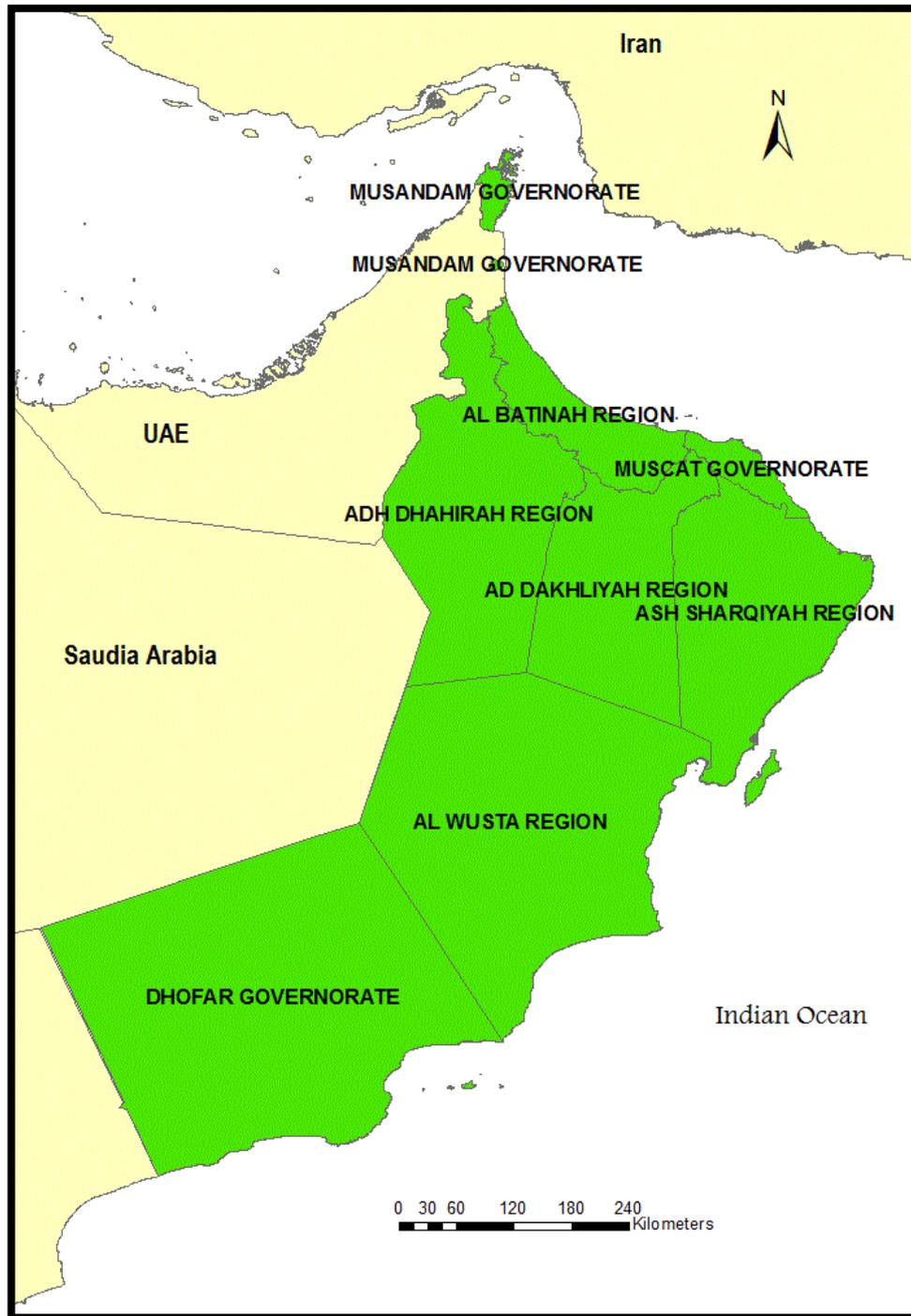
Region	2013	2014
Asia	38083	38497
Africa	4977	5040
Central America	4290	4340
South America	18475	18726
North America	21440	21821
Europe	18430	18950
Oceania	1283	1329
Total Global Production	108991	110717

## **1.2 The Sultanate of Oman**

The Sultanate of Oman covers a total land mass of 309,500 square kilometres of the south-eastern corner of the Arabian Peninsula. It lies between 16°'40 - 26°'20 N and 51°'50- 59°'40 E. It is bordered by the Arabian Gulf, Gulf of Oman, the Arabian Sea, the Indian Ocean, Saudi Arabia, Yemen, and United Arab Emirates (Figure 1.1). It has more than 3165 km of coastline.

The Sultanate has varied types of topographical features, however, desert and valleys accounts for 82% of the Oman land mass (Figure 1.2), mountainous terrain accounts for 15% and the coastal plain, 3 percent. It has different administrative regions most of which are in the more populous, northern part of the country.

**Figure 1.1** Sultanate of Oman's different geographical regions and governorates



**Figure 1.2** Different topographical environments of Oman (A) rocky valley environment in Northern regions, (B) lush tropical environment in the monsoon-affected region of Dhofar in southern Oman (C) desert environment



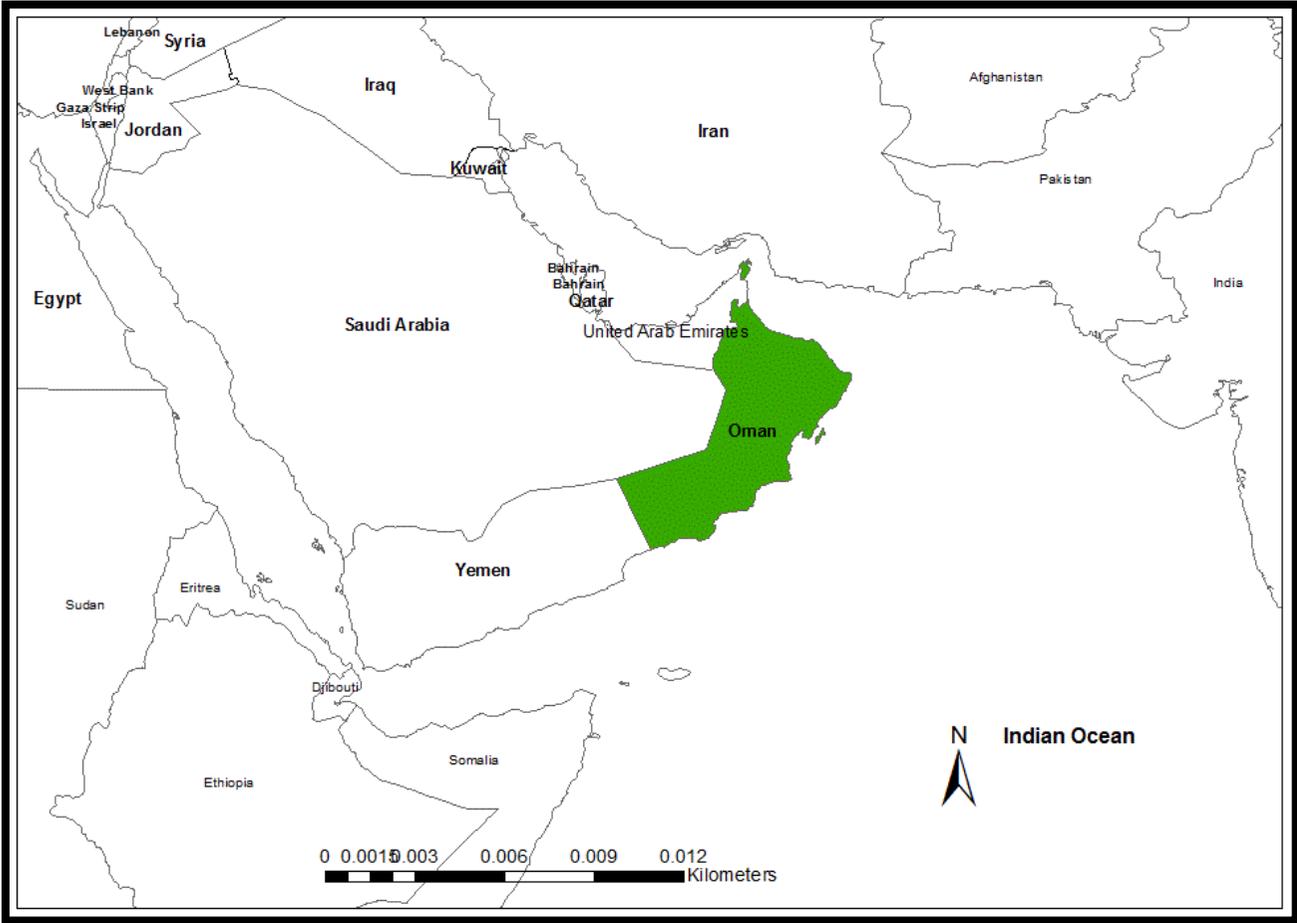
The majority of the desert has a very hot dry summer. However the coastal areas of the northern regions have hot, humid weather, especially during the summer season. Dhofar Governorate (the most southern region) has a typical strong monsoon climate and receives warm winds from the Indian Ocean (Figure 1.2). Oman winter weather generally is pleasant and enjoyable.

As a part of the land bridge between Asia and Africa, Oman plays an important role for avian migration and nesting. Barr al Hikman, the large pristine coastal wetland in Al Wusta region is a key wintering and stopover site for migratory birds within the West Asian - East African and Central Asian Flyways.

The country is also a part of the Middle East region (ME), which is a central area connecting the old world three continents (Figure 1.3). The ME can play an important role in the spread of diseases from one continent to another.

According to the National Centre for Statistics (NCSI) Oman has undergone a very rapid increase in population and by 2014 the country's population rose to nearly 4 million (The National Centre for Statistics (NCSI), 2014), which is almost double the number 10 years ago. However, almost fifty percent of the population live in just two geographical regions in the north; namely Muscat Governorate and Al Batinah region (The National Centre for Statistics (NCSI), 2014).

**Figure 1.3.** Oman location map



### **1.3 Poultry production in Oman**

The rapid increase of Oman's population in the last four decades, coupled with the increase in the average Omani family's income, has led to a huge production gap in poultry meat, table eggs and fertilized eggs. This gap has encouraged investment in poultry production, particularly in the last 15 years, giving rise to an enormous increase in the net local poultry production. For example, during the two year period 2010 to 2012, table egg production rose from 183 million eggs annually to 236 million eggs. Also, in the same period the net local poultry meat production almost doubled, rising from 24 million kg to 43 million kg annually (Anon, 2013a).

However, according to the Oman agriculture census 2012/13, this remarkable increase in the production in local poultry meat still only accounts for around 34% of the local demand. Similarly, local layer farms produce around 44% of the local consumption requirements of table eggs and nearly more than 60% of the fertilized eggs produced locally. According to the global poultry trends web site Oman is ranking 41 place of the Asian countries in chicken meat production (Anon, 2015b). The high price of poultry feeds and the presence of poultry diseases stand as the major obstacle to increasing poultry production in Oman.

Table 1.2: shows the number of farms and the biosecurity levels of different broiler poultry systems in Oman. Oman poultry production comprises three basic compartments; integrated commercial farms (system 1), medium and small poultry producers (system 2 & 3), and backyard sector (system 4). In the broiler farming the recorded annual local production is nearly 43 million kilogram (Anon, 2013a). Eighty-five percent of this local poultry meat production comes from the integrated poultry farms. The rest is from the production of the medium and small commercial

producers. Of the 303 production farms shown in the Oman agriculture census 2012/13, just two broiler farms (A'saffa and Sohar) are considered to be integrated broiler farms (Table 1.2). However, a third broiler farm, called Arzat farm in the southern region (Dhofar governorate), is also producing around one and a half million birds annually.

The agriculture census 2004 stated that local backyard poultry constitutes more than one million birds, however, due to the efforts of the H5N1 contingency plan implemented from 2004 onward a considerable reduction on the backyard poultry has been noted, both in terms of the number of farms and birds. The plan's target was to actually minimize the numbers of both water fowl farming and uncaged backyard poultry. During 2012, the Department of Rural Women's Development estimated the number of backyard poultry flocks to be around 10,000 flocks, with a mean size of 50 birds. Most of these backyard flocks comprise chickens, however, other species, like ducks, geese, turkeys and guinea fowls may be present too. Although backyard farming is widely distributed in most of the regions of Oman, their participation in meat production seems to be very low.

Integrated farms strictly apply high standard biosecurity measures and vaccinate against all diseases likely to affect their flocks and for which vaccines are available. The enterprises usually have most of the production cycle compartments. This type of production needs very specialized personnel and equipment which carry a very high investment cost.

On the other side, the medium and small poultry producers vary greatly in their biosecurity measures. The medium poultry farms mostly apply biosecurity measures very similar to those applied in integrated farms. However, the small producers have

very low biosecurity measures, and vaccinate against Newcastle disease only. For both medium and small producers, the enterprise covers only one step of the production chain, mainly rearing birds from one day old to the marketing age. This leads to high interaction between different farms that may cause leakage in the biosecurity process.

In all the previous production types except for the backyard, birds are kept indoors in big numbers with flooring that is covered with few centimetres in depth of litter (wood shavings or straw) which could facilitate pathogen infections (Madelin and Wathes, 1989).

In Oman, the backyard poultry farmers do not vaccinate their birds or apply significant biosecurity measures. This type of rearing is very cheap and does not require much attention. Although some farmers keep the birds within outdoor cages, they occasionally let them forage outside, particularly during day-time. These actions increase the chances of contact between poultry birds of different farms and with wild birds. Furthermore, breeding more than one species of poultry bird on the same holding can mean increasing the possibility of disease introduction (Koch and Elbers, 2006).

Backyard and the small producers are the ones mainly affected by avian diseases and suffer losses from them; they also may play a major role in the spread of the diseases to other farms. Therefore, in this thesis I chose this poultry sector to examine the epidemiology of the viral respiratory diseases circulating in Oman.

**Table 1.2** The number of farms and the biosecurity levels of different broiler poultry systems in Oman

System type	Number of farms	Number of birds	Type of production compartment	Biosecurity level
System 1	3	>1 million bird	Broiler birds Hatcheries Breeder birds Slaughterhouses	Excellent biosecurity
System 2	66	18000 to 1000,000 bird	Broiler birds	Vary from poor to excellent
System 3	~ 220	<18000 birds	Broiler birds	Poor to fair
System 4	~ 10000	1 to 2000 local birds	Birds for different purposes	Poor

## **1.4 Poultry viral respiratory diseases**

Avian influenza (AI) and other viral respiratory diseases such as those caused by Newcastle disease (ND), infectious bronchitis (IB), infectious laryngotracheitis (ILT) and avian metapneumovirus (aMPV) viruses are major constraints for poultry production globally (Jones, 2010). The wide distribution of Newcastle disease (ND) and increasing incidence of avian influenza outbreaks has a great negative impact on local poultry production and international trade (Hafez, 2005; Marangon and Busani, 2006). IB is considered to be the most important respiratory disease in some Middle Eastern countries such as Jordan (Gharaibeh, 2007). Less attention is given to ILT and aMPV in the Middle East in general and in Oman in particular.

Most of the control efforts for these diseases are based on vaccination and biosecurity. In Oman where NDV is endemic and clinical signs have been seen most frequently in the last decade (Anon, 2014b), live and killed vaccines are widely used in commercial farms. Also, vaccination is used for IBV, ILT, low pathogenic AI (H9N2), and aMPV. Generally the level of vaccine usage is greatly affected by many factors such as production type, biosecurity level, disease patterns, vaccine availability and potential losses from the disease (Marangon and Busani, 2006). Nevertheless vaccination should go in parallel with the good management and biosecurity in order to minimize and control infectious disease (Hafez, 2005).

Table 1.3 shows the distribution of viral poultry diseases worldwide and its presence in Oman.

**Table 1.3** The distribution of viral poultry disease worldwide and its presence in Oman

The disease	The causative agent	Global distribution	In Oman
Highly pathogenic avian influenza	Influenza A viruses of the family <i>Orthomyxoviridae</i> (H5 and H7)	Asia, Africa and North America	Not reported
Low pathogenic avian influenza	Influenza A viruses of the family <i>Orthomyxoviridae</i>	Globally distributed	H9N2 since ~20 years
Newcastle disease	Avian paramyxovirus type 1	Worldwide except North America and Australia	Endemic
Avian infectious bronchitis	Coronavirus type 3	Worldwide	Endemic
Avian metapneumovirus	<i>Paramyxoviridae</i> family	Worldwide	Endemic
Infectious laryngotracheitis	<i>Herpesviridae</i>	Worldwide	Not reported
Fowl pox	Avipox virus genus	Worldwide	Not reported

## 1.5 Avian influenza viruses (AIV)

Avian influenza is caused by influenza A viruses of the family *Orthomyxoviridae* (Hafez, 2005). Haemagglutinin (HA) and neuraminidase (NA) are the two surface antigens used to classify AI viruses, of which there are 18 and 11 identified types respectively (Tong et al., 2013). Nearly all of these types have been identified in birds (Capua and Alexander, 2004; Alexander, 2007; Tong et al., 2012). Bird migration and the trade in live birds are the main two ways for spreading the virus globally (Kilpatrick et al., 2006; Alexander, 2007; Grund et al., 2011; Tong et al., 2012; Afifi et al., 2013).

Avian influenza disease complex produces a wide range of clinical signs in birds. Highly pathogenic viruses are usually characterised by a very high mortality and morbidity in poultry (both may reach 100%). They cause sudden death in chickens and turkeys, particularly in young ages with no clinical signs or with recumbency, depression and comatose state. The remaining birds may show dehydration, decreased feed intake and severe depression and death. In laying birds, egg production decreases almost to zero within 3 to 5 days. Birds that survive the acute phase of the disease occasionally show torticollis, paresis, paralysis and convulsion and rolling in circular movements (Swayne and Suarez, 2000).

However, both low pathogenic and highly pathogenic avian influenza viruses may replicate in water fowl birds without showing clinical signs and cause sub-clinical, or mild to moderate clinical manifestations (Keawcharoen et al., 2008).

Generally avian influenza viruses differ greatly in the clinical signs they cause according to the virus strain, type, age and immune status of the birds and the presence of coinfection with other pathogens.

Therefore, these viruses are classified according to virulence into two main groups; low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) (Capua and Alexander, 2007; Mohamed et al., 2009; Swayne, 2009; Martins, 2012). The latter group which was termed fowl plague (Hafez, 2005) causes huge mortality in affected flocks that may reach 100%. It is caused by AI viruses with H5 and H7 subtypes (Capua and Alexander, 2004; Hafez, 2005). Moreover, those viruses have the ability to cross the species barrier infecting and causing fatality in other species including humans and some other mammals (Hafez, 2005; Riedel, 2006).

The huge increase in the frequency of HPAI outbreaks in the last two decades, particularly the biggest ever AI outbreak of H5N1 (Swayne, 2012) which has led to the deaths of hundreds of millions of birds worldwide (Kilpatrick et al., 2006) and has more than 50% case fatality rate in humans (Afifi et al., 2013), has raised the alertness of the international organizations such as World Health Organization (WHO), World Organisation for Animal Health (OIE) and Food and Agriculture Organization (FAO) to act quickly and coherently with local governments to identify and get rid of any new outbreaks and provide help to the affected countries.

### **1.5.1 High pathogenic *versus* low pathogenic viruses**

The crucial difference between HPAIV and LPAIV lies in the cleavability of hemagglutinin (HA) protein (Garcia et al., 1996; Post et al., 2012). HPAI viruses have the ability of systemic replication, whereas the LPAI viruses are more specific and local. This difference may lead to huge variations in clinical signs. To date all HPAIV are restricted in the H5 and H7 subtypes (Alexander, 2007a). These two

subtypes have the ability or the potential to mutate from the low pathogenic to the high pathogenic status, especially if they circulate widely in poultry flocks. The previous classification of avian influenza by the World Organisation for Animal Health (OIE) i.e. notifiable avian influenza viruses (NAIV) then further divided in to high pathogenic notifiable avian influenza (HPNAI) and low pathogenic notifiable avian influenza (LPNAI) (Anon, 2010) caused some confusion with the scientific use of “avian influenza”. This classification has been removed and the terms HPAI, H5/H7 LPAI and influenza A are widely used now. The latter indicates any influenza virus from birds that is H1–H18 (Anon, 2015).

The outbreaks in poultry and other birds of the HPAI and H5/H7 LPAI are notifiable and subject to official control.

### **1.5.2 General epidemiology**

Although AIV is one of the earliest diseases shown to be “ultra-filterable” and hence caused by viral pathogens (Alexander, 2007a), it took more than 50 years to show the link between the pathogenic subtypes and the milder forms (Alexander, 2007a). Most of the AIV subtypes have been identified in wild birds, especially water fowl; however, these viruses do not appear to cause clinical disease in these birds nor in poultry. Poultry outbreaks have usually originated from both direct and indirect contact with wild birds (Alexander, 2007a). Mammals, with the exception of swine, which are instrumental in swine influenza dissemination to neighbouring turkey farms, have no direct role in AIV epidemiology (Alexander, 2007a). It is not possible to predict the time of occurrence of a highly pathogenic outbreak, however, the wider circulation of the low pathogenic viruses (particularly H5 and H7) in

poultry flocks gives a higher chance of a mutation to virulent viruses (Alexander, 2007a).

Wild birds play minimal role in the local spread of AIV once it gets established in poultry flocks. AIV is highly contagious in poultry and it is produced in high volume in the infected birds' secretions and faeces (Anon, 2014a). The highly pathogenic subtypes are not equally fatal for different types of birds. This therefore gives a chance for some poultry types such as ducks and geese to disseminate the virus relatively unnoticed and for longer periods.

The sport of falconry plays a role in virus transmission in countries where this hobby is present, such as in Saudi Arabia and UAE. In both countries HPAI H5N1 has been found infecting falcons and their prey (Monne et al., 2008; Naguib et al., 2015).

The ecology of the AI viruses is very complicated involving many host species of birds and mammals. All types of poultry rearing, wet markets (selling places of live birds and other susceptible animals), places of poultry slaughtering, swine farms, the live birds trade and migratory bird movements (Ma et al., 2009) all contribute to the complexity of AIV ecology. Moreover, rodents and insects may play a role in the mechanical transmission of the virus between farms (Hafez, 2005). Persistence of the AIV in the wet environment such as surface water, mud and soil may last many months which gives the opportunity of the infection of newly hatched birds (Rohani et al., 2009).

Transmission of the AIVs from one flock to another is usually through the movement of infected birds, or mechanically, through contaminated equipment, egg trays, feed trucks, and service crews (Hafez, 2005).

Though vertical transmission is not ruled out, the infected chick embryo is unlikely to hatch. However, the possibility of transmission of the virus with infected shell remains (Hafez, 2005). Airborne transmission does not play a major role except when birds are in close proximity (Hafez, 2005; Anon, 2014a). The virus may survive for a longer period in cold and frozen media (Hafez, 2005).

The low pathogenic viruses are generally quicker in spreading from infected to non-infected birds. This phenomenon may be attributed to the high fatality of the highly pathogenic viruses which kill the birds before they produce high amounts of virus (Alexander, 2007a).

### **1.5.3 Prevention, control and eradication**

Prevention, and if the virus is introduced, eradication, are usually the ultimate aims of both veterinary authorities and poultry producers in each country when dealing with AI outbreaks. However, the ways of reaching this goal and the seriousness of the actions needed against the outbreak are mainly based on the virulence of the causative virus. Eradication policy and the killing of animals are commonly used with the suspicion or confirmation of emergency diseases that have high zoonotic potential or great economic impact (Hafez, 2005).

The HPAIV are the most devastating viruses causing huge financial losses and trade bans and have a high zoonotic potential. Therefore both international organisations such as the World Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO) and World Health Organization (WHO), and local veterinary authorities in most of the countries around the world adopt a contingency plan to control and eradicate any AI outbreak (Martin et al., 2009).

OIE, FAO and WHO agree that the earliest detection, followed by quick action, are the best way to contain HPAIV before it spreads to other premises. Therefore, continued surveillance and, stamping out of infection at the affected premises, with proper disposal measures, are essential (Martin et al., 2009).

The emergency response to HPAI and H5/H7 LPAI notifiable suspicions includes establishing a protection zone around the infected holding surrounded by a surveillance zone. Once the outbreak is confirmed all the birds on the vicinity should be killed without delay and on the spot (Anon, 2008a).

The use of mass vaccination to control highly pathogenic avian influenza subtypes is still being debated (Anon, 2008a). However, many countries incorporate emergency vaccination in their HPAI contingency plans. The time spent between the detection and diagnosis of the first incursion and the application of mass vaccination is crucial for the success of a vaccination campaign (Capua and Marangon, 2003). Most of the countries that employed this method, such as Egypt, China, and Indonesia, were forced to shift to a mass vaccination policy because they were late to respond to initial infection and the virus spread widely.

#### **1.5.4 The situation in Oman**

H5N1 outbreaks have been observed threatening both human and bird populations over the last decade, but have not been detected in Oman. Nevertheless veterinary authorities have increased their active surveillance in order to detect any new incursion as soon as is possible. The first phase of the preparedness contingency plan was fully imposed, incorporating sampling of all suspected birds, importation of required protective clothing and acquisition of disinfection chemicals, importation of

the H5N2 vaccine as a stock for emergency vaccination and completing simulation exercises (Anon, 2008c).

There has been no previous published work identifying other avian influenza viruses in Oman, however H9N2 has circulated in the Oman poultry industry since the late 1990s as well as in other Middle East countries (Fusaro et al., 2011a). Some poultry production farms add the H9N2 vaccine to their bird vaccination programmes. The vast majority of the production farms, especially small farms and the backyard sector have not been vaccinating.

### **1.5.5 Zoonosis**

Historically avian influenza viruses were the causative pathogens of several pandemics (Table 1.4) (Ma et al., 2009; Shoham, 2011). Around twelve documented humans AI outbreaks were due to AI viruses during the last two centuries.

During the last two decades several different subtypes (H5N1, N7H7, N9H7 and H9N2) have been reported infecting humans and causing deaths. These incidences again raised the pandemic potential of avian influenza (Lee and Saif, 2009). These viruses did not attain the ability of person to person transmission, however the H5N1 outbreak is still ongoing and increases the probability of generating a human adapted version of the virus (Ma et al., 2009). The Egyptian clade 2.2.1 of H5N1, which is circulating in backyard poultry, has acquired more abilities to infect humans (El-Zoghby et al., 2012). Also the reassortment of the H5N1 with other human influenza viruses generate hybrid viruses with higher virulence (Li, 2010). Laboratory-induced genetic modifications for the H5N1 virus have made the viruses transmissible between ferrets (Murillo, 2012).

Swine appear to be the mixing vessel for avian influenza viruses due to their susceptibility to both human and avian influenza viruses. Co-infection of pigs with two different subtypes of AIV may lead to reassortment and the generation of a new virus comprising genome segments of both. Although the origin of 1918 pandemic virus is still debated as being of purely avian origin or with mammalian assortment, the 1957 and 1968 virus proved to have originated from pig farms (Ma et al., 2009).

**Table 1.4** Influenza pandemics since the 16<sup>th</sup> Century

Century	Number of of outbreaks	Area of occurrence	Antigenic subtype
16 <sup>th</sup> Century	3	Asia and Africa	Unknown
18 <sup>th</sup> Century	3	(Russia/USA, Americas, China	Unknown
19 <sup>th</sup> Century	6	(2 in Russia, China, Panama, Kazakhstan and one unknown)	H1N1, H2N2, H3N8
20 <sup>th</sup> Century	6	4 in China, 1 in USA and 1 in Mexico	H2N8, H1N1, H2N2, H3N2

## **1.6 Newcastle disease virus (NDV)**

Newcastle disease virus is an enveloped negative-sense, single stranded RNA virus called avian paramyxovirus type 1 (APMV 1) which causes a highly contagious bird disease (Alexander, 2000; Mase et al., 2002; Madadgar et al., 2013; Samuel et al., 2013). In countries affected by this disease, outbreaks lead to losses both directly, by loss of production (mortality and culling) and indirectly, through trade restrictions (Hafez, 2005). This disease is a major hindrance for backyard poultry production, especially in poorer areas. For example, 90% of village chickens died each year due to ND disease in Nepal (Spradbrow, 1992; Alexander, 2001).

The ND viruses are classed in a single serotype group (Samuel et al., 2013), however, in terms of virulence the NDVs are categorized into 5 different groups, viscerotropic, neurotropic, mesogenic, lentogenic and asymptomatic enteric. The first two groups are highly virulent with mortality reaching almost 100%. The viscerotropic group affects mainly the intestines of the birds, with lethal haemorrhages, while the neurotropic group causes severe, often fatal, neurological and respiratory clinical signs. The mesogenic virus group is less pathogenic, affecting both the nervous and the respiratory systems with moderate clinical signs. The lentogenic group causes mild respiratory lesions, whereas the asymptomatic enteric group is non-virulent viruses mainly replicating in the gut of the birds (Alexander, 2000; Hafez, 2005). Clear mucus discharge, gasping and green watery faeces are seen in viscerotropic ND (Suarez, 2013). In neurotropic forms the birds may seem excitable in the first 3 to 4 days of infection followed by head or muscular tremor, torticollis and paralysis of wings or legs (Suarez, 2013). Factors such as the host species (Madadgar et al., 2013), age of host, co-infection with other pathogens, environmental stress, route of infection and immune status of the infected birds

affect the severity of the clinical signs (Parede and Young, 1990; Hafez, 2005; Suarez, 2013). These factors may narrow the demarcation between the different groups. Recent molecular characterisation of the NDV genome has divided the ND viruses into two main classes (I and II) which then subdivide into 9 and 15 genotypes respectively (Samuel et al., 2013). NDV class I genotypes are mainly low pathogenic NDV (Suarez, 2013). In contrast, NDV class II split into high and low virulence genotypes (Suarez, 2013). Genotypes V, VI, VII and VIII are currently responsible for causing most outbreaks around the world (Farooq et al., 2014). Some of these genotypes are further divided e.g. genotype VII sub-divides into eight subgenotypes (VIIa–VIIh) (Farooq et al., 2014).

The main clinical signs observed in infected birds are depression, diarrhoea, prostration, oedema of the head and wattles, nervous manifestations like paralysis and neck torticollis, and respiratory distress (Alexander, 2000). NDV has the ability to infect more than 250 bird species (Heiden et al., 2014). Annually there are recurrent outbreaks of the highly pathogenic NDV in different parts of the world. Both mutations of the low virulence viruses and the transmission of the virus by wild and migratory birds play an important role in this reoccurrence (Alexander, 2001; Mase et al., 2002; Madadgar et al., 2013).

### **1.6.1 General epidemiology**

ND virus can spread between farms by physically moving infected birds or by mechanical methods such as shared poultry services crews, and rendering-truck (Hafez, 2005). However, the trade in international pet birds and the migration of

wild birds contribute to transmitting the virus between different geographical areas (Hafez, 2005).

There is a closely related paramyxovirus which infects only *columbiformes* called pigeon paramyxovirus type 1 virus, which probably originated in the Middle East in the late 1970s (Mase et al., 2002). This virus also causes neurological clinical signs in the pigeons similar to that produced by NDV in other birds.

Although the NDV is a major problem for the poultry producer in developing countries, nevertheless, even the countries that are able to control the disease by vaccination and biosecurity suffer losses due to the high cost of control measures (Alexander, 2000).

### **1.6.2 Epidemiology of Newcastle disease in Oman**

In Oman, Newcastle disease viruses are endemic, and all production farms vaccinate against this pathogen infection regularly. However, despite this regular vaccination, clinical manifestations are still often seen both in production farms and backyard poultry, over the last decade (Anon, 2014b). Between 2012-2014, about 90 NDV outbreaks had been reported to the OIE by the Omani veterinary authorities (Anon, 2015a), most of them showing velogenic viscerotropic clinical signs (Ahmed et al., 2012).

## **1.7 Avian infectious bronchitis virus (IBV)**

IBV is one of the most challenging respiratory disease pathogens of birds (Jones, 2010). It belongs to *coronavirus* type 3. The disease, which has a very high morbidity rate reaching 100%, but, comparatively low mortality, was firstly described in young chicks in 1931 (Schalk and Hawn, 1931). After nearly a decade the first IBV vaccine was produced namely the M41 serotype, however, several years later new antigenically different serotypes were discovered (Jackwood and De Wit, 2013). The early work of IBV identification was based on serology, therefore the IBV was categorised as serotypes. During the 1990s and onward molecular identification techniques developed rapidly and were used widely for the identification of different isolates, thus, the term “genotype” started to dominate in the IBV identification field (Jackwood et al., 1997; Keeler et al., 1998).

The level of morbidity is greatly affected by factors such as management, environment, immune status of the birds, age of the birds, the presence of secondary infections (Ganapathy, 2009) and virulence of the IBV genotype (Jackwood and De Wit, 2013).

IBV is an important respiratory disease in chickens, generally characterized by increased oculo-nasal secretion and excess mucus in the trachea affecting the growth rate of the bird (Grgic et al., 2008). The virus also affects the reproductive epithelia causing reduction in the egg production, quality and fertility. Genotypes causing renal inflammation are the most likely to cause high fatality (Cook et al., 2001). The virus can also replicate in the alimentary tract but without causing clinical signs. The clinical signs infection with IBV depends on the breed of chicken and IBV genotype (Cavanagh, 2007).

Secondary infections are common complicating factor in disease progression (Jackwood, 2012) particularly in small chicks. Chickens and pheasants are the only natural hosts for IBV (Ignjatovic and Sapats, 2000). Infected and recovered birds shed the virus and contaminate the environment (Ignjatovic and Sapats, 2000). The high probability of spontaneous mutation in its unstable S1 gene and the genetic recombination leads to continuous diversification of the virus (Jones, 2010). There is none or minimal protection between different genotypes (Jones, 2010; Jackwood, 2012). The number of commercially produced genotypes for the purpose of vaccination is limited. As a result, there is the constant need to have vaccination strategies using currently available live and killed vaccines, also development of new vaccines where appropriate.

### **1.7.1 General epidemiology**

The IBV is highly infectious and spread between flocks in the same area between birds, within the same flock, mostly by the aerosol or ingestion, however, mechanical transmission may also play a role (Ambali and Jones, 1990; Cavanagh, 2007). Migratory birds may transmit IBV between different geographical areas. Contaminated litter, foot-wear, clothing, utensils, equipment and personnel could cause horizontal transmission. Factors such as virus genotype, breed, age, immune status and co-infection with other organisms like *Mycoplasma gallisepticum* and *E. coli* have influence on the severity of the disease caused by IBV infection (Cavanagh, 2007). The virus has been recovered from one day-old chicks from infected hens and from the semen of cockerels showing the possibility of vertical

transmission (Ignjatovic, 2000) and the virus can be secreted in faeces of infected birds for a long period (Ignjatovic, 2000).

The distribution of different genotypes around the world has been reviewed in depth (Jackwood, 2012). Arkansas and Ark-like are the most common isolates in the USA (Jackwood et al., 2005). This genotype is still evolving and raises questions, such as the effectiveness of the protection conferred by currently available vaccines (Jackwood, 2012). Other commonly detected genotypes in the USA are Conn and Mass (Jackwood et al., 2005).

In the last three decades, several new isolates have been reported from California State, namely the Californian variant (Moore et al., 1998). Also, two nephropathogenic strains “PA/Wolgemuth/98 and PA/171/99” were identified in Pennsylvania (Ziegler et al., 2002; Jackwood, 2012).

Many unique viruses were identified in Mexico and Brazil such as MX/BL56-19/UNAM/96 (MX/5697/99), MX/UNAM-97/97, MX/07484/98, and MX/7277/99 (MX/1765/99) (Jackwood, 2012).

Viruses previously identified outside Central and South America, such as Arkansas-type, Q1-type, Conn (EU526403), Mass (EU526411), MX/47/UNAM/01 (EU526405), Belgium/B1648/95, CA/1737/04 and 793B type have now been detected in this region (Villarreal et al., 2010; Jackwood, 2012; Sesti et al., 2014).

Mass-type viruses, B/D274/84 and E/D3896/84, Netherlands/D207/79, and Netherlands/D1466/79 were the earliest IB viruses isolated in Western Europe. The 793B (793B/4/91/91) which emerged in Europe in 1991 (Parsons et al., 1992) is one of the most widely detected viruses across the world (Jackwood, 2012).

Italy-02 (Italy/Italy-02/497/02) was the most wide spread IB virus in Europe (Worthington et al., 2008); however, reports for around 10 years showed that it has been declining from all countries, with the exception of Spain. QX-like types are the most important IB viruses circulating in Europe due to their high pathogenicity (Jackwood, 2012). Mass and 793B, followed by D274, and finally QX are the most dominant IB viruses in Eastern Europe.

Several genotypes have been detected in Middle Eastern countries such as Iran/793B/19/08, Iraq/Sul/01/09, Israel/720/99, Israel/885/00, IS/1494/06, Egypt/Beni-Seuf/01, Egypt/Mass/F/03, Egypt/F/03, D3128, D274, D-08880, Egypt/D/89, CK/CH/LDL/97I, CK/CH/SCYA/10ICk/Eg/BSU-2/2011, Ck/Eg/BSU-3/2011 and QX-like (Abdel-Moneim et al., 2006; Ababneh et al., 2012a; Abdel-Moneim et al., 2012; Amin et al., 2012; Jackwood, 2012; Awad et al., 2014a; Al-Shekaili et al., 2015; Hosseini et al., 2015).

The control of IBV mainly relies on vaccination with the homologous genotype. As different IBV genotypes may provide partial or no cross protection against each others. It is essential to investigate and identify the circulating field genotypes. Although some commercial poultry farms in Oman include IBV vaccine in their vaccination programme, no previous studies reported this disease in Oman.

## **1.8 Avian metapneumovirus (aMPV)**

Avian metapneumovirus (aMPV), previously referred to as avian pneumovirus (APV) causes rhinotracheitis and swollen head syndrome in turkeys and chickens, respectively. Field infections are commonly more severe than experimental aMPV infection due to the involvement of secondary infection such as *E. coli*. It can cause high economic losses, particularly in turkey farms (Jones and Rautenschlein, 2013). This virus belongs to the *Paramyxoviridae* family (Njenga et al., 2003; Jones, 2010; Kwon et al., 2010). The virus has mainly an upper respiratory affinity, however sometimes it causes reproductive tract inflammation leading to drops in egg production in layers and breeders (Kwon et al., 2010). Young turkey poults show snicking, rales, sneezing, nasal discharge, conjunctivitis, swollen infraorbital sinus, and submandibular oedema. Older birds show coughing and head shaking (Jones and Rautenschlein, 2013). Both egg numbers and quality could drop due to aMPV infection in laying hens with increased incidence of uterine prolapse (Jones and Rautenschlein, 2013).

Morbidity can be as high as 100%, however mortality may range from 0.5% in adult turkeys to 80% in young poults (Buys et al., 1989).

### **1.8.1 General epidemiology**

Although both local and global transmission of the aMPV is still unclear (Jones and Rautenschlein, 2013), migratory birds are believed to serve as a reservoir and transmits the virus between different geographical areas (Turpin et al., 2008), whereas the local wild birds transmit the virus locally (Gharaibeh and Shamoun, 2012). Movement of the live birds, personnel, trucks, equipment and contaminated

water have all been implicated in transmission of the virus (Jones and Rautenschlein, 2013). The vertical transmission of aMPV has not been demonstrated experimentally.

The first reports of aMPV was from South Africa in 1978 (Buys and Preez, 1980), then the virus was detected in France (Giraud et al., 1986) and UK (McDougall and Cook, 1986) followed by reports of the virus from other countries of Europe (Baxter-Jones et al., 1989). The virus also reported from Africa (Morley and Thomson, 1984), Middle East (Banet-Noach et al., 2005), Far East (Lu et al., 1994; Tanaka et al., 1996), South America (Santos et al., 2012), Central America (Jones, 1996) and in the USA (Senne et al., 1997). The disease has global distribution in poultry-producing regions with few exceptions (Anon, 2009). Nowadays it is considered to be a major disease threat in both turkeys and chickens in many parts of the world (Kwon et al., 2010).

There are four known aMPV subtypes: A, B, C and D (Jones, 2010). Subtypes A and B, the most widely spread subtypes, were both identified in the Middle East (Banet-Noach et al., 2005) and subtype B is still the most commonly detected subtype in some countries in the Middle East (Gharaibeh and Algharaibeh, 2007; Al-Shekaili et al., 2015) and indeed world-wide (Jones, 2010). Both subtype A and B vaccines give good cross protection against each other (Cook et al., 1995), however, recently, reports from Brazil (Villarreal et al., 2009) and Italy (Cecchinato et al., 2009) suggest that the protection is not complete. Management practises which reduces stress and elimination of adventitious pathogens contribute in reduction of aMPV infections.

aMPV subtype C is mainly found in turkey farms in USA (Cha et al., 2013), however it has been isolated also in France (Toquin et al., 2006) and in Korea (Lee et al., 2007). The aMPV subtype D has been only reported in France (Bayon-Auboyer et al., 2000).

There are no previous reports of this virus in Oman poultry.

## 1.9 West Nile virus (WNV)

West Nile was diagnosed in Africa during 1937 (Komar, 2003), however, it was not reported with huge outbreaks. During the 1950s, it started to be identified occasionally in children with fever in the Middle East (Komar, 2003; Sejvar, 2003). Encephalitis caused by WNV was first seen in the early 1960s, in Egypt and France (Sejvar, 2003). The number of outbreaks reported from Middle East, Africa and Europe increased in the second half of the last century (Sejvar, 2003).

In late August 1999 the disease was reported in United States for the first time (Sejvar, 2003), in New York City. Molecular work suggests that the source of the WNV that entered the USA is the Middle East (Sejvar, 2003). The virus has moved across North America very rapidly causing the biggest ever WN meningoencephalitis outbreak ever recorded anywhere (Sejvar, 2003).

West Nile virus is a mosquito-borne pathogen, in the *Flavivirus* genus, which infects humans, birds, horses and some other mammals (Hubalek and Halouzka, 1999; Petrovic et al., 2013). This virus is a member of the Japanese encephalitis (JE) virus serocomplex which includes some important zoonotic viruses like JE virus, St. Louis encephalitis (SLE) virus, and Murray Valley encephalitis virus (Lanciotti et al., 2000).

Birds are the natural host, amplifying the virus and the mosquito-bird-mosquito transmission cycle (mainly involving *Culex* species of mosquitoes) is the usual infection cycle (Campbell et al., 2002; Austin et al., 2004; Frost et al., 2012). The virus generally produces sub-clinical infection in birds, nevertheless some bird species such as crows and pigeons show neurological clinical signs and deaths more

than others (Hubalek and Halouzka, 1999; Bernard et al., 2001; Figuerola et al., 2008; Petrovic et al., 2013).

The mammalian hosts such as man and horses are dead end hosts of the pathogen because the virus is not produced in their blood in sufficient amount to infect a susceptible mosquito (Rossi et al., 2010); nevertheless, they may develop deadly disease. In humans the majority (80%) of cases goes unnoticed, and the other 20% may show generalised unspecific clinical signs which maybe misdiagnosed with other viral diseases. Less than 1% develop neuroinvasive disease (encephalitis, meningitis, or flaccid paralysis) (Hayes and Gubler, 2006). This virus is distributed across the world (Campbell et al., 2002; Kramer et al., 2007; Petrovic et al., 2013).

### **1.9.1 General epidemiology**

Based upon genomic studies five lineages of WNV have been detected in the different parts of the world (Rossi et al., 2010). Lineage Ia is the most virulent and wide spread genotype. It has been recorded in the Middle East, Europe, Africa and America. The lineage Ib is found in Australia and lineage II is mainly found in Africa. Less is known about lineages III, IV and X (Rossi et al., 2010).

The prevalence of WNV is poorly studied in most of the countries (Gubler, 2002). Migratory birds have been found with higher prevalence than local birds in some areas like Europe, suggesting that they may play a role in dissemination of the virus between different geographical areas (Petrovic et al., 2013). Yet local birds are thought to play an important role in WNV maintenance within local areas (Petrovic et al., 2013).

The high production of antibodies against the disease suggests that birds like chickens are good sentinels for disease monitoring (Savage et al., 1999; Otte J et al., 2007; Yapici et al., 2012).

### **1.9.2 Vector preference**

The ability to infect different mosquito species, the abundance of different mosquito species and their feeding pattern play an important role determining the importance of different mosquito species as vectors of WNV. Different mosquito species have variable ability of acquiring and transmitting the West Nile infection. Around 65 mosquito species have been found to have the ability to transmit WNV. However, *Culex* mosquitoes are the main vector throughout the world. Species which have the ability of feeding on a wide range of birds and mammals may be more efficient vectors. For example, in the western United States *C. tarsalis* was found to be the major WNV vector, but other *Culex* mosquitos such as *C. stigmatosoma*, *C. thriambus*, *C. quinquefasciatus*, *C. nigripalpus*, and *C. pipiens* were also shown to possess the capability of transmitting the virus (Colpitts et al., 2012). *Aedes* mosquitoes also participate in West Nile transmission but they are not considered primary vectors. There is strong evidence that the virus is also transmitted vertically from one mosquito generation to another (Colpitts et al., 2012). Yet, argasid (soft) and amblyommine (hard) ticks play a part of the vector role in some areas (Hubalek and Halouzka, 1999).

### **1.9.3 West Nile virus in Oman**

In Oman there has only been a single recorded WNV outbreak, back in 2003, affecting a number of horses in the Muscat area (Ali, 2003). Since then there have been no other reports of the disease. However, a study done of 750 horse sera found 19.2% seropositivity for WNV in the neighbouring country of United Arab Emirates (Wernery et al., 2007). It was not clear why there are no further reports for the disease both in humans and equines in Oman.

## **1.10 Aim of the thesis**

This thesis explores the epidemiology of avian influenza and certain other viral respiratory diseases in Omani backyard poultry flocks. Backyard poultry was chosen as this sector is not practising vaccination for any of the diseases investigated in this thesis.

The first study (chapter 3) reviews the occurrence and the epidemiology of respiratory viral diseases affecting poultry in the Middle East area. A systematic literature search was used to identify the gaps of knowledge on these diseases in this region.

The second study (chapter 4) describes a survey of the backyard poultry to investigate the seroprevalence and attempted molecular detection of both avian influenza and Newcastle disease viruses in Oman.

Chapter 5 identifies the risk factors associated with the intensity of the infection of both avian influenza and Newcastle disease viruses in the backyard poultry farms.

Chapter 6 identifies the circulating genotypes of avian infectious bronchitis and avian metapneumoviruses in Omani backyard poultry flocks.

In chapter 7, and from the same sera in chapter 4, I describe using the backyard birds as sentinel animals for West Nile virus presence. The prevalence of West Nile virus in Oman was investigated, and potential mosquito vectors were identified.

Chapter 8 describes the management and health characteristics of broiler production poultry farms in Oman using data gathered by a predesigned questionnaire.

In Chapter 9, I complete the thesis with a discussion and suggestions of possible future work.

## **2- Chapter two: Materials and methods**

The materials and methods used in more than one chapter are described in this chapter. Materials and methods related to specific chapters are given the respective individual chapters.

## **2.1. Sample size calculation**

Serum and oro-pharyngeal swabs were taken from backyard poultry flocks from all regions and governorates of Oman, between mid-June and the end September 2012. Although, the total backyard poultry population was previously reported in the 2004 agriculture census as more than one million birds, due to the AI contingency plan and the concerns of farmers regarding the global spread of H5N1 outbreaks starting in 2004, the number of both birds and flocks has reduced. In early 2012 the backyard poultry was estimated by the Ministry of Agriculture and Fisheries / Department of Rural Women Development to be approximately 10,000 poultry flocks with a median size of 50 birds per flock.

The required sample size was based on detecting expected AIV and NDV prevalence of 30% and 70%<sup>1</sup> respectively, with 95% confidence and 5% precision. A two-stage cluster sampling method was used, with a between cluster variance of 0.165 estimated from a study of AIV in poultry flocks in another Middle Eastern country (Gharaibeh and Algharaibeh, 2007) using the following equation (Thrusfield, 2005).

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<sup>1</sup> Al shekaili personal communication

$$G = (1.96)^2 \{n V_c + P \exp (1 - P \exp)\} / n d^2$$

Where

G= desired number of flocks

n= mean of flock size = 50

P exp= expected prevalence (30% and 70%)

d = precision 0.05

V<sub>c</sub> = between flock cluster variance = .165

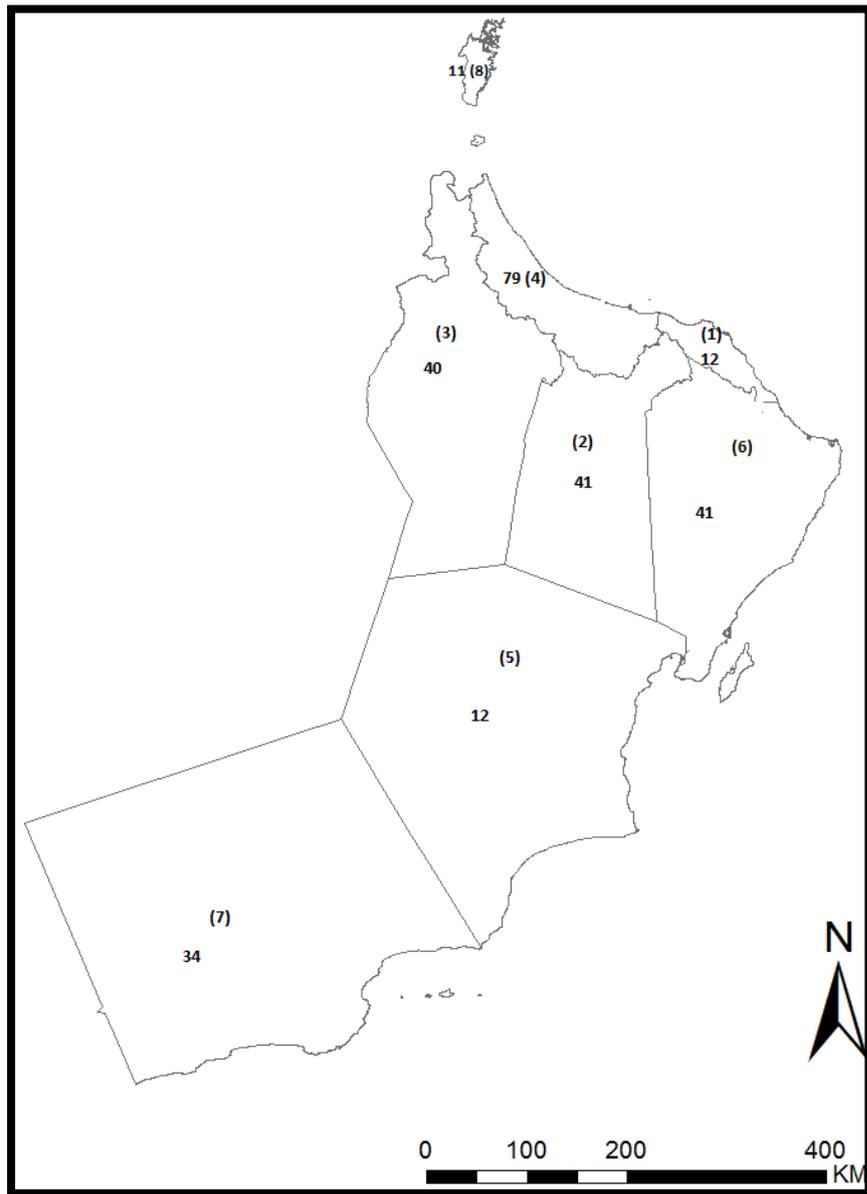
The desired number of flocks to be sampled was then stratified by region according to the percentage of poultry farms, percentage of poultry, percentage of people and percentage of backyard poultry present in each region (Table 2.1) and Figure 2.1 show the estimated flock numbers to be sampled from each region.

The local veterinarians and animal production engineers in each state helped in selecting the farms meeting the sampling criteria within their state territories.

**Table 2.1** Stratification of the total required 269 backyard farms calculated from Department of Rural Women Development estimation (10000 flocks) for each Oman region using 2004 data percentages.

The region	Poultry numbers and percentages from the total farms (%)	farm and from Oman	Poultry numbers and percentages (%) of the region from the total poultry Oman	People numbers and percentages of the region from the total Oman population	Percentage of backyard poultry from the total poultry birds in the region	Estimated Backyard numbers	Estimated Backyard percentages of the region backyard from the total Oman backyard	Used weight percentage for each region	The required Sampled farms
Al Batinah region	9222 (42%)		2616806 (15%)	772590 (28%)	16%	418689	40.30%	30	79
Adh Dhahirah region	4410 (20%)		725084 (4%)	224581 (9%)	16%	118189	11.40%	15	40
Muscat governorate	781 (4%)		480987 (3%)	775878 (28%)	8%	37998	3.70%	5	12
Ad Dakliyah region	787 (4%)		3685144 (22%)	315318 (11%)	8%	298497	28.80%	15	41
Ash Sharqiyah region	5713 (26%)		311689 (2%)	341788 (12%)	48%	148364	14.30%	15	40
Dhofar governorate	171 (1%)		9172359 (54%)	248660 (9%)	0	9172	.88%	12	34
Al Wusta region	115 (1%)		707 (0%)	50837 (2%)	100%	707	0.07%	4	12
Musandam governorate	484 (2%)		6273 (0%)	31425 (1%)	100%	6273	0.60%	4	11
<b>Total</b>	<b>21683</b>		<b>16999049</b>	<b>2761077</b>		<b>1037889</b>			<b>269</b>

**Figure 2.1** Estimated flock numbers to be sampled from each region, number between brackets are referring to the region names, 1= Muscat Governorate, 2 = Ad Dakhliyah, 3 = Adh Dhahirah, 4 = Al Batinah, 5 = Al Wusta, 6 = Ash Sharqiyah, 7 = Dhofar Governorate, 8 = Musandam Governorate



### **2.1.1. Sampling Criteria**

The main aim of this study was to perform a nation-wide survey for AIV and other viral respiratory diseases such as the NDV, IBV and aMPV viruses from backyard poultry in Oman.

A randomised sampling was done by selecting a number of backyard birds from the total population of Omani backyard flocks. A sampling frame work was done in order to reduce bias with more detailed inclusion and exclusion criteria.

#### **2.1.1.1. Criteria for inclusion**

The presence of backyard poultry, i.e. chickens that are not reared commercially as pure breed broilers, layers and breeders, and poultry of any other species (only chickens are reared commercially in Oman).

- Apparently healthy birds were sampled to avoid bias, as most farmers would prefer to test their sick birds, and hide the healthy ones. To avoid bias , our sampling concentrated on healthy birds only
- Adult birds (>~3 months old) were chosen to avoid targeting younger birds since they are easier to catch, and also sampling adult birds allowed about 2 mls of blood to be taken, which was needed for a number of serological assays. The bleeding process and volume of blood that can be collected from young chicks is very limited, and sometimes chicks may die.

#### **2.1.1.2. Criteria for exclusion:**

Commercial poultry breeds.

Game birds, not normally used for production

Show birds

Clinically ill birds

Exclusively young birds (< 3 months old)

If more than two farms were sampled from the same village.

If the farm is less than 1 km away from another sampled farm.

Where only one bird species was present, ten healthy adult (older than 3 months) birds were selected randomly and sampled, unless there were fewer than ten available, in which case all were sampled. A small number of premises had two or more species present. On some of these, only one species was made available by the owner and so just one species was sampled as before. On others, a maximum of two species were sampled (up to a maximum of 10 birds each). In my analysis, these are treated as separate flocks on the same farm. On no farms were three or more species sampled, even if they were present.

## **2.2. Sampling**

A total of 2350 birds were sampled from 243 flocks in 238 backyard farms. Figure 2.2 shows the distribution of these sampled backyard farms. Table 2.2 show the number of sampled birds from the different bird species.

### **2.2.1. Blood**

Blood (1-2 ml) was collected from the wing (brachial) vein of each bird using a new disposable syringe and needle (Figure 2.3. A); samples were transferred into anticoagulant-free transport tubes<sup>2</sup> labelled individually. The samples were transported in a cool box with ice. In the laboratory, serum was separated from blood samples and stored at -20 °C until tested by ELISA.

### **2.2.2. Oropharyngeal swabs**

Oropharyngeal samples were taken from each bird using sterile wooden swabs<sup>3</sup> for viral genomic identification (Figure 2.3. B). The swabs from all of the birds in each flock were wrapped together with tape and labelled. The samples were transported in a cool box with ice and cotton. The swabs of each flock/species were pooled in 1.5 ml distilled water (Figure 2.4. A) and then 80 - 100 µl of the mixture was inoculated onto the centre of sampling rings on an FTA card<sup>4</sup> using a sterile pipette and then left for one hour to dry at room temperature (22 °C), kept away from direct

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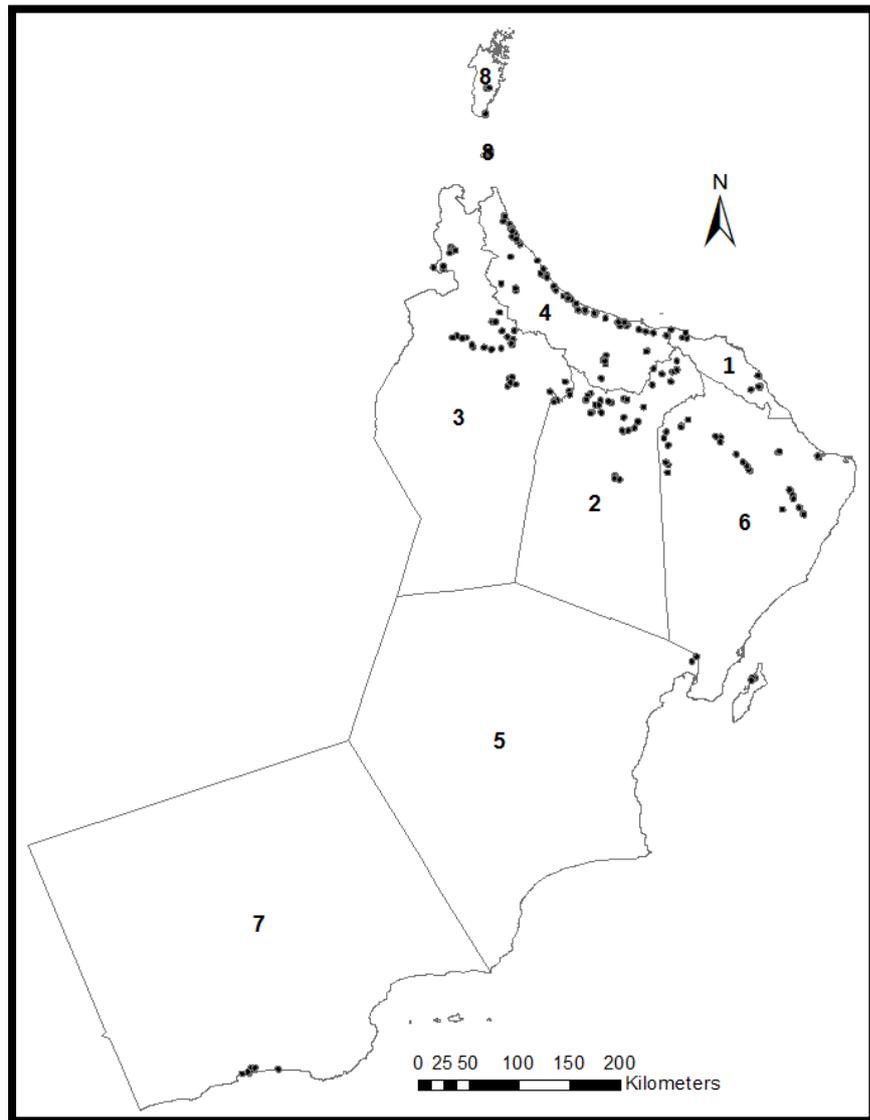
<sup>2</sup> Genomia, Czech Republic

<sup>3</sup> Medical wire and equipment Co. Ltd

<sup>4</sup> QIAGEN, UK

sunlight. Each FTA card circle was labelled individually (Figure 2.4. B). FTA cards were stored in a sealed plastic bag at 4 °C and then transported to the University of Liverpool, UK, for processing and analysis.

**Figure 2.2** Distribution of sampled farms from each region, numbers are referring to the region names, 1= Muscat Governorate, 2 = Ad Dakhliyah, 3 = Adh Dhahirah, 4 = Al Batinah, 5 = Al Wusta, 6 = Ash Sharqiyah, 7 = Dhofar Governorate, 8 = Musandam Governorate



**Table 2.2** The number of flocks sampled, the number and type of each poultry species sampled in the eight regions of Oman.

Region	Number of flocks	Total number of birds	Hens	Turkeys	Ducks	Geese	Guinea fowls
Al Batinah region	82	792	659	30	88	5	10
Adh Dhahirah region	47	461	451	10	0	0	0
Muscat governorate	13	129	110	9	10	0	0
Ad Dakliyah region	39	383	353	0	30	0	0
Ash Sharqiyah region	36	355	333	0	10	0	10
Dhofar governorate	7	70	70	0	0	0	0
Al Wusta region	8	72	72	0	0	0	0
Musandam	11	88	84	0	4	0	0
Total	243	2350	2132	49	142	5	20

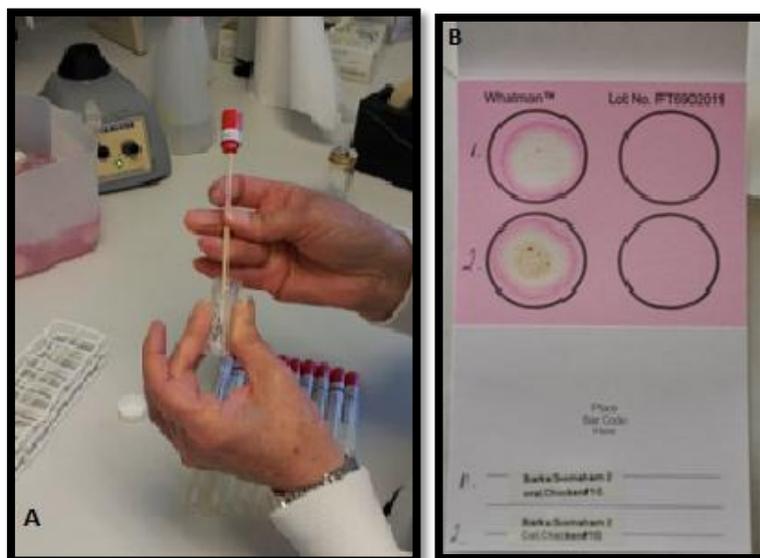
**Table 2.3** The number of birds sampled from each bird species from Oman backyard poultry

Bird species	Number of birds sampled
Chickens	2134
Ducks	142
Turkeys	49
Geese	5
Guinea fowls	20

**Figure 2.3** (A) Blood samples (1-2 ml) were collected from the wing (brachial) vein, (B) Oropharyngeal swab samples were taken using sterile wooden swab



**Figure 2.4** The swabs of each flock/species were pooled in 1.5 ml distilled water (A), then 80 -100  $\mu$ l is inoculated onto respective sampling circles on to the FTA card (B)



### 2.2.3. Data

Farm information like date of sampling, owner name, location of the farm (village, state, and region), type of birds in the farm, flock size, number and type of sampled birds, type of housing, and source of water were recorded for each farm (Appendix 1). The spatial coordinates of the location were recorded using a GPS<sup>5</sup> and the altitude of farms were obtained by feeding the GPS-recorded farm latitude and longitude into the Google Maps Elevation API (<https://developers.google.com/maps/documentation/elevation/>). Detailed farms data in Appendix 3.

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<sup>5</sup> Garmin GPS MAP 62s, USA

## 2.3. Serology

### 2.3.1. Detection of AIV and NDV antibodies

Antibodies to the nucleoprotein of AIV in chicken and turkey samples were detected by indirect enzyme-linked immune-sorbent assay (ELISA) using BioChek commercial kit<sup>6</sup>. Procedure recommended by the manufacturer was followed, including calculation of antibody titres. AIV antibodies in other species were examined using the IDEXX Ab multispecies ELISA kit<sup>7</sup>. Similarly, antibodies to NDV in chicken and turkey samples were detected using an indirect ELISA kit (BioChek). The duck and geese serum samples were not tested for NDV antibodies, as the kits were not validated for sera from these species.

For both AIV and NDV, all steps were carried out at room temperature and the ELISA plate was adapted to room temperature for around 30 minutes before use.

For BioChek ELISA kits, the sera were diluted 1:500 by adding 1 µl of sample to 0.5 ml of sample diluent and mixed well by vortexing. After the addition of 100 µl positive and negative control samples to the indicated wells of the ELISA plate, 100 µl of diluted sample was added to the appropriate well of the plate. The plate was incubated at 22 °C for 30 minutes and then washed 4 times with wash buffer at 350 µl /well/wash. After addition of 100 µl/well of conjugate reagent (Anti-chicken IgG labelled with the enzyme alkaline phosphatase), the plate was covered again and

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<sup>6</sup> Gouda, The Netherlands

<sup>7</sup> Hoofddorp, The Netherlands

incubated at room temperature for 30 minutes and then washed as in the previous step to remove unreacted conjugate. Next, 100 µl/well of substrate reagent was added and the plate was incubated for 15 minutes. Substrate development was stopped with stop solution (100 µl/well) (sodium hydroxide in diethanolamine buffer). The intensity of the yellow colour was directly related to the amount of antibody present in the sample. Optical density (ODs) was determined by measurement of absorbance at 405 nm with a microplate reader<sup>8</sup>. Based on the ODs the sample to positive (S/P) ratios were calculated and used to express the mean (S/P) ratio per group.

Samples with antibody levels above the thresholds defined by the kit manufacturer were classified as positive; all other samples were classified as negative.

### **2.3.2. WNV antibody detection**

Sample preparation for WNV antibody testing was modified from that described above, because of limited funds for the analysis and the expectation that the prevalence of WNV would be low. Instead of testing all individual samples, samples from the same flock were initially pooled and then tested. If the pool was found to be positive, individual samples were then tested; if the pool was negative, no further testing was undertaken.

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<sup>8</sup> Thermo Scientific, Multiskan FC, Finland

### 2.3.2.1. Sample preparation

Each flock's samples were pooled (10 µl of serum from each bird) in an Eppendorf tube<sup>9</sup> and tested as a single sample. If the pool was positive, 3 to 5 birds' serum samples were randomly selected from the respective flock and individually tested.

### 2.3.2.2. ELISA

Fifty µl of the serum were diluted with a similar amount of diluent. Using the ID Screen West Nile Competition Multi-species Elisa kit<sup>10</sup> the serum incubated for 90 minutes at 22 °C. This step was followed by washing of the plates and addition of 100 µl/well conjugate and incubated at 22 °C for 30 minutes. Next, the washing step was repeated, followed by addition of 100 µl/well of the substrate for 15 minutes in the dark at 22 °C. Substrate development was stopped with stop solution (100 µl/well) (sodium hydroxide in diethanolamine buffer). The intensity of the colour was related to the amount of antibody present in the sample. Optical density (ODs) was determined by measurement of absorbance at 450 nm with a microplate reader. Based on the ODs the sample to negative (S/N) ratios were calculated and used to express the mean (S/N) ratio per group.

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<sup>9</sup> Elkay laboratory products Ltd UK

<sup>10</sup> ID.vet Innovative Diagnostics, France

## **2.4. Molecular Testing**

### **2.4.1. RNA extraction from FTA cards**

RNA extraction was performed using a Qiagen, QIAamp Viral RNA Mini Kit<sup>11</sup> according to the manufacturer's instructions.

#### 2.4.1.1. Elution of RNA from FTA cards

One circle from each FTA card was cut out using sterile scissors and forceps and placed in a bijou containing 800 - 1000 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), vortexed and incubated at room temperature for 10 min. The supernatant was used to extract RNA.

#### 2.4.1.2. RNA Extraction

One hundred and forty µl of supernatant was added to 560 µl of a viral lysis buffer (AVL) plus carrier RNA in an Eppendorf, vortexed and incubated at room temperature (22 °C) for 10 min. 560 µl of 100% ethanol was added, vortexed and pulse centrifuged. Then 630 µl of sample was transferred to a spin column, centrifuged at 8000 rpm for 1 minutes and the flow through discarded. This was repeated once more for the remaining sample. The column was then washed with a wash buffer 1 (AW1), a strong protein denaturant and centrifuged at 13,000 rpm for 1 min, followed by a final wash with wash buffer 2 (AW2) at 13,000 rpm for 3 min,

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<sup>11</sup> Qiagen Ltd, Germany

discarding the flow through and a final spin at 13,000 rpm for 1 min. Finally the viral RNA was eluted from the membrane with RNase free water and stored at -20 °C until required (detailed protocol in Appendix 2).

#### **2.4.2. Reverse transcriptase polymerase chain reaction (RT-PCR)**

RT-PCR was performed on the extracted RNA for the detection of NP and F genes of AIV and NDV, respectively using both primers and cycle conditions as previously published (Aldous et al., 2003; Wei et al., 2006). Published nested PCR protocols were used for the detection of partial S1 and G genes of both IBV and aMPV (Cavanagh et al., 1999). The PCR primer oligonucleotides used for the four viruses are shown in Table 2.4.

**Table 2.4** Reverse transcription polymerase chain reaction (RT-PCR) oligonucleotides

Virus		Oligo	Sequence (5' to 3')	Gene	Product size (bp)	Position in sequences			
AIV	Single step	NP – F	AGRTACTGGGCHATAAGRAC	NP	-	-			
	PCR	NP – R	ATTGTCTCCGAAGAAATAAG						
NDV	Single step	MSF-1R	GACCGCTGACCACGAGGTTA	F	18	-			
	PCR	MSF-2F	AGTCGGAGGATGTTGGCAGC		2	-			
IBV	PCR 1	SX1+	CACCTAGAGGTTTG T/C T A/T GCAT	S1	393	677-698			
		SX2-	TCCACCTCTATAAACACC C/T TT			1148 -1168			
	Nested PCR 2	SX3+	TAATACTGG C/T AATTTTTTCAGA			705 - 725			
		SX4-	AATACAGATTGCTTACAACCA CC			1075-1097			
	aMPV	PCR 1	G6-			CTGACAAATTGGTCCTGATT	G		422-441
			G1+			GGGACAAGTATC T/C C/A T/G AT			1- 17
Nested PCR 2		G5-	CAAAGAAGCCAATAAGCCCA	401 to 419					
		G8+A	CACTCACTGTTAGCGTCATA	268	152-171				
		G9+B	TAGTCCTCAAGCAAGTCCTC		361	68 to 87			

#### 2.4.2.1. Reverse transcription (RT) reaction

The RT reaction mixture included superscript II RT and one of the outer (negative) oligonucleotides (Appendix 2). The mixtures were then taken to a separate workstation where 5 µl of the mixture was placed into a 0.5 ml pre labelled clip top Eppendorf tube<sup>12</sup>, after which 2 drops of mineral oil<sup>13</sup> were added. To this, 0.5 µl of RNA was placed under the oil. Positive and negative controls were included in each run. This was thoroughly mixed by vortexing and then centrifuged for 10 seconds at 5000 rpm. The tubes were placed in a thermocycler<sup>14</sup> and run under the following conditions: 42<sup>0</sup> C for 1 hour, 72 °C for 10 minutes and then held indefinitely at 8 °C. Immediately after the RT mixture, the following was performed:

#### 2.4.2.2. Nested PCR 1

For all four viruses, the total volume mixture for PCR 1 reaction was 20 µl. Sufficient PCR reaction mixture was made for all the samples including a positive and negative control for a particular run in a 1.5 ml clip top Eppendorf tube (Appendix 2). The PCR reaction mixture was thoroughly vortexed before dispensing 20 µl aliquots beneath the oil layer in each tube containing the RT-PCR product. The tubes were placed in a thermo-cycler and run under the following conditions: 94 °C for 15 seconds followed by 35 cycles of 94 °C for 10 seconds, 50 °C for 20 seconds, and 72 °C for 40 seconds and then held indefinitely at 8 °C.

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<sup>12</sup> Elkay Laboratory Products UK (Ltd), Basingstoke, Hampshire, UK

<sup>13</sup> Sigma-Aldrich, UK

<sup>14</sup> GeneAmp PCR system 9700

**3- Chapter Three: Systematic research on the  
epidemiology of avian influenza and other viral  
respiratory diseases of poultry in the Middle East**

### **3.1. Abstract**

Avian influenza and other respiratory diseases such as Newcastle disease, avian infectious bronchitis, avian metapneumovirus and infectious laryngotracheitis are major respiratory viral infections of poultry. In the Middle East, the numbers of highly pathogenic avian influenza outbreaks has risen sharply in the last two decades. Newcastle disease remains endemic with continuous outbreaks, new and harmful variant infectious bronchitis viruses circulate thorough out the region, avian metapneumovirus is widespread and infectious laryngotracheitis is detected in many production flocks. Despite the heavy presence of viral respiratory diseases, there is a lack of comprehensive knowledge on the epidemiology of these diseases in the context of Middle East.

A systematic search review on the epidemiology of the viral respiratory diseases in the different Middle East countries was undertaken Information for the review came from published articles that were found in the Liverpool University electronic library and genomic sequences of respiratory viruses that were uploaded onto Genbank using the Emerging Infectious diseases database (EID2, University of Liverpool). Abstracts were used to categorize the articles to avian and non-avian studies.

The search for scientific papers found 283 relevant articles, most of them focussed on avian influenza (192/283, 67.8%) particularly HPAI H5N1 (120/192, 62.5%). More than 56% of the LPAI articles were from just two countries Iran and Israel. ND signs were reported in most of the ME countries in the last 8 years. Very few studies were found on economically important viral respiratory diseases such as ILT, IB and aMPV infection (2.1%, 8.1%, and 2.5% respectively). The EID2 database found

2741 sequencing from bird species from all the ME countries, most of them were for influenza A viruses (2183/2741, 79.6%) done in Iran (1377/2183, 63%), and Jordan (468/2183, 21.4%). Since the beginning of the HPAI H5N1 outbreak in 2003, ten Middle East countries have been affected by the disease. All of the affected countries eradicated the disease except Egypt where the disease spread rapidly and widely. Low pathogenic H9N2 has been circulating in the ME for more than two decades. There is a paucity of information about the IBV, aMPV and ILT in the ME.

While the continuous circulation of H5N1 poses the biggest challenge to the Middle East poultry, the region is still suffering with huge losses due to arrival of genotype VII of NDV, the emergence of many variant IBVs and co-infection with aMPV and ILT. A proper epidemiological and/or surveillance study in each ME country would assist in identification of risk factors that could be used for a better disease control strategies.

### **3.2. Introduction:**

Viral respiratory diseases of poultry, such as avian influenza (AI), Newcastle disease (ND), infectious bronchitis (IB), infectious laryngotracheitis (ILT) and avian metapneumovirus (aMPV) infection, are major constraints on global poultry production (Jones, 2010). The high mortality in poultry caused by highly pathogenic AI (HPAI) and the virulent ND virus, together with effort to contain their outbreaks in the affected areas, usually lead to tremendous economic losses making them the most economically-important poultry diseases worldwide (Williams and Peterson, 2009). Furthermore, while low pathogenic avian influenza (LPAI) strains cause fewer losses compared to HPAI strains, they may complicate the poultry health situation by facilitating co-infection in poultry flocks with other respiratory diseases, such as IB and even other highly pathogenic avian influenza viruses such as H5N1 (Swayne and King, 2003; Capua and Alexander, 2004).

Although IB, ILT and aMPV diseases cause less mortality than AI and ND, nevertheless they greatly reduce the productivity of the surviving birds (Jones, 2010). In particular, IB virus (IBV), the causative agent of IB, is endemic in almost all countries in the world and more importantly new variant IBVs are emerging constantly including those not efficiently controlled by available vaccine genotypes. Moreover, a number of genotypes have recently been isolated which cause severe kidney lesions (Jones, 2010; Feng et al., 2012). ILT infection has been reported world-wide with varying degree of severity of lesions. Occasionally intermittent unexpected outbreaks of ILT in intensive poultry industry happen due to differences in control practices (Bagust et al., 2000). aMPV with its 4 subtypes is widely

distributed, and its control is solely dependent on live attenuated vaccines (Jones, 2010).

Some strains of AI viruses, notably those with haemagglutinin surface antigens H5, H7 and H9, (Trampuz et al., 2004) viruses are very important zoonotic pathogens. In the last two decades the number of zoonotic AI outbreaks has increased drastically. The ability of the circulating virus H5N1 to infect humans and cause high mortality leads to fears of it acquiring the ability of human to human transmission and causing a new pandemic (Kelly et al., 2008; Lee and Saif, 2009; Williams and Peterson, 2009).

### **3.3. Research justifications**

The Middle East (ME) is located in the central area between the three old world continents, Asia, Africa and Europe. This makes it a central area connected to, and affected by, the diseases spread in those three continents. The presence of important bird migration routes (Black Sea/Mediterranean, East Asia/East Africa, and Central Asia) over the Middle East countries (Gerloff et al., 2013), and the extensive trade in live birds within the Middle East put this region at high-risk of entry of avian viral respiratory diseases.

Also, fast expanding poultry farming in this region has allowed importation of fertile eggs and young chicks from all over the world, creating an ideal ‘melting- pot’ for infectious pathogens. Despite this significance, the numbers of published articles on viral respiratory diseases in the region are low, and this chapter appears to be the first review on avian respiratory viral pathogens focussing on the Middle East.

In this chapter, a systematic research on peer-reviewed publications and other information related to the epidemiology of important avian respiratory pathogens in the Middle East was undertaken. The main aim was highlight the potential knowledge gaps in the Middle East in comparison to other parts of the world. The main focus was on AI, particularly; the HPAI H5N1, as much published information available on this disease/pathogen, followed by other viral infection such as NDV, IBV, ILT and aMPV.

### **3.4. Literature searches**

I investigated the availability of two sources of scientific information: published scientific papers and gene sequences uploaded to public databases. I accessed the University of Liverpool Electronic library which searches over 500 databases in all subjects; particularly relevant to this study are Scopus, Web of Science, Science Direct, PubMed and Medline. Scopus covers articles published since 1823 and Web of Science covers the articles published since 1898. I used the search terms such as birds, respiratory diseases, viral and Middle East and then I repeated the search, replacing *Middle East* with the name of each Middle East country in turn (Oman, Yemen, United Arab Emirates Saudi Arabia, Qatar, Bahrain, Kuwait, Iraq, Iran, Turkey, Syria, Lebanon, Jordan, Israel, Egypt and West bank and Gaza) and for each disease and country. I read the abstracts of the found articles to examine their relevance to the study. I accessed gene sequence data from the Emerging Infectious diseases database (EID2, University of Liverpool). EID2 uses the metadata from millions of gene sequences uploaded to Genbank, and provides spatially-referenced information on the occurrence of pathogens. My systematic searches did not find all published scientific papers in viral respiratory diseases in the ME; for example, articles published in local journals or the journals outside Liverpool University search engines are not covered in this study and the discussion of epidemiology excluded the papers not covering this topic.

### **3.4.1. Results for the literature searches**

The search for scientific papers found 283 relevant articles (Table 3.1), nearly all of them published in the last 15 years. Most of them focussed on avian influenza (192/283, 67.8%) particularly HPAI H5N1 (120/192, 62.5%).

Due to the extensive circulation of H5N1 in both humans and birds in Egypt and the detection of some LPAI genotypes such as H7 and H9, it was not surprising that the majority of these AI studies were done in Egypt (90/192, 46.9%). Despite the circulation of the LPAI H9N2 in the region for around two decades, there is lack of knowledge about the true prevalence in most of the ME countries. More than 56% of the LPAI articles were from just two countries i.e. Iran and Israel. Despite the endemic status of ND in ME countries, where more than 6000 outbreaks were reported to the OIE from the ME region countries during the period 2012-2014 (Anon, 2015a), and the continued notification of the disease to the World Organisation of Animal Health (OIE) in the last 8 years (Anon, 2014a), only 52 studies on ND were found, most of them in Egypt and Israel. Very few studies were found on other economically important endemic viral respiratory diseases such as ILT, IB and aMPV infection (2.1%, 8.1%, and 2.5% respectively).

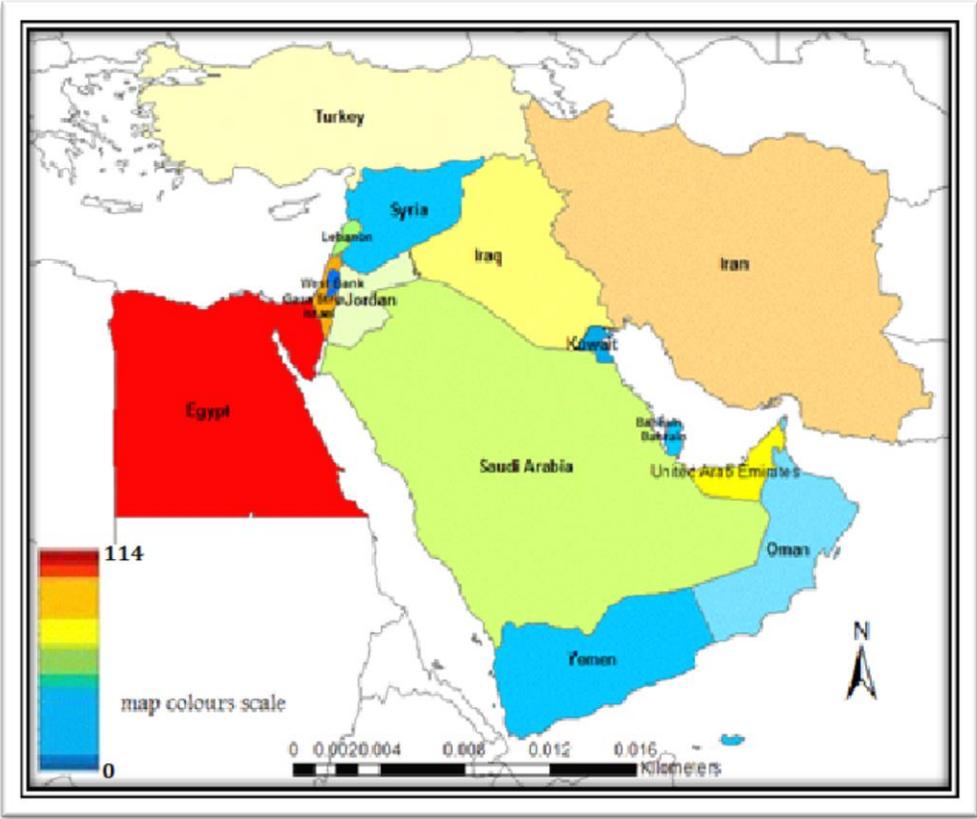
There were no published articles from Bahrain or Gaza and West Bank on any of the searched diseases. Moreover, only one or two articles was found from each of Oman, Qatar, Yemen, Syria, and Kuwait.

Figure 3.1 (A & B) shows the number of scientific articles and genomic sequencings on the above mentioned diseases from different Middle East countries, Figure 3.2

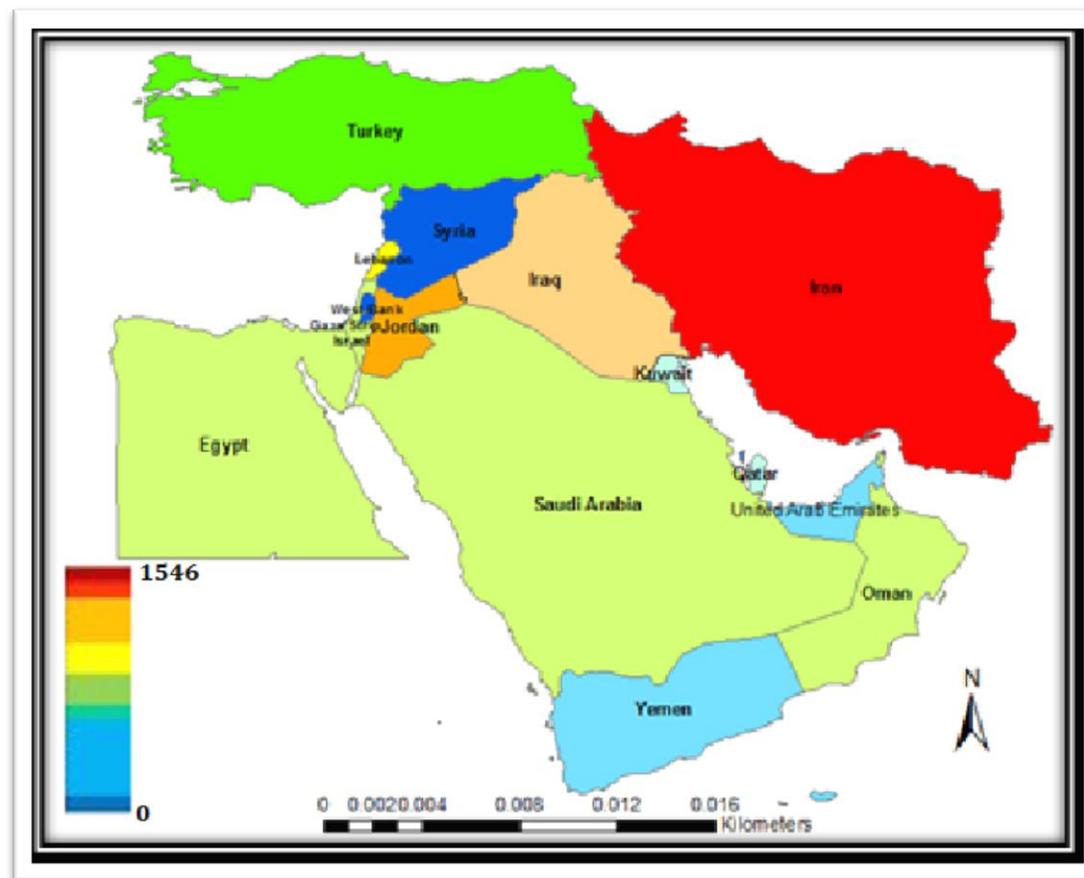
plots the number of genomic sequences *versus* the number of published articles from Middle East countries.

The EID2 database found 2741 sequencing from avian species from all the ME countries. Most of the genomic sequences were for influenza A viruses (AIV) (2183/2741, 79.6%). The majority of the AIV genomic work was done in Iran (1377/2183, 63%), and Jordan (468/2183, 21.4%). NDV genomic sequences were mainly found from Iraq (115/278, 41.4%). Despite the importance of genomic work for identification of the circulating IBV genotypes, fewer than 270 genomic sequences were found from all the ME countries. Most of these IBV sequences were submitted from Iran and Iraq (188/263, 71.5%). Ten genomic sequences were submitted for aMPV just from two countries, Israel and Iran. Seven genomic sequences were found for ILT from all the Middle East countries. Table 3.2 show the number of gene sequencings from each ME countries found in EID2 data base.

With the exception of Egypt, Israel and Iran, it is clear that there is a gap of knowledge of avian influenza and Newcastle disease in most of the ME countries. There is little published information on ILT, IB and aMPV infection in any ME countries. Generally Iran (1546/2741, 56.4%), and Jordan (535/2741, 19.5%) are the most active countries in genomic sequencing.



**Figure 3.1 (A)** Middle Eastern countries coloured according to the total number of poultry viral disease articles published from each country



**Figure 3.1 (B)** Middle Eastern countries coloured according to the total number of poultry viral genomic sequences uploaded into GenBank

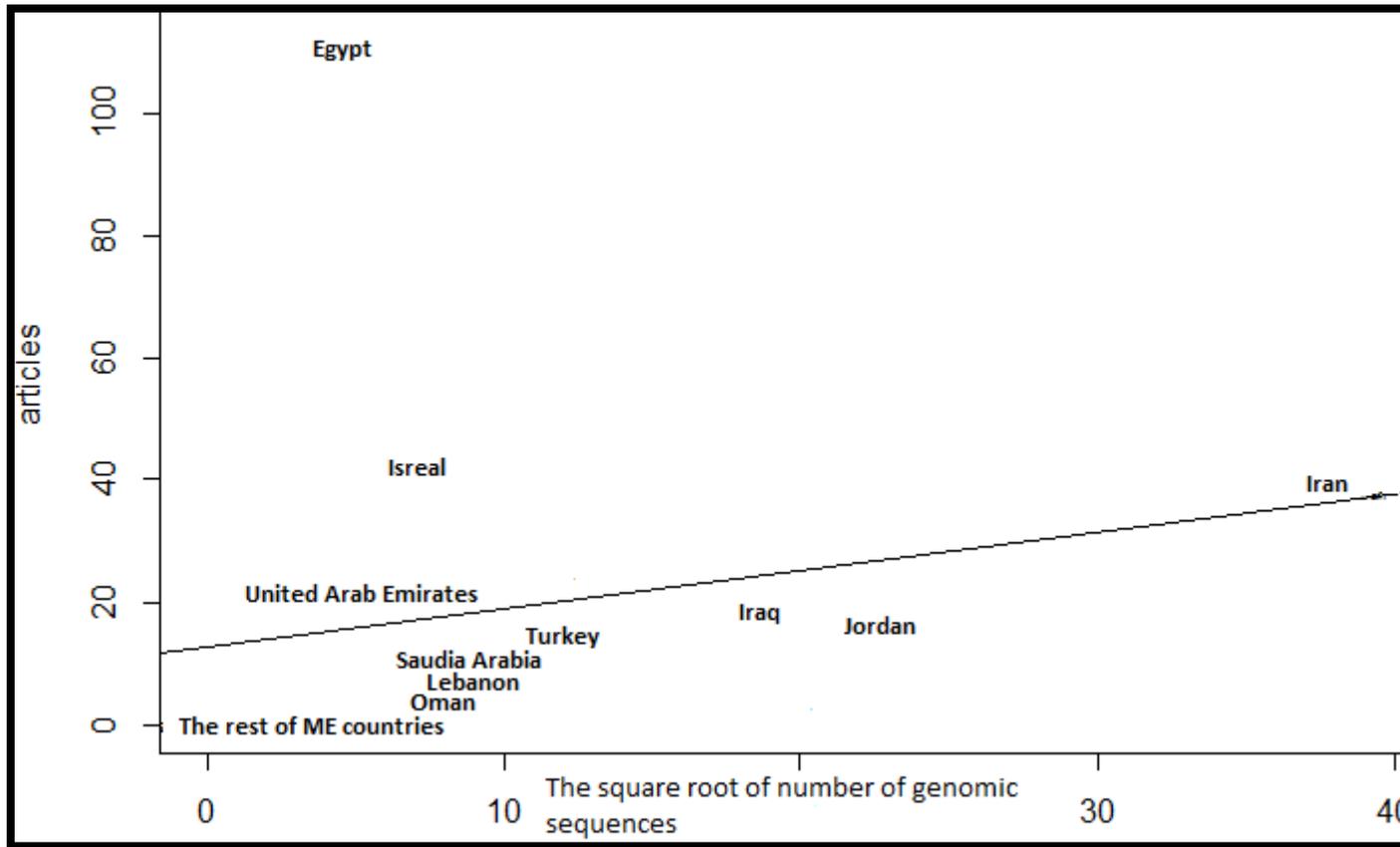
**Table 3.1** Published papers on poultry viral diseases from Middle Eastern countries until January 2015.

The country	H5N1	LPAI	Total AI	ND	IB	aMPV	ILT	Total
Turkey	10	2	12	2	0	0	1	15
Egypt	82	8	90	14	6	1	3	114
UAE	8	5	13	6	1	1	0	21
Kuwait	1	0	1	0	0	0	0	1
Saudi Arabia	5	0	5	3	2	0	0	10
Bahrain	0	0	0	0	0	0	0	0
Iraq	1	4	5	7	5	0	0	17
Yemen	1	0	1	0	0	0	0	1
Qatar	0	0	0	1	0	0	0	1
Oman	0	0	0	0	1	1	0	2
Iran	0	30	30	6	2	0	0	38
Jordan	0	5	5	2	4	2	0	13
Lebanon	0	6	6	0	0	0	1	7
Israel	12	11	23	11	5	2	1	42
Syria	0	1	1	0	0	0	0	1
Gaza and West Bank	0	0	0	0	0	0	0	0
Total	120	72	192	52	26	7	6	283

**Table 3.2** Number of gene sequencings from each ME countries found in EID2 data base.

The country\The pathogen	Influenza A viruses	ND	IB	aMPV	ILT	Total
Turkey	55	2	2	0	0	59
Egypt	9	25	0	0	0	34
United Arab Emirates	3	1	0	0	0	4
Kuwait	1	0	0	0	0	1
Saudi Arabia	0	1	34	0	0	35
Bahrain	0	0	0	0	0	0
Iraq	200	115	81	0	0	396
Yemen	0	0	3	0	0	3
Qatar	1	0	0	0	0	1
Oman	0	0	31	0	0	31
Iran	1377	52	107	4	6	1546
Jordan	468	67	0	0	0	535
Lebanon	65	1	0	0	0	66
Israel	4	14	5	6	1	30
Syria	0	0	0	0	0	0
Gaza and West Bank	0	0	0	0	0	0
Total	2183	278	263	10	7	2741

**Figure 3.2** The square root of number of genomic sequences vs the number published articles from Middle Eastern countries



### **3.5. Avian influenza (AI) in The Middle East**

AI is a disease complex caused by influenzavirus type A (family *Orthomyxoviridae*) infection in birds. This disease is reviewed in detail in Chapter 1.

#### **3.5.1. Epidemiology of HPAI H5N1**

Since the beginning of the HPAI H5N1 outbreak in 2003 in South East Asia, ten Middle East countries have been affected (Anon, 2013b). Turkey was the first affected country in the region, notifying the H5N1 HPAI outbreak in a backyard turkey flock in October 2005, followed by Kuwait which detected the virus in a single migratory flamingo one month later. Throughout the period from the second half of December 2005 until April 2006 there were frequent reports of new backyard and wild bird foci in the eastern provinces of Turkey. Poultry culling was used to prevent disease spread into Iraqi Kurdistan and Iran (Neumann et al., 2012; Anon, 2013b). Nevertheless, in January 2006 the virus was isolated from a cat in northern Iraq and shortly after the first human case in the Middle East was diagnosed in that country (Anon., 2012). The virus was detected in Iran for the first time during February 2006, in a dead swan (Anon., 2012). In December 2005 the RNA of the H5N1 virus was detected in a common teal (*Anas crecca*) captured in the Nile Delta region of Damietta in Egypt (Gerloff et al., 2013). This was followed by Egypt notifying its first H5N1 outbreak in its domestic poultry on the 17<sup>th</sup> Feb 2006 (Kandeel et al., 2010). A series of outbreaks were reported from the West Bank and Gaza Strip from the last week of February 2006 through the following two months. In this period Israel and Jordan also detected the virus in their poultry flocks (Brown, 2010; Anon., 2012).

By spring 2006 eight Middle East countries (Turkey, Egypt, Kuwait, Israel, West Bank and Gaza, Iran, Iraq and Jordan) had reported the disease in their territories (Anon., 2012; Neumann et al., 2012). The following year, Turkey reported H5N1 in a backyard poultry flock in February, followed by the first detection of H5N1 in the poultry of the Arabian Gulf (backyard poultry farm and in a zoo) in Kuwait (Anon., 2012). Saudi Arabia reported the disease in March 2007 in two "backyard" flocks of Houbara bustards (*Chlamydotis undulata*) and falcons (*Falco peregrinus*) (Lu et al., 2010). This was followed by a wave of outbreaks in four poultry farms near the capital Ar Riyad from October 2007 to January 2008, affecting nearly 5 million birds of different species and breeding systems (Williams and Peterson, 2009; Brown, 2010; Lu et al., 2010; Anon., 2012). In the beginning of 2008 there was a new outbreak in Israel affecting petting zoo birds in Haifa (Anon., 2012).

Egypt reported 579 outbreaks in the first year of infection alone and by the middle of January 2008 the disease had spread to the commercial and backyard poultry of 17 governorates (Kandeel et al., 2010; Anon., 2012). On the 7th of July 2008 the Egyptian veterinary service declared the country endemic for H5N1.

In January 2008, after around one year of the absence of the disease in Iran and Turkey, there were two reports from backyard poultry flocks (Anon., 2012). The disease reappeared in a new province (Sinop) in Anatolian Turkey in February 2008 and in Edirne, in European Turkey (Anon., 2012), a month later. The virus detected from most of the Middle East countries revealed high molecular similarity to each other (Shimon et al., 2010).

In most Middle East outbreaks migratory wild birds were the suspected source of the virus introduction; however it was clearly not always the case, such as in the first Saudi H5N1 outbreak detected in Houbara bustards and falcons (Monne et al., 2008).

H5N1 clade 2.2 was the genotype introduced in all Middle East countries (Yingst et al., 2006; Shimon et al., 2010; Neumann et al., 2012). However, in Egypt this clade was quickly replaced with clade 2.2.1 which further diversified to two main groups, the first one circulating in human and backyard poultry and the other group circulating in production poultry farms (Abdelwhab and Hafez, 2011). The forces driving this diversification may be adaptation to a new host and co-circulation with other human influenza viruses for the first group; and vaccination and maternal immunity for the second group (Abdelwhab and Hafez, 2011; Cattoli et al., 2011; Ibrahim et al., 2011)

### **3.5.2. Control of H5N1**

Despite re-incursions of the virus, control measures applied in affected countries, such as culling of birds in infected zones, strengthened biosecurity in poultry farms, quarantine (Lu et al., 2010; Rahman et al., 2013) and emergency vaccination succeeded in eradicating H5N1 from all countries in the region except Egypt, where the disease spread rapidly and widely (Peyre et al., 2009; Abdelwhab and Hafez, 2011). The failure in the Egyptian case was due to widespread backyard poultry breeding in close proximity to production farms and in the very crowded areas of the Nile delta (Abdelwhab and Hafez, 2011; Cattoli et al., 2011; Grund et al., 2011). The Egyptian veterinary authority was enforced to amend its control measures to mass vaccination, heightened awareness of biosecurity, increased surveillance and

culling of infected birds to decrease the viral load in the environment, and reduce the risk of virus spreading to both humans and birds (Peyre et al., 2009; Abdelwhab and Hafez, 2011). Both poultry production sectors and zoo birds were included in the vaccination campaign (Swayne et al., 2011). Despite the application of mass vaccination for production and backyard poultry, the virus was continuously detected from those vaccinated birds with higher intensity from the backyard in comparison to the production poultry (Hafez et al., 2010).

Egypt was not the only country using H5 vaccine in the Middle East; Israel, Kuwait and United Arab Emirates used the vaccine but used only in Ostrich farms and for zoo birds.

Despite the extensive use of vaccination in all types of birds in Egypt, H5N1 remained endemic, perhaps because of viral evolution. Antigenic drift to evade the immune system and/or to adapt to a new host, antigenic recombination and reassortment are the main forces driving virus evolution (Kim et al., 2010). In addition, the extensive circulation of the virus within and between species helps promote diversification of the circulating viruses (Abdelwhab and Hafez, 2011). Therefore, updated vaccines used in each geographical area and poultry sector are necessary to improve the vaccination campaign (Abdelwhab and Hafez, 2011).

### 3.5.3. Other avian influenza viruses

Low pathogenic avian influenza H9N2, the most probable candidate for a new pandemic strain in humans (Fusaro et al., 2011), has been the most widely circulating AI virus strain in the Middle East for nearly two decades (Monne et al., 2007; Homayounimehr et al., 2010; Fusaro et al., 2011). This virus can infect humans and pigs and sometimes it shares high similarity with highly pathogenic subtypes such as H7N3 (Fusaro et al., 2011; Bashashati et al., 2013) and H7N9 (Wang et al., 2014). Scientists believe that H9N2 could transmit its ability to cross the species barrier to other subtypes, as recently happened in H7N9 and H10N8 human outbreaks in China (Wang et al., 2014).

The earliest incursions of H9N2 in poultry in the Middle East are believed to have originated from Far East countries (G1 like and Y280 like viruses) except for a few Iranian isolates (Monne et al., 2007; Pazani et al., 2011; Arafa et al., 2012b). Antigenic drifts and shifts then diversified the circulating clades in the Middle East. Even LPAI virus can cause considerable losses to poultry producers, especially when it co-infects with other pathogens like mycoplasma, *Escherichia coli*, and *Ornithobacterium rhinotracheale* (Toroghi and Momayez, 2006) IBV and H5N1, increasing mortality and complicating their control programmes (Nili and Asasi, 2003; Homayounimehr et al., 2010; Seifi et al., 2010; Fusaro et al., 2011; Pazani et al., 2011; Arafa et al., 2012b). Also, the immune-suppression effect of infectious bursal disease virus increase the pathogenicity of H9N2 in the affected flocks (Motamed et al., 2013).

The genetic evolution of the virus in each region may have the biggest effect in the diversity of this pathogen rather than mixing with new incursions of the virus (Fusaro et al., 2011; Bashashati et al., 2013). Possibly due to the limited number of

H9N2 isolates uploaded from some Middle East countries to the Genbank, and low number of published papers from some Middle East countries the true status of these groups of viruses is still not clear (Fusaro et al., 2011). Since the first detection of the H9N2 subtype in the Middle East from Saudi Arabia and Iran in 1998 (Alexander, 2003) the virus has spread and been identified in most countries in the region (Alexander, 2003; Monne et al., 2007; Butt et al., 2010; Watanabe et al., 2011). Four distinct groups of H9N2 (A-D) are circulating in the Middle East, however group C and D are more restricted to Iran and the United Arab Emirates (Fusaro et al., 2011; Abdel-Moneim et al., 2012).

Studies of antibody responses against LPAI in poultry farm workers in Lebanon found evidence of exposure of workers to low pathogenic subtypes, such as H4 and H11. It seems likely that those two viruses were acquired from the poultry and silent outbreaks of those viruses were circulating in some of the Lebanese farms (Kayali et al., 2011a).

Studies of AI viruses in wild birds in the Egyptian delta region found a prevalence of 9.5 % of 15 different subtypes, mostly related to Eurasian viruses circulating in the wild birds (Gerloff et al., 2013).

In the period of 2000-2001 H1N7 and H6N3 were isolated from Israeli poultry farms (Perk et al., 2006). No other Middle East country has reported these viruses. Prior to this only H9N2 was detected in this country.

The H7N9 subtype, which was detected in April 2013 in China and Malaysia causes serious illness in humans, has not at present been reported from any country in the Middle East (Anon, 2013b).

#### **3.5.4. Future of HPAI H5N1 and LPAI**

For a variety of reasons, it seems there is no light at the end of the tunnel for the H5N1 outbreak in Egypt, at least for the near future. The main reasons for this pessimism are (i) the virus is still circulating in humans, poultry and wild birds despite mass vaccination in Egypt (Peyre et al., 2009); (ii) most risk factors for AI transmission and persistence remain present in Egypt (Neumann et al., 2012); (iii) the co-circulation of H9N2 and H7 viruses hinders the control efforts and gives the possibility of viral re-assortment and the generation of a new virus (Monne et al., 2008); (iv) both clade 1 and 2 have shown a tendency, though mutation, to acquire the ability to bind human-like receptors when they are passaged for several times in the ferrets (Herfst et al., 2012; Imai et al., 2012) and (v) the virus is widely spread in the highly populated Nile delta. (vi) furthermore, in Egypt the human circulating clade lacked the HA154–156 glycosylation sites, which means it has a higher ability to infect humans (Neumann et al., 2012).

Furthermore the wide circulation of the virus (Abdelwhab et al., 2010) increases the chance of the spill over of the Egyptian H5N1 virus to migratory wild birds, which may induce a new wave of H5N1 outbreaks in the Middle East or even could extend it to other parts of the world.

To eradicate AI from Egypt, particularly H5N1, efforts should not rely on vaccination alone. Current vaccines and vaccination strategies reduce mortality and morbidity and reduce virus shedding, but do not stop the infection and the viral excretion and do not give protection against the recent viruses (Rahman et al., 2013). Enforcement of biosecurity, education of the public, culling of the birds in the infected farm zone and monitoring of the evolution of infection should work alongside other efforts (Capua and Marangon, 2006). It has been clearly learned

from the H7N9 and H5N1 outbreaks that control of H9N2, thereby decreasing its circulation in birds and swine, will decrease the chance of assortment with highly pathogenic subtypes such as H5 and H7, which otherwise may produce a new lineage capable of infecting and killing humans (Wang et al., 2014). Control efforts in Egypt should therefore address all AI strains, and not just H5N1.

Other ME countries should pay more attention to the epidemiology and control of H9N2 in particular and other LPAIs.

## **3.6. Epidemiology of other poultry respiratory viruses in the Middle East**

### **3.6.1. Newcastle disease**

Newcastle disease is a highly contagious respiratory disease caused by an enveloped negative-sense, single stranded RNA virus called avian *paramyxovirus* type 1 (APMV 1) (Alexander, 2000; Mase et al., 2002; Madadgar et al., 2013; Samuel et al., 2013). This disease is reviewed in detail in Chapter 1.

#### **3.6.1.1. Epidemiology in the Middle East countries.**

Historically, the Middle East was the origin of the second and third ND panzootics in the late 1960s and late 1970s respectively (Mase et al., 2002). The genotypes V and VI were the main genotypes involving in the 2<sup>nd</sup> panzootic. The pigeon paramyxovirus type 1 virus has been widely found in the Middle East countries from the late 1970s (Weisman et al., 1984; Wernery et al., 1992; Semeka et al., 2013b). This was followed by the circulation of genotype VII from the early 1980s and the genotypes VIIb and VIIc caused outbreaks in the Middle East in the mid-1990s (Yu et al., 2001). Recently, the virulent lineage Vd, the most prominent genotype in Africa and China, was detected in Jordan and Israel (Ababneh et al., 2012b). Clinical signs of the disease have been reported in most Middle Eastern countries in the last 8 years (Anon, 2014a) and more than 6000 NDV outbreaks (most of them from Iran) were reported to the OIE during the period 2012-2014 (Anon, 2015a).

Mohamed et.al (2009) and Ababneh et.al (2012) found a close relation between the NDV genome of the Egyptian and Jordanian isolates and that which circulates in China. This could be due to involvement of migratory birds between the Middle

East and the Far East regions (Ababneh et al., 2012b). Likewise, wider circulation of those genotypes could explain the similarity in available studied genes between the Egyptian isolate and some Malian viruses (Mohamed et al., 2009), and in Iranian genotypes and genotypes circulating further east in India and Pakistan (Shabbir et al., 2013).

Genotype VI was reported in several Middle Eastern countries, such as Iraq, Israel, Lebanon, and Saudi Arabia (Aldous et al., 2003).

The importation of live birds, as well as fertile hatching eggs, plays an important role in the epidemiology of NDV. This is particularly the case where the art of falconry has great popularity, such as in Saudi Arabia and United Arab Emirates. In these countries isolated NDV were mostly sub-lineage IVc. It was first seen in imported birds and then found its way to local birds (Shabbir et al., 2013).

Recently the genotype VIIId has been reported in Egypt for the first time (Radwan et al., 2013). The virus had been detected previously in other ME countries such as Saudi Arabia, Jordan, and Israel (Radwan et al., 2013).

Studies on the sero-prevalence of ND, both in Oman (chapter 4 and Shekaili et al, 2015) and from Iran (Hadipour, 2009) found that the ND prevalence is around 40% in backyard poultry, the only non-vaccinating poultry sector.

### **3.6.2. Avian infectious bronchitis (IB)**

IB, caused by a type 3 coronavirus, is one of the most challenging avian respiratory diseases (Masters, 2006). The disease has a very high (up to 100%) morbidity rate, although mortality is comparatively low. The losses in production are due to respiratory, urogenital and alimentary tract lesions (Cavanagh, 2001; Awad et al., 2014c). Chapter 1 reviewed this disease in detail.

### **3.6.3. Epidemiology in the Middle East**

In some Middle East countries, such as Jordan, IBV is believed to be the main pathogen causing respiratory diseases in chickens (Gharaibeh, 2007). Although 793/B and M41 are the most widespread IB viruses, the majority of genotypes are area-specific (Seyfi Abad Shapouri et al., 2004).

IBV has been recognised in Egypt since the 1950s, the first IB variant shown by neutralisation tests was closely related to the D3128 variant then Mass variant was also identified (Abdel-Moneim et al., 2006). Nowadays, a number of European (Mass 41, D3128, D274, D-08880, 793/B) and one Israeli genotype (IS/885/00) have been isolated from Egyptian poultry. Moreover, two Egyptian variants were also detected, variant 1 represented by Egypt/Beni-Suef/01, Ck/Eg/BSU-1/2011, Ck/Eg/BSU-4/2011, and Ck/Eg/BSU-5/2011, and variant 2, represented by Ck/Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011 (Abdel-Moneim et al., 2012).

In Israel, IBV was recognised in the early 1990s (Meir et al., 2004). Numbers of global and regional genotypes were identified such as Mass41, Egyptian variants 1 and 2, IS/720/99, (de Witt et al., 2010), IS/1494/06 (Kahya et al., 2013), and IS/885/00 (Meir et al., 2004). In Jordan, D274 and 4793B IB (Roussan et al., 2008),

CK/CH/LDL/97I genotypes (Ababneh et al., 2012a) were detected circulating in the poultry. In Iraq both CK/CH/LDL/97I (Ababneh et al., 2012a) and QX geotypes were reported infecting poultry (Mahmood et al., 2011). The IS/1494/06 was also detected in broiler farms in Turkey (Kahya et al., 2013).

Mass, 793/B, IS720, Variant 2, QX, IR-I and IR-II (Hosseini et al., 2015) and QX genotypes (Bozorgmehri-Fard et al., 2013) were identified in Iran. IBV CK/CH/LDL/97I (Ababneh et al., 2012a), Mass 41, 793/B, IS/885/00, IS/1494/06, CK/CH/LDL/97I and D274 genotypes were isolated from poultries in the Arabian Gulf countries (Ababneh et al., 2012a; Al-Shekaili et al., 2015).

The control of the disease mainly relies on surveillance, identification of the circulating genotypes, and vaccination. (Jackwood, 2012). Commercial live attenuated vaccines used in the Middle East are mainly 793/B and Massachusetts serotypes either as monovalent vaccine or in combination with D274. In layer farms booster vaccination with inactivated vaccines (3-6 weeks before transfer to laying houses) is practised to give higher and longer humoral immune responses. These vaccine when strategically used provides protection against most of the circulating virulent IBVs. Nevertheless, certain IBV genotypes is still causing considerable losses in vaccinated and non-vaccinated flocks. In Oman, a considerable number of small broiler farms with low biosecurity measures are not vaccinating against this virus.

The presence of other pathogens such as *M. gallisepticum* and *E. coli* exacerbates the losses due to IB infection (Cavanagh, 2007). Therefore, control of all other possible co-infecting pathogens will assist in IBV control too.

### **3.7. Avian metapneumovirus (aMPV)**

Avian metapneumovirus (aMPV) , a virus that belongs to the *Paramyxoviridae* family, causes of turkey rhinotracheitis and swollen head syndrome in chickens (Njenga et al., 2003; Jones, 2010). The disease is reviewed in chapter 1.

aMPV was first identified in Middle East more than 30 years ago (Banet-Noach et al., 2005), however, I found few studies describing the disease prevalence in the region. Both subtypes A and B have been isolated from non-vaccinated flocks in the area (Banet-Noach et al., 2005; Al-Shekaili et al., 2015).

In Jordan, Gharaibeh and Algharaibeh (2007) detect aMPV subtype B in broiler, layer, and broiler breeder chicken poultry farms (Gharaibeh and Algharaibeh, 2007). In Israel, Banet-Noach (2005) using PCR found 44% of turkey farms infected both aMPV subtype A and B, but the majority of the farms infected with subtype B (Banet-Noach et al., 2005). Al-shekaili et al., (2015) found aMPV subtype B circulating in Oman backyard poultry (Al-Shekaili et al., 2015) . In Egypt, Abdel-Azeem et.al (2014) reported aMPV subtype A outbreak in turkeys farm (Abdel-Azeem et al., 2014).

### **3.8. Infectious laryngotracheitis (ILT)**

Infectious laryngotracheitis virus (ILTV) is an alpha-herpes virus affecting mainly chickens and pheasants. It has a wide range of clinical signs ranging from subclinical infection to per acute with high mortality. The virus solely affects the respiratory tract. Although the virus has many strains it has a single serotype which helps greatly in control.

As with other herpes viruses, ILT becomes latent in the trigeminal ganglia (Williams et al., 1992) of the recovered birds (Bagust et al., 2000). This latency interspersed with episodes of virus shedding leading to redevelopment of the disease (Williams et al., 1992). Factors such as stress or onset of lay may evoke virus shedding. Even the early use of live vaccine was hindered by this phenomenon leading to infection in naïve flocks (Kotiw et al., 1995; Jones, 2010). Molecular techniques can be used to differentiate the vaccinal virus from the field viruses (Jones, 2010).

There are few reports of this disease in the Middle East. The first of the virus report in Egypt was Tantawi et al. (1983), who reported the presence of ILT infection in layers flocks at different locations in Egypt in 1982/83. Shehata et al., 2013 reported 4 isolates of ILT detected from a vaccinated broiler chickens from different Egyptian governorates in the period 2007-2010. Gulacti et.al (2007) detected the disease in number of layers hens in an amateur breeder unit in Elazig, Turkey. Deaths of 9 out of 18 birds within a week with ILT clinical signs, the diagnosis was confirmed with PCR (Gulacti et al., 2007).

### **3.9. Future of NDV, IBV, aMPV and ILTV in the Middle East**

Biosecurity is the term used to describe all measures aimed at the prevention of the introduction and/or the spread of diseases. NDV has been circulating in the Middle East for more than four decades. Farms using vaccination together with biosecurity measures are able to minimise the devastating effect of ND (Alexander, 2001). However, all Middle Eastern countries have extensive rural backyard poultry producers, many of which are located not far from commercial farms. For these producers, vaccination is rarely used, and biosecurity levels are low. These producers therefore present a continual risk to commercial production farms. There is also regular contact between backyard poultry and wild migratory birds. Annual bird migration and the location of most Middle Eastern countries in more than one bird migration route make the eradication of this disease very difficult.

Moreover, factors such as the virus' ability to infect many different bird species, its wide range of clinical signs, limited detection especially in live bird markets and quarantine stations and the ability of low pathogenic genotypes to mutate into high pathogenic ones may lead to the continuous circulation of the virus in the Middle East (Alexander et al., 2004).

Measures such as the planned movement of new commercial production farms away from other production farms (East et al., 2006; Anon, 2008), backyard poultry farms and residential areas could help in minimizing NDV outbreaks in the production farms. Increased awareness of the biosecurity measures and improved vaccination practices among poultry farm producers could also improve the situation.

IBV, aMPV and ILTV receive less attention from researchers in the Middle East. Therefore given the paucity of information describing their status at the current time,

these viruses will continue to circulate and may pose a threat to efficient poultry production. Extra work is needed to study the prevailing viruses circulating in each country, the modes of transmission, risk factors and the best means of control.

Generally, there is a need for improvements in conventional and molecular diagnostic services for detection of these viruses in the ME countries, either individually or in collaboration with each other. In addition, the use of available vaccines should be optimised with the use of proper vaccine genotypes, good cold chain, proper administration, and improved farm management (Marangon and Busani, 2006).

### **3.10. Conclusion**

AI, ND, IB, aMPV and ILT, important viral respiratory diseases of poultry, have all been reported in Middle Eastern countries. Some attract more attention than others, either due to their zoonotic potential or due to their virulence. HPAI H5N1 the most serious pathogen which has been reported in ten countries is still circulating extensively in Egypt despite the vaccination efforts. This circulation will continue presenting various threats, such as spill over of the infection to migratory birds which will expand the affected geographical area, or the virus may again the ability of human to human transmission.

Although, H9N2 has been detected in the region for more than 20 years the complete picture of LPAI viruses is not known in most of the countries. Newcastle disease is widely seen in the area with extensive use of vaccine for its control. Less attention is given to IBV, aMPV and ILT in almost all the Middle East countries.

More research work is needed to address the situation and help develop ways of control and eradication. In the meantime in most Middle Eastern countries, where there is political instability and/or lack of resources and, poverty, these diseases will continue to circulate among poultry.

**4- Chapter Four: Sero-surveillance for avian influenza  
and Newcastle disease viruses in backyard poultry in  
Oman**

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## **4.1. Abstract**

Avian Influenza (AI) and Newcastle disease (ND) are the most important reportable poultry diseases worldwide. Low pathogenic AI (H9N2) and ND viruses are known to have been circulating in the Middle East, including in Oman, for many decades. However, detailed information on the occurrence of these pathogens is almost completely lacking in Oman. As backyard poultry are not vaccinated against either virus in Oman, this sector is likely to be the most affected poultry production sector for both diseases. Here, in the first survey of AI and ND viruses in backyard poultry in Oman, I report high flock-level sero-prevalence of both viruses. Serum and oropharyngeal swabs were taken from 2,350 birds in 243 backyard flocks from all regions and governorates of Oman. Information was recorded on location, type of bird and housing type for each sampled farm. Individual bird serum samples were tested using commercial indirect antibody detection ELISA kits. Pooled oropharyngeal samples from each flock were inoculated onto FTA cards and tested by RT-PCR. Samples came from chickens (90.5%), turkeys (2.1%), ducks (6.2%), guinea fowl (0.8%) and geese (0.4%). The bird-level sero-prevalence of antibody to AI and ND viruses was 37.5% and 42.1% respectively, and at the flock level it was 84% and 90.2% respectively. There were from one to ten AIV and NDV positive birds in each positive flock, with a means of 4.3 and 4.6 and a SDs of 3 and 2.8, respectively. All oropharyngeal samples were negative for both viruses by RT-PCR, consistent with a short duration of infection. This study demonstrates that eight or nine out of ten backyard poultry flocks in Oman are exposed to AI and ND viruses, and may present a risk for infection for the commercial poultry sector in Oman, or wild birds which could carry infection further afield.

## **4.2. Introduction**

Avian influenza (AI) and Newcastle disease (ND) are the most important reportable poultry diseases worldwide (Malik et al., 2004; Wang et al., 2008). Both are highly contagious viral diseases affecting a wide range of bird species. Avian influenza and Newcastle disease are caused by influenza A virus (AIV) and Newcastle disease virus (NDV) respectively. The AIV belong to the *Orthomyxoviridae* family while the NDV is one of the avian paramyxovirus serotype-1 (APMV-1) viruses of the *Paramyxoviridae* family (Swayne and King, 2003). Both viruses and their epidemiology in Oman and the Middle East were reviewed in chapter 1 and 3.

### **4.3. Research justification**

There is a particular paucity of information from Oman, with almost no published studies of avian respiratory viruses of any species, except on infectious bronchitis virus (IBV) and avian metapneumovirus (Al-Shekaili et al., 2015), chapter 6. This is despite Oman's geographic situation, between the horn of Africa and southern Asia, and its importance as a site for migrating wild birds. Most poultry production in Oman is carried out in commercial farms, which vaccinate against NDV and LPAI H9N2 (albeit at a lower intensity). Despite this, clinical cases of both diseases are still observed annually in backyard birds. The backyard flocks are bred for household consumption and vaccination against either disease is not practiced within this sector. To date, the HPAI has not been detected in poultry or wild birds in Oman.

## **4.4. Materials and Methods**

### **4.4.1. Sampling method**

Serum and oropharyngeal swabs were taken from 2350 birds of 243 backyard flocks from all regions and governorates of Oman, between mid-June and the end of September 2012 as described in chapter 2.1 and 2.2.

### **4.4.2. Detection of AIV and NDV antibodies**

Antibodies to the nucleoprotein of AIV in chicken and turkey samples were detected by indirect ELISA using a commercial kit (BioChek Ltd., Gouda, Holland) as described by the manufacturer (chapter 2.3.1). Nucleoprotein antibodies in other bird species were examined using the IDEXX Ab multispecies ELISA kit (IDEXX, USA). Similarly, antibodies to NDV in chicken and turkey samples were detected using an indirect ELISA kit (BioChek, Gouda, Holland); however, the duck and geese serum samples were not tested as the kits have not been validated for these species.

#### **4.4.3. RNA extraction from FTA cards**

RNA extraction was performed using a Qiagen, QIAamp Viral RNA Mini Kit<sup>15</sup> according to the manufacturer's instructions and RT-PCR was carried out as described in chapter 2.4.1.

#### **4.4.4. Reverse transcriptase polymerase chain reaction (RT-PCR)**

RT-PCR was performed on each of the AIV and NDV RNA extracts amplifying NP and F genes for these viruses respectively (Appendix 2). Both primers and cycle conditions were as previously published (Aldous et al., 2003; Wei et al., 2006) (chapter 2.4.3). Table 2.4 shows the RT-PCR oligonucleotides used for all PCR tested viruses.

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<sup>15</sup> Qiagen Ltd, Germany

## 4.5. Results

### 4.5.1. Flocks and ELISA results

Figure 2.2 shows the distribution of the sampled farms from different regions and Table 4.1, 4.2 presents number of flocks and birds of each poultry species seen in different regions during the sampling.

A total of 2350 birds, from five backyard poultry species (chickens, turkeys, ducks, geese and guinea fowl) were sampled from 243 flocks on 238 farms. Eighty-nine percent (213/238) of the sampled backyard farms had chickens only. The sampled flocks were chickens (90.5 %), turkey (2.1%), duck (6.2%), guinea fowl (0.8%) and geese (0.4%).

In term of bird numbers; chickens comprised approximately 96% of all poultry present at the visited farms (Table 4.1). Ducks were the second most common species found in the visited farms, forming nearly 2.5% of the total; the main duck species were Muscovy (*Cairinia moschata*) and mallard (*Anas platyrhynchos*). The other three poultry species accounted for less than two percent of the total birds. Only chickens were found in the farms visited in the Al Wusta region. Three regions (Musandam, Adh Dhahirah and Dhofar) had a small percentage of farms with turkey or guinea fowl hens. Al Batinah region had the highest number of mixed bird flocks, reaching more than 19% of the total. The number of ducks (both birds and flocks) was the second highest in that region. The mean flock sizes were 149 birds for chickens (standard deviation, SD = 211), 28 for turkeys (SD= 58.7), 49 for ducks (SD = 32.9), 36 for guinea fowl (SD = 43.4) and 5 for geese (which were just

represented in one flock). The mean flock size for all bird types was 139 birds (SD = 203.8).

While Dhofar governorate had the smallest number of flocks, it had the greatest mean flock size, reaching 377 birds (SD = 352.3). The second largest mean flock size was 240 birds at Adh Dhahirah region (SD = 339.4). In other regions mean flock sizes were fewer than 130 birds.

The most prevalent type of housing for the backyard birds is semi-closed houses (227/238; 95.3%), particularly in the northern regions. In the Dhofar governorate and Al Wusta region birds tend to be kept outdoors, scavenging during daylight hours.

The bird and flock-level ELISA results for AIV and NDV, by region, are shown in Table 4.3 and 4.4. The seroprevalence of antibodies to AIV and NDV in birds was 37.5% and 42.1% respectively. There were from one to ten AIV and NDV positive birds in each positive flock, with means of 4.3 and 4.6 and SDs of 3 and 2.8 respectively. The flock-level seroprevalence was 84% (SD = 31.57) and 90% (SD = 31.23), respectively. Mean within-flock seroprevalences were 37.6% and 43.4% respectively. Figure 4.1 and 4.2 show the percentages of AIV and NDV positive birds and flocks in each Oman region.

The percentages of birds and flocks of each species that were positive for AIV and NDV are shown in Table 4.5 and 4.6, respectively. There was no significant difference between the percentages of flocks of different species serologically positive for AIV (Fisher Exact,  $p > 0.5$

**Table 4.1** Number of birds of each poultry species present in farms visited in different regions of Oman in 2012

Region	Number of chickens	Number of ducks	Number of Turkeys	Number of Guinea fowls	Number of geese	Percentage of other birds than chickens
Al Batinah region	8259	556	133	100	5	8.8
Musandam governorate	641	4	0	0	0	0.6
Ash Sharqiyah region	4160	80	1	100	0	4.2
Ad Dakliyah region	4773	210	0	10	1	4.4
Adh Dhahirah region	10516	0	30	0	0	0.3
Dhofar governorate	2605	0	0	7	0	0.28
Al Wusta region	321	0	0	0	0	0
Muscat governorate	1600	13	9	0	0	1.3
Total	32875	854	173	217	6	3.7

**Table 4.2** Number of flocks of each poultry species present in farms visited in different regions of Oman in 2012

Region	chickens	ducks	Turkeys	Guinea fowls	geese	Percentage of other flocks than chickens
Al Batinah region	69	10	4	2	1	19.8
Musandam governorate	11	1	0	0	0	8.3
Ash Sharqiyah region	35	1	1	1	0	7.8
Ad Dakliyah region	37	4	0	1	1	13.9
Adh Dhahirah region	47	0	1	0	0	2.1
Dhofar governorate	7	0	0	1	0	12.5
Al Wusta region	8	0	0	0	0	0
Muscat governorate	11	1	1	0	0	15.4
Total	255	17	7	5	2	11.1

**Table 4.3** Bird level of avian influenza virus (AIV) and Newcastle disease virus (NDV) serological results in different regions of Oman

Region	Total number tested		AI positive birds		ND positive birds	
	birds		Numbers	Percentage	Numbers	Percentage
	AI/ND <sup>16</sup>					
Al Batinah region	792/689		298	37.6	256	37.2
Musandam governorate	88/84		30	34.1	20	23.8
Ash Sharqiyah region	355/335		114	32.3	188	56.1
Ad Dakliyah region	383/353		147	38.4	144	40.8
Adh Dhahirah region	461/461		175	40	276	59.9
Dhofar governorate	70/70		8	11.4	9	12.9
Al Wusta region	72/72		37	51.4	14	19.4
Muscat governorate	129/119		72	55.8	46	38.6
Total	2350/2262		881	37.5%	953	42.1%

<sup>16</sup> Total birds numbers displayed as the number of birds tested for AIV/NDV, 792/689 represents 792 birds tested for AIV and 689 birds tested for NDV.

**Table 4.4** Flock level of avian influenza virus (AIV) and Newcastle disease virus (NDV) serological results in different regions of Oman

Region	Total number tested flocks		AIV positive flock		NDV positive flock	
	AIV	NDV	Numbers	Percentage	Numbers	Percentage
Al Batinah region	82	71	68	82.9	64	90.1%
Musandam governorate	11	10	9	81.8	8	80%
Ash Sharqiyah region	36	34	30	88.2	32	94.1%
Ad Dakliyah region	39	36	33	84.6	33	91.7%
Adh Dhahirah region	47	47	38	80.9	46	97.9
Dhofar governorate	7	7	5	71	5	71.4%
Al Wusta region	8	8	8	100	5	62.5%
Muscat governorate	13	12	13	100	11	91.7%
Total	243	226	204	84%	203	90.0%

**Table 4.5** Number of avian influenza virus (AIV) and Newcastle disease virus (NDV) positive birds from each bird type

Bird Species	Number of tested birds		AIV positive birds		NDV of positive birds	
	AIV	NDV	<i>n</i>	%	<i>n</i>	%
Chickens	2134	2134	827	38.8	938	44
Ducks	142	NA	35	24.6	NA	
Turkeys	49	49	15	30.6	15	30.6
Geese	5	NA	2	-	NA	
Guinea Fowls	20	NA	2	10	NA	

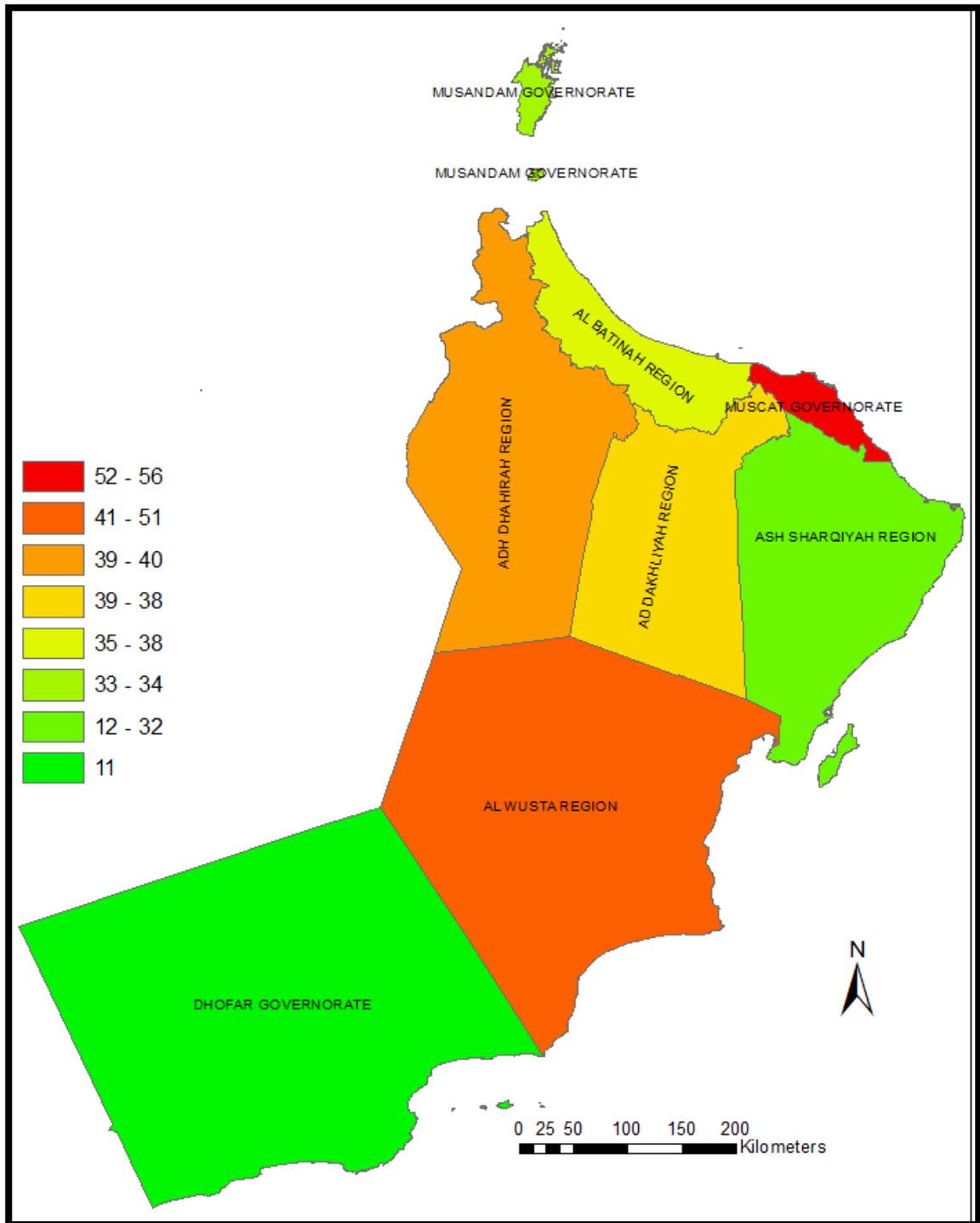
NA – not applicable, as ducks, geese and guinea fowl were not tested for NDV

**Table 4.6** Number of avian influenza virus (AIV) and Newcastle disease virus (NDV) positive flocks from each bird type

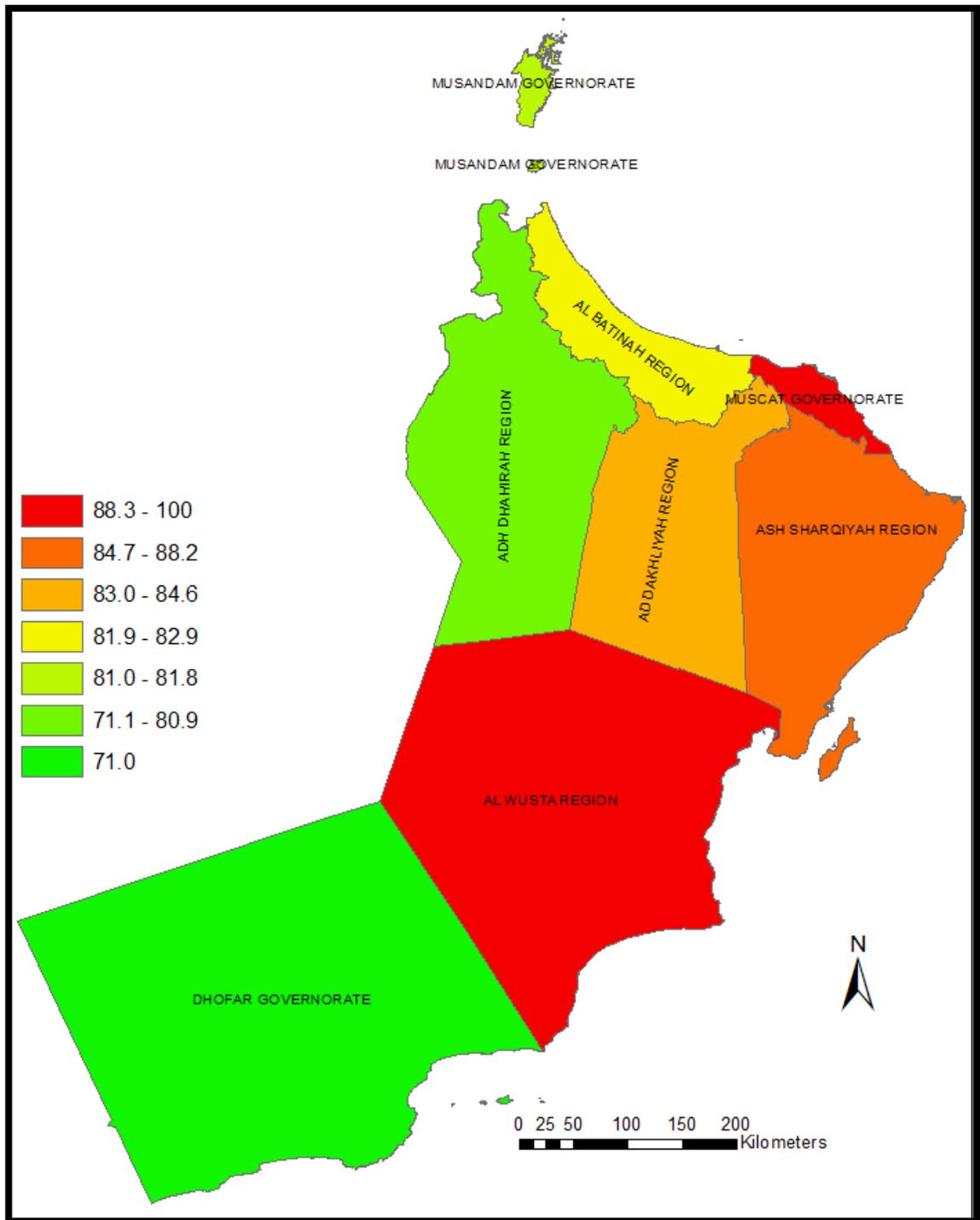
Bird Species	Number of tested flocks		AIV positive		NDV of positive	
	AIV	NDV	<i>n</i>	%	<i>n</i>	%
Chickens	220	220	185	84.4	199	90.5
Ducks	15	NA	12	80	NA	
Turkeys	5	5	4	80	4	80
Geese	1	NA	1	-	NA	
Guinea Fowls	2	NA	2	100	NA	

NA – not applicable, as ducks, geese and guinea fowl were not tested for NDV

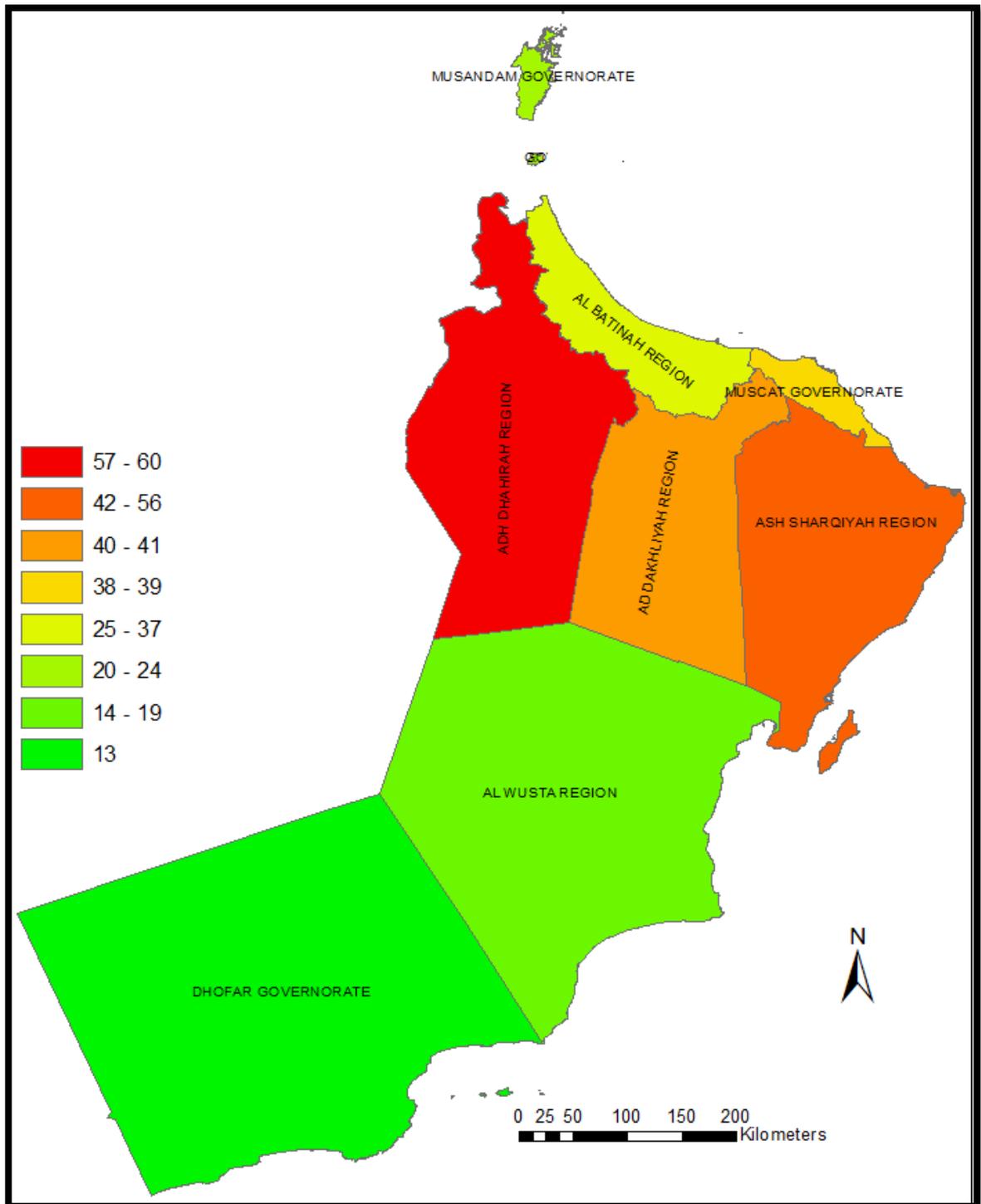
**Figure 4.1** (A) percentages of birds positive for avian influenza virus (AIV) in the backyard poultry in each region indicated by colour zones. The lowest percentage was 11.



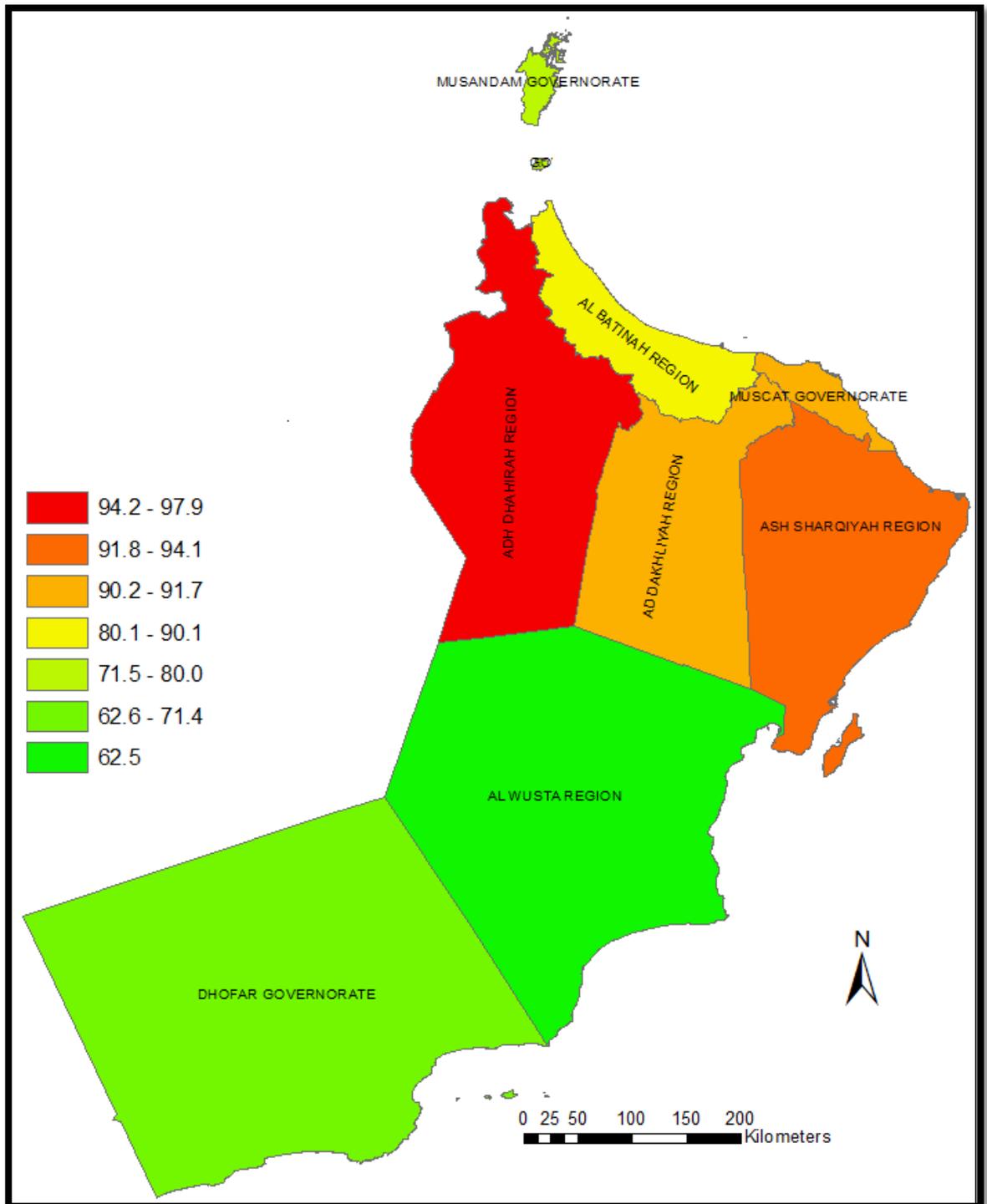
**Figure 4.1 (B)** percentages of flocks positive for avian influenza virus (AIV) in the backyard poultry in each region indicated by colour zones



**Figure 4.2 (A)** Percentages of birds positive for Newcastle disease virus (NDV) in the backyard poultry, in each region indicated by colour zones



**Figure 4.2 (B)** Percentages of flocks positive for Newcastle disease virus (NDV) in the backyard poultry in each region indicated by colour zones (the lowest percentage was 62.5)



#### **4.5.2. AIV and NDV RT-PCR**

All PCR results for genome detection of both pathogens from the FTA cards were negative. A positive control was included in all PCR reactions and it gave an expected band size on the agarose gel. RNA extracts from the same cards were also analysed for IBV and aMPV (chapter 7), where a good number of positive flocks were detected.

## **4.6. Discussion**

This is the first investigation into the seroprevalence of AIV and NDV in Oman on a national scale. The results show a widespread exposure to both viruses in backyard poultry in Oman. Nearly 84% and 90% of the sampled flocks, and about 4 of 10 sampled birds, had serological evidence of previous infection with AIV and NDV respectively. The high prevalence of seropositive flocks reported here may be due to the circulation of low pathogenic viruses of both types producing mild or no clinical signs in infected birds.

The free ranging of backyard birds presents a high risk for AIV (Terregino et al., 2007) and NDV (Schelling et al., 1999) transmission between wild birds and poultry in both directions. Although the majority of the visited backyard farms rear their birds in wire-netted enclosures, there are often gaps or damage in poultry houses that may allow the entry of wild birds. As such, the chance of contact with wild birds is higher in the backyard poultry rearing system than those raised in commercial farms where the whole house is concealed against entry of wild birds.

Backyard poultry health status is important for the birds' owners, the nearby commercial poultry farms and human health authorities (Madsen et al., 2013), as the diseases affecting the backyard flocks could spill-over to commercial poultry or people. There have been only a few previous studies investigating the epidemiology of AIV and/or NDV in Middle Eastern countries, and the majority of these studies targeted HPAI H5N1 in commercial poultry farms (Al-Natour and Abo-Shehada, 2005; Aamir et al., 2007; Banet-Noach et al., 2007; Al-Azemi et al., 2008; Monne et al., 2008; Fereidouni et al., 2010; Hafez et al., 2010; Ababneh et al., 2012b; Arafa et

al., 2012a; El-Shesheny et al., 2012; Watanabe et al., 2012; Hassan et al., 2013; Madadgar et al., 2013). This is the one of the first studies to measure the seroprevalence of AIV and NDV in backyard poultry on a national scale in a Middle East country.

I found high (80-90%) flock-level seroprevalences of both AIV and NDV, suggesting that there is widespread exposure of backyard poultry in Oman to both viruses. Possible explanations include exposure to wild birds, introduction of new birds onto existing flocks, co-mixing with neighbouring poultry and feeding of uncooked poultry waste. Studies elsewhere have found lower flock-level seroprevalences. For example, in New Zealand, 20.8% (5/24) of backyard poultry flocks were found to be seropositive for avian influenza viruses (Zheng et al., 2010). In Maryland, USA, 23.1% (9/39) of backyard flocks were seropositive for AIV (Madsen et al., 2013) and in Côte d'Ivoire the ND seropositive was 19.8% (Couacy-Hymann et al., 2012). Factors such as testing method, species and age of birds, climate condition, time of year, farming practises and migratory bird routes contribute to the difference between locations (Madsen et al., 2013).

In contrast to the high flock-level seroprevalences, I detected much lower (~40%) within-flock seroprevalences of both viruses. Similar, low within-flock seroprevalences have been reported elsewhere in the Middle East. Similar to this study, Saadat et al. (2014) found bird-level seroprevalences of AIV (39%) and NDV (40.1%) in Iran. A second study near the Caspian Sea in Iran, however, reported higher seroprevalences (~73%) of H9N2 seropositivity in backyard chickens (Hadipour, 2010). There are several possible explanations for why fewer than half of

the birds in exposed flocks in this study were seropositive. First, in backyard poultry flocks in Oman, the density of birds is less than in commercial premises, with less contact between individuals and less sharing of air space; this may mean that there is less transmission between birds than that which occurs under more intensive production. Second, some exposed birds may deal with the respiratory infection with mucosal immunity with little or no seroconversion, while other surviving birds may have seroconverted strongly. The humoral antibody response is dependent on the host (eg. age, immune status), agent (eg. virulence, dosage, co-infections) and environment (eg. stocking density, air quality, ventilation). Third, especially if the initial exposure was to a low dose of virus, immunity may wane over time and older, exposed, birds may become seronegative. Fourth, if the circulating viruses are highly pathogenic, many exposed birds may die, and be replaced by unexposed stock. Fifth, variation in the level of genetic resistance between different breeds and species of poultry has been reported (Kapczynski et al., 2013).

There was a marginally significant difference in the seroprevalence of AIV in terms of birds and chicken flocks between different regions of Oman (Table 7). The Muscat Governorate and Al Wusta region had the highest seroprevalences of AIV. The high AIV prevalence in Muscat Governorate may be attributable to the presence of many known risk factors for the disease, such as high population density (Gilbert et al., 2008; Moriguchi et al., 2013), presence of water bodies (Fournie et al., 2012), high road density (Ward et al., 2008), the presence of live bird markets (Kung et al., 2007) and the presence of a large number of wild birds (Senne et al., 2006). In Al Wusta region, the high AIV seroprevalence in terms of both birds and flocks may be attributable to the presence of the Barr Al Hikman wetland areas used for nesting of

migratory birds annually (Terregino et al., 2007). The wetland birds such as gulls, terns, and waders are a major natural virus reservoir of AIV (Olsen et al., 2006). By contrast, the Dhofar governorate in the south of Oman showed the lowest seroprevalences of AIV and NDV in chickens. This may be due to the small number of backyard flocks in this region.

I found no evidence of very recent or current AIV or NDV infections in any of the flocks, as all the PCR results (for the detection of viral RNA) were negative. However, the RNA of two other viruses (IBV and aMPV) were detected on a number of the same FTA cards, confirming that the sampling, transportation and laboratory methods did not have any adverse effects on the RNA (Al-Shekaili et al., 2015). Previous studies have demonstrated the stability of AIV (Abdelwhab et al., 2011) and NDV (Awad et al., 2012) RNA on FTA cards. Similar findings were reported for NDV in New Zealand (Dunowska et al., 2013) and H5N1 in Egypt (Kayali et al., 2011b). The negative results may be due to the low chance of finding pathogenic viruses in samples taken from clinically healthy birds, or due to the short duration of the shedding of the AI viruses (Latorre-Margalef et al., 2009; Lebarbenchon et al., 2010) in comparison to IB virus (de Wit et al., 2011). Regarding the NDV, it could be that exposure of the backyard birds to the virus, may have triggered immune responses that contributed to the decrease in the duration of virus shedding (Miller et al., 2009). It is also possible that the snapshot sampling method that I used, with all samples taken during the hot summer months, may have reduced the likelihood of detecting either virus although the seasonality of transmission of these viruses in Oman is not known. Another explanation could be that, the sampling of adult birds (> 3 months) may give a chance to adult birds to get rid of the infection with

acquiring better immunity (Deibel et al., 1985). For the molecular detection, the pooled oropharyngeal swabs from each flock was tested using PCR. The process of pooling the samples might dilute the virus if present and reduce the chance of RNA detection, particularly with the use of FTA card. Both the AIV and NDV PCR were established in our laboratory. However for NDV F gene detection, there is no particular test validated to detect all types of NDV pathotypes. In recent years, most laboratories have switched from the conventional RT-PCR to the use of rRT-PCR using probes to target the most conserved parts (Suarez, 2013; Tombari et al., 2013).

In view of the negative PCR results for both viruses, measuring the mean Abs response for each flock is a useful tool to see any evidence of recent infections.

In future, if this work is to be repeated, it would be best to include young birds in the sampling frame, as this group of birds is more likely to have an active infection/disease. Also, it would be recommended to target respiratory ill birds and use virus isolation techniques to replicate the virus or more sensitive PCR protocol (eg. real-time RT-PCR) to obtain more accurate results.

Against a background of a paucity of information on AIV and NDV prevalence and their risk factors in Oman and other Middle East countries, this study provides evidence of a high serological prevalence of NDV and AIV in backyard poultry flocks in Oman and provides some preliminary information about risk factors that may help to target disease control measures and/or avian flu contingency planning.

**5- Chapter Five: Risk factors affecting the epidemiology  
of avian influenza and Newcastle disease viruses in  
backyard poultry in Oman**

## **5.1. Abstract**

Avian influenza (AI) and Newcastle disease (ND) are the most reportable viral poultry diseases. Previous researches studied the risk factors associated with prevalence of both diseases, especially the highly pathogenic avian influenza H5N1. In this chapter, I tried to examine some previously identified risk factors for their association with percentage of positivity for AIV and NDV in the backyard poultry in Oman. I utilized the serological results from chapter 4. Variables deemed to have a possible association with the probability of a chicken flock being positive for NDV or AIV prevalence were as follows: Region (all Oman regions and governorates), Altitude in meters (continuous), Flock size (continuous), distance of the flock from the nearest production poultry farm, nearest main road and nearest surface water (lines or areas) (continuous), Flock composition. For NDV, the sample AIV seropositivity result (continuous) was also included. There was a regional effect on both diseases; however there was no North-South pattern. There was a highly significant association between the presence of AIV and NDV infection. Furthermore as reported elsewhere, there was a negative association between the farm altitude and the AIV intensity of infection, The flock size was marginally negatively associated with the NDV infection rate.

## 5.2. Introduction

Avian influenza (AI) and Newcastle disease (ND) are the most important reportable poultry diseases caused by the *Orthomyxoviridae* (Alexander, 2000a) and paramyxovirus type 1 (Alexander, 2000b) respectively. These two viruses and their epidemiology in Oman and the Middle East were revised in chapter 1 and 3.

In the past two decades the number of AI outbreaks has increased dramatically, leading to huge economic losses and fear of zoonotic involvement. In addition, some low pathogenic strains such as H9N2 have become endemic in Asia and Middle East (Fusaro et al., 2011). Newcastle disease is widespread, affecting all types of poultry farming, particularly backyard poultry.

Control programmes usually differ according to the type pathogen to be controlled, and the presence of different risk factors affecting the pathogen's spatial and temporal distribution (Marangon and Busani, 2006). Therefore, prevention and control of disease is facilitated by understanding of spatial distribution of the disease and its risk factors that give rise to this distribution.

With the recent advance in Geographical Information Systems (GIS), spatial models can be developed to predict and map the distribution of diseases based on the available information of their risk factors. These maps help those responsible for developing control programmes to prioritize certain areas for surveillance (Hay et al., 2006).

A recent serological study done in Oman backyard poultry (the only non-vaccinating sector for both diseases) for both AIV and NDV prevalence found a prevalence of

38.8% and 40.8% respectively (Al shekaili et al., 2015 (accepted), Chapter 4). Production poultry farms vaccinate against NDV and less extensively for H9N2 genotype of the AI infection.

Previous studies identified a number of AIV and NDV risk factors such as poultry farm density (East et al., 2006; Pfeiffer et al., 2007; Gilbert et al., 2008; Loth et al., 2010), area population (Pfeiffer et al., 2007; Gilbert et al., 2008) type of poultry production (Brown et al., 2006; East et al., 2006; Gilbert et al., 2006; Pfeiffer et al., 2007; Gilbert et al., 2008; Sharkey et al., 2008; Rasamoelina Andriamanivo et al., 2012; Wang et al., 2013), presence of migratory birds and wild birds (Brown et al., 2006; East et al., 2006; Koch and Elbers, 2006; Munster et al., 2006; Otim et al., 2007; Gilbert et al., 2008; Peterson and Williams, 2008), presence of fighting cocks in the area (Gilbert et al., 2006; Tiensin et al., 2009; Paul et al., 2010; Villarreal et al., 2010), distance to main roads (Boender et al., 2007; Ward et al., 2008; Iglesias et al., 2011), density of backyard poultry in the area (Gilbert et al., 2006; Koch and Elbers, 2006; Tiensin et al., 2009; Paul et al., 2010; Iglesias et al., 2011; Rasamoelina Andriamanivo et al., 2012; Wang et al., 2013), distance to the nearest poultry farm (East et al., 2006; Nishiguchi et al., 2007), presence of live bird markets in the area (Kung et al., 2003; Gilbert et al., 2006; Lefrancois et al., 2010; Villarreal et al., 2010; Rasamoelina Andriamanivo et al., 2012) and distance to the nearest surface water bodies (Gilbert et al., 2006; Iglesias et al., 2011; Chaka et al., 201

### **5.3. Research Justifications**

This chapter aims to study the association of a number of risk factors with intensity of AIV and NDV serological results in Oman backyard poultry. This study used data generated during a previous study testing the prevalence of both diseases in Oman backyard poultry (Chapter 4). An attempt was made to measure the magnitude and direction of previously identified risk factors such as population density, flock size, the altitude of the farm, presence of fighting cocks in the area, distance of the farm to the nearest production poultry farm, main road and surface water (line or area) and the regional effect on each virus distribution.

## **5.4. Materials and Methods**

### **5.4.1. Data sources**

#### **5.4.1.1. Infection intensity**

The levels of exposure of Oman backyard flocks to the AI and ND viruses were obtained from chapter 4 data (Shekaili et al., 2015), taking the percentage of flock infection as a measurement for the intensity of the infection in each farm. Two hundred forty three backyard flocks were examined from 238 farms using ELISA in chapter 4 (Appendix 3). However, because the number of flocks of birds other than chickens is very small (15 duck flocks, 1 flock of geese, 2 flocks of guinea fowl and 5 flocks of turkeys) the power to detect statistically meaningful effects in groups of birds other than chickens is low. The decision was hence taken to restrict formal statistical modelling to chicken flocks only, of which there were 220 in the data set.

#### **5.4.1.2. Explanatory variables**

A set of risk factors applicable for AIV and NDV were obtained from the published literature as follows. The PubMed database was searched for articles using Medical Subject Heading MESH term “avian influenza” and “risk factors” and MESH term “Newcastle disease” and “risk factors”. Table 5.1 shows the risk factors being found in the PubMed database search for AIV and NDV risk factors.

**Table 5.1** A list of risk factors previously identified for avian influenza and Newcastle disease viruses found in the PubMed database search

The risk factor	AIV	NDV	Direction
Population density	Yes	Yes	Positive
Agriculture activities density in the area	Yes	Yes	Positive
Poultry farm density	Yes	Yes	Positive
The prevailing type of poultry production (Industrial or commercial 2,3 or backyard)	Yes	Yes	Backyard has a higher risk to attract avian influenza than commercial farm and the Industrial farms have the lowest risk
Presence of migratory birds and wild birds	Yes	Yes	Positive
Distance to water bodies	Yes	Yes	Negative
Type of marketing (horizontal contact between farms)	Yes	Yes	Inside farm slaughter houses and own truck distributors have lower risk
Number of visits to each production poultry farm	Yes	Yes	Positive
Presence of fighting cocks hobby in the area	Yes	Yes	Positive
Density of backyard poultry in the area	Yes	Yes	Positive
General biosecurity in the farm	Yes	Yes	Negative
Number of waterfowl in the area	Yes		Positive
Presence of rice farming	Yes	Yes	Positive
Presence of life bird marks	Yes	Yes	Positive
Vegetation	Yes	Yes	Positive
Road density	Yes	Yes	Positive
Direct distance to the nearest case	Yes	-	Negative

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farm			
Free grazing ducks	Yes	-	Positive
Flock vaccination	Yes	Yes	Negative
Increasing age of the bird	-	Yes	Positive
Number of owners	Yes	Yes	Positive
Presence of different bird species	Yes	Yes	Positive
Humidity	-	Yes	Negative
Presence of free range chickens on the farm	Yes	Yes	Positive
Purchasing of restocking chickens from the market and neighbourhood	Yes	Yes	Positive
Avian species present	Yes	Yes	Quail and water fowl positive with AIV, chickens and guinea fowl positive with NDV
Frequency of cleaning of poultry houses	-	Yes	Negative
Larger flock size	-	Yes	Positive
Birds drinking water source	Yes	Yes	Open surface water has more risk
Sex of birds	-	Yes	Female have more risk
Multi-age production practices	Yes	Yes	Positive

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Then selecting the most appropriate quantifiable risk found in Oman. The selected risk factors and their source of information are shown in Table 5.2.

To obtain the sampled farms' distance to the nearest different risk factors (roads, water line and areas, coast, and production farms), the farm locations were plotted on a digital map of Oman obtained from the ministry of agriculture and fisheries Oman. Roads, water lines (Wadis i.e. temporary rivers), water areas (temporary lacks), coastline and production poultry farms were obtained from various sources (Table 5.2) using ArcGIS<sup>17</sup>. Within ArcGIS, the shortest distance between each farm and each feature (road, water line and area, coast and production poultry farm) were calculated using the near, proximity, analysis tool of ArcMap tools (Figure 5.2).

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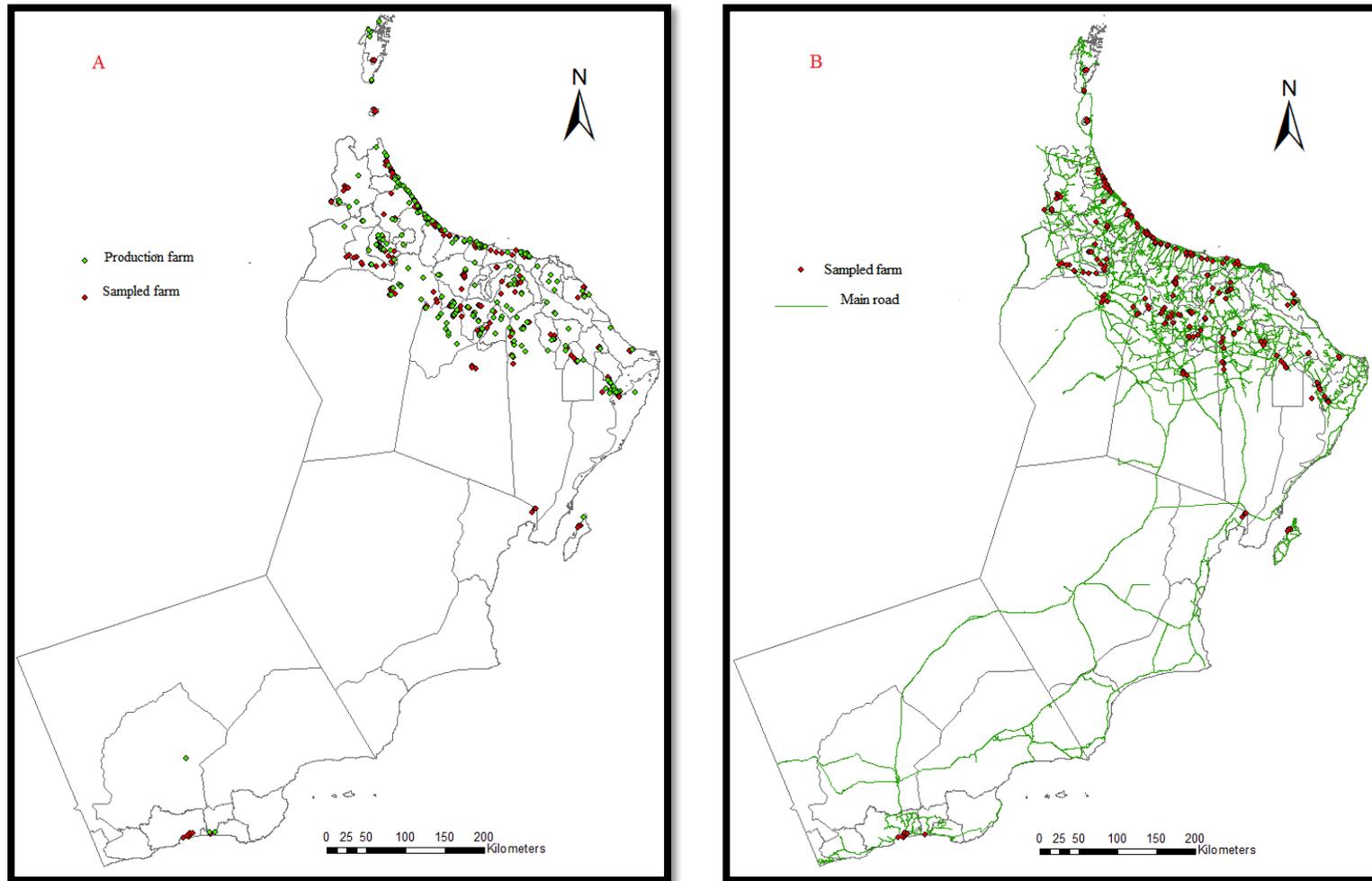
<sup>17</sup> ArcGIS Desktop 10, ESRI

**Table 5.2** The source of data for the studied risk factors in Oman

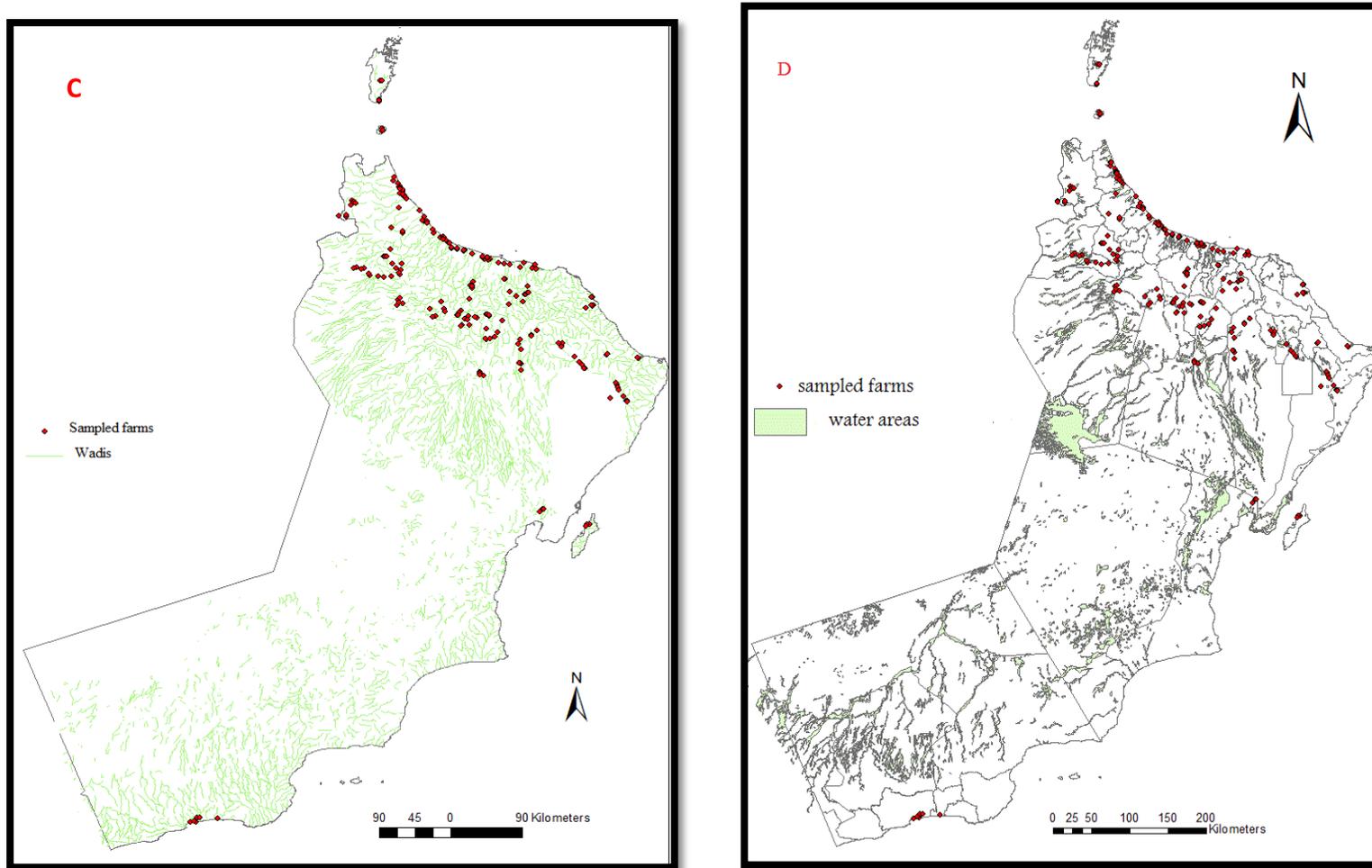
<b>Risk factor</b>	<b>The source of data</b>
Distance to surface water (Wadis) <sup>18</sup>	Electronic map from Ministry of Oman Agriculture and Fisheries
Distance to roads	Electronic map from Ministry of Oman Agriculture and Fisheries
Distance to the nearest production poultry farms	Oman agriculture census 2012.
Presence of wild birds	From a questionnaire study done for production broiler poultry farms (estimated), Chapter 8
Presence of fighting cocks in the region	From a questionnaire study done for production broiler poultry farms (estimated), Chapter 8
Flock size	Chapter 4
Type of birds in the farm	Chapter 4

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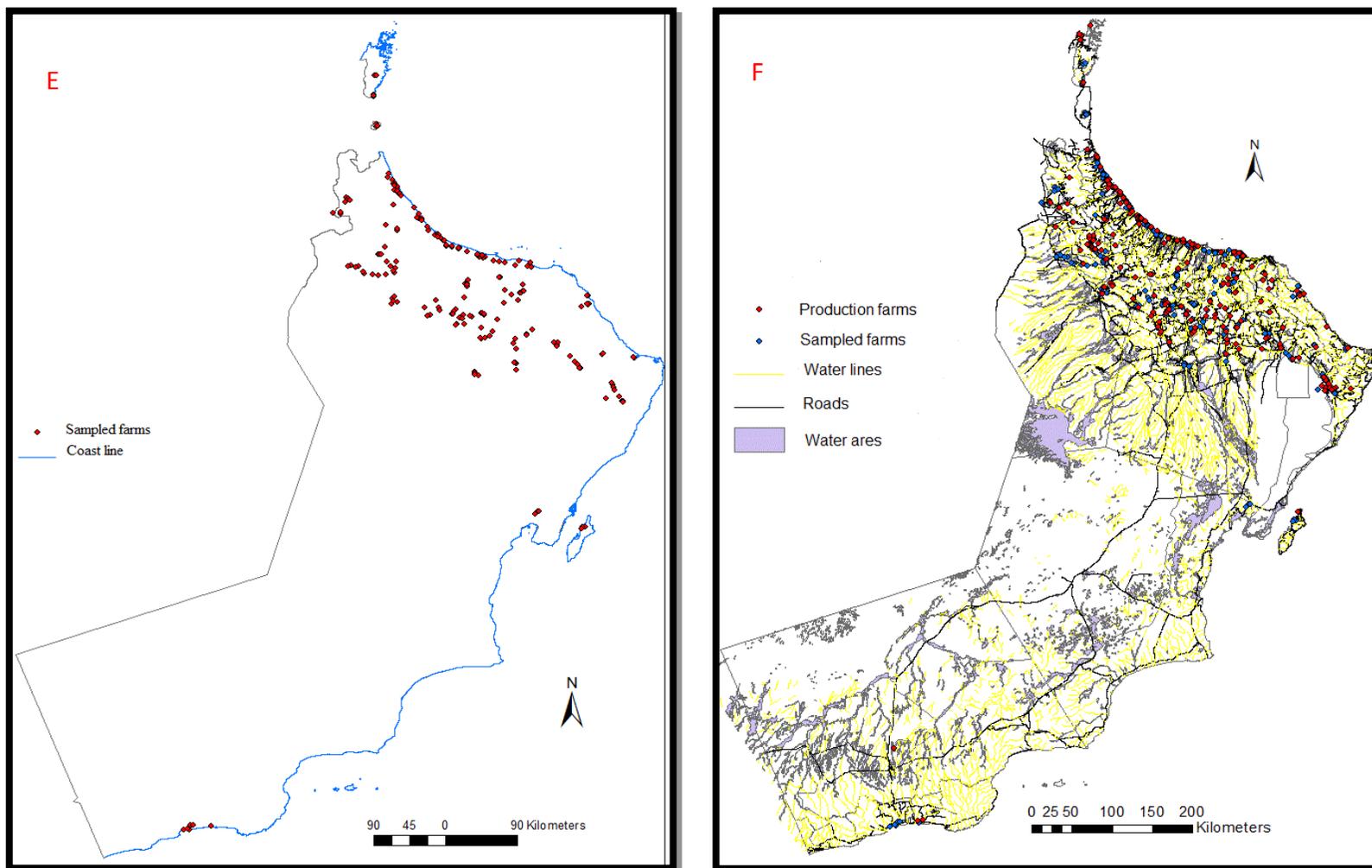
<sup>18</sup> Wadis are temporary rivers



**Figure 5.1** Plotting of location of sampled backyard farms with other risk factors such as production farms (A), roads (B) in Oman map



**Figure 5.1** Plotting of location of sampled backyard farms with other risk factors such as Wadis (C), water areas (temporary lakes) (D) in Oman map



**Figure 5.1** Plotting of location of sampled backyard farms with other risk factors such as coast line (E) and (F) most of the variables plotted together in Oman map

### 5.4.2. Statistical methods

Variables deemed to have a possible association with the probability of a chicken flock being positive for NDV or AIV prevalence were as follows: Region (factor with eight levels: al Batinah, Musandam, Ash Sharqiyah, Ad Dakhliyah, Adh Dhahirah, Dhofar, Al Wusta, Muscat), Altitude in metres (continuous), Flock size (continuous), distance of the flock to the nearest production poultry farm, nearest main road, nearest coast line and nearest surface water (lines or areas) in kilometres (continuous). Flock composition (chickens only, or chickens and other species present). For NDV, the sample AIV seropositivity result (continuous) was also included.

Exploratory analyses in the form of smoothed scatter plots were used together with Generalised Additive Models (GAMs) to assess the relationship between the logit-transformed prevalence and quantitative explanatory variables. GAMs (Wood, 2001) modify the general linear model (GLM) to allow the investigation of relationships between predictor and arbitrary smooth functions of explanatory variables, which are often non-linear.

Because the outcome of interest was the seroprevalence of NDV or AIV in chicken flocks, I fitted a binomial GLM with a logit link to model the relationship between the probability of being positive for NDV or AIV and explanatory variables, transformed where necessary, using quasi-likelihood to allow for potential over-dispersion. Since all explanatory variables are at the flock level, I have a single observation per flock (the number of birds positive,  $k$ , out of number of birds sampled,  $n$ ) and so a GLM-based analysis is appropriate, with quasi-likelihood providing a pragmatic approach to allowing for the over-dispersion which may be evidenced as a result of dependence within farms. Initially I investigated, using graphical approaches and GAMs as described, whether the assumption of a linear relationship between logit-transformed seroprevalence and tested variables (only in the case of the outcome NDV seroprevalence, AI sample prevalence), seemed reasonable. If the estimated degrees of freedom (edf) associated with the smoothed function of each continuous variable in the GAM was close to 1, a linear relationship was assumed to be

appropriate (Wood, 2001); if it was not, I considered transformations of the associated explanatory variable which might improve linearity. Following exploratory analysis, the binomial GLM including all potential explanatory variables, transformed or otherwise, was then fitted, and an analysis of deviance based upon the F test (appropriate because the dispersion parameter is estimated when quasi-likelihood is used, rather than being fixed at 1 in the traditional binomial GLM) was used to determine which variables should be retained in the model. Insignificant variables were removed by backwards selection. The removal of each successive variable was determined by a deviance reduction test as described, with a 5% critical value being used for comparison. Region was included as a fixed effect because the study regions include all the regions of interest. For the explanatory variable Flock composition, chickens only were set as the baseline.

Generalised linear models were fitted using the routine `glm` in R (R core team (2014), available at <http://www.r-project.org>) and GAMs were fitted using the `mgcv` package (Wood, 2001), also in R. R codes are in Appendix 4.

## 5.5. Results

### 5.5.1. Risk factors for NDV seropositivity

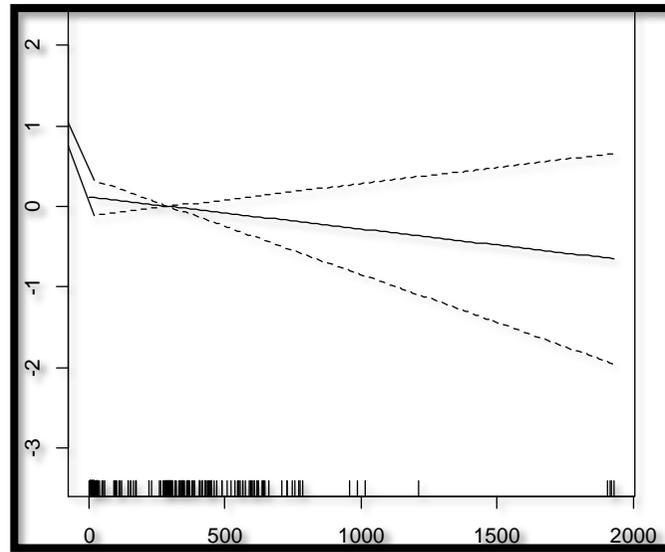
The initial exploration using scatterplots and GAMs suggested that the relationship between logit-transformed seroprevalence and (distance to nearest road and nearest water area) are closer to linear, when variables are square root transformed (the GAM including a smoothed function of distance to nearest road and nearest water area had an edf of 5.27 and 1.91, respectively, while a smoothed function of the square root of both of them gave an edf of 1 and 1.71, respectively). For all other terms, both scatter plots and estimated edfs suggest that their direct inclusion as parametric linear terms in a GLM is appropriate. Figure 5.2 shows the GAM plotting of the NDV seroprevalence with explanatory variables

I hence model the sero-prevalence as a function of altitude, flock size, percentage of birds AIV positive, flock composition ('farm only has chickens' versus 'farm also has other birds', with 1 = multiple species present, 0 = multiple species not present), presence of fighting cocks in the state, distances of the flock to the nearest poultry production farms, surface water (wadis) and the nearest coast line. Also, the square-root of the distance to the nearest road and surface water area (temporary lakes) and region are included in a binomial GLM model.

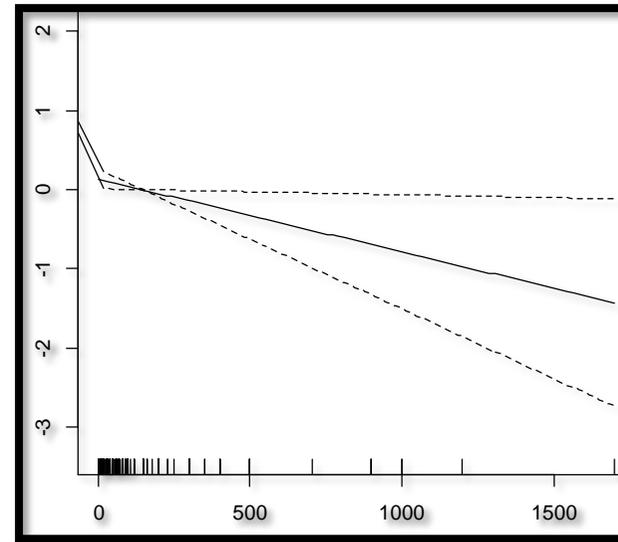
Over-dispersion in this first model was evidenced by the fact that the ratio of the residual deviance and degrees of freedom in the model is 3.51 (when no over-dispersion is present this ratio should be 1), validating the choice of a quasi-likelihood approach to model fitting. The only terms which reduced the residual deviance by a statistically significant amount were region ( $F_{1,219} = 6.95$  for which I obtain  $p < 0.001$ ), and the proportion of birds on the same premises positive for Avian Influenza ( $F_{1,208} = 28.94$  and I again obtain  $p < 0.001$ ). The effect of flock size is marginal ( $F_{1,210} = 3.77$ , which yields a p-value of 0.054). I fitted a further model including only these terms and the analysis of deviance table for this model is presented in Table 5.2.

The model coefficients, standard errors and 95% confidence intervals around parameter estimates are summarised in Table 5.3.

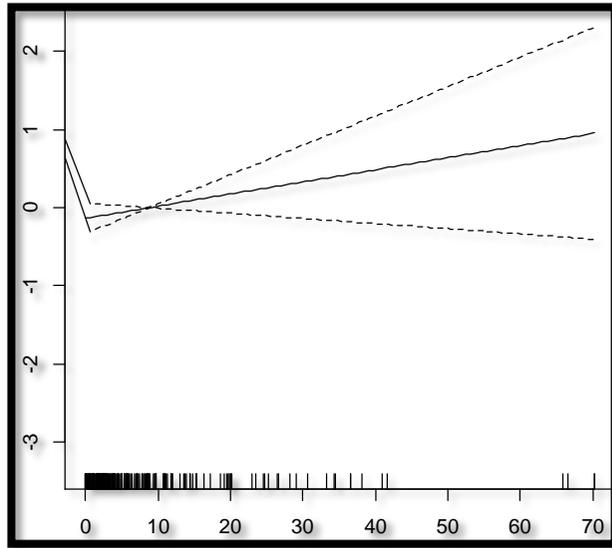
**Figure 5.2** The GAM plot of the NDV seroprevalence with the continuous explanatory variables showing linear or close to linear relations (A-J). The solid line represents the fitted line (the smooth), the dashed lines represent 95% CI and the rugged plot on the x axis represent the data points



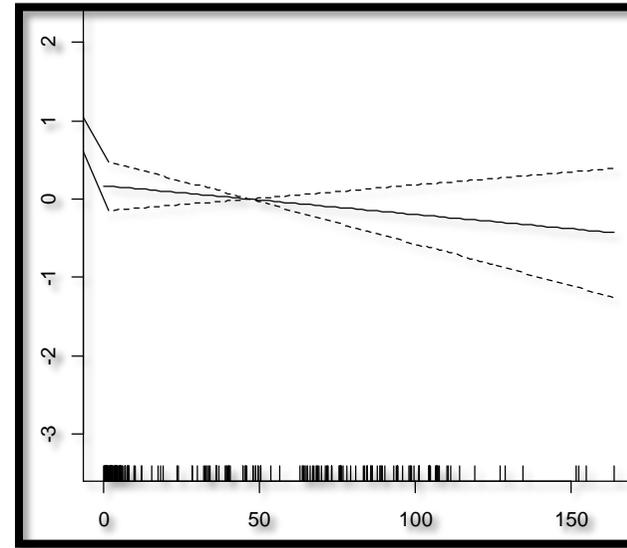
**A.** GAM plot of NDV seroprevalence and the altitude (edf =1)



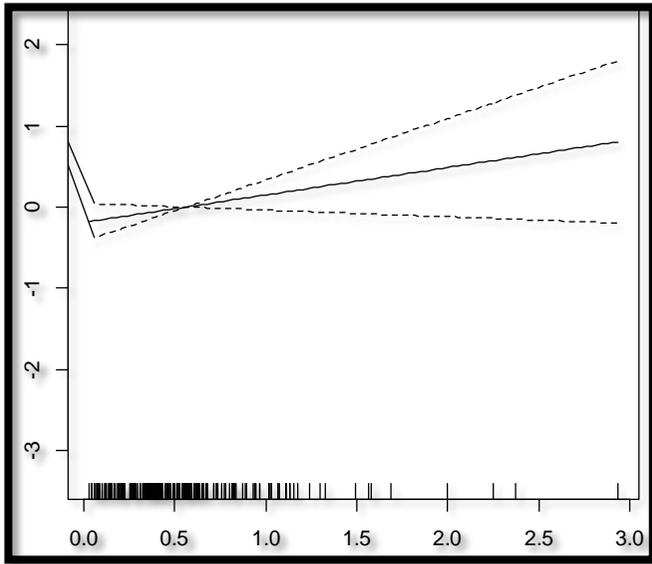
**B.** GAM plot of NDV seroprevalence and the flock size (edf =1)



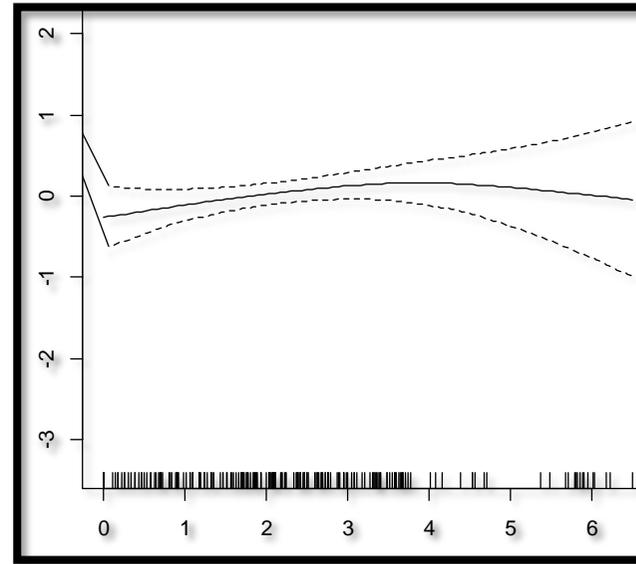
**C.** GAM plot of NDV seroprevalence and distance to near production farm (edf =1)



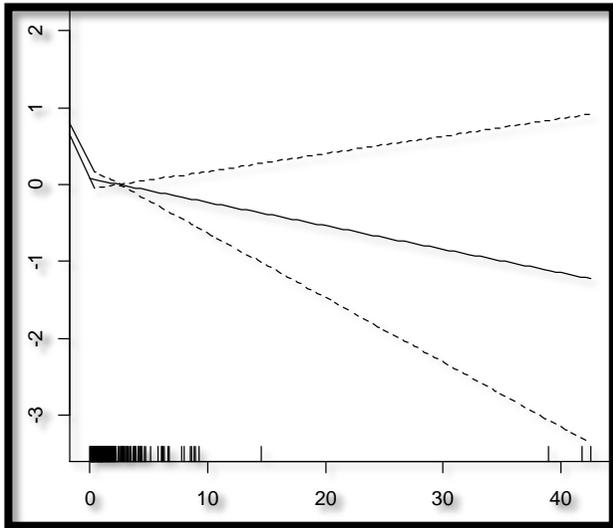
**D.** GAM plot of NDV seroprevalence and distance to the near coast line (edf =1)



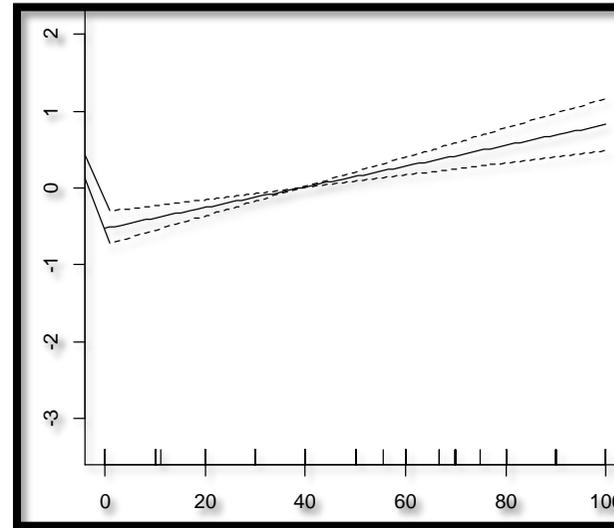
**E.** GAM plot of NDV seroprevalence and square-root distance to the nearest road (edf =1)



**F.** GAM plot of NDV seroprevalence and the square-root of distance to the nearest water area (edf =1.71)



**G.** GAM plot of NDV seroprevalence and the distance to the nearest water line (edf =1)



**J.** GAM plot of NDV seroprevalence and AIV positive percentage (edf =1)

**Table 5.3** Analysis of deviance for the reduced model for Newcastle disease virus (NDV) prevalence in Oman backyard poultry flocks fitted using quasi likelihood methods.

Variable	Degrees of freedom	Deviance explained by term	Residual degrees of freedom	Residual deviance	F-statistic	$P(F > f)$
Null model (constant mean)			219	991.8		
Region	7	149.37	212	842.4	7	<0.001
AI percent positive	1	93.6	210	738.7	30	<0.001
Flock size	1	10.07	211	832.3	3	0.07

There was statistically significant evidence of a regional effect. Taking Ad Dakhliyah region, as the baseline, there was evidence ( $p < 0.05$ ) that chicken flocks in three of the regions (Ash Sharqiyah, Al Wusta and Al Dhahira) may have a higher seroprevalence of NDV antibodies than chicken flocks in Ad Dakhliyah. Furthermore there was marginal evidence ( $p < 0.1$ ) that flocks in one of the other regions (Musandam) is likely to have a higher proportion of chickens positive than those in Ad Dakhliyah. There was no evidence of a consistent North-South gradient in the magnitude of these effects. NDV seropositive chicken flocks are significantly more likely to be AIV seropositive as well. There is also marginal negative effect of the flock size on the NDV seropositive level.

**Table 5.4** Coefficients and standard errors from the reduced model for Newcastle disease virus (NDV) prevalence in backyard poultry flocks in Oman, the Ad Dakhliyah region was the reference for the statistical comparison of different regions; avian influenza virus (AIV) seropositivity is a continuous variable (number of positive birds/ number of sampled birds).

Variable	Coeff	SE(Coeff)	t-value	$P(T> t )_j$
Intercept	-0.8348	0.22708	-3.676	<0.001
Ad Dakhliyah	Ref.			
Al Wusta	-1.3530	0.5646	-2.396	0.0174
Ash Sharqiyah	0.70411	0.27657	2.546	0.011
Dhofar	-0.99257	0.66366	-1.496	0.13626
Al Dhahira	0.84993	0.26228	3.240	0.001
Al Batinah	-0.2620	0.2421	-1.082	0.28
Muscat	-0.4180	0.4056	-1.031	0.303
Musandam	-0.8115	0.49118	-1.652	0.100
Flock size	-0.00078	0.0004	-1.936	0.054
AI Positivity	0.0145	0.00268	5.424	<0.001

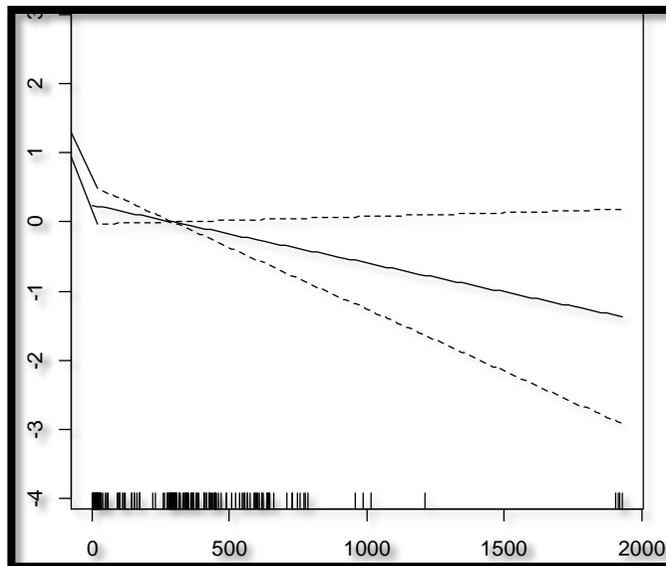
### 5.5.2. Risk factors for AIV seropositivity

A similar analysis to build a model describing the relationship between the proportion of chickens positive for AIV and flock-level explanatory variables was conducted. Exploratory analysis using scatterplots and GAMs suggested that the inclusion of flock size, presence of fighting cocks in the state and distances of the flock to the nearest (poultry production farms, main road, surface water (pounds/lacks), and altitude directly as parametric linear terms in a GLM was reasonable (from the GAM the edf associated with smoothed functions of each variable was close to 1 in all cases). The relationship between logit-transformed seroprevalence of AIV and distance to nearest production farm is closer to linear, when variables are square root transformed (the GAM including a smoothed function of distance to nearest production farm had an edf of 1.32, while a smoothed function of the square root gave an edf of 4.03), therefore the normal value of distance to the nearest production farm was included in the GLM model. The distance to the nearest water line was significantly non linear in both normal and transformed value, therefore, I exclude it from the GLM model (the GAM including a smoothed function of distance to nearest water line had an edf of 2.08, while a smoothed function of the square root gave an edf of 2.81). GAM plotting for the AIV seropositivity and explanatory variables is shown in Figure 5.3.

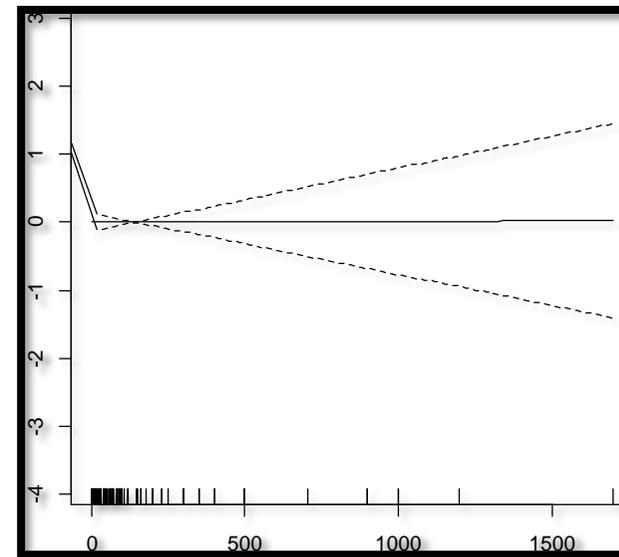
Region and an indicator of whether or not multiple species were present on the same premises were again included as factors (Ad Dakhliyah was baseline in the former, and 1 = multiple species present, 0 = multiple species not present in the latter). Again the potential presence of over-dispersion suggested the need for a quasi-

likelihood-based approach to model fitting, and a subsequent analysis of deviance to assess the contribution made by each of the variables to the model was again conducted with a series of F tests. From the full model (including flock size, altitude, region, presence of fighting cocks in the state, distance to the nearest (poultry production farms, main road, surface water (pounds or lacks) and coast line, and flock composition as explanatory variables), the presence of over-dispersion, confirming the need for a quasi-likelihood approach to fitting, was again evidenced by the fact that the ratio of the residual deviance and degrees of freedom in this model is 4.8. Variables which may be associated with proportion of birds seropositive for AIV are region ( $F_{1,212} = 1.95$  giving  $p = 0.059$ , and altitude ( $F_{1,209} = 3.2$  with  $p = 0.065$ ). Both Regions and altitude variables are significant at the 10% level but not the 5% level. Fitting a second model, therefore, which included only these terms and again conducting analysis of deviance based upon a series of F tests, provides the output in Table 5.4.

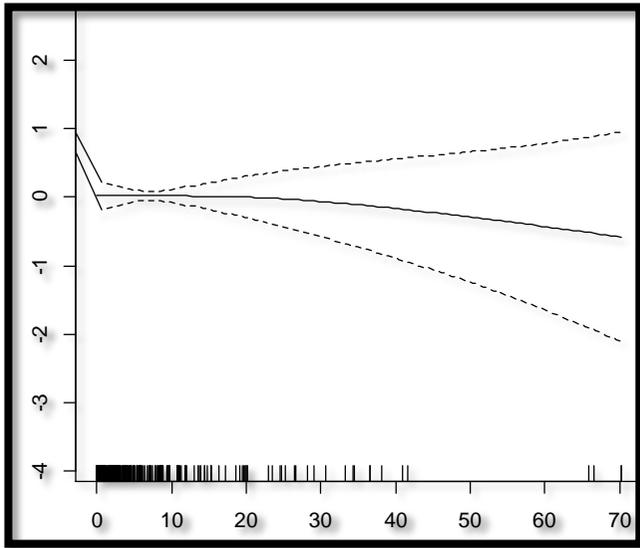
**Figure 5.3** The GAM plot of the AIV seroprevalence with the continuous explanatory variables showing linear or close to linear relations (A-F). The solid line represents the fitted line (the smooth), the dashed lines represent 95% CI and the rugged plot on the x axis represent the data points



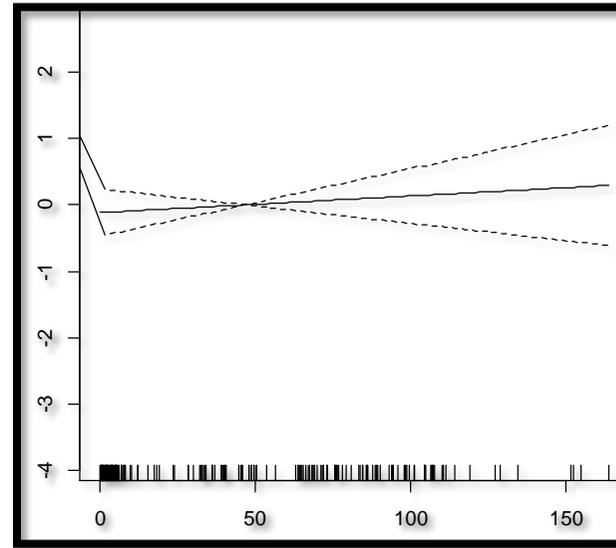
**A.** GAM plot of AIV seroprevalence and the altitude (edf =1)



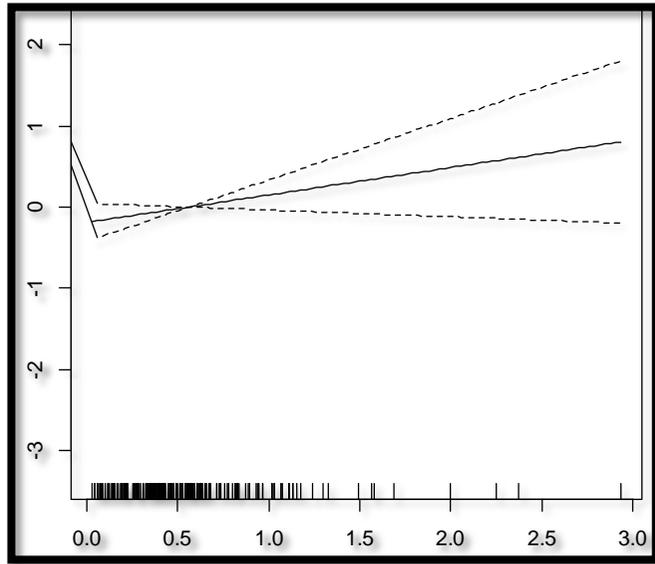
**B.** GAM plot of AIV seroprevalence and the flock size (edf =1)



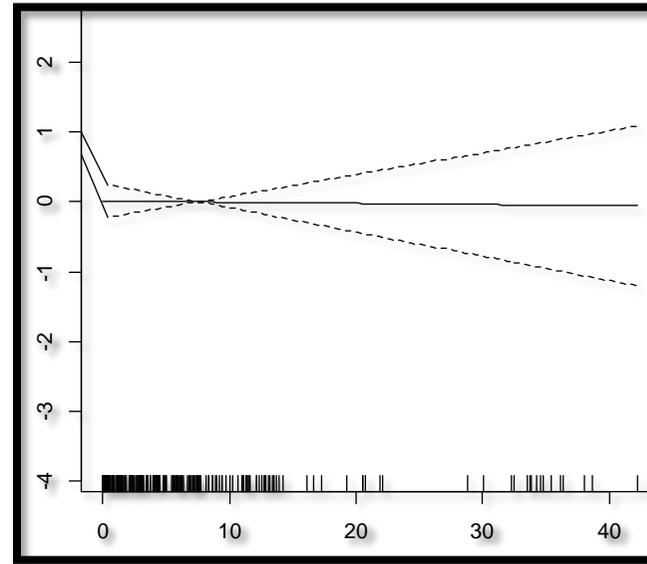
**C.** GAM plot of AIV seroprevalence and distance to near production farm (edf = 1.32)



**D.** GAM plot of AIV seroprevalence and distance to the near coast line (edf = 1)



**E.** GAM plot of NDV seroprevalence and sqrt distance to the nearest road (edf =1)



**F.** GAM plot of NDV seroprevalence and distance to the nearest water area (edf =1)

**Table 5.5** Analysis of deviance for the reduced model for avian influenza virus (AIV) prevalence in Oman backyard poultry flocks fitted using quasi likelihood methods

Variable	Degrees of freedom	Deviance explained by term	Residual degrees of freedom	Residual deviance	F-statistic	P(F > f)
Null model (constant mean)			219	1061.76		
Region	7	54.789	212	1006.97	2.0085	0.0573
Altitude	1	13.512	211	993.45	3.4211	0.065

The summary of this reduced model is given in Table 5.5. There was statistical evidence of a regional effect. Taking the most central province, Ad Dakhliyah, as the baseline, Ash Sharqiyah also was marginal significantly lower than Ad Dakhliyah region, there was evidence that flocks in all regions have experience higher proportions of birds seropositive for AIV than those in Dhofar. There was no clear systematic North-South effect. There was a negative effect of altitude on the seroprevalence of AIV.

**Table 5.6** Coefficients and standard errors from the reduced model for avian influenza virus (AIV) seroprevalence in backyard poultry flocks in Oman. The Ad Dakhliyah region was the reference for the statistical comparison of different regions; altitude (m) is a continuous variable.

Variable	Coeff	SE(Coeff)	t-value	$P(T> t )j$
Intercept	0.076	0.3261	0.234	0.8156
Ad Dakhliyah	Ref.			
Al Wusta	0.0855	0.5638	0.152	0.8654
Ash Sharqiyah	-0.478	0.399	-1.409	0.1603
Dhofar	-2.2	0.8100	-2.5	0.013
Adh Dhahirah	-0.101	0.2940	-0.343	0.731
Al Batinah	-0.323	0.336	-0.963	0.337
Muscat	0.409	0.494	0.828	0.408
Musandam	-0.478	0.534	-0.895	0.372
Altitude	-0.0008	0.0004	-1.779	0.076

## 5.6. Discussion

The migration of wild birds and the international trade in live birds are the main ways implicated of both AIV and NDV transmission between different geographical regions. However, numerous factors play role in the transmission, maintenance and the infection intensity level within local areas. The interactive effect of these factors usually form a local micro-environment that favours the pathogen propagation and dissemination in some areas and inhibit it in others.

Risk factors previously identified affecting the AIV intensity of infection such as a high fighting cocks density, commercial poultry flocks, human population densities (Gilbert et al., 2006; Gilbert et al., 2008; Tiensin et al., 2009), distance of the farm to the main road and river (water streams) (Ward et al., 2008; Martin et al., 2011) land elevation (Gilbert et al., 2006; Gilbert et al., 2008) were all studied investigating HPAI H5N1 virus. However most of those risk factors apply to low pathogenic avian influenza too (Nishiguchi et al., 2007; Tombari et al., 2013). In this study I tried to correlate the applicable weighed variables with both AIV and NDV serological results done for nation-wide backyard poultry in Oman during 2012 summer (chapter 4). No viral RNA was detected in the PCR testing of sampled birds; therefore the AIV genotypes circulating in Oman backyard poultry were not fully identified, though the H9N2 had been identified in Oman and in the neighbouring Middle East countries poultry since more than 2 decades. Each AIV genotype virus has its own characteristics of infectivity and survivability in the environment. Also, each genotype has a bird's tropism. These viral identities lead

us to suggest that viruses circulating in Oman backyard poultry may have different risk factors than these found in previously studied areas.

Although there was a regional effect, there was no evidence of a consistent North-South gradient in the magnitude of the regional effects. Taking Ad Dakhliyah region, as the baseline, there was evidence ( $p < 0.05$ ) that chicken flocks in three of the regions (Ash Sharqiyah, Al Wusta and Al Dhahira) may have a higher seroprevalence of NDV antibodies than chicken flocks in Ad Dakhliyah. Furthermore there was marginal evidence ( $p < 0.1$ ) that flocks in one of the other regions (Musandam) is likely to have a higher proportion of chickens positive than those in Ad Dakhliyah. There is no clear reason for this difference particularly in case of Al Dhahira and Ash Sharqiyah was almost all risk factors seem to be similar to that in Ad Dakhliyah. However, in Al Wusta this higher seroprevalence could be attributed to the type of housing (foraging outside all the day time) and the presence of Barr Al Hikman wet land area where huge number of wild and migratory birds area resting.

The NDV seropositive chicken flocks are significantly more likely to be AIV seropositive as well. This is most probably attributed to the level of biosecurity adopted in the farms. Low biosecurity was found to be a common risk factor for both viruses in other studies (East, 2007; Iglesias et al., 2011). My study finding lends supports to these previous findings. Similar to AIV, most of the risk factors such as population and agriculture density, presence of migratory birds, distance to water bodies, and distance to main roads are applicable to NDV as well.

Although, a previous study done in backyard chicken flocks in Eastern Shewa zone, Ethiopia (Chaka et al., 2013) found a positive association between the flock size and the NDV intensity, this study result find marginally negative significant association. This could be attributed to the level of biosecurity in Oman backyard poultry where the farms having big numbers of birds tend to keep their birds all the day indoors.

Again for the AIV modelling results, there was statistical evidence of a regional effect. There was evidence that flocks in all regions experience higher proportions of birds seropositive for AIV than those in Dhofar. Ash Sharqiyah also was marginally significantly lower than Ad Dakhliyah region (base line region). Again, there was no clear systematic North-South effect. There was a negative effect of altitude on the seroprevalence of AIV. This result is consistent with previous studies on HPAI risk factors (Mannelli et al., 2006; Pfeiffer et al., 2007; Gilbert et al., 2008; Loth et al., 2011; Martin et al., 2011). The reason behind this association is still unclear, although the negative correlation of altitude with high population density and intensive agriculture activities may be one of the reasons, since both in South East Asia and in Oman, both the population and agriculture activities are higher in low-lands areas. Moreover, the presence of wild birds such as sea birds in low-lands near coastal areas may contribute to this result or the level of connectivity of farms in flat low-land areas is more than in hilly areas.

The number of poultry farms (both production and backyard) is a risk factor for the prevalence of AIV (East et al., 2006; Pfeiffer et al., 2007; Gilbert et al., 2008; Loth et al., 2010). Although Dhofar has the biggest mean flock size the number of backyard poultry flocks was low in comparison to the other regions. Also, there are just four

poultry production farms located in remote areas, this situation could be the reason of the low prevalence of the AIV in the backyard chickens in this region.

This chapter provides the first work carried out, to the best of our knowledge, to identify the risk factors affecting the distribution of both AIV and NDV in Oman poultry. With the efforts of the government to encourage poultry production by increasing both farm and bird numbers, it will be helpful to know some of the risk factors for these two important poultry viral diseases. Also, against a background of a paucity of information on AIV and NDV risk factors in Oman and other Middle Eastern countries, this study provides some preliminary information about the risk factors that may help in preparing disease control measures and/or avian flu contingency planning.

Similar studies will build better knowledge about the risk factors and will improve ways of control.

**6- Chapter Six: Molecular detection of infectious  
bronchitis and avian metapneumoviruses in Omani  
backyard poultry**

This chapter was published in a peer-reviewed journal :

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## **6.1. Abstract**

Infectious bronchitis virus (IBV) and avian metapneumovirus (aMPV) are economically important viral pathogens infecting chickens globally. Identification of endemic IBV and aMPV strains promotes better control of both diseases and prevents production losses. Oropharyngeal swab samples were taken from 2350 birds within 243 different backyard flocks in Oman. Swabs from each flock were examined by reverse transcription polymerase chain reaction (RT-PCR) using part-S1 and G gene primers for IBV and aMPV respectively. Thirty-nine chicken flocks (39/243, 16%) were positive for IBV. Thirty two of these were genotyped and they were closely related to 793/B, M41, D274, IS/1494/06 and IS/885/00. 793/B-like IBV was also found in one turkey and one duck flock. Five flocks were positive for aMPV subtype B. Though no disease was witnessed at the time of sampling, identified viruses including variant IBV strains, may still pose a threat for both backyard and commercial poultry in Oman.

## **6.2. Introduction**

Infectious bronchitis virus (IBV) and Avian metapneumovirus (aMPV) are highly contagious viral pathogen of chickens. Both viruses and their epidemiology were reviewed in chapter 1 and 3.

### **6.3. Research justifications**

There is a particular paucity of information from Oman, with almost no published studies of avian respiratory viruses for any species. This is despite Oman's geographic location (between the horn of Africa and southern Asia), its importance as a site for migrating wild birds and the presence of large commercial poultry production farms. These farms produce the majority of the Omani poultry requirements; however census data in 2004 reports there were around 25,000 backyard flocks bred for household consumption (Anon, 2004a). Due to the avian influenza contingency plan implemented between 2004 and 2012, this number has been reduced to nearly 10,000 flocks (Rural Women Development Department, *personal communication*, 2012). Maintaining a good health status of backyard flocks is crucial for both the flock owners and the owners of nearby commercial flocks (McBride et al., 1991). Backyard poultry in Oman are not vaccinated against IBV or aMPV.

This is the first study on the prevalence of IBV genotypes and aMPV subtypes within backyard poultry flocks in Oman.

All the sampling work was done by me for all the Omani regions and governorates in summer 2012. Molecular work was done at the University of Liverpool laboratories.

## **6.4. Materials and methods**

### **6.4.1. Sampling method**

Oropharyngeal swabs were collected from a total of 243 backyard flocks (2350 birds) from 238 backyard farms within all regions and governorates of Oman and preserved in the FTA cards, from June to September 2012. The swabs of each flock/species were pooled in 1.5 ml distilled water and then 80 - 100  $\mu$ l of the mixture was inoculated onto the centre of sampling rings on an FTA card<sup>19</sup> as described in chapter 2.2.

Table 2.2 and Figure 2.2 (chapter 2) show the distribution and location of the sampled flocks from different Oman regions.

Samples were transported to the University of Liverpool, UK, for processing and analysis.

Data such as sampling date, farm location (village, state, and region), species of birds, flock size, species of sampled birds, housing conditions, and water sources were recorded for each farm. Spatial coordinates of the location were recorded using GPS (Garmin GPSMAP 62s, USA) (chapter 2).

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<sup>19</sup> Sigma Aldrich, Dorset, UK

**Table 6.1** The estimated total number of flocks in each region, the number of flocks sampled, the number and type of each poultry species sampled in the eight regions of Oman.

Region	Estimated total number of flocks <sup>20</sup>	Number of flocks	Total number of birds	Hens	Turkeys	Ducks	Geese	Guinea fowls
Al Batinah region	4200	82	792	659	30	88	5	10
Adh Dhahirah region	2000	47	461	451	10	0	0	0
Muscat governorate	400	13	129	110	9	10	0	0
Ad Dakhliyah region	400	39	383	353	0	30	0	0
Ash Sharqiyah region	1600	36	355	333	0	10	0	10
Dhofar governorate	140	7	70	70	0	0	0	0
Al Wusta region	120	8	72	72	0	0	0	0
Musandam governorate	140	11	88	84	0	4	0	0
Total	10000	243	2350	2132	49	142	5	20

<sup>20</sup> Figures kindly provided by Rural Woman Development Department, Oman

#### **6.4.2. RNA extraction from FTA cards**

One circle from each FTA card was removed using sterile scissors and forceps and placed in a bijoux containing 800–1000 µl of TE buffer (10mM Tris–HCl, 0.1mM EDTA, pH 8.0), vortexed and incubated at room temperature for 10 min (Abdelwhab et al., 2011). The supernatant was then used to extract viral RNA. RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen Ltd, Hilden, Germany) according to the manufacturer's instructions and stored at –20 °C until required (chapter 2.4).

#### **6.4.3. aMPV RT-PCR**

RT-PCR was performed on each of the 243 RNA extracts using both primers and cycle conditions as previously published (Cavanagh et al., 1999) and as described in chapter 2. A 268 base pair (bp) band corresponds to type A, whereas a 361 bp band is type B. Positive isolates were typed by comparison of amplicon size to an aMPV type B positive control (chapter 2.4). This PCR protocol has previously been shown to detect up to  $10^{1.56}$  and  $10^{1.51}$   $CD_{50}$ / ml viral concentration of aMPV type A and B respectively from FTA cards and one log higher from TOC medium (Awad et al., 2014b). Subtypes C and D were not examined in this study.

#### **6.4.4. IBV RT-PCR and amplicon sequencing**

All samples were subjected to RT-PCR to detect IBV positive flocks. Primers and cycle conditions were as previously described (Cavanagh et al., 1999) and as described in chapter 2.4.

Table 2.4 (in chapter 2) shows the reverse transcription polymerase chain reaction (RT-PCR) oligonucleotides used for both viruses Ribonucleic acid (RNA) detection in our study.

#### **6.4.5. Reverse transcription (RT) reaction**

The RT reaction mixture included superscript II RT and one of the outer (negative) oligonucleotides (Appendix two). The mixtures were then taken to a separate workstation where 5 µl of the mixture was placed into a 0.5 ml pre labelled clip top Eppendorf tube, after which 2 drops of mineral oil were added. To this, 0.5 µl of RNA was placed under the oil. Positive and negative controls were included in each run. This was thoroughly mixed by vortexing and then centrifuged for 10 seconds at 5000 rpm. The tubes were placed in a thermocycler and run under the following conditions: 42 °C for 1 hour, 72 °C for 10 minutes and then held indefinitely at 8 °C. Immediately after the RT mixture, the following was performed:

#### **6.4.6. Nested PCR 1**

For both viruses, the total volume mixture for PCR 1 reaction was 20 µl. Sufficient PCR reaction mixture was made for all the samples including a positive and negative control for a particular run in a 1.5 ml clip top Eppendorf tube (Appendix 2). The PCR reaction mixture was thoroughly vortexed before dispensing 20 µl aliquots beneath the oil layer in each tube containing the RT-PCR product. The tubes were placed in a thermo-cycler and run under the following conditions: 94 °C for 15 seconds, followed by 35 cycles of 94 °C for 10 seconds, 50 °C for 20 seconds, and 72 °C for 40 seconds and then held indefinitely at 8 °C.

#### **6.4.7. Nested PCR 2**

The total volume of the reaction mixture for one PCR 2 reaction was 24.5  $\mu$ l (Appendix 2). Sufficient PCR reaction mixture was made for all the samples including a positive and negative control for a particular run in a 1.5 ml clip top Eppendorf tube. The PCR mixture was thoroughly vortexed before dispensing 24.5  $\mu$ l aliquots into labelled 0.5 ml clip top Eppendorf tubes, and overlaid with two drops of mineral oil. Then 0.5  $\mu$ l of the PCR 1 product was added beneath the oil layer, tubes were flick-mixed, quickly spun and finally placed in a thermo-cycler and run under the following conditions: 94 °C for 15 seconds followed by 35 cycles of 94 °C for 10 seconds, 50 °C for 20 seconds, and 72 °C for 40 seconds and held indefinitely at 8 °C.

#### **6.4.8. Gel Electrophoresis**

Agarose gels (1.5%) were prepared by dissolving agarose in 1x TBE buffer (Tris borate-EDTA). Once dissolved the agarose solution was cooled by immersing the flask in a beaker of cold water and stained with 2  $\mu$ l nucleic acid solution (Redsafe<sup>TM</sup>)<sup>21</sup>. The solution was then poured into a gel mould (12cm x 9cm) Hybaid Electro-4 gel tank<sup>22</sup> (Appendix 2).

For each sample, 10  $\mu$ l of reaction mix was added to 4  $\mu$ l of loading buffer. After mixing, the 14  $\mu$ l was added to each of the wells in the gel. A 100 base pair ladder

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<sup>21</sup> iNtRON Biotechnology, Inc

<sup>22</sup> Hybaid Ltd, Middlesex, UK

(Molecular marker)<sup>23</sup> was included for amplicon analysis. Gels were run at 75V for 55 minutes in 1x TBE buffer. The gels were analysed using UV transillumination.

#### **6.4.9. DNA sequencing of IBV**

The positive IBV PCR products was cleaned up prior to sequencing by adding 10 µl of PCR II product to appropriately labelled 0.5 ml PCR tubes. To each tube 0.1 µl of exonuclease I (EXO)<sup>24</sup> and 0.66 µl of shrimp alkaline phosphatase (SAP) were added. Tubes were flick mixed, pulse centrifuged and placed in a thermo-cycler for 30 minutes at 37 °C, 10 minutes at 80 °C and 4 °C hold. At the end of the cycle the tubes were pulse centrifuged before transferring 7 µl in to clean, labelled tubes to which 3.7 µl of RNase and DNase free water was added and 1.3 µl of IBV Sx4-oligo. The tubes were sealed with parafilm and sent to Source Bioscience, Nottingham for sequencing.

#### **6.4.10. Phylogenetic analysis and nucleotide comparison**

Sequences were initially analysed in ChromasPRO v1.7.3 (<http://technelysium.com.au/>) to confirm good quality read data. Alignments were carried out in MEGA6 (Tamura et al., 2013) using Clustal W (Thompson et al., 1994). Following alignment, BLAST searches were conducted to confirm isolate identification. Obtained IBV sequences were compared against reference strains for S1 retrieved from GenBank (National Centre of Biotechnology Information). Reference strains used throughout this study were UK/3/91 (Z83977), UK/4/91

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<sup>23</sup> Amersham Pharmacia Biotech, UK

<sup>24</sup> 78250, Affymetrix

(JN600614), 793/B (Z83979), CR88121 (JN542567), IS/1494/06 (EU780077), IS/885/00 (AY279533), Eg/1212B (JQ839287), Q1 (AF286302), QX (AF193423), M41 (GQ219712) and D274 (X15832).

Maximum likelihood analysis was utilised to infer evolutionary trees of both isolate and reference sequences, with default settings and 1000 bootstrap re-sampling. BLAST was utilised for nucleotide and amino acid identity comparisons between representative isolates within identified clusters.

## **6.5. Results**

### **6.5.1. Detection of aMPV**

From the total of 243 flocks sampled during the study, five tested aMPV positive (2.06%). All five samples were from chickens and all were identified as aMPV subtype B. The positive samples came from northern regions of Oman; three from the Ad Dhahirah region (Albrimi, Ibri and Dank), one from the Ash Sharqiyah region (Snaw) and the fifth from the Muscat Governorate (Qurayat). Four of the infected flocks were located within the mainland, with a single flock from the coastal town of Qurayat (Figure. 6.1. A).

### **6.5.2. Detection and genotyping of IBV**

From the 243 flocks assayed using RT-PCR, a total of 39 tested positive for IBV (16.05%) (Figure 6.1. B). Of the 39 isolates sequenced, it was possible to determine the genotypes of 32 (82.05%) using BLAST (Table 6.2). A total of five genotypes were represented within the samples. The majority of samples showed greatest homology to genotype 793/B ( $n = 26/39$ ; 66.67%), with the remaining isolates relating closely to M41 ( $n = 2/39$ ; 5.12%), D274 ( $n = 2/39$ ; 5.12%), IS/1494/06 ( $n = 1/39$ ; 2.56%) and IS/885/00 ( $n = 1/39$ ; 2.56%). All 32 sequenced samples were submitted to GenBank and assigned accession numbers (Figure. 6.2).

The Al Wusta region demonstrated the highest flock prevalence rate (37.5%) followed by the Dhofar Governorate (28.5%). The 793/B-like genotype was identified from all regions and governorates, except the Muscat Governorate which

remained the only region to have no IBV positive flocks. Dhofar Governorate was the only infected region to demonstrate a single genotype (793/B), while the other infected regions had a presence of  $\geq 2$  genotypes. All IBV infected flocks consisted were chickens flocks, with the exception of one duck and one turkey flock.

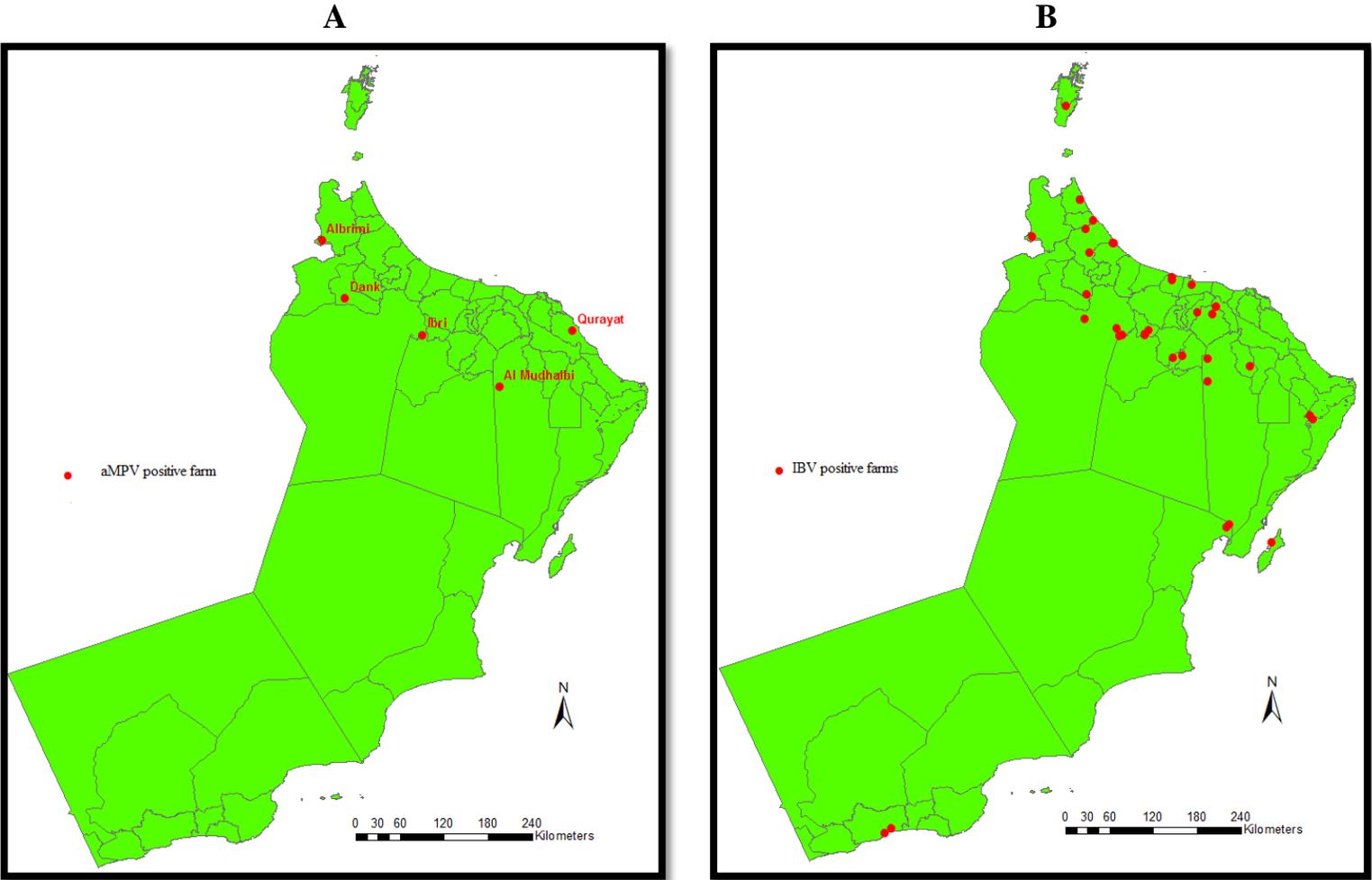
Genotype 793/B was detected in the duck and turkey flocks from Ad Dakhliyah and Al Batinah regions respectively. The 793/B genotype was also detected within chicken flocks from these regions.

### **6.5.3. Phylogenetic analysis of IBV isolates**

Results from the maximum likelihood analysis demonstrate that the isolates formed five distinct clusters (Figure 6.2), relating to genotypes of strains previously reported. Representative isolates from each of the five clusters were chosen for comparison of nucleotide and amino acid similarities (Table 6.3).

The majority of the 26 isolates clustering with UK/4/91 had between 96% and 100% nucleotide homology (resulting in 92–100% amino acid similarity). The IS/885/00-like strain had a higher variation from the reference isolate with 90% nucleotide homology. The three other genotypes demonstrated minimal nucleotide variation from the reference strains: IS/1494/06-like had 99% homology, M41-like had 99% homology and D274-like had 98% homology.

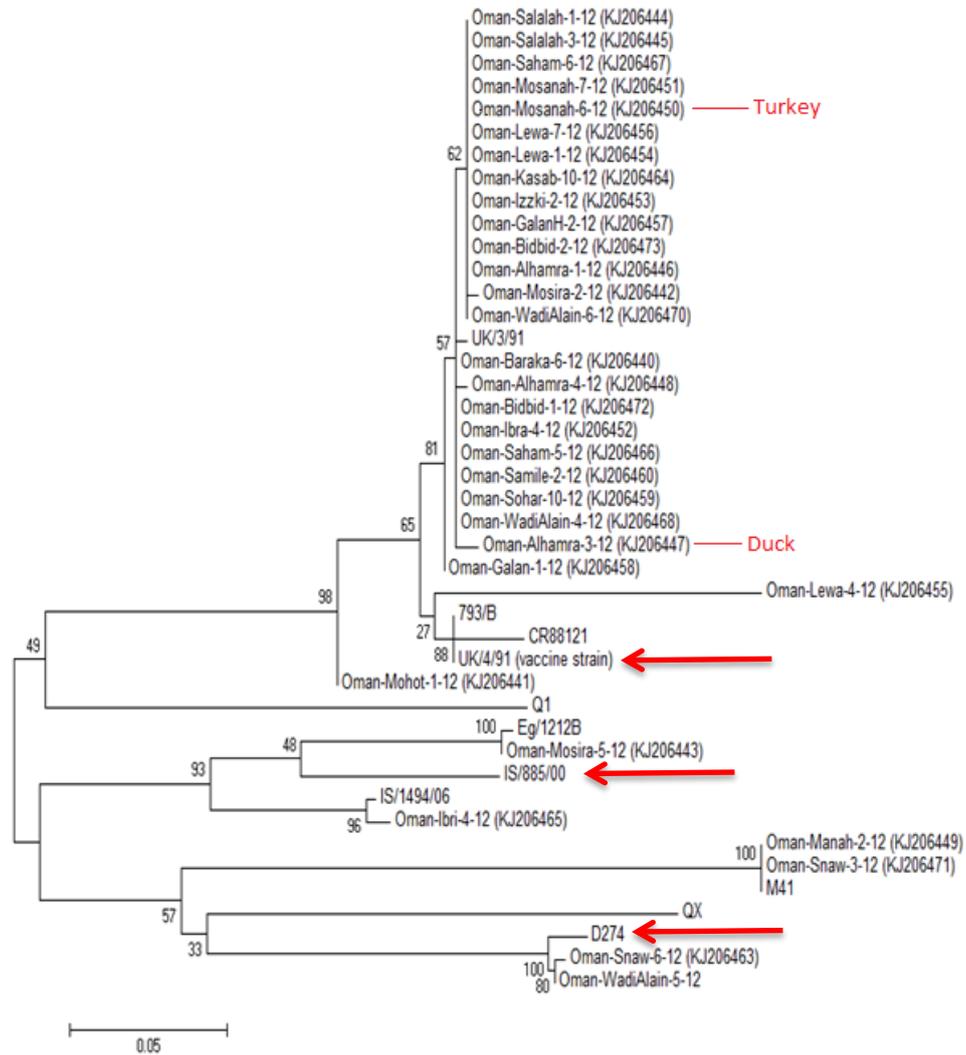
**Figure 6.1:** (A) Locations aMPV positive flocks (n=5). (B) Locations of IBV positive flocks (n=39)



**Table 6.2** Prevalence of identified genotypes within each sampled region from 39 IBV positive isolates.

Region	Total number of flocks	Total number of flocks with IBV+ isolations	Number of IBV positive isolates					Non-interpretible isolates <sup>25</sup>
			793/B-like	M41-like	D274-like	IS/885-like	IS/1494-like	
AL BATINAH REGION	81	12 (14.81%)	9	-	-	-	-	3
ADH DHAHIRAH REGION	47	7 (14.89%)	2	-	1	-	1	3
MUSCAT GOVERNORATE	13	0	-	-	-	-	-	0
AD DAKHLIYAH REGION	41	9 (21.95%)	7	1	-	-	-	1
ASH SHARQIYAH REGION	35	5 (14.29%)	3	1	1	-	-	0
DHOFAR GOVERNORATE	7	2 (28.57%)	2	-	-	-	-	0
AL WUSTA REGION	8	3 (37.5%)	2	-	-	1	-	0
MUSANDAM GOVERNORATE	11	1 (9.09%)	1	-	-	-	-	0
<b>TOTAL</b>	<b>243</b>	<b>39</b>	<b>26</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>7</b>
OVERALL PREVALENCE OF IBV GENOTYPE (%)		16.05	10.70	0.82	0.82	0.41	0.41	
NUCLEOTIDE IDENTITY RANGE OF ISOLATES COMPARED TO GENOTYPE (%)			91-98	99-100	98-99	90	99	

<sup>25</sup> Isolate was sequenced but either failed or returned poor sequence data



**Figure 6.2** Maximum likelihood analysis of 32 isolates (accession numbers included in brackets) obtained from the regions of Oman, and IBV reference strains from GenBank, the isolates obtained from the turkey and duck flocks are highlighted (Oman-Mosanah-612 and Oman-Alhamra-3-12) and arrows indicating the vaccine strains.

## 6.6. Discussion

I present the first study to identify the circulating IBV genotypes and aMPV subtypes in backyard flocks in Oman. For this study, an epidemiologically representative number of samples were collected from different regions of Oman and transferred to the University of Liverpool on FTA cards. The cards inactivate genomic material and allow for RNA extraction once back in the laboratory, proving useful for analysing field isolates away from the point of sampling. The successful use of the cards for both IBV and aMPV has been previously described (Moscoso et al., 2005; Awad et al., 2014b). Using RT-PCR and direct amplicon sequencing, I identified the prevalence of both viruses within backyard flocks in relation to location and poultry species.

Thirty-nine flocks were identified as IBV positive, with an overall prevalence of 16.04%. This finding is lower than previously reported in production farms within neighbouring countries, for example 58.8% and 42.8% in Jordan and Iran respectively (Seyfi Abad Shapouri et al., 2004; Roussan et al., 2009). However both studies sampled chickens within a higher density environment, which may have contributed to the higher prevalence rate compared with the backyard flocks.

In this study, 793/B was the dominant IBV genotype infecting backyard flocks, with an overall prevalence of 66.67% in IBV positive flocks. The presence of 793/B in commercial flocks has previously been reported in other Middle East countries such as Iran, Jordan and Israel (Meir et al., 2004; Seyfi Abad Shapouri et al., 2004; Roussan et al., 2008). In the last few years, 793/B vaccines and strains closely-related to 793/B, have been detected in the Omani commercial chicken (K.

Ganapathy, unpublished data, 2010). The 793/B isolates detected within the backyard flocks formed a close phylogenetic cluster, indicating that potentially the same 793/B strain may be circulating between different Omani backyard flocks and regions. Oman-Lewa-4-12 was distinct from other 793/B-type isolates, with only 92% identity with the vaccine strain, which shows that it could be a virulent 793/B virus circulating in this flock.

The Mass serotype was the first to be isolated in the 1930s (Jackwood, 2012) and adopted for use in early IBV vaccines (de Wit et al., 2011; Jackwood, 2012). Strains belonging to the D274 serotype were first isolated in the Netherlands (Davelaar et al., 1984) and later developed as a vaccine. In the current study, I detected M41-like and D274-like infections within four chicken flocks at a low prevalence of 0.82% each. Both genotypes have been previously reported in commercial flocks in the Middle East (Seyfi Abad Shapouri et al., 2004; Roussan et al., 2008; de Wit et al., 2011), albeit at a much higher prevalence than witnessed during this study. Despite no clinical disease being identified in the M41 or D274 positive birds, both viruses are known for their ability to cause disease in chickens (Bourogaa et al., 2009; Feng et al., 2012).

IBV genotypes IS/1494/06 and IS/885/00 were originally isolated in Israel and are currently circulating in a number of Middle East countries (Meir et al., 2004; Kahya et al., 2013). Here, for the first time, I report the detection of these important Middle East variant IBV in backyard flocks. Even though a low prevalence was witnessed in this study, their importance cannot be ignored due to their ability to cause severe respiratory, reproductive and renal diseases (Kahya et al., 2013). These viruses pose

a threat not only to backyard flocks but also to the commercial poultry industry in Oman and possibly further afield in the region.

As in most ME countries, commercial live attenuated vaccines are used in Oman to control IBV are mainly 793/B and Massachussetts serotypes either as a monovalent vaccine or with D274. In layer farms the booster vaccine consists of inactivated vaccines. Most of these vaccines genotypes were detected in this study, although the majority of the IBV isolates (26/39 isolates) clustering with UK/4/91 had between 96% and 100% nucleotide homology (resulting in 92–100% amino acid similarity). Higher variation was seen in the IS/885/00-like strain which had 90% nucleotide homology with the reference isolate. The M41-like strain had 99% homology and the D274-like one had 98% homology with respective vaccine strain. It appears that the majority of the isolates detected are associated with field viruses.

It is of interest to note that IBV genotype 793/B was detected in a flock of Muscovy ducks from the Ad Dakhliyah region and in a turkey flock in the Al Batinah region. This presence suggests that common circulating IBV genotypes (such as 793/B) could potentially establish an infection in both ducks and turkeys. Using RT-PCR, IBV has previously been detected in apparently healthy ducks in China and Nigeria (Feng et al., 2012; Semeka et al., 2013a). Despite previous reports having identified a turkey coronavirus closely related to IBV (Breslin et al., 1999; Cavanagh et al., 2001), to date there has been only one report of IBV infecting turkeys (Semeka et al., 2013a). The turkey flock was raised in a mixed bird flock farm with chickens and other birds, which allowed the chance for incidental infection from the chickens, or just mechanical harbouring of the virus. However, the duck flock was raised indoors

in isolation from the other birds, there are some chickens in nearby farm that run freely. Up to date there has been little evidence that these two species cause a real threat to commercial birds. To the best of my knowledge, this appears to be the first report of an IBV-like detection in both duck and turkey flocks in the Middle East.

The presence of the RNA of pathogenic IBV genotypes in clinically healthy birds could be explained by a long period of virus shedding (weeks to months) after the birds' recovery, or that the backyard birds breeds are more resistant to IBV infection. Also, adult chickens infected with IBV, in particularly in single infection, likely to have little or no clinical signs.

All five aMPV positive samples from this study were of subtype B and were isolated from five different states within four northern regions, highlighting the sporadic distribution pattern of aMPV in the backyard flocks. Although subtype A was not identified in this study, co-circulation of both subtypes A and B within commercial flocks in the Middle East has been previously reported (Banet-Noach et al., 2005). This appears to be the first detection of aMPV within backyard flocks in Oman. With its involvement in respiratory and reproductive disorders (Georgiades et al., 2001; Jones, 2010), the circulation of this pathogen might pose a threat to backyard and commercial poultry in Oman. As subtype C has only previously been reported in France (Toquin et al., 2006), Korea (Lee et al., 2007) and the US (Cha et al., 2013), and subtype D is only present in France (Bayon-Auboyer et al., 2000), neither of the subtypes were investigated during this study.

In Oman there are many small broiler producers (>1000 birds) with very low biosecurity measures scattered in close proximity to backyard farms. The backyard

flocks have a greater chance of coming into contact with wild birds which could transmit the virus biologically or mechanically. It was not possible to sample the production poultry farms at the same time as sampling the backyard ones. Although it would be a good idea to identify the IBV and aMPV genotypes circulating commercial chickens to try to find out if there is any link between the two production systems.

This study demonstrates the complex epidemiology of both IBV and aMPV in backyard flocks in Oman. The reasons for the predominant detection of 793/B-like over other IBV genotypes and the presence of a single aMPV subtype (subtype B) are unknown.

Further to this, the potential sources of these viruses in the sampled flocks are not known. The detection of 793/B in duck and turkey flocks highlights the possible role of these birds as potential mechanical or biological carriers. With the expanding commercial poultry industry and the increasing role of backyard poultry in Oman, it is essential to improve our understanding on the epidemiology of IBV and aMPV strains for better control of these pathogens.

## **7- Chapter Seven: Seroprevalence of West Nile virus in backyard poultry in Oman**

## 7.1. Abstract

This is the first study to estimate the prevalence of West Nile virus in Oman utilising backyard poultry as a sentinel animals. WN is zoonotic, mosquito-borne viral disease caused by West Nile virus (WNV). Disease is mainly seen in humans and horses, and wild birds generally act as reservoirs. Local birds, such as poultry, can be used as sentinel animals, warning of the presence of the WN virus. WN virus has been known for more than 60 years to cause humans outbreaks in the Middle East. To date, however, only a single outbreak of WN has been reported in horses in March 2003 in Oman, despite the known presence of vector species of mosquito. I undertook a survey of antibody to WN virus in poultry in Oman. Serum samples were collected from 2,350 birds in 243 backyard flocks from all governorates of Oman. Sampled flocks mainly comprised chickens but a few had ducks, geese, turkey or guinea fowl. Samples were tested by ELISA to detect antibodies against WN virus. The total flock prevalence was 45% and the total bird prevalence was 21%. All tested species showed positive ELISA results. Mosquito species recorded on WN positive farms were *Culex quinquefasciatus*, a known vector of WNV, and *Anopheles stephensi*, a malaria vector. The study concluded that WNV is endemic in Oman.

## **7.2. Introduction:**

West Nile fever is a re-emerging disease, caused by a mosquito-borne virus (West Nile virus, WNV) which infects humans, horses, some other mammals and many species of bird (Hubalek and Halouzka, 1999; Petrovic et al., 2013). This virus epidemiology is reviewed in chapter 1.

The Middle East possibly plays a pivotal role in WN epidemiology. It connects Africa (the historical source of the virus) with Europe and Asia, and migratory birds and air flights into and from this area put it at continuous threat of disease introduction or dissemination (Douglas et al., 2007). The virus that entered the USA in 1999 is believed to have originated in Israel (Peterson et al., 2003). Despite this, there have been very few studies of WNV in the Middle East.

### **7.3. Research justifications**

There has only been one report of WN in Oman; a limited outbreak occurred in horses in March 2003 (Ali, 2003). There have been no subsequent reports in horses, nor any cases in humans. However, a study done in 2007 of 750 horse sera in neighbouring United Arab Emirates, after suspicion of a clinically affected horse, found 19.2 % positivity (Wernery et al., 2007) suggesting that the virus is still circulating in the region.

There is also only limited information on the presence of possible WNV mosquito vectors. One study reported six *Culex* mosquito species present in Oman (Harbach, 1985): *C. laticinctus*, *C. perexiguus*, *C. quinquefasciatus*, *C. sinaiticus*, *C. sitiens* and *C. tritaeniorhynchus*.

Here I report the first study of WNV seroprevalence in backyard poultry in Oman. The high production of antibodies against WNV means that chickens are good sentinels for WN disease monitoring (Savage et al., 1999; Otte J et al., 2007; Yapici et al., 2012). I also report the first study of mosquito species present in backyard poultry premises in Oman, as an indication of the possible vector species that are transmitting the virus.

## **7.4. Materials and Methods**

### **7.4.1. Sampling of poultry**

Serum samples were collected from a total of 243 backyard flocks (2350 birds) from 238 farms within all regions and governorates of Oman, from June to September 2012 as described in chapter 2.1.

### **7.4.2. Detection of West Nile virus antibodies**

Detection of WNV antibodies (Abs) by ELISA is the most widely used method to confirm previous or current WNV infection (Rossi et al., 2010). To maximise efficiency and minimise costs, I adopted the strategy of testing pooled samples of the ten bird sera from each flock first, and only testing individual sera from those pools found positive. According to the manufacturer (IdVet ID Screen© West Nile Competition, IdVet, Montpellier, France), during ELISA kit validation studies it was found that the kit detects 22/25 positive samples with dilution of 1:16; so I expect a 1:10 dilution to detect a least 90% of pools with at least one positive in them. Aliquots (10 µl) of the serum from all the sampled birds in a flock were pooled and tested as one sample using the commercial ELISA kit (IdVet ID Screen© West Nile Competition, IdVet, Montpellier, France), following manufacturer's instructions as described in chapter 2.3.

In order to better understand the epidemiology of WNV in Oman, particularly the vectors present in poultry premises, in contact with birds and potentially able to transmit WNV to humans and horses, I did a sampling survey for mosquitoes in sixteen previously identified WNV positive farms.

### **7.4.3. Mosquito Sampling**

#### **7.4.3.1. Selection of sampling farms**

Sixteen farms previously identified as WNV positive were selected randomly from 3 northern regions (Al Batinah, Adh Dhahirah, and Ad Dakhliyah). Figure 7.1 show the location of the sampled farms.

Data such as sampling date, farm location (village, state, and region), species of birds, flock size, species of sampled birds, housing conditions, weather and type of plantation were recorded for each farm (Appendix 1).

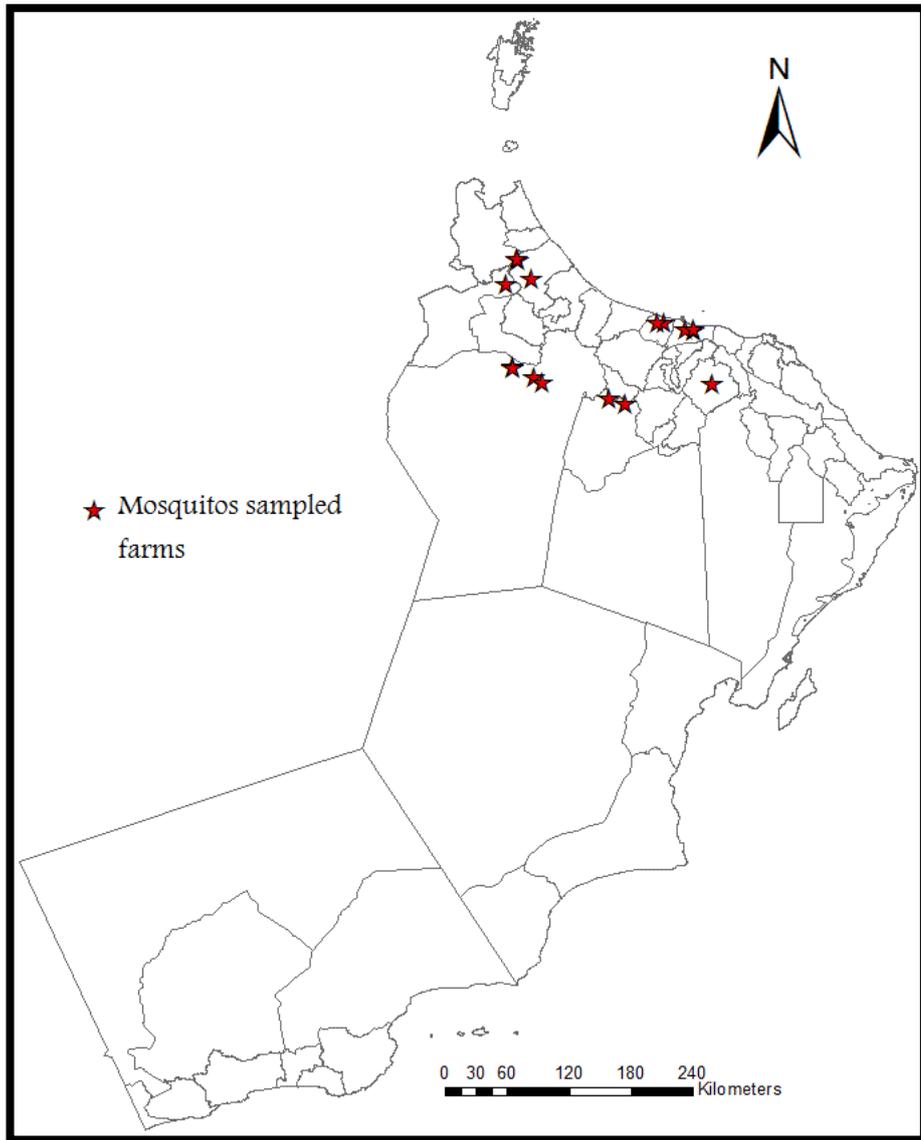
#### **7.4.3.2. Criteria for farm selection**

The criteria used for selecting the backyard farms to be sampled for mosquitoes were that

1. The farm should be one of the previously sampled backyard farm in chapter 2
2. Identified positive for WNV
3. Still have poultry in the farm

#### 7.4.3.3. Random jittering of the mosquito sampled farms

The map of the sampled farms (Figure 7.6) was jittered (Random movement of farms location) using Microsoft EXCEL by randomly adding 0.1 to the degree numbers of the longitude reading of the sampled farms, then plotting the farms on Oman electronic map using ArcGIS. The jittering noise is within 11.1 km.



**Figure 7.1** Map of the mosquitos sampled backyard farms in Oman

#### 7.4.3.4. Mosquito Trapping

A CDC light-trap with a standard 6V 100mA incandescent bulb was hung in each selected farm, about 1.5 metres from the floor just outside the poultry houses (Figure 7.2).



**Figure 7.2** CDC light trap hanging around 1.5 m height, near the backyard poultry house (A & B)

#### 7.4.3.5. Mosquito Identification

Collected mosquitoes were placed directly in a cool box containing dry ice and later killed using insecticide, pinned and labelled with site and region information. All samples were brought to University of Liverpool for identification using published keys (Harbach, 1985; Jayson I, 1992) and confirmed by the key's author (RH).

Keys for *Culex quinquefasciatus* mosquito identification according to (Harbach, 1985)

- 1- Hindungues (the distal part of the claw) is small and inconspicuous
- 2- No post-spiracular setae
- 3- Anterior surfaces of fore- and midfemora and all tibiae entirely dark-scaled
- 4- Abdominal terga with basal pale bands
- 5- Wing entirely dark-scaled
- 6- Subcosta intersects costa before level of furcation of  $R_{2,3}$

## **7.5. Results:**

### **7.5.1. Serological results**

One hundred and ten flocks from the 243 sampled flocks were found positive for WN antibody (110/243, 45%). Those 110 positive flocks were distributed in all the Sultanate regions and governorates of Oman. The highest flock-level seropositivity was in Ad Dakhliyah region with more than 54% seropositive, followed by Al Wusta, Adh Dhahirah region and Al Batinah with 50, 49 and 45% seropositive respectively. Musandam governorate had the lowest flock-level seropositivity (9 %) (Table 7.1 & Figure 7.3). Despite these differences, there was no significant difference in seroprevalence between regions (Fisher Exact Test,  $P = 0.18$ ). WNV seropositivity was detected in flocks of all five poultry species. The single geese flock and both guinea fowl flocks were seropositive, as were half of the duck (7/14) and turkey (2/4) flocks and 98 of 222 chicken flocks (44%). The overall flock prevalence was 45%.

A total of 433 individual samples were tested from the 110 positive flocks, and 88 were positive (20.3%) (Table 7.1). The numbers positive/tested for the different species were: guinea fowl, 3/8 (37.5%); ducks, 8/35, 20.9%; chickens, 77/377, 20.4%), geese (1/5, 20%) and turkeys 0/8 (0%).

**Table 7.1** Percentage WNV seropositivity of flocks and birds in different regions of Oman

Region	Positive flocks %	Number of birds		Percentage of positive birds
		Tested	positive	
Dhfar Governorate	28.6	10	4	40
Al Wusta region	50	18	6	33.3
Adh Dhahirah region	48.9	91	21	23
Ad Dakhliyah region	56.4	94	23	24.5
Ash Sharqiyah region	43	55	6	11
Musandam Governorate	9.1	4	2	50
Al Batinah region	45	144	23	16
Muscat Governorate	38.4	17	3	17.6
Total	45	433	88	20.3

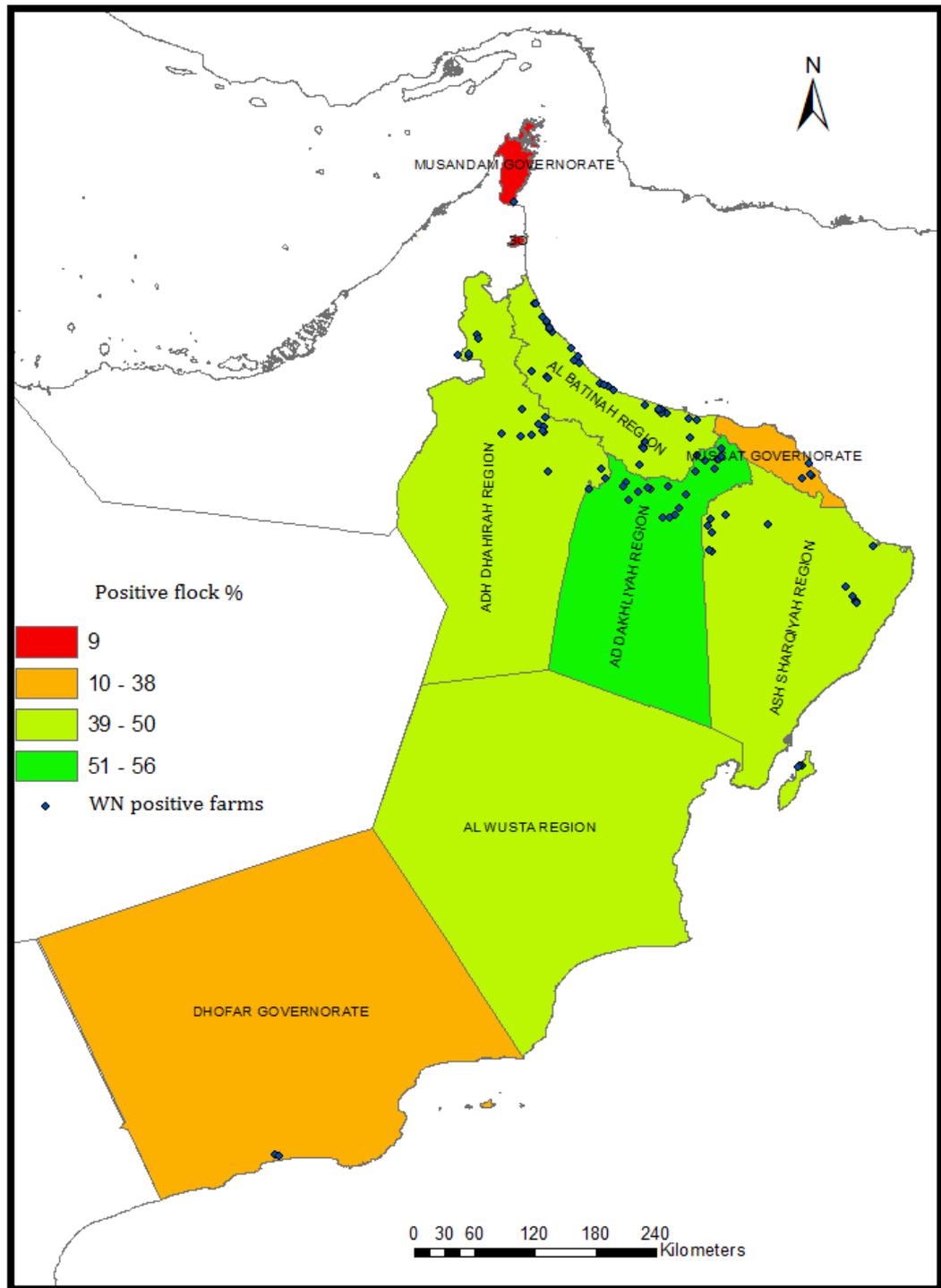
**Table 7.2** Percentage of birds positive for West Nile virus antibodies in backyard poultry in Oman

Species	<i>n</i> of birds tested	Positive birds	
		<i>n</i>	%
Chicken	377	77	20.4
Turkey	8 <sup>a</sup>	0	0
Ducks	35	8	20.9
Geese	5	1	25
Guinea fowl	8	3	37.5
Total	433	89	20.76

A: 8 birds were tested from the 2 positive turkey flocks (8 from 20 birds) and they were all negative

**Table 7.3** Percentage of flocks positive for West Nile virus antibodies in backyard poultry in Oman

Species	<i>n</i> of flocks tested	Positive flocks	
		<i>N</i>	%
Chicken	222	98	44
Turkey	4	2	50
Ducks	14	7	50
Geese	1	1	100
Guinea fowl	2	2	100
Total	243	110	45.3



**Figure 7.3** Distribution of backyard farms positive for West Nile virus antibodies and the percentage of seropositivity in each regions in Oman

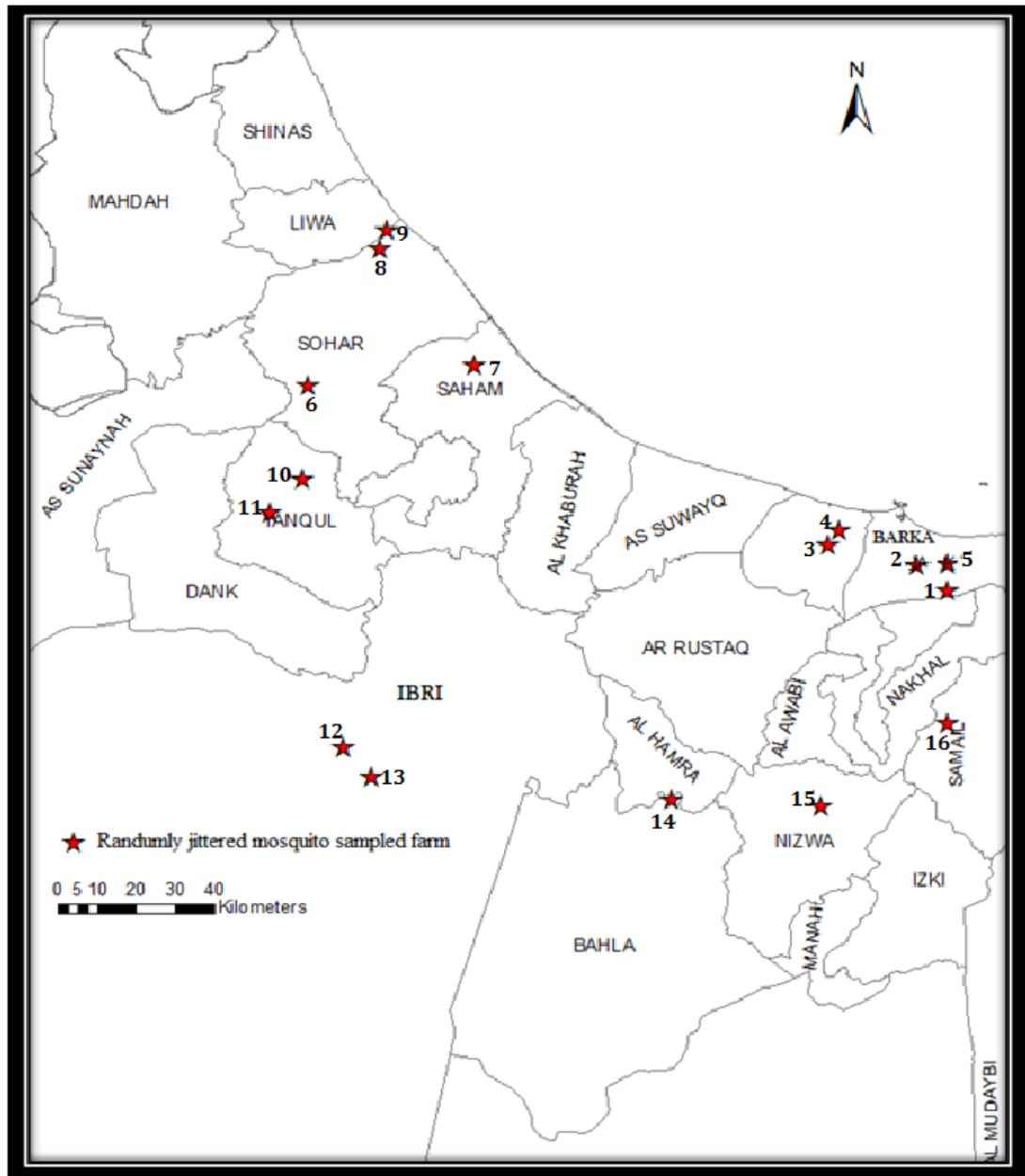
### 7.5.2. Mosquito trapping results

Mosquito trap catches were very small at the sampled poultry houses. I caught 14 mosquitoes in 6 premises and none at the remaining 8 premises. Thirteen of the mosquitoes were *Culex quinquefasciatus* (Figure 7.5) and one was identified as *Anopheles stephensi*.

Figure 7.6 show the randomly jittered (11.1 km) location of the mosquito sampled backyard farms which were previously found positive for West Nile Virus (WNV) and Table 7.4 show the number of mosquitoes trapped from each sampled farm.



**Figure 7.4** Photo of a *Culex quinquefasciatus* mosquito ( Leica microscope, UK)



**Figure 7.5** Randomly jittered (11.1 km) location of the mosquito sampled backyard farms which were previously found positive for West Nile Virus (WNV).

**Table 7.4** Number and species of the trapped mosquitos in the three sampled regions

Farm number	Region	WNV result	Number of mosquitoes	Species of mosquitoes
Farm 1	Al Batinah	0/3	0	Nil
Farm 2	Al Batinah	1/3	1	<i>Culex quinquefasciatus</i>
Farm 3	Al Batinah	¼	2	<i>Culex quinquefasciatus</i> & <i>Anopheles stephensi</i>
Farm 4	Al Batinah	0/3	2	<i>Culex quinquefasciatus</i>
Farm 5	Al Batinah	2/5	1	<i>Culex quinquefasciatus</i>
Farm 6	Al Batinah	1/3	0	Nil
Farm 7	Al Batinah	1/3	0	Nil
Farm 8	Al Batinah	0/3	0	Nil
Farm 9	Al Batinah	0/5	0	Nil
Farm 10	Adh Dhahirah	1/5	0	Nil
Farm 11	Adh Dhahirah	4/5	1	<i>Culex quinquefasciatus</i>
Farm 12	Adh Dhahirah	2/5	7	<i>Culex quinquefasciatus</i>
Farm 13	Adh Dhahirah	2/5	0	Nil
Farm 14	Ad Dakhliyah	2/4	0	Nil
Farm 15	Ad Dakhliyah	1/3	0	Nil
Farm 16	Ad Dakhliyah	0/5	0	Nil

## 7.6. Discussion

Birds develop lifelong immunity if they survive WN infection (Yapici et al., 2012), and poultry can therefore be used as sentinel animals to monitor the circulation of the virus in a given area (Weingartl et al., 2003; Yapici et al., 2012). There have been no previous studies in Oman investigating the prevalence of the virus in birds.

Oman has reported just a single outbreak of WNV in 2003 WN in 19 horses in the town of Al-Seeb at Muscat governorate on the north coast of Oman (Anon, 2003).

Since that time there have been no further outbreaks reported in horses in Oman, nor any cases in humans. It was not clear why there have been no further outbreaks. However, a serological survey done in United Arab Emirates (geographically very close to the north of Oman) found one of each five horses has a serological response to the WN antigen (Wernery et al., 2007).

I did not attempt to find WNV itself in poultry, as the duration of viraemia is short. Instead, I attempted to detect antibody to WNV, as a marker of historical infection.

However, as WNV is within the Japanese encephalitis (JE) virus serocomplex, it might cross-react serologically with other members of this group (Figuerola et al., 2008); in other words, antibodies to other members of the JE virus serocomplex might yield the same ELISA results. Nevertheless, the ELISA is widely believed to be the most suitable method for WNV serological screening (Weingartl et al., 2003; Sotelo et al., 2011). This is particularly so in birds, as many different species can be infected and the use of competitive or blocking ELISAs is the preferred way to overcome the technical difficulties of multispecies diverse immunoglobulins and associated antigenic differences (Sotelo et al., 2011).

Without significantly compromising sensitivity and specificity, it is essential to find ways of reducing the cost of testing large numbers of samples, particularly in surveys (Cahoon-Young et al., 1989). In this study I utilise the ability of a commercial kit (IdVet ID Screen© West Nile Competition, IdVet, Montpellier, France) to detect up to 22 positive samples from 25 total positives using 1:16 serum dilutions (according to the manufacturer).

Therefore, I expect to have detected 90-100% of the pooled samples containing at least one positive individual sample. From our results it was obvious that this method is effective, detecting 110 pooled serum flocks from the total 243 sampled flocks.

My findings show that WNV is widespread in Oman, present in all regions, with an overall flock prevalence of 45% (Table 7.1). As in this randomly sampled backyard birds survey only about 1 in 5 birds had detectable WNV antibody within infected flocks, it is likely that the true flock-level prevalence is even higher, as only unexposed birds may have been sampled in a proportion of infected flocks. Ad Dakhliyah, Al Wusta and Adh Dhahirah regions were the regions with the highest flock prevalences, and Musandam governorate was the lowest.

Domestic chicken (*Gallus gallus domesticus*) develop robust immune responses to WNV that eliminate the virus. The rise of antibodies take place between 5 and 10 days post-infection and may remain protective for life (Pérez-Ramírez et al., 2014). Though, after nearly 6 weeks post-infection the level of immunity will differ between different birds (Reisen and Hahn, 2007). Together with the pooling of samples, this further suggests that the true prevalence of WNV in Oman is higher than the 45% found in this study.

The intrinsic ability of the mosquito vector to transmit the virus (called ‘vector competence’) and its feeding preferences both play major roles in the spread of WNV infection: transmission requires a vector competent mosquito to blood feed on a suitable host (Apperson et al., 2004; Molaei et al., 2006). Different mosquito vector species have different transmission abilities for WNV and different feeding patterns. In Oman, most studies of mosquitoes have focused on the anopheline vectors of malaria, and very little information is available on culicines, the group of mosquitoes that contains the major vectors of WNV. One study listed 12 *Anopheles* mosquito species identified in Oman (Jayson I, 1992), while there is only one previous study identifying 6 species of culicines (Harbach, 1985).

The distribution of mosquitoes can be precisely predicted when accounting for the contribution of environmental and climatic factors (Bisanzio et al., 2011). In the mosquitoes trapped in this study, the timing was poorly chosen, since the Malaria Control Department has reported that in Oman, a higher number of mosquitoes are found during the warmer months (April to July). In Saudi Arabia, Alahmed (2012) and El-Badry et al, (2010) found the mosquitoes activity all-over the year, however there was an increase in the mosquitoes activity with the increase in temperature reaching the peak in June, where the temperature was around 35 °C and the lowest activity was in January, when the temperature was 15 °C. His results also show that *Culex* mosquitoes were the most abundant mosquitoes species (Alahmed, 2012, El-Badry et al, 2010). The low numbers of mosquitoes trapped in this study could be attributed to the time of sampling (January) when the temperatures are usually low (~ 5 to 20 °C). Also, this study result was in agreement with Alahmed (2012) finding *Culex* mosquitoes as the most abundant.

There was a golden opportunity to examine the presence of the WNV RNA in the trapped mosquitos using PCR, although, I did no't do this due to lack of time and funds. Future work could focus more on sampling mosquitos at their abundance peak time and testing them for the presence of the WNV.

In conclusion, despite the low reporting rate of WN disease both in humans and horses, backyard poultry are at a high exposure level in all Oman regions and governorates.

## **8- Chapter Eight: Biosecurity and flock health practices in commercial broiler farms in Oman**

## 8.1. Abstract

Biosecurity and flock health practices are key factors determining the level of productivity in modern poultry farming. A questionnaire survey designed to investigate the management, biosecurity and health practices in Oman broiler production poultry farms was distributed through the Ministry of Agriculture and Fisheries animal production engineers. These engineers visited the farms that met the study criteria in their areas and completed the questionnaires for broiler poultry farms. A total of 69 questionnaires were completed from across Oman. These farms produce around 95% of the total poultry meat produced annually in Oman.

Ash Sharqiyah and Ad Dakhliyah region had the highest number of respondent farms, 24 and 19, respectively. No responses were received from, Al Wusta, Musanadam and Muscat. Just three farms responded from the most southern governorate of Dhofar, these three farms produce 82% of the total meat production from this region. The three integrated large farms produce more than 85% of the total poultry meat in Oman. The majority of the farms (46/69, 66.7%) use closed houses with evaporating/cooling fans and cooling pads; the rest use the natural air flow to cool their houses. Both the general biosecurity level and the managers' knowledge of biosecurity concepts range from only fair to good. However, the bigger farms were judged to have implemented very high and significantly better biosecurity measures ( $p=0.001$ ), and their owners/managers have significantly more knowledge ( $p=0.001$ ). All the farms vaccinate against Newcastle disease and 35/69 (50.7%) also reported vaccinating against Gumboro disease; vaccination against other diseases was reported in fewer than 15% of the farms.

## **8.2. Introduction**

Biosecurity is a series of management practices designed to reduce the potential for the introduction or spread of disease-causing pathogens within and between farms (Donaldson, 2008; Mahmoud et al., 2014). Therefore, it is a crucial part of health management, helping to determine the level of production and profitability of poultry farms (Sims, 2007; Sharma, 2010). Although biosecurity implementation may lead to extra costs in the short term, it is intended to reduce losses in the long term (Sharma, 2010).

Biosecurity starts as early as the choice of the farm's location, which should be based on a sound scientific knowledge of the best ways of production and disease control. Factors such as farm location and house structure will greatly influence the ability to apply biosecurity measures (Appleby et al., 1992; Sharma, 2010). For example, ideally it is always preferable that the new poultry farm be located away from other production poultry farms and backyard poultry to reduce the chance of infectious disease, so if the owner does not plan for the best place in advance he or she will not get the chance to correct it later.

Biosecurity can be categorized into three types; (i) conceptual, which includes the choice of the location of the farm, distance between farms, connectivity with roads and the proximity of surface water; (ii) structural, including physical facilities and fencing that protect the birds from contact with wild birds and mammals, providing good uncontaminated food and water, providing facilities for the disinfection and showering of workers, and the means of the hygienic disposal of dead birds and manure; and (iii) operational, such as the existence of standard operating protocols to be followed during day-to-day work for both visitors and workers (Sims, 2007;

Mahmoud et al., 2014). All three types contribute to achieving the goals of biosecurity measures:; isolation, traffic control and sanitation (Sharma, 2010).

According to their biosecurity level, poultry farms have been categorized by the Food and Agriculture Organization (FAO) into four types. System 1: integrated industrialized poultry production enterprises with highly sophisticated biosecurity levels. System 2: intensive commercial enterprises practising moderate to high-level biosecurity. System 3: commercial farms with relatively poor biosecurity levels. System 4: backyard village-level, scavenging chickens for local consumption where there is no or minimal biosecurity measures (Anon, 2004b; Mahmoud et al., 2014).

In the last two decades poultry production in Oman has increased sharply because of the growing human population and increased export opportunities. For example, table egg production increased from 183 million eggs in 2010 to 236 million eggs in 2012 and poultry meat production increased from 24 million kg to nearly 43 million kg during the same period (Anon, 2013a).

Despite these increases in the amount of poultry production, according to the Oman agriculture census of 2012/13, local poultry meat production only meets 34% of the local poultry meat needs.

Most small farms import fertilised eggs from different sources (India, Jordan, Holland, USA and Saudi Arabia). However, a few integrated farms have their own breeding flocks. These farms apply very sophisticated biosecurity measures and vaccinate against all possible diseases for which commercial vaccines are available. The importation of fertilised eggs is facilitated by a few distribution companies. Also, nearly 60% of the table eggs are imported from different sources (India, Jordan, Holland and Saudi Arabia). Local table egg production companies are few

in number, although all of them apply good biosecurity measures and good vaccination programmes. In general all the farms that produce eggs (fertilised or table eggs) have qualified management with responsible veterinary poultry consultants.

For the purpose of regulating poultry farm production in terms of its effect on neighbouring people's health and other poultry production poultry farms' disease status, as well as regulating poultry meat marketing, all farms planning to produce more than 3000 birds per cycle should obtain permits from the Ministry of Agriculture and Fisheries and the Ministry of Municipalities. These permits bodies make sure that new farms have no or minimal effect on the neighbouring houses because of the high production of ammonia (environmental pollution) or that they are not too close to other poultry production farms by measuring the distance from the farms to the nearest house and to the nearest production poultry farm. These permits allow the farmer to buy and sell products in the market with his own trademark. Farms with fewer than 3000 birds do not require any permission; however, they are not authorized to market their birds in official markets since they do not have a trademark. These farmers usually slaughter their birds manually on their farm and sell them to neighbouring residents.

In Oman, local poultry meat comes mainly from integrated production farms (system 1), followed by the intensive commercial enterprises of system 2 (Anon, 2013a). For example, a farm such as A'Saffa produces around 18 million broilers annually.

The most commonly reared broiler chicken breed is the Cobb 500 breed, which has an average market weight at 35 days of 1.7 Kg (Cobb 500 performance & nutrition supplements). The government, which is aiming at achieving complete self-

sufficiency for eggs and poultry meat, supported the investment in this sector through a system of land and money grants as well as cheap electricity.

### **8.3. Research Justifications**

Many pathogens, such as avian influenza H9N2 and the viruses that cause Newcastle disease, avian infectious bronchitis, avian metapneumovirus and infectious laryngotracheitis, have been diagnosed in poultry flocks in Oman. All these pathogens are controlled by the use of proper biosecurity measures as well as the vaccination of the birds. Both vaccination and biosecurity measures, such as dipping the wheels of cars entering the farms, having fences or walls around the farms, having a place inside the farm to change clothes, not sharing the farm's equipment with other farms, and appropriate means of disposal of dead birds and waste, are all essential practices for poultry farmers.

However most of the production farms are located in agricultural areas where many backyard flocks are also present. Also, a number of small broiler producers have backyard birds in their farms with very little biosecurity measures and most of the farms labourers usually live on the same site, share a lot of their kitchen waste. All of these practises may lead to spread of pathogens present in backyard flocks into the production sector.

Here, a questionnaire was designed to investigate the nature of commercial broiler farming in terms of management, health and biosecurity practices. The questionnaire covered areas like management, biosecurity and health in order to build a better understanding of the production systems and their attitudes, as well as the implementation of biosecurity measures.

## **8.4. Methodology**

### **8.4.1. Study area**

Oman is an Arab country found in the southeastern corner of the Arabian Peninsula, between 16° and 28° N latitude, and 52° and 60° E longitude (Figure 1.1). The total number of residents is more than 4 million people.

### **8.4.2. Data collection**

In the 2012/2013 Oman agriculture census there were 303 identified production poultry farms (Figure 8.1). However, there is no available detailed data about the actual numbers and types of birds on these farms or their purpose of production. However, these 303 farms include all the broiler, layer and breeder farms seen during the census in summer 2012 and include any farm with any number of chickens >50 birds kept indoors all the time and fed for one purpose of production (Agriculture census 2012-13).

From my experience most of these farms produce small numbers of birds (<18000 birds/ annually) avoiding the Ministry of Agriculture and Fisheries and the Ministry of Municipalities requirement for farms producing more than 3000 birds/cycle of a minimum distance of 1.5 Km to the nearest block of houses.

These farms have very low biosecurity measures and only vaccinate against the Newcastle disease virus; therefore, they could be categorized as commercial farms with relatively low biosecurity measures, i.e. system 3 farms.

Production broiler farms feed the official market, i.e. selling their products to the public through supermarkets and other officially designated points. In this study I targeted broiler farms which produce (>18000/ birds annually) and which work with

official permission from the governing authorities. Theoretically, the number of poultry production farms and their distribution can be found at both the Ministry of Agriculture and Fisheries (Animal production department) and the Ministry of Municipalities (Health affair department), who produce the permits; however, I did not find any record of the farms in these two places. Therefore, the questionnaire (both Arabic and English versions are in Appendix 1) was distributed with an official letter via the Directorate of Animal Health to the animal production engineers of the Ministry of Agriculture and Fisheries, who are usually in regular contact with poultry farmers in their various states. These engineers visited the farms producing more than 18000 birds annually and completed the questionnaire.

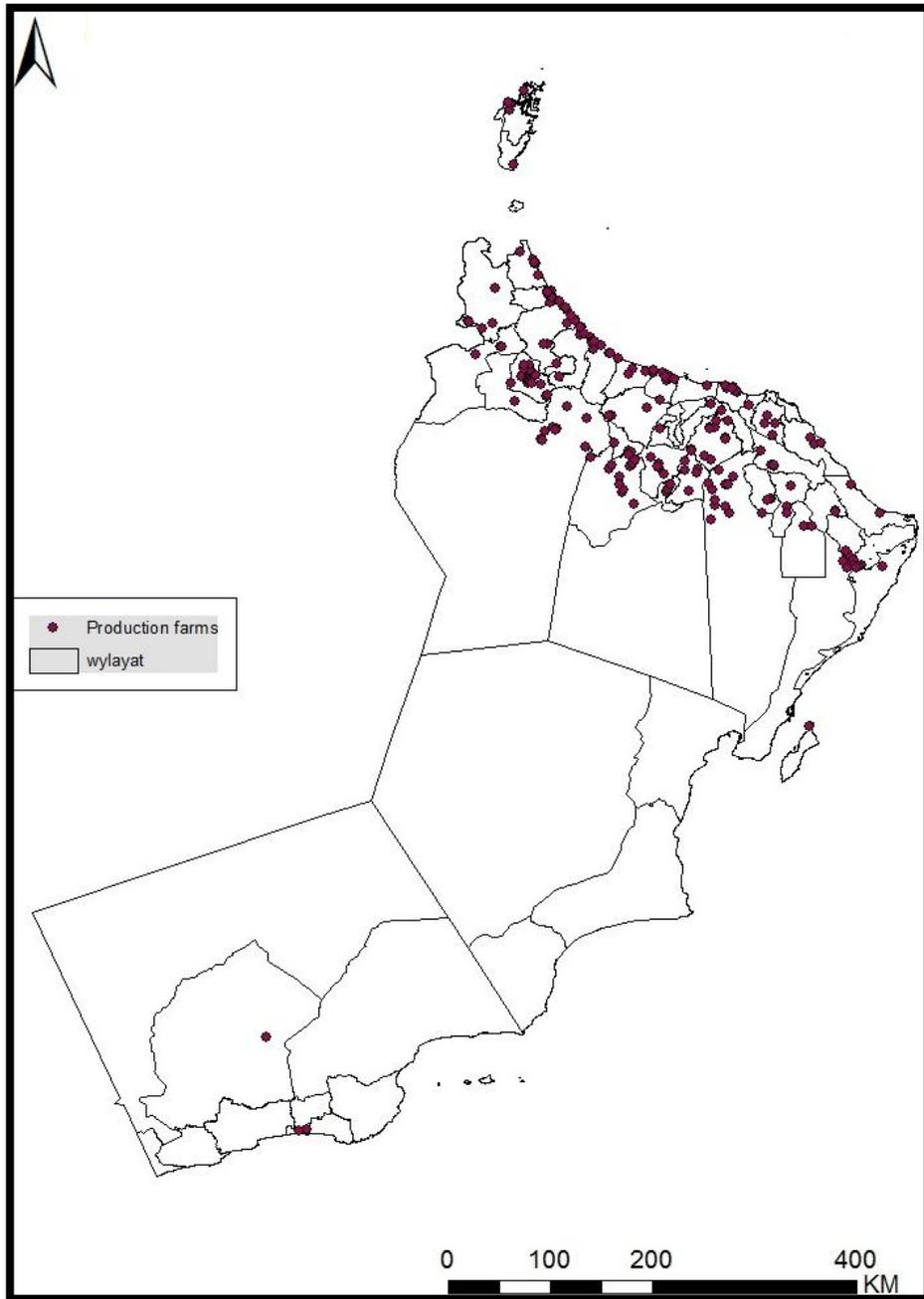
The biosecurity level was judged according to the presence of a wall or fence around the farm, the gate to control entry, requirement to have permission to enter in advance, the presence of a wheel disinfectant dip, presence of a room to change clothes before going to the birds houses, the presence of farm animals and other birds on the farm and the general level of hygiene.

Both the general biosecurity level and the managers' knowledge of biosecurity concepts, which ranged from poor to excellent (Table 8.1), were quantitatively measured by the visiting animal engineers.

**Table 8.1** The scores for both general farm biosecurity and the managers' knowledge of biosecurity concepts

Biosecurity level	Poor	Fair	Good	Excellent
Score	1	2	3	4

**Figure 8.1** locations of 303 production poultry farms reported in the 2012/13 census produced by Ministry of Agriculture and Fisheries, Oman.



### **8.4.3. Statistical Analysis**

Ordinal Logistic Regression was used to measure the relation between different variables using Minitab 17 software <http://www.minitab.com/en-us/downloads/>.

## **8.5. Results**

### 8.5.1 Survey response

Sixty-nine broiler production farms responded to the questionnaire. Tables 8.2 and 8.3 summarize the questionnaire results.

The responding farms produce around 95% of the total annual recorded poultry meat in Oman. Ash Sharqiyah and Ad Dakhliyah had the highest number of respondent farms with 24 and 19, respectively.

Three regions, Al Wusta, Musanadam and Muscat, did not respond to the questionnaires. Although only 3 farms in Dhofar responded, they reported the highest number of birds, nearly 20 millions annually. This large number, about 82% of the total number of birds in all the respondent farms, is due to the presence of the biggest broiler production farm, A'Saffa, which produces around 18 million birds annually.

### 8.5.2 Production characteristics

All the respondent farms (100%) answered the questions on production characteristics. Four of these broiler farms (3/69, 4.3%) are integrated farms with most of the links in the production chain, i.e. they have breeder stocks, hatcheries and slaughterhouses. Two of these integrated farms have breeding birds for their own production and one just has hatcheries to incubate the purchased fertilized eggs.

### 8.5.3 Housing

Just one broiler farm (1/69, 1.4%) uses cages for the rearing of the birds, otherwise all the other farms keep their birds loose on the litter (68/69, 98.6%). The majority

of the farms (46/69, 66.7%) use closed houses with evaporating pads, the rest use the natural air flow ventilation cooling methods in their houses.

#### 8.5.4 Management and biosecurity

##### 8.5.4.1 All in all out policy

About 27% (16/43) of the farms apply an “all in all out policy,” whereby all new chicks are brought onto the farm on the same day and are slaughtered or marketed at the same time. The remainder keep birds of different ages, i.e. the ages of the birds in each production house are different from the others; in this way they maintain a continuous production line.

##### 8.5.4.2 Presence of cock fighting in the area

Four farms (4/69, 5.8%) reported the occurrence of cock fighting in their local area, and twenty seven (27/69, 39.1%) of the respondent farms reported the presence of backyard birds in their neighbourhood.

##### 8.5.4.3 Source of the birds' drinking water

Fifty-eight farms (58/69, 84.1%) answered the question on the source of the birds' drinking water, forty-two farms (72.4%) have their own wells to provide water for their birds, fourteen (24.2%) use public water networks and two (3.4%) still use surface water (Falajs), see figure 8.2.

##### 8.5.4.4 Marketing

Fifty-three farms (53/69, 77%) answered the question on marketing. Thirty-five of them (35/53, 66%) have their own slaughterhouses within the production site.

However, 15 farms (15/53, 28.3%) reported transporting their birds to slaughterhouses belong to other producers and three farms (5.7%) reported that they sell their birds alive in the markets.

**Figure 8.2** Falajs are small rivers used mainly for palm tree farms irrigation in the North of Oman



#### 8.5.4.5 Understanding and application of Biosecurity

Although all the respondent farms have either concrete walls or fences surrounding them, (8/53, 15.1%) have permission to allow entrance on their farm, (25/53, 47.2%) have a place for changing clothes, and both the general biosecurity level and the managers' knowledge of biosecurity concepts range between fair and good (Table 8.1). The bigger farms were judged to have applied significantly better biosecurity measures ( $p=0.001$ ) and that their owners/managers have significantly better knowledge ( $p=0.001$ ). Just nine of the farms have facilities for wheel dipping vehicles at their farm entrance (9/53, 17%), mostly on the bigger farms ( $p= 0.001$ ).

#### 8.5.4.6 Disposal methods of dead birds

Fifty-five respondent farms (55/69, 80%) answered the questions regarding the method of disposing of dead birds. Burial, open burning and closed garbage pens are the most common ways of disposing of dead birds (34.5%, 30.1% and 23.6%), respectively. However, three farms (5.4%) reported using closed incinerators and another three use open waste pens.

#### 8.5.4.7 Disinfection

Although all the farms claimed to disinfect between the bird production cycles, only fifty-six (81.2%) of the farms reported on the types of disinfectants used on their farms. Dettol is the most common disinfectant used by the responding farms (21/56, 37.5%), although other disinfectants like Formalin (25%) and Virkon (18%) are also used. Twenty-eight farms mentioned the frequency of disinfection between breeding cycles (40.6%). The disinfection is generally done twice between each restocking (21/28, 75%). Six farms (21.4%) disinfect just once between every two bird cycles

and one farm reported disinfecting three times between every two successive bird cycles.

#### 8.5.5 Vaccination programme

All the farms vaccinate against Newcastle disease. In addition, 35/69 (50.7%) reported vaccinating against Gumboro disease, and 8 (12%) against the avian influenza virus H9N2. Avian infectious bronchitis (IBV) and Marek's disease were reported to be vaccinated against in 5 and 4 (7% and 6%) farms, respectively. The biggest farm in Oman, A'Saffa, is the only farm that reported vaccinating against aMPV. The larger farms were significantly more likely to report vaccination than smaller farms for all vaccination types except Newcastle disease, which is carried out on farms of all sizes, and aMPV, which was reported by just one farm.

Just 7 farms (7/69, 10.1%) provided their disease record, and they mainly reported respiratory clinical signs.

**Table 8.2** Number of responding commercial broiler farms from each Omani region and their total numbers of birds produced annually.

Region	Number of farms	Total number of birds (million)
Al Batinah	14	1.63
Adh Dhahirah	9	0.821
Muscat	0	0
Ad Dakhliyah	19	1.138
Ash Sharqiyah	24	0.733
Dhofar	3	19.71
Al Wusta	0	0
Musandam	0	0

**Table 8.3** Characteristics of commercial broiler farms in Oman

Question	Response
Production types present in the 69 respondent broiler farms:	
Type and number of birds:	24.03 million birds/ annually, all farms have just chickens
Total annual poultry meat production in Oman (Oman Agriculture Census 2012/13):	43 Million kg/ annually
Poultry meat produced by the responding farms (mean bird weight 1.7 kg):	40.85 million kg (95%) of the total annual poultry meat production annually
Farm size:	<50000 birds: 39 farm 50000 < > 100000 birds: 11 farms 100000 <> 500000 birds: 17 farms >500000 birds: 2 farms
Rearing type:	Litter: 68 Caged: 1
Presence of the farm on a migration route:	Yes: 31 No: 38
Housing type:	Closed: 45 Semi closed: 21 Open: 2
Ventilation type:	Evaporating cooling/fans and cooling pads: 56 Natural air circulation: 11
All in, all out policy:	Yes: 16 No: 43
The owner has other birds:	Yes: 6 No: 56
Workers live within farm site:	Yes: 35 No: 26
Using the ND vaccine:	All farms
Using the infectious bronchitis vaccine:	5 farms
Using the Gumboro vaccine:	35 farms
Using the avian influenza H9N2 vaccine:	

	8 farms
Using the aMPV vaccine:	
	1 farm
Disease records:	
	7 farms recording respiratory clinical signs
Using their own trucks:	
	Yes: 35
	No: 23
Source of water:	
	Well: 42
	Public network: 14
	Surface water (falaj): 2
Means of selling production:	
	In-farm slaughterhouse: 35
	Off-farm slaughterhouse: 15
	Life bird market: 3
Presence of contact of the farm birds with wild birds:	
	All claimed no contact
Presence of farm animals on the farms (sheeps, goats and cattle):	
	Yes: 18
	No: 50
Presence of fighting cocks in the area:	
	Yes: 4
	No: 65
Presence of backyard neighbourhood:	
	Yes: 27
	No: 42
Means of waste removal:	
	Closed incineration: 3 farms
	Open burning: 17 farms
	Burial: 19 farms
	Closed garbage pens: 13 farms
	Open garbage pens: 3 farms
Type of disinfectant used:	
	Dettol: 21 farms
	Formalin: 14 farms
	Virkon: 10 farms
	Mixed: 11 farms
Frequency of disinfection annually:	
	Once between every two successive cycles: 6 farms
	Twice between every two successive cycles: 21 farms
	Three times between every two successive cycles: 1 farm
Number of owner visits per week:	
	1-4 times: 19 farms

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5-7 times: 25 farms  
>7 times: 5 farms  
Distance of the farm to a main road  
>1 km: 19 farms  
1-3 km: 25 farms  
< 3 km: 5 farms  
Presence of wheels disinfectant dip at the farm entrance:  
Yes: 9 farms  
No: 44 farms  
Presence of a fence or wall around the farm:  
All the responding farms  
Presence of a place for changing clothes:  
Yes: 25 farms  
No: 28 farms  
Having permission to enter the farm:  
Yes: 8 farms  
No: 45 farms  
Manager's level of biosecurity understanding:  
Poor: 3 farms  
Fair: 28 farms  
Good: 18 farms  
Excellent: 4 farms  
General biosecurity level:  
Poor: 3 farms  
Fair: 25 farms  
Good: 18 farms  
Excellent: 3 farms

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## 8.6. Discussion

More than 300 poultry farms were identified in Oman during the Agriculture Census 2012/13, most of them in the northern regions. The exact number of broiler farms that produce >18000 annually was not provided by the Department of Statistics in the Ministry of Agriculture and Fisheries for unknown reasons. However, in the 1980s the Ministry of Agriculture and Fisheries, during trials to encourage the modernization of poultry farming, built many small (~1000 birds) semi-closed poultry houses in different regions, some of which are still functioning, albeit with some modifications. In this study and due to the lack of any evidence of the total number of broiler farms meeting the study criteria, I have converted the number of birds produced by the responding farms using the average bird weight at 30 to 40 days for the most prominent broiler chicken breed (Cobb 500) and compared it to the annual total reported poultry meat production in Oman. The 69 responding farms made up around 95% of the total annual meat production.

At planning stage of this study, managers and owners who are directly responsible for production were interviewed, and a questionnaire was designed. To be simple and straightforward, the questionnaire was simply asking the responders to answer Yes/No, sometimes with multi choices or short answers. In this study, the questionnaires were completed by Agriculture Engineers from the Ministry of Agriculture. The engineers were instructed to judge the farms according to what they could see, although some questions needed to be answered by the managers or the owners alone. These questions may have got biased answers when the managers or owners gave what they think is the correct answer, and not what they are actually doing.

Poultry meat other than chicken is not popular in Oman; therefore, it is not surprising that all the responding farms produce chicken meat only.

In this study, I found that most of the respondent farms (56/68; 83.58%) use closed housing systems with evaporating/cooling pads is well justified in a hot country like Oman, where the temperature in most of the regions regularly reaches over 40 °C in summer months. Achieving the ideal environment for birds depends on the appropriate management of poultry houses. Birds perform better in a controlled environment compared to that in naturally ventilated houses (Glatz and Pym, 2013). However, depending on the farm's capacity and financial power, the farmers either choose conventional housing, which mainly provides shade and protection from predators for the birds, or unconventional housing, which controls the micro-environment to allow maximum bird production. In areas where there are very high temperatures, high humidity or huge variations between day and night temperatures, the use of a closed system is vital (Shuaib, 2007), otherwise in areas with cooler temperatures and lower humidity, using semi-closed housing yields good results (Shuaib, 2007).

Although a small number of broiler farms still use surface water, most are using water from well or public water supplies, which has been or later treated with Chlorine. Contaminated drinking water has been demonstrated to be a source of infection in poultry flocks (Shane, 2000) in many countries. A number of pathogens such as *Campylobacter* (Shane, 2000) and the avian influenza virus (Sivanandan et al., 1991) are transmitted to poultry flocks through the consumption of contaminated drinking water. Surface water is more commonly contaminated with pathogens due to usage by other animals and birds. The provision of drinking water is not a major source of infection in Oman as there is little use made of surface water.

Biosecurity aims to decrease the introduction of infection into poultry production farms in three steps: (i) Segregation, or keeping infected materials away from reared poultry birds, (ii) cleaning to remove most of the contaminated materials and (iii) disinfection to destroy the diseases causing pathogens (Wanaratana et al., 2010). The presence of fences or walls around the farm, the presence of a secure gate, requiring visitors to get permission to visit the farm, and limiting the number of visitors and cars entering the farm, all serve this aim. Our study found that large farms certainly apply all these measures; however, most small and medium farms just have fences around them. As in other countries, these small-medium farms are likely to become as source of poultry infectious agents (Bagust, 2013).

Moreover, each farm has a unique risk profile for each disease determined by the presence and interaction of a complicated network of risk factors. These risk factors include the prevalence of infection in the area and the level of disease preventive measures applied on the farm. Therefore, some studies highlight the key role of management and biosecurity in the control of diseases in production poultry farms (East et al., 2006; Sims, 2007; Gunn et al., 2008). This study results show that most of the farms have low biosecurity and their owners or managers lack a good level of knowledge of biosecurity measures; however, the larger farms have significantly better management teams. This result could explain the continuous detection of many pathogens, particularly in small farms.

All farms claim to clean and disinfect; however, the effectiveness of disinfection and cleaning will be different from farm to farm. The individual experience of proper cleaning (physical removal) and the use of the proper concentration of disinfectant will affect this step. Factors such as use of both dry and wet cleaning to allow the removal of all litter, and to satisfy the required contact times for the disinfection

products in order to get the best effect are essential. Moreover, the water temperature, pH and interaction with soap affect the effectiveness of the disinfectant compounds.

The clinical signs of viral diseases such as Newcastle disease have been commonly seen in both production farms and backyard poultry in Oman in recent years (Anon, 2014b) and particularly in the last three years (Anon, 2015a). Biosecurity on poultry farms will be a key component of defence against this diseases (East, 2007). The majority of the respondent farms are categorized according to the FAO categorization to system 2, although the biosecurity is relatively low (fair 25 and poor 3). Just 3 farms have an excellent level of biosecurity, while 21 were assessed as good.

The international trade in poultry meat and eggs gives the opportunity for the global dissemination of more than one hundred poultry pathogens such AIV, NDV and IBD (Cobb, 2011). Therefor the OIE advises member countries to ban the importation of poultry products from countries/areas affected with HPAIV and vNDV (OIE, 2015). However, many trade-transmitted poultry pathogens are not listed by the OIE, and some countries do not report their disease status regularly, either due to a delay in the diagnosis of some outbreaks or a lack of transparency. Therefore most of the importing countries, such as Oman are at continues risk. Therefore, most of the poultry farms have applied the vaccine programmes that were adopted in the country of the source of the chicks. Moreover, the live bird trade creates a very high risk of introducing AIVs and NDV in particular, which can establish infection in many birds with various clinical signs and levels of severity.

The reason for vaccination against NDV in all the farms responded could be due to the wider circulation of Newcastle disease and its devastating effect on poultry production accounts for all the respondent farms vaccinating against this disease. Other diseases such as H9N2 avian influenza, and infectious bronchitis are vaccinated against less frequently. The Gumboro vaccine is the second most commonly-used vaccine by the respondent farms; more than half of them use the vaccine against this immune-suppressing virus. It would ideal for greater use of viral vaccines and vaccination in Oman, as it has been reported that It seems that vaccination is the key component in the control of this resilient poultry pathogens (Nawathe and Lamorde, 1982).

This study has highlighted a number of risk factors associated with commercial broiler farms in Oman. At the beginning of this study, all types of poultry production farms were target and was expected to response, but I very low number of completed questionnaires were returned. Therefore it was decided that all other poultry other than those from broilers farms are excluded from the analysis. Knowledge and know-how on the biosecurity, vaccination, management and flock health could be further improved for a better a control of infectious disease and for better production. The Ministry of Agriculture and Fisheries could play a greater role on educating the producers, particularly the small-medium holders, and enforcing existing rules.

## **9- Chapter Nine: General discussion and future work**

This chapter elaborates on the most significant epidemiological findings reported in the thesis study chapters. Then, based on my findings, I will propose some future work.

Poultry meat is one of the leading expanding sectors world-wide. This due to the short money recirculation and the less area needed. In Oman, the government (Ministry of Agriculture and Fisheries) starts to encourage farmers to rear poultry in a commercial manner since the early 1980s by building hundreds of small (> 1000 birds) open poultry houses for both broiler and layer production. However, with the repaid increase in both, Oman population and money income the demined for poultry production increased, encourage investors to build bigger farms and increase the production. Nevertheless, until 2014 the local poultry production has a huge production gap in broiler, layer and fertilized eggs. The fluctuation of the poultry food price and the infectious diseases seems to be the most hindrance for the poultry farmers.

Infectious poultry disease status in each area is an important factor determining the profitability of poultry production in particular in commercial farms. Those diseases are usually circulating between farms through different means of transmission. Farms applying low biosecurity measures are usually affected more and participate more in transmission of these viruses to other farms particularly backyard poultry farms. For these reasons and that the backyard poultry are not vaccinating against any type of diseases, I selected this sector to investigate some viral respiratory diseases in Oman.

Many factors may account for the introduction of new pathogens into Oman poultry farms. The most important is the importation of poultry by-products (meat, table

eggs and fertilised eggs) from many countries to fill the production gap. This huge importation presents a continuous risk for the introduction of disease-causing pathogens into Oman. Moreover the importation of live birds (mainly show birds) has a high risk of pathogen transmission. The geographical location in Oman in two main migratory pathways may lead to the accidental introduction of some pathogens through migrating wild birds. The increase in people's movements between countries and the movement of commodities may participate in pathogen transmission.

Locally, many risk factors play a part in the dissemination of pathogens between and within farms, particularly viral ones. One of the most important risk factors is the presence of production poultry farms, particularly small broiler farms close to backyard poultry. The sampling process was a good opportunity to see the backyard sector more closely. Many small production broiler farms were just few metres from backyard birds, and labourers working in small broiler farms have their own backyard birds. A number of the sampled backyard farm birds were totally free to graze outdoors all the time and some just during the day time. These practices lead to a greater chance of contact between these birds and wild or migratory birds. Even in farms where birds are caged there were chances for pigeons and sparrows to enter the cages and feed with the backyard birds. The biosecurity was generally low in the backyard farms, although some had a relatively large flock (> 500 birds) and are not applying any type of vaccination. The main source of backyard birds was in-farms breeding, although there were some bigger backyard farms that sold their birds to their neighbours. To the best of my knowledge there are a few live backyard markets in Oman, although the number of bird markets is growing, particularly via the use of internet web pages and social media groups. This connectivity points to a chance of disease transmission within backyard flocks or with other poultry sectors.

One of the earliest difficulties I faced was a lack of published information, and it was obvious from the beginning that there was a knowledge gap of all viral respiratory diseases published in peer reviewed journals in Oman. Therefore, I decided to look at the bigger picture of these viruses in the ME. I did systematic searches of two sources of scientific information: published scientific papers and gene sequences uploaded to public databases. I accessed the University of Liverpool Electronic Library and the gene sequence data from the Emerging Infectious Diseases Database (EID2, University of Liverpool). I know that I will not cover all published articles, as many are published in local journals or journals which can not be found online. For epidemiological analysis, only those articles focusing on the epidemiology of viral respiratory pathogens in the ME were included.

Despite the importance of avian influenza and other avian respiratory viral diseases such as Newcastle disease, avian infectious bronchitis, infectious laryngotracheitis and avian metapneumovirus in term of both poultry production and human health, the true prevalence of these diseases in most of the ME countries is not known and there is a gap in the knowledge on most of these respiratory diseases in most Middle Eastern countries (chapter 3).

It seems that the socio-economic costs caused by highly pathogenic avian influenza in Egypt encouraged Egyptian scientists to explore the disease more than other diseases which endanger poultry farming there. Neither the published articles nor the genomic sequences uploaded to the gene bank are sufficient to give a complete epidemiological picture of these studied diseases in the Middle East. To my knowledge, chapter 3 is the first review addressing the epidemiology of these viruses in the Middle East area. Genomic sequences, in particular for the AIV and IBV genotypes, forms an important consideration in designing effective control strategies

through proper selection of vaccine and vaccination programmes. We should know the circulating low pathogenic genotypes of the AIV (eg. H9N2 clade), not just to reduce their direct effect, but also to avoid their interaction with the control programs of any HPAIV, if it has been detected. Researchers should look for both articles and genomic sequences in order to reach a better epidemiological understanding of the studied diseases in the Middle East.

Diseases such as Newcastle disease, avian infectious bronchitis, infectious laryngotracheitis and avian metapneumovirus should be paid more attention by all the Middle Eastern countries in order to understand their epidemiology better and to find the best methods of control and eradication. In Oman in particular, despite the circulation and vaccinations used in the production poultry farms of ND and AI (H9N2), the viral and epidemiological information is extremely scarce. The IBV, ILT and aMPV are even ignored by the vaccination programs of almost all the production farms.

Then, I did a two-stage cluster random sampling survey of backyard birds taking blood (serum) samples for serological testing, and oropharyngeal swabs. The serum samples were tested using ELISA for three disease viruses (AIV, NDV and WNV) in the Veterinary Research Centre in Oman. Both the oropharyngeal swabs for each flock were pooled and ~100 µl was poured into a FTA card and transported to Liverpool University Lab where the oropharyngeal swab cards were tested for the presence of the RNA of four viruses (AIV, NDV, IBV and aMPV) using PCR protocols. The sampling itself took around 4 months during the hottest months of the summer (June to September 2012).

Chapter 4 described a surveillance study carried out on both avian influenza and Newcastle disease in Oman backyard poultry. This study tried to exploit the absence of vaccination in the backyard poultry in order to investigate the field viruses' immunological responses using antibody detecting ELISA with trials of identifying the genotypes of circulating viruses using PCR. Also, this study utilized FTA card technology to overcome sample transportation difficulties. The serological results for both studied diseases were almost similar to those found in backyard poultry in the neighbouring country of Iran which could be attribute to the geographical, environmental, type of rearing and type of viruses circulating in both countries. The study concluded the endemic nature of the two studied viruses in Oman backyard poultry; however, due to an unknown reason we could not find any positive PCR results, which means that the complete picture of the circulating genotypes will remain unknown.

The negative PCR results could be attributed to many possible explanations. The first is the choice of adult healthy birds for the sampling. However, to do a random sampling survey you have to minimize the bias causes as much as you can. If I had told the owners of the farms visited that I a'm sampling the birds for the presence of diseases without giving him/her a guidelines he/she will bring the sickest birds or the youngest as they are the easiest targets he/she will find. This approach would also have made it impossible to estimate the prevalence of exposure/disease in Oman, as it would not have been random.

The second possible explanation is the pooling of swabs in distilled water and then inoculating 100 µl of the water into the FTA card, which may lead to the dilution of the virus content if present. The third explanation is the FTA card preservation and

transportation, which may lead to the partial distraction of the viral genome in the FTA card. The fourth explanation is, that the RNA extraction step done incorrectly.

However, these explanations are not reasonable, since two other viruses were detected in some of the same FTA cards and previous study has proven the validity of the FTA cards in the transportation of the other two viruses.

The most reasonable causes of the negative results were one of the following:

- The PCR method used was not the most sensitive, and the labs recently changed to the use of (rRT-PCR) which has better sensitivity.
- The true absence of both RNA viruses, as I was sampling clinically healthy birds.

For avian influenza in particular, where there is huge difference between different genotypes in terms of effect and the control eradication process, it is important to further identify the viruses genotypes using different sampling approaches (targeting sick and targeting young ages birds) in order to identify the circulating genotypes.

With the essential need for understanding the factors attributed to the transmission and spread of the AI and ND, chapter 5 utilized the serological data in chapter 4 to study the association of a number of previously identified risk factors with the intensity of the AI and ND infections in Oman backyard farms using GLM model. The serological results show a regional effect on both diseases; however there is no north south pattern. Also, there is a highly significant association between the presence of AIV and NDV infection which could be attributed the fact that most of the risk factors applied to AIV, such as population and agriculture density, presence of migratory birds, distance to water bodies, distance to main roads and general biosecurity, are applicable to NDV too.

Furthermore; as previously reported there is negative association between the farm altitude and the AIV intensity of infection; our results were similar to these findings.

The reason behind this association is still unclear, although the negative correlation of altitude with high population density and intensive agriculture activities may be one of the reasons, since both in South East Asia and in the Oman, population and agricultural activities are more intensive in low-lands areas. Also, it could be that the level of connectivity in flat low-land areas is more than in hilly areas.

The flock size was marginally negatively affecting the NDV infection rate. Further studies on the risk factors of these diseases in the commercial poultry farms or inclusion of more variables may improve the knowledge of transmission and control of these diseases.

Chapter 6 described a molecular survey of the field circulating IBV and aMPV genotypes in Oman backyard poultry flocks. RT-PCR was done for the pooled oropharyngeal swabs poured into FTA cards (chapter 2), followed by genomic sequencing. This study shows the practicality of the use of FTA cards technology in transmitting IBV and aMPV samples. This study found 5 IBV genotypes dominated by the 793/B like and aMPV subtype B circulating in the backyard poultry flocks. Also, the IBV RNA was isolated from turkey and duck backyard flocks. Although the presence of RNA material of IBV in turkey flocks may be surprising, it could be an accidental infection whereby the turkeys catch the IBV virus from the chickens birds running in the same farm without establishing an infection. The duck flock was more isolated, reared indoors, but still there is a big chance of getting the virus accidentally from a neighbouring chicken flocks.

This result indicates the needs for the further investigation of the roles of turkeys and duck in IBV epidemiology. More studies are needed to investigate the IBV and aMPV genotypes circulating in production farms and the probable link between the viruses of these two sectors.

To my knowledge this is the first published study for both economically important viruses in Oman poultry. Similarly the IBV and aMPV genotypes affecting production farms should be identified to improve the vaccination programs for these two pathogens.

The presence of representative random samples from Oman backyard poultry, encourage me to test the prevalence of one of the important zoonotic human diseases. The West Nile disease virus was diagnosed as causing humans illness in some Middle Eastern countries more than 70 years ago. This disease attracted huge attention after its arrival in America in 1999. Since the only outbreak reported in Oman in 2003, which affected a number of horses in the Omani capital area there have been no further reports. However, a serological survey on 750 horses done in the neighbouring UAE found a 19.2% positivity result. Chapter utilized the backyard birds as sentinel for the presence of the virus. I adopted a new testing method (testing pooled flock serum then testing some random bird sera from the positive pooled tested flocks) without compromising the sensitivity or specificity. This method greatly reduce the coast and labour work particularly in a national survey as I did.

The results show that 45% of the sampled backyard flocks were positive for WNV. Also, this study identified *Culex quinquefasciatus* near some positive backyard flocks. This culicine mosquito is a main WNV vector in America. It is still unclear

why there have been no further reports of this disease in Oman, either in humans or in horses.

More studies are needed to identify the circulating genotype, since there are remarkable differences between the clinical effects of the different WNV genotypes. It seems to me that there is under-reporting of this disease in humans since most of the clinical signs are similar to other viral diseases. Some cases of WN fever might, for example, be confused with other causes of encephalitis, such as dengue. Further studies are essential to investigate the level of exposure of human and horses to the virus. In addition more studies are required of the probable vectors that play a role in the WNV epidemiology in Oman.

The close interaction between the backyard poultry sector with other poultry production sectors gives rise to the idea of exploring the management, biosecurity and health practises applied in the production poultry farms using a questionnaire-based study. I started collecting information about the number of farms for each sector from the Ministry of Agriculture census, but I found that the census used different definitions of production poultry farms than those I was using. Then I decided to distribute the questionnaire through the animal production engineers in the government agriculture devolving center in each state. These engineers are usually in daily contact with poultry farmers and know their locations. However, the number of expected responses was unknown to me at this stage. There were 70 responding farms (69 broiler, one layer), so I decided to limit my analysis to the broiler farms.

In the last few years there has been a big increase in poultry production in Oman. Nevertheless, there are still big production consumption gaps in all aspects of poultry

production. Management, biosecurity and health practices are vital to improve this sector and there by minimize losses. I tried to analyze the biosecurity and health practices in Omani broilers production farms using a questionnaire completed by 69 broiler production farms (chapter 8),

The result shows that the level of application and understanding of the biosecurity is generally low. Good understanding of biosecurity concepts with enforcement of the application of biosecurity in broiler farms is required to improve the production.

In term of health practics all of the farms vaccinate against NDV and at a lower intensity for the Gumboro, IBV and H9N2 viruses.

With the continuous circulation of Newcastle disease in these vaccinated farms, the Omani veterinary authority has reported many outbreaks to the OIE in the last three years, the reason for these outbreaks could be poor biosecurity measures, which helps in further spread of viruses to other backyard and commercial flocks. The reason behind the failure of the vaccine to control the virus in infected flocks should be identified. Many aspects related to the vaccine and vaccination process such as the cold chain, the administration of the vaccine and the water source should also be examined. The Ministry of Agriculture and Fisheries should pay more attention to the poultry producers' education and biosecurity enforcement in all types of farms.

Although most of the questionnaire's questions were completed by qualified engineers after personal visits to the farms, some questions, such as those on the daily practices and vaccine programs, were answered by the owners or managers. These individals might have given the best answers they could think of, but not always the true answer. .

To conclude, the thesis shows the endemic status of the LPAI, ND, IB, aMPV and the WN viruses in Oman. The lack of knowledge of these pathogens including the genotypes/subtypes in Oman.. Also, the thesis highlight the weakness of the biosecurity measures applied in broilers farms in Oman.

## 10- Chapter ten: References

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## **11- Appendice**

### 11.1. Appendix 1 Data collection forms

#### Form of Sample Collection from the Backyard farms

The sample serial Number : 1 barka

The date of collection of the sample: 18/6/12

The region of the farm : Albatenah

The state and The village : Barka / Algabat

The owner name : Majdi Metwali A njar

Number of samples taken and type of birds: 4 adult chickens

Number of birds in the farm : 4

Type of birds in the farm : Local chickens

Type of sampled birds : Serum, oropharyngeal and cloacae swabs

Housing open

Type of food : local

Why of watering: Indoor traditional

Source of water: well

Long:                      Lati:                      Altitude:

Appendices

The questionnaire used for the investigation of management, biosecurity and health practises applied in Oman production poultry farm (Arabic and English version)



تحديد مناطق الخطر لدخول انفلونزا الطيور الى مزارع الدواجن بالسلطنة وانتشارها بقطعان الدواجن عزيزي المشارك يهدف هذا الاستبيان لتحديد المناطق الاكثر خطورة لدخول مرض انفلونزا الطيور للقطعان الدواجن. وبأذن الله سيتم تحليل نتائج الاستبيان التعرف على المناطق الاشد خطورة لدخول المرض واستيطانه والممارسات التي قد تؤدي الى هذا الانتشار لا سمح الله، لذا نرجو منك التعاون معنا علما بان نتائج الدراسة ستكون لصالح مربى قطاع الدواجن حيث سترشد صانعي القرار لكيفية حماية الدواجن ضد هذه الافة.

الاستبيان سيكون في ثلاث فقرات

الفقرة الاولى: معلومات عامة عن المزرعة (اسم المالك ، الموقع، .... الخ)

الفقرة الثانية: معلومات حول نظم الانتاج

الفقرة الثالثة: معلومات حول الوضع الصحي والامن الحيوي

لذا نرجو:

- قراءة الأسئلة بتأني
- للإشارة للإجابة التي تعتقد بصحتها في المربع المخصص للإجابة X وضع علامة
- للأسئلة التي لا يوجد بها خيارات للإجابة يرجى كتابة الاجابة حسب المطلوب
- اذا كانت لديك اية ملاحظات الرجاء اضافتها في نهاية السؤال

## معلومات عامة عن المزرعة التجارية

		١,٢- المحافظة			رقم المزرع 1.1-
		١,٤- القرية			١,٣- الولاية
		١,٦- قراءة الجي بي اس لموقع المزرعة			١,٥- اسم المالك
				١,٧- هاتف المالك: الايمل (ان توفر)	
				١,٨- الطقس العام بالمنطقة	
مزرعة منفردة بقطر ٣كم	من ٢-٤ مزارع بقطر ٣كم		اكثر من ٥ مزارع بقطر ٣كم		١,٩- كثافة مزارع الدواجن بالمنطقة (تقديرية)
ارضي		اقفاص		١,١٠- نوع التربية بالحظائر	
صحراوية	وادي	جبلية	ساحلية	١,١١- الطبيعة الجغرافية للمنطقة	
فدان				١,١٢- المساحة الكلية للمزرعة	
لا	نعم		١,١٣- هل المنطقة بخط هجرات الطيور		

## معلومات عن مكونات الانتاج

مفتوحة	نصف مقفلة	مقفلة	٢,١- نوعية حظائر التربية	
			٢,٢- نظام التهوية بحظائر الدواجن	
			٢,٣- انواع الطيور بالمزرعة	
			١- دجاج ٢- بط ٣- اوز ٤- رومي ٥- سمان ٦- نعام ٧- حمام ٨- دجاج افريقي	
فقاسة	امهات	لاحم	بياض	٢,٤- نوع التربية
طير			٢,٥- اعداد الطيور بالمزرعة - مساحة الحظائر	
			٢,٦- مصدر الطيور بالمزرعة	
لا	نعم		٢,٧- وجود اعمار مختلفة من الطيور	
لا	نعم		٢,٨- هل يوجد مزارع طيور اخري لدي صاحب المزرعة	
لا	نعم		٢,٩- هل يسكن العمال بالمزرعة	
لا	نعم		٢,١٠- هل يوجد لدى اي شخص يعمل بالمزرعة طيور اخرى	
عامل			٢,١١- عدد العمال بالمزرعة	
داخل المزرعة		خارج المزرعة		٢,١٢- مكان تناول الوجبات اليومية للعمال
			٢,١٣- ما هو اتجاه الريح في المنطقة	

## معلومات عن صحة الدواجن واجراءات الصحة الحيوية

التحصين المستخدم		المرض		٣,١- برنامج التحصين في المزرعة
العلاجات	تاريخ الاصابة		المرض	٣,٢- السجل العلاجي
شاحنات الشركة		شاحنات المزرعة		٣,٣- نقل الاعلاف
مياه سطحية	بئر خاصة	شبكة محلية	شبكة حكومية	٣,٤- مصدر المياه
يصرف الي خارج المزرعة		يجمع داخل المزرعة		٣,٥- كيفية تصريف المياه
للتسويق الحي	مسلخ خارج المزرعة	مسلخ داخل المزرعة		٣,٦- تسويق الدواجن
كل سنة	شهريا	اسبوعيا	يومية	٣,٨- معدل ملاحظة الطيور البرية حول المزرعة
لا	نعم			٣,٩- وجود تماس بين الطيور البرية وطيور المزرعة
لا	نعم			٣,١٠- وجود حيوانات برية بالمزرعة (ثعالب- كلاب- قطط... الخ)

لا	نعم				٣,١١- وجود حيوانات اليفة بالمزرعة (كلاب.....الخ)				
					٣,١٢- اذا كانت الاجابة للسؤال السابق نعم ماهي انواع الحيوانات الموجودة بالمزرعة				
لا	نعم				٣,١٣- وجود معارض للطيور بالمنطقة				
كم					٣,١٤- اذا كانت اجابة السؤال السابق نعم، كم يبعد هذا المعرض				
لا	نعم				٣,١٥- وجود مسابقات مناقبة الديوك				
لا	نعم				٣,١٦- وجود مسابقات الحمام				
لا	نعم				٣,١٧- وجود تربية طيور منزلية حول المزرعة				
كم					٣,١٨- اذا كانت اجابة السؤال السابق نعم، كم تبعد اقرب حيازة بها دواجن منزلية				
لا	نعم				٣,١٩- وجود سوق طيور حية بالمنطقة				
كم					٣,٢٠- اذا كانت اجابة السؤال السابق نعم، كم يبعد عن المزرعة				
سلة النفايات العامة	سلة نفايات مقفلة	حرق مفتوح	حرق فرن	دفن	٣,٢١- طرق التخلص من الطيور النافقة				
					٣,٢٢- طرق التخلص من مخلفات الدواجن والفرشة (في كومة السماد، الاستخدام المباشر كسماد، ..... الخ)				
					٣,٢٣- انواع المطهرات المستخدمة (قائمة بالمطهرات الاكثر استخداما بالمزرعة				
					٣,٢٤- ما هي اوقات التطهير (مرة بين كل دورتين او مرتين بين كل دورتين)				
شركات الخدمات	الاطباء البيطريين	الاصدقاء	صاحب المزرعة	٣,٢٥- الزيارات					

Appendices

				عددها كل اسبوع
كم	٣,٢٦- كم تبعد المزرعة عن الشارع الرئيسي			
٣,٢٧- ما هو تقييمك لمستوى المعلومات الصحية والامن الحيوي لدى العاملين بالمزرعة				
ضعيف	مقبول	جيد	ممتاز	
٣,٢٨- هل اجراءات الامن الحيوي التالية مطبقة بالمزرعة				
لا	نعم	اخذ تصريح زيارة للمزرعة		
لا	نعم	وجود سور او شبك حول المزرعة		
لا	نعم	وجود مغطس تطهير الاطارات عند بوابة المزرعة		
لا	نعم	وجود مكان لتغيير الملابس		
لا	نعم	اهتمام العمال بنظافة المزرعة		
٣,٢٩- التقييم العام للامن الحيوي بالمزرعة				
ضعيف	مقبول	جيد	ممتاز	
٣,٣٠- انواع الزراعات حول الحظائر				



## Modelling of Avian influenza Risk factors in Oman Poultry Farms

Dear participant: This questioner is designed for modelling the risk factors of introduction and establishment of Avian Influenza Virus in the Omani poultry farms. The results will be beneficial in defining the risk areas and risk practises which may lead to propagation of any single avian influenza outbreak when it happens. Therefore both the farmers and the veterinary authorities will benefit from its result, so please answer this questioner as precise as possible.

The questioner is divided in to 3 main parts:

- 1- Part one: General Information about the farm (location, Owner. Etc...)
- 2- Part two: Information about the production components
- 3- Part three: Information's about heath and bio-security measures.

### Please Notes:

- 1- Please read question carefully.
- 2- Please uses a **cross** 'X' to indicate your choice(s) for each question.
- 3- For questions where no answers have been given please **write** your answer in the space provided.
- 4- Please add any relevant comments you wish to make at the end of the questionnaire.

## 1- General Information

1.1- Farm number:		1.2- The Region	
1.3- The State:		1.4- The Village	
1.5- Owner name:		1.6- The GPS location	
1.7- Owner contacts:	GSM: Email(if applicable):		
1.8- Weather in the area:			
1.9-			
1.10- Area population density:	Low (rural areas)	Medium (small cities)	High (big cities)
1.11- Type of Housing the birds:	Caged	lose	
1.12- The geographical type of the area:	Costal	Desert	Valley Hilly
1.13- Total area of the farm:	Squire meter		
1.14- Is the area in a main migration route :	Yes	no	
1.15- Area density of poultry birds per km <sup>2</sup> in the 25 km radius:	Low	moderate	high

## 2- Information about the production components

2.1- Type of Production System:	Close	Semi close	Open
2.2- Type of ventilation in the birds houses:			
2.3- Type of birds in the farm:	1-	2-	3-
	4-	5-	6-
2.4- Type of production:	Layers	broilers	Breeders Hatchery
2.5- Number of birds in the farm:	Bird/annually		
2.6- Source of birds in the farm:			
2.7- Presence of different ages in the farm:	Yes	No	
2.8- Dose the owner have another poultry farm of any size:	Yes	No	

2.9- Do the labours live in the farm:		Yes	No
2.10 Do any of the labours have his own birds:		Yes	No
2.11- Number of labours:			
2.12- Source of labours food:	From outside the farm	From inside the farm	

### 3- Information about the birds health and biosecurity

3.1- Vaccination programme:	Bird disease		Vaccine used	
3.2- Disease history of the farm:	The disease	Date	Ways of control	
3.3- Food supplies:		Own trucks	Supplier trucks	
3.4- Source of water:	Government network	Local network	Well	Service water

3.5- Where do the drainage of the waste water go:	Collected inside the farm		Drained outside the farm	
3.6- Marketing system for broilers:	In farm Slaughter house	Out farm slaughter house		Life bird market
3.7- Marketing system for eggs:	Own trucks		Distributer trucks	
3.8- Frequency of noticing of wild birds around the farm (migratory water fowls):	Daily	weekly	monthly	annually
3.9- Presence of contact between wild birds and the poultry in the farm:	Yes		no	
3.10- Presence of wild animals (stray cats and dogs) in the farm:	Yes		no	
3.11- Presence of mammals in the farm:	Yes		no	
3.12- If yes, what type of mammals are they:				
3.13- Presence of bird exhibitions in the area:	Yes		no	
3.14- How far the exhibition place from the farm:	Km			
3.15- Presence of fighting cock practises in the locality:	Yes		no	
3.16- Presence of pigeons race computations:	yes		no	
3.16- Presence of back yard farming in the area:	Yes		no	
3.17- How far is the nearest backyard farm from this farm and in which direction:	Km			
3.18- wind direction in the area:				

3.18- Presence of live birds market in the area:		Yes		no	
3.19- If yes how far is it from the farm:		Km			
3.20- Died birds disposal ways:	Incinerate	Burial	Open burning	Close garbage pen	Open garbage pen
3.21- Waste disposal ways: (list the most frequent ways)					
3.22- Types of disinfections used (list of disinfections used):					
3.23- Frequency of disinfection:					
3.24- Visitors:	Owner	Friends	vets	Food suppliers	
Frequency of visits per week					
3.25- How far the farm is from the main roads:		Km			
3.26- How much knowledge do the owner and workers have about avian flu pre-cautionary measures and bio-security (clinical signs/ notification / bio-security/ contingency plan)					

Poor	Fair	Good	excellent
<p>3.27- The bio-security measures application in the farm: (please comment and tick the box which you think is the correct)</p> <ul style="list-style-type: none"> <li>• Presence of wheel disinfectant dip in the farm entrance</li> <li>• Presence of a fence around the farm</li> <li>• Presence of a place changing clothes</li> <li>• Having permition to inter the farm</li> <li>• General heath and hygiene of the workers</li> </ul> <ul style="list-style-type: none"> <li>• General score of the bio-security of the farm</li> </ul>			
Poor	Fair	Good	excellent
<p>3.28- Type of plants in and around the poultry farms:</p>			

The study is disgnd by Thunai Al shekaili as a part of his PhD work.



Mosquitos Sampling farms form	
<b>Farm number:</b>	<b>Farm region:</b>
<b>Owner name:</b>	
<b>Farm state:</b>	<b>Farm village:</b>
<b>Farm longitude:</b>	<b>Farm latitude:</b>
<b>Farm altitude:</b>	
<b>Type of species of birds present in the farm:</b>	
<b>Particular farm features:</b>	
<b>Vegetation type:</b>	
<b>Vegetation percentage:</b>	
<b>Presence of animals manure:</b>	
<b>Amount of animals manure:</b>	
<b>General hygiene in the farm:</b>	
<b>Weather condition:</b>	
<b>Wind direction:</b>	
<b>Other information:</b>	

## 11.2. Appendix 2 Protocols and Reagents

Reconstitute stock primers according to delivery details, Dilute stock oligos 1:10 to give working solutions

### IBV Oligos

Working Oligo	Stock Oligo		Sigma Water
SX4-	10µl		90 µl
	A	B	
SX1+	10 µl	10 µl	80 µl
SX3+	10 µl	10 µl	80 µl
SX2-	10 µl	10 µl	80 µl

### aMPV Oligos 50

Working Oligo	Stock Oligo		Sigma Water
G6-	10 µl		90 µ
G5-	10 µl		90 µl
G8+A	10 µl		90 µl
G9+B	10 µl		90 µl
	A	B	
G1+	10 µl	10 µl	80 µl

**NDV Oligos**

Working Oligo	Stock Oligo	Sigma Water
MFS+	10 µl	90 µl
MSF-	10 µl	90 µl

**AIV Oligos**

Working Oligo	Stock Oligo	Sigma Water
NPF+	10 µl	90 µl
NPR-	10 µl	90 µl

10mM dNTP's Working Solution

dATP, dCTP, dGTP, dTTP at 100mM 51

Take 20µl of each dNTP, total volume 80µl

Add 120µl of sigma water to give 200µl of 10mM dNTP's

**RT-PCR reaction for IBV/aMPV**

5X Buffer 51	1 µl
DTT	0.5 µl
dNTP 51	0.25 µl
Ultra-pure Water	2.13 µl
RNase inhibitor	0.12 µl
Superscript II (200 µ/ µl) 51	0.25 µl
Negative Oligo (10 pmoles/µl) (IBV SX2-) (aMPVG6-) (MFS-)	0.75 µl

## Appendixes

### Nested PCR 1 protocol

PCR Supermix	19 $\mu$ l
+ Oligo (IBV SX1+) (aMPV G1+) (MSF-)	0.5 $\mu$ l
- Oligo (IBV SX 2- ) (aMPV G6-) (MSF+)	0.5 $\mu$ l

### Nested PCR 2 protocol

PCR Supermix	23.5 $\mu$ l
+ Oligo (IBV SX3+) (aMPV G8-A)	0.5 $\mu$ l
- Oligo (IBV SX 4- ) (aMPV G9+B)	0.5 $\mu$ l
- Oligo (aMPV G5-0 (aMPV)	0.5 $\mu$ l

### Loading Buffer

Ficol	3 g
1XTBE	20 ml
10x Tris-borate-EDTA (TBE)	

This was purchased at 10X concentration and diluted to 1X concentration when required.

### Gel preparation

1.5% agarose 53	0.58 g
TBE	35 ml

## **QIAamp viral RNA Extraction**

### **Equilibrate samples and buffers to RT**

#### **Addition of carrier RNA to buffer AVL**

- Add 310µl Buffer **AVE** to the tube containing 310µg lyophilized carrier RNA
- Dissolve the carrier RNA completely
- If extracting ~55 samples add the 310µl reconstituted carrier RNA to 31ml Buffer **AVL**
- This is stable in the fridge for 48h. Look out for precipitation. To dissolve any precipitate heat @ 80°C for no more than 5min (no more than 6 times)
- If extracting smaller numbers of RNA see table in booklet for amounts and storage of reconstituted carrier RNA

#### **Protocol**

- 1- Add 560µl of prepared buffer **AVL** containing reconstituted carrier RNA into 1.5ml eppendorf tubes
- 2- Add 140µl of sample
- 3- Vortex for 15sec
- 4- Incubate @ RT for 10min
- 5- Pulse centrifuge
- 6- Add 560µl of 100% ethanol
- 7- Vortex for 15sec

- 8- Pulse centrifuge
- 9- Label spin columns
- 10- Carefully remove 630 $\mu$ l from tube (step 6) into spin column
- 11- Centrifuge @ 8000 rpm for 1min, discard flow through
- 12- Place spin column in clean collection tube and repeat steps 10 and 11
- 13- Place spin column in clean collection tube add 500 $\mu$ l of buffer **AW1**
- 14- Centrifuge @ 8000rpm for 1min, discard flow through
- 15- Place spin column in clean collection tube add 500 $\mu$ l of buffer **AW2**
- 16- Centrifuge @ 13,000rpm for 3min, discard flow through
- 17- Centrifuge @ 13,00rpm for 1min
- 18- Place spin column in clean 1.5ml eppendorf tube add 60 $\mu$ l of **Sigma water**
- 19- Incubate @ RT for 1min
- 20- Centrifuge @8000rpm for 1min, discard spin column and save flow through (RNA)
- 21- Store RNA @ -20 $^{\circ}$ C

### **11.3. Appendix 3 Sampled Flocks Data**

Table of the sampled Oman backyard farms information and results

<i>n</i>	Region	State	<i>n</i> AI P	<i>n</i> ND P	<i>n</i> sampled	AI%	ND%	Pop <sup>26</sup> .	RW B <sup>27</sup>	SP <sup>28</sup>	RF <sup>29</sup>
1	Adh Dhahirah	Mahda	6	10	10	60	100	6,197	Low	Medium	No
2	Adh Dhahirah	Mahda	9	1	10	90	10	6,197	Low	Medium	No
3	Adh Dhahirah	Mahda	10	4	10	100	40	6,197	Low	Medium	No
4	Adh Dhahirah	Mahda	2	8	10	20	80	6,197	Low	Medium	No
5	Adh Dhahirah	Mahda	9	9	10	90	90	6,197	Low	Medium	No
6	Adh Dhahirah	Mahda	7	3	10	70	30	6,197	Low	Medium	No
7	Adh Dhahirah	Al Buraymi	1	4	10	10	40	82,889	Low	Medium	No
8	Adh Dhahirah	Al Buraymi	0	8	10	0	80	82,889	Low	Medium	No
9	Adh Dhahirah	Al Buraymi	0	0	10	0	0	82,889	Low	Medium	No
10	Adh Dhahirah	Al Buraymi	1	7	10	10	70	82,889	Low	Medium	No
11	Adh Dhahirah	Al Buraymi	1	6	10	10	60	82,889	Low	Medium	No
12	Adh Dhahirah	Al Buraymi	4	9	10	40	90	82,889	Low	Medium	No
13	Adh Dhahirah	As Sunaynah	4	5	10	40	50	478	Low	Medium	No
14	Adh Dhahirah	As Sunaynah	4	9	10	40	90	478	Low	Medium	No

<sup>26</sup> Population<sup>27</sup> Region wild birds<sup>28</sup> State plantation<sup>29</sup> Presence of fighting cocks in the region

Appendixes

15	Adh Dhahirah	As Sunaynah	9	10	10	90	100	478	Low	Medium	No
16	Adh Dhahirah	As Sunaynah	2	3	10	20	30	478	Low	Medium	No
17	Dhofar	Salalah	3	3	10	30	30	278,552	High	High	No
18	Dhofar	Salalah	1	0	10	10	0	278,552	High	High	No
19	Dhofar	Salalah	0	0	10	0	0	278,552	High	High	No
20	Dhofar	Salalah	0	1	10	0	10	278,552	High	High	No
21	Dhofar	Salalah	1	3	10	10	30	278,552	High	High	No
22	Dhofar	Mirbat	2	1	10	20	10	14,034	High	High	No
23	Dhofar	Mirbat	1	1	10	10	10	14,034	High	High	No
24	Al Wusta	Muhut	2	1	10	20	10	15,068	High	High	No
25	Al Wusta	Muhut	2	1	10	20	10	15,068	High	High	No
26	Al Wusta	Muhut	12	7	12	100	58.3	15,068	High	High	No
27	Al Wusta	Masirah	2	3	10	20	30	12,344	High	High	No
28	Al Wusta	Masirah	7	3	10	70	30	12,344	High	High	No
29	Al Wusta	Masirah	6	0	10	60	0	12,344	High	High	No
30	Al Wusta	Masirah	2	0	4	50	0	12,344	High	High	No
31	Al Wusta	Masirah	6	0	10	60	0	12,344	High	High	No
32	Adh Dhahirah	Ibri	8	5	10	80	50	131,601	Low	Low	No
33	Adh Dhahirah	Ibri	0	3	3	0	100	131,601	Low	Low	No

Appendixes

<b>34</b>	Adh Dhahirah	Ibri	2	9	10	20	90	131,601	Low	Low	No
<b>35</b>	Adh Dhahirah	Ibri	3	7	10	30	70	131,601	Low	Low	No
<b>36</b>	Adh Dhahirah	Ibri	8	6	10	80	60	131,601	Low	Low	No
<b>37</b>	Adh Dhahirah	Ibri	6	4	10	60	40	131,601	Low	Low	No
<b>38</b>	Adh Dhahirah	Ibri	5	2	9	55.5	22.2	131,601	Low	Low	No
<b>39</b>	Adh Dhahirah	Ibri	4	9	10	40	90	131,601	Low	Low	No
<b>40</b>	Adh Dhahirah	Ibri	7	9	10	70	90	131,601	Low	Low	No
<b>41</b>	Adh Dhahirah	Ibri	9	7	10	90	70	131,601	Low	Low	No
<b>42</b>	Adh Dhahirah	Ibri	6	5	10	60	50	131,601	Low	Low	No
<b>43</b>	Adh Dhahirah	Ibri	9	9	10	90	90	131,601	Low	Low	No
<b>44</b>	Adh Dhahirah	Ibri	0	1	10	0	10	131,601	Low	Low	No
<b>45</b>	Adh Dhahirah	Ibri	1	8	10	10	80	131,601	Low	Low	No
<b>46</b>	Adh Dhahirah	Ibri	2	8	10	20	80	131,601	Low	Low	No
<b>47</b>	Adh Dhahirah	Ibri	0	4	10	0	40	131,601	Low	Low	No
<b>48</b>	Adh Dhahirah	Yanqul	7	3	10	70	30	20,149	Low	Medium	No
<b>49</b>	Adh Dhahirah	Yanqul	2	6	10	20	60	20,149	Low	Medium	No

Appendixes

50	Adh Dhahirah	Yanqul	0	6	10	0	60	20,149	Low	Medium	No
51	Adh Dhahirah	Yanqul	3	4	10	30	40	20,149	Low	Medium	No
52	Adh Dhahirah	Yanqul	2	3	10	20	30	20,149	Low	Medium	No
53	Adh Dhahirah	Yanqul	4	2	10	40	20	20,149	Low	Medium	No
54	Adh Dhahirah	Yanqul	5	8	10	50	80	18,834	Low	Low	No
55	Adh Dhahirah	Yanqul	0	3	10	0	30	18,834	Low	Low	No
56	Adh Dhahirah	Yanqul	1	4	10	10	40	18,834	Low	Low	No
57	Adh Dhahirah	Yanqul	0	1	10	0	10	18,834	Low	Low	No
58	Adh Dhahirah	Yanqul	0	5	10	0	50	18,834	Low	Low	No
59	Adh Dhahirah	Yanqul	4	9	10	40	90	20,149	Low	Medium	No
60	Adh Dhahirah	Yanqul	2	10	10	20	100	20,149	Low	Medium	No
61	Adh Dhahirah	Yanqul	1	10	10	10	100	20,149	Low	Medium	No
62	Adh Dhahirah	Yanqul	5	10	10	50	100	20,149	Low	Medium	No
63	Ad Dakhliyah	Nizwa	4	1	10	40	10	96,128	Low	Medium	No
64	Ad Dakhliyah	Nizwa	7	5	10	70	50	96,128	Low	Medium	No
65	Ad Dakhliyah	Nizwa	6	6	10	60	60	96,128	Low	Medium	No
66	Ad Dakhliyah	Nizwa	6	5	10	60	50	96,128	Low	Medium	No

Appendixes

67	Ad Dakhliyah	Nizwa	6	4	10	60	40	96,128	Low	Medium	No
68	Ad Dakhliyah	Samail	0	4	10	0	40	63,505	Low	Medium	No
69	Ad Dakhliyah	Samail	2	2	10	20	20	63,505	Low	Medium	No
70	Ad Dakhliyah	Samail	1	4	10	10	40	63,505	Low	Medium	No
71	Ad Dakhliyah	Samail	1	3	10	10	30	63,505	Low	Medium	No
72	Ad Dakhliyah	Samail	0	7	10	0	70	63,505	Low	Medium	No
73	Ad Dakhliyah	Manah	7	3	10	70	30	18,632	Low	Low	No
74	Ad Dakhliyah	Manah	8	0	10	80	0	18,632	Low	Low	No
75	Ad Dakhliyah	Manah	8	1	10	80	10	18,632	Low	Low	No
76	Ad Dakhliyah	Manah	1	4	10	10	40	18,632	Low	Low	No
77	Ad Dakhliyah	Al Hamra	1		10	10	0	21,853	Low	Medium	No
78	Ad Dakhliyah	Al Hamra	4	4	10	40	40	21,853	Low	Medium	No
79	Ad Dakhliyah	Al Hamra	3	8	10	30	80	21,853	Low	Medium	No
80	Ad Dakhliyah	Al Hamra	4	8	10	40	80	21,853	Low	Medium	No
81	Ad Dakhliyah	Bahla	0	1	3	0	33.3	68,582	Low	Low	No
82	Ad Dakhliyah	Bahla	2	1	10	20	10	68,582	Low	Low	No

Appendixes

83	Ad Dakhliyah	Bahla	7	3	10	70	30	68,582	Low	Low	No
84	Ad Dakhliyah	Bahla	7	3	10	70	30	68,582	Low	Low	No
85	Ad Dakhliyah	Nizwa	0	0	10	0	0	96,128	Low	Medium	No
86	Ad Dakhliyah	Nizwa	3	3	10	30	30	96,128	Low	Medium	No
87	Ad Dakhliyah	Nizwa	1	1	10	10	10	96,128	Low	Medium	No
88	Ad Dakhliyah	Nizwa	0	1	10	0	10	96,128	Low	Medium	No
89	Ad Dakhliyah	Adam	6	10	10	60	100	21,960	Low	Low	No
90	Ad Dakhliyah	Adam	1	0	10	10	0	21,960	Low	Low	No
91	Ad Dakhliyah	Adam	8	8	10	80	80	21,960	Low	Low	No
92	Ad Dakhliyah	Adam	0	2	10	0	20	21,960	Low	Low	No
93	Ad Dakhliyah	Izki	9	9	10	90	90	48,893	Low	Medium	No
94	Ad Dakhliyah	Izki	5	6	10	50	60	48,893	Low	Medium	No
95		Izki	2	1	10	20	10	48,893	Low	Medium	No
96	Ad Dakhliyah	Izki	1		10	10	0	48,893	Low	Medium	No
97	Ad Dakhliyah	Bid bid	3	5	10	30	50	28,474	Low	Medium	No
98	Ad Dakhliyah	Bid bid	8	10	10	80	100	28,474	Low	Medium	No
99	Ad Dakhliyah	Bid bid	9	9	10	90	90	28,474	Low	Medium	No

Appendixes

<b>100</b>	Ad Dakhliyah	Bid bid	3	1	10	30	10	28,474	Low	Medium	No
<b>101</b>	Ad Dakhliyah	Bid bid	3	9	10	30	90	28,474	Low	Medium	No
<b>102</b>	Ash Sharqiyah	Al Mudaybi	0	8	10	0	80	91,254	Low	Low	No
<b>103</b>	Ash Sharqiyah	Al Mudaybi	3	4	10	30	40	91,254	Low	Low	No
<b>104</b>	Ash Sharqiyah	Al Mudaybi	2	1	10	20	10	91,254	Low	Low	No
<b>105</b>	Ash Sharqiyah	Al Mudaybi	9	10	10	90	100	91,254	Low	Low	No
<b>106</b>	Ash Sharqiyah	Al Mudaybi	6	10	10	60	100	91,254	Low	Low	No
<b>107</b>	Ash Sharqiyah	Al Mudaybi	6	10	10	60	100	91,254	Low	Low	No
<b>108</b>	Ash Sharqiyah	Al Mudaybi	4	10	10	40	100	91,254	Low	Low	No
<b>109</b>	Ash Sharqiyah	Al Qabil	8	10	10	80	100	21,114	Low	Low	No
<b>110</b>	Ash Sharqiyah	J. Bani Bu A.	1	7	10	10	70	76,444	Low	Medium	No
<b>111</b>	Ash Sharqiyah	J. Bani Bu A.	1	8	10	10	80	76,444	Low	Medium	No
<b>112</b>	Ash Sharqiyah	J. Bani Bu A.	0	4	10	0	40	76,444	Low	Medium	No
<b>113</b>	Ash Sharqiyah	J. Bani Bu A.	1	4	10	10	40	76,444	Low	Medium	No
<b>114</b>	Ash Sharqiyah	J. Bani Bu H.	4	4	10	40	40	36,403	Low	Medium	No
<b>115</b>	Ash Sharqiyah	J. Bani Bu H.	3	9	10	30	90	36,403	Low	Medium	No
<b>116</b>	Ash Sharqiyah	J. Bani Bu H.	1	9	10	10	90	36,403	Low	Medium	No

Appendixes

117	Ash Sharqiyah	Ibra	8	9	10	80	90	42,330	Low	Medium	No
118	Ash Sharqiyah	Ibra	2	1	10	20	10	42,330	Low	Medium	No
119	Ash Sharqiyah	Ibra	4	5	10	40	50	42,330	Low	Medium	No
120	Ash Sharqiyah	Ibra	3	4	10	30	40	42,330	Low	Medium	No
121	Ash Sharqiyah	Badiyah	1	9	10	10	90	32,921	Low	Low	No
122	Ash Sharqiyah	Badiyah	2	4	10	20	40	32,921	Low	Low	No
123	Ash Sharqiyah	Badiyah	0	3	10	0	30	32,921	Low	Low	No
124	Ash Sharqiyah	Badiyah	1	4	10	10	40	32,921	Low	Low	No
125	Ash Sharqiyah	Al Kamil	7	5	10	70	50	28,037	Low	Low	No
126	Ash Sharqiyah	Al Kamil	0	1	5	0	20	28,037	Low	Low	No
127	Ash Sharqiyah	Al Kamil	6	1	10	60	10	28,037	Low	Low	No
128	Ash Sharqiyah	Al Kamil	1	0	10	10	0	28,037	Low	Low	No
129	Ash Sharqiyah	Al Mudaybi	1	6	10	10	60	91,254	Low	Low	No
130	Ash Sharqiyah	Al Mudaybi	2		10	20	0	91,254	Low	Low	No
131	Ash Sharqiyah	Al Mudaybi	1		10	10		91,254	Low	Low	No
132	Ash Sharqiyah	Al Mudaybi	4	0	10	40	0	91,254	Low	Low	No

Appendixes

<b>133</b>	Ash Sharqiyah	Sur	7	5	10	70	50	90,306	Low	Low	No
<b>134</b>	Ash Sharqiyah	Sur	10	9	10	100	90	90,306	Low	Low	No
<b>135</b>	Ash Sharqiyah	Sur	5	5	10	50	50	90,306	Low	Low	No
<b>136</b>	Ash Sharqiyah	Wadi Bani Khalid	0	5	10	0	50	10,517	Low	Low	No
<b>137</b>	Ash Sharqiyah	Wadi Bani Khalid	0	4	10	0	40	10,517	Low	Low	No
<b>138</b>	Musandam	Daba Al Bayah	1	4	10	10	40	7,252	Low	Low	No
<b>139</b>	Musandam	Daba Al Bayah	0		4	0	0	7,252	Low	Low	No
<b>140</b>	Musandam	Daba Al Bayah	5	1	10	50	10	7,252	Low	Low	No
<b>141</b>	Musandam	Daba Al Bayah	7	1	10	70	10	7,252	Low	Low	No
<b>142</b>	Musandam	Madha	4	3	10	40	30	2,445	Low	Low	No
<b>143</b>	Musandam	Madha	4	2	10	40	20	2,445	Low	Low	No
<b>144</b>	Musandam	Madha	2	1	10	20	10	2,445	Low	Low	No
<b>145</b>	Musandam	Madha	4	0	10	40	0	2,445	Low	Low	No
<b>146</b>	Musandam	Khasab	0	2	3	0	66.7	21,602	Low	Low	No
<b>147</b>	Musandam	Khasab	2	6	10	20	60	21,602	Low	Low	No
<b>148</b>	Musandam	Khasab	1	0	1	100	0	21,602	Low	Low	No

Appendixes

149	Al Batinah	Barka	3	1	4	75	25	103,629	High	High	yes
150	Al Batinah	Barka	9	9	10	90	90	103,629	High	High	yes
151	Al Batinah	Barka	4	9	10	40	90	103,629	High	High	yes
152	Al Batinah	Barka	7	4	10	70	40	103,629	High	High	yes
153	Al Batinah	Barka	6	5	10	60	50	103,629	High	High	yes
154	Al Batinah	Barka	7	7	10	70	70	103,629	High	High	yes
155	Al Batinah	Barka	1	1	10	10	10	103,629	High	High	yes
156	Al Batinah	Al Musanaah	9	2	10	90	20	74,528	High	High	yes
157	Al Batinah	Al Musanaah	3	0	5	60	0	74,528	High	High	yes
158	Al Batinah	Al Musanaah	2		10	20	0	74,528	High	High	yes
159	Al Batinah	Al Musanaah	8	2	10	80	20	74,528	High	High	yes
160	Al Batinah	Al Musanaah	10		10	100	0	74,528	High	High	yes
161	Al Batinah	Al Musanaah	6	6	10	60	60	74,528	High	High	yes
162	Al Batinah	Al Musanaah	1		10	10	0	74,528	High	High	yes
163	Al Batinah	Al Musanaah	1		10	10	0	74,528	High	High	yes
164	Al Batinah	Al Musanaah	8	7	10	80	70	74,528	High	High	yes

Appendixes

165	Al Batinah	Ar Rustaq	8	6	10	80	60	95,435	High	Medium	yes
166	Al Batinah	Ar Rustaq	10	8	10	100	80	95,435	High	Medium	yes
167	Al Batinah	Ar Rustaq	6	3	10	60	30	95,435	High	Medium	yes
168	Al Batinah	Ar Rustaq	2	4	10	20	40	95,435	High	Medium	yes
169	Al Batinah	Ar Rustaq	2	7	10	20	70	95,435	High	Medium	yes
170	Al Batinah	Ar Rustaq	2	5	10	20	50	95,435	High	Medium	yes
171	Al Batinah	Ar Rustaq	1	8	10	10	80	95,435	High	Medium	yes
172	Al Batinah	Ar Rustaq	3	6	10	30	60	95,435	High	Medium	yes
173	Al Batinah	Al Khaburah	10	7	10	100	70	60,232	High	High	yes
174	Al Batinah	Al Khaburah	10	8	10	100	80	60,232	High	High	yes
175	Al Batinah	Al Khaburah	10	6	10	100	60	60,232	High	High	yes
176	Al Batinah	Al Khaburah	7	0	10	70	0	60,232	High	High	yes
177	Al Batinah	Al Khaburah	10	9	10	100	90	60,232	High	High	yes
178	Al Batinah	Al Khaburah	9	9	10	90	90	60,232	High	High	yes
179	Al Batinah	Al Khaburah	0		10	0	0	60,232	High	High	yes
180	Al Batinah	Al Khaburah	0		10	0	0	60,232	High	High	yes
181	Al Batinah	Al Khaburah	4		10	40	0	60,232	High	High	yes

Appendixes

<b>182</b>	Al Batinah	Saham	3	9	10	30	90	122,607	High	High	yes
<b>183</b>	Al Batinah	Saham	0	1	10	0	10	122,607	High	High	yes
<b>184</b>	Al Batinah	Saham	8	4	10	80	40	122,607	High	High	yes
<b>185</b>	Al Batinah	Saham	6	5	10	60	50	122,607	High	High	yes
<b>186</b>	Al Batinah	Saham	5	6	10	50	60	122,607	High	High	yes
<b>187</b>	Al Batinah	Saham	5	3	10	50	30	122,607	High	High	yes
<b>188</b>	Al Batinah	Saham	1		10	10	0	122,607	High	High	yes
<b>189</b>	Al Batinah	Saham	5	0	5	100	0	122,607	High	High	yes
<b>190</b>	Al Batinah	Saham	3	0	10	30	0	122,607	High	High	yes
<b>191</b>	Al Batinah	Sohar	0	2	10	0	20	181,867	High	High	yes
<b>192</b>	Al Batinah	Sohar	2	1	10	20	10	181,867	High	High	yes
<b>193</b>	Al Batinah	Sohar	2	3	10	20	30	181,867	High	High	yes
<b>194</b>	Al Batinah	Sohar	2	4	10	20	40	181,867	High	High	yes
<b>195</b>	Al Batinah	Sohar	2	1	5	40	20	181,867	High	High	yes
<b>196</b>	Al Batinah	Sohar	3	3	10	30	30	181,867	High	High	yes
<b>197</b>	Al Batinah	Sohar	2	1	10	20	10	181,867	High	High	yes

Appendixes

<b>198</b>	Al Batinah	Sohar	2	2	10	20	20	181,867	High	High	yes
<b>199</b>	Al Batinah	Sohar	3	0	10	30	0	181,867	High	High	yes
<b>200</b>	Al Batinah	Sohar	9	5	10	90	50	181,867	High	High	yes
<b>201</b>	Al Batinah	Sohar	3	5	5	60	100	181,867	High	High	yes
<b>202</b>	Al Batinah	Liwa	0	2	10	0	20	36,680	High	Medium	yes
<b>203</b>	Al Batinah	Liwa	1		8	12.5		36,680	High	Medium	yes
<b>204</b>	Al Batinah	Liwa	1	1	10	10	10	36,680	High	Medium	yes
<b>205</b>	Al Batinah	Liwa	1	3	10	10	30	36,680	High	Medium	yes
<b>206</b>	Al Batinah	Liwa	0	4	10	0	40	36,680	High	Medium	yes
<b>207</b>	Al Batinah	Liwa	4	2	10	40	20	36,680	High	Medium	yes
<b>208</b>	Al Batinah	Liwa	1	2	10	10	20	36,680	High	Medium	yes
<b>209</b>	Al Batinah	Liwa	2	1	10	20	10	36,680	High	Medium	yes
<b>210</b>	Al Batinah	Liwa	0	2	10	0	20	36,680	High	Medium	yes
<b>211</b>	Al Batinah	Liwa	0	0	10	0	0	36,680	High	Medium	yes
<b>212</b>	Al Batinah	Liwa	0	3	10	0	30	36,680	High	Medium	yes
<b>213</b>	Al Batinah	Shinas	0	1	10	0	10	59,973	High	Medium	yes
<b>214</b>	Al Batinah	Shinas	0	1	10	0	10	59,973	High	Medium	yes

Appendixes

<b>215</b>	Al Batinah	Shinas	0	3	10	0	30	59,973	High	Medium	yes
<b>216</b>	Al Batinah	Shinas	0	4	10	0	40	59,973	High	Medium	yes
<b>217</b>	Al Batinah	Shinas	0	3	10	0	30	59,973	High	Medium	yes
<b>218</b>	Al Batinah	Shinas	1	1	10	10	10	59,973	High	Medium	yes
<b>219</b>	Al Batinah	Shinas	1		10	10	0	59,973	High	Medium	yes
<b>220</b>	Al Batinah	Shinas	1		10	10	0	59,973	High	Medium	yes
<b>221</b>	Al Batinah	Shinas	1	1	10	10	10	59,973	High	Medium	yes
<b>222</b>	Al Batinah	As Suwayq	3	1	10	30	10	136,847	High	Medium	yes
<b>223</b>	Al Batinah	As Suwayq	3	0	10	30	0	136847	High	Medium	yes
<b>224</b>	Al Batinah	As Suwayq	3	3	10	30	30	136847	High	Medium	yes
<b>225</b>	Al Batinah	As Suwayq	2	4	10	20	40	136847	High	Medium	yes
<b>226</b>	Al Batinah	As Suwayq	4		10	40	0	136847	High	Medium	yes
<b>227</b>	Al Batinah	As Suwayq	2	4	10	20	40	136847	High	Medium	yes
<b>228</b>	Al Batinah	As Suwayq	7	4	10	70	40	136847	High	Medium	yes
<b>229</b>	Al Batinah	As Suwayq	7	7	10	70	70	136847	High	Medium	yes
<b>230</b>	Muscat	As Suwayq	4	4	10	40	40	136847	High	Medium	yes

Appendixes

<b>231</b>	Muscat	Qurayyat	2	0	10	20	0	46,562	Medium	Low	No
<b>232</b>	Muscat	Qurayyat	10	5	10	100	50	46,562	Medium	Low	No
<b>233</b>	Muscat	Qurayyat	10	1	10	100	10	46,562	Medium	Low	No
<b>234</b>	Muscat	Qurayyat	9	2	10	90	20	46,562	Medium	Low	No
<b>235</b>	Muscat	Qurayyat	10	3	10	100	30	46,562	Medium	Low	No
<b>236</b>	Muscat	Qurayyat	7	0	10	70	0	46,562	Medium	Low	No
<b>237</b>	Muscat	Qurayyat	1		10	10	0	46,562	Medium	Low	No
<b>238</b>	Muscat	As Seeb	7	9	10	70	90	310,673	Medium	Medium	No
<b>239</b>	Muscat	As Seeb	4	5	10	40	50	310,673	Medium	Medium	No
<b>240</b>	Muscat	As Seeb	2	4	10	20	40	310,673	Medium	Medium	No
<b>241</b>	Muscat	As Seeb	3	6	10	30	60	310,673	Medium	Medium	No
<b>242</b>	Muscat	As Seeb	1	7	10	10	70	310,673	Medium	Medium	No
<b>243</b>	Muscat	As Seeb	6	4	10	60	40	310,673	Medium	Medium	No

Appendixes

<b><i>n</i></b>	<b>F-Birds</b>	<b>FZ<sup>30</sup></b>	<b>WN p<sup>31</sup></b>	<b>WN%</b>	<b>IBV<sup>32</sup></b>	<b>aMPV<sup>33</sup></b>	<b>T Birds<sup>34</sup></b>	<b>NPF<sup>35</sup></b>	<b>NWL<sup>36</sup></b>	<b>NR<sup>37</sup></b>	<b>NWA<sup>38</sup></b>	<b>NCL<sup>39</sup></b>
<b>1</b>	Chicken	500	Y	0/5	0	0	chicken	20.237	1.584	0.201	7.17	68.38
<b>2</b>	Chicken	200	N	0	0	0	chicken	20.058	1.156	0.273	6.78	68.86
<b>3</b>	Chicken	230	N	0	P <sup>40</sup>	0	chicken	19.769	0.888	0.128	6.25	67.28
<b>4</b>	Chicken	1000	Y	0/5	0	0	chicken	17.264	0.887	0.043	5.98	68.99

<sup>30</sup> Flock size

<sup>31</sup> WN positive flock

<sup>32</sup> IBV results

<sup>33</sup> aMPV result

<sup>34</sup> Tested birds

<sup>35</sup> Nearest production farm

<sup>36</sup> Nearest water line

<sup>37</sup> Nearest road

<sup>38</sup> Nearest water area

<sup>39</sup> Nearest coast line

<sup>40</sup> Positive for IBV but not identified

Appendixes

5	Chicken	50	N	0	P	0	chicken	14.405	0.653	0.506	2.83	72.2
6	Chicken	1700	N	0	0	0	chicken	19.965	1.171	0.124	4.32	65.62
7	Chicken	200	N	0	0	0	chicken	15.327	0.358	0.42	3.1	86.32
8	Chicken turkey	730	Y	0/3	0	0	turkey	10.965	6.099	0.452	11.1	94.36
9	Chicken	1200	Y	1/3	0	aMPV B	chicken	1.928	0.663	0.228	1.72	86
10	Chicken	9	Y	3/3	0	0	chicken	10.965	6.099	0.452	11.1	94.36
11	Chicken	180	Y	0/5	793/B	0	chicken	0.417	2.045	2.5	1.76	84.55
12	Chicken	900	N	0	0	0	chicken	10.911	6.054	0.396	11.04	94.32
13	Chicken	150	N	0	0	0	chicken	19.724	3.646	5.607	0	108
14	Chicken	200	N	0	0	0	chicken	23.564	4.068	3.981	0.25	111.5
15	Chicken	100	N	0	0	0	chicken	28.18	2.802	2.841	0	114.4
16	Chicken	160	N	0	0	0	chicken	33.31	0.566	0.177	0.45	119.4
17	Chicken	500	N	0	783/B	0	chicken	36.628	1.645	1.373	42.26	0.23
18	Chicken GF <sup>41</sup>	407	N	0	0	0	chicken	29.149	2.099	0.315	34.76	0.091

<sup>41</sup> Guinea fowls

Appendixes

<b>19</b>	Chicken	700	N	0	783/B	0	chicken	26.411	1.236	0.459	32.28	3.439
<b>20</b>	Chicken	900	Y	3/5	0	0	chicken	26.577	0.116	0.692	32.53	5.08
<b>21</b>	Chicken	30	Y	1/5	0	0	chicken	22.916	0.467	2.225	28.85	4.11
<b>22</b>	Chicken	50	N	0	0	0	chicken	30.673	2.848	0.683	36.38	1.137
<b>23</b>	Chicken	25	N	0	0	0	chicken	2.428	5.093	0.205	4.82	0.666
<b>24</b>	Chicken	12	N	0	783/B	0	chicken	66.668	1.822	0.037	7.78	17.61
<b>25</b>	Chicken	15	N	0	793/B	0	chicken	70.262	4.084	0.601	4.4	12.23
<b>26</b>	Chicken	15	N	0	0	0	chicken	65.932	1.014	0.402	7.18	18.28
<b>27</b>	Chicken	50	N	0	0	0	chicken	70.262	4.084	0.601	4.4	12.23
<b>28</b>	Chicken	100	Y	3/5	0	0	chicken	10.817	0.25	0.123	6.07	3.283
<b>29</b>	Chicken	120	Y	3/5	0	0	chicken	11.909	2.518	0.131	9.24	0.363
<b>30</b>	Chicken	3	Y	1/4	0	0	chicken	14.467	3.163	0.035	10.3	1.098
<b>31</b>	Chicken	9	Y	1/5	IS/885	0	chicken	14.772	3.006	0.263	10.7	0.867
<b>32</b>	Chicken	30	N	0	0	0	chicken	2.39	3.722	0.011	0.5	110.9
<b>33</b>	Chicken	3	N	0	0	0	chicken	5.695	1.814	0.369	0.06	104.6
<b>34</b>	Chicken	90	N	0	0	0	chicken	10.703	0.366	0.28	13.47	48.09
<b>35</b>	Chicken	30	N	0	1494/	0	chicken	4.456	0.725	0.018	3.56	104.7

Appendixes

36	Chicken	25	Y	2/5	0	0	chicken	5.711	1.783	0.395	0.03	104.6
37	Chicken	60	N	0	0	0	chicken	0.681	0.487	0.134	4.93	101.3
38	Chicken	9	N	0	0	0	chicken	5.71	1.785	0.393	0.03	104.6
39	Chicken	40	Y	0/5	0	0	chicken	5.704	1.8	0.38	0.05	104.6
40	Chicken	25	Y	0/3	0	0	chicken	8.758	1.705	0.122	5.87	49.61
41	Chicken	30	Y	0/3	0	0	chicken	13.995	0.314	0.247	5.73	78.27
42	Chicken	50	Y	1/3	0	0	chicken	19.106	0.684	0.169	21.89	63.22
43	Chicken	70	N	0	793/B	0	chicken	0.456	1.546	0.344	0.14	93.12
44	Chicken	200	N	0	D274	0	chicken	2.997	2.123	0.113	5.45	98.65
45	Chicken	80	Y	0/5	793/B	aMPV B	chicken	1.826	0.125	0.327	3.57	101.2
46	Chicken	40	Y	4/4	0	0	chicken	8.825	0.102	0.04	8.99	86.03
47	Chicken	100	N	0	0	0	chicken	9.546	0.747	0.321	5.8	89.12
48	Chicken	60	Y	1/5	0	0	chicken	2.762	0.19	0.676	0.47	71.04
49	Chicken	300	Y	4/5	0	0	chicken	5.064	0.39	0.404	3.73	63.8
50	Chicken	40	Y	0/3	0	0	chicken	7.854	1.394	0.537	0	73.42
51	Chicken	20	N	0	0	0	chicken	2.839	1.474	0.145	0	73.38
52	Chicken	70	Y	0/5	793/B	0	chicken	3.809	1.3	0.46	0.53	75.95
53	Chicken	200	Y	0/3	0	0	chicken	3.505	0.557	0.079	1.37	75.53

Appendixes

<b>54</b>	Chicken	65	Y	2 / 5	0	0	chicken	13.565	2.581	0.293	0	107.2
<b>55</b>	Chicken	150	N	0	0	0	chicken	15.345	2.926	0.891	0	107.1
<b>56</b>	Chicken	200	N	0	0	aMPV B	chicken	2.033	2.383	0.244	2.99	98.74
<b>57</b>	Chicken	160	Y	0 / 5	0	0	chicken	6.403	2.163	0.872	0.02	94.3
<b>58</b>	Chicken	80	Y	0 / 3	0	0	chicken	15.324	0.356	0.417	3.11	86.31
<b>59</b>	Chicken	30	N	0	0	0	chicken	0.019	1.297	0.087	12.89	75.92
<b>60</b>	Chicken	230	Y	1 / 5	0	0	chicken	0.018	0.915	0.464	12.38	75.5
<b>61</b>	Chicken	200	N	0	0	0	chicken	0.046	1.559	0.074	10.03	73.1
<b>62</b>	Chicken	350	N	0	0	0	chicken	1.964	0.335	0.162	17.28	63.92
<b>63</b>	Chicken	900	N	0	0	0	chicken	2.235	0.767	0.528	20.49	94.01
<b>64</b>	Chicken	80	Y	0 / 3	0	0	chicken	1.774	2.18	1.285	22.09	83.64
<b>65</b>	Chicken	30	Y	0 / 3	0	0	chicken	1.54	0.523	0.394	19.29	83.28
<b>66</b>	Chicken	20	N	0	0	0	chicken	7.034	0.577	0.034	11.37	84.45
<b>67</b>	Chicken	40	N	0	0	0	chicken	8.667	1.27	5.059	12.78	95.91
<b>68</b>	Chicken	100	Y	1 / 5	0	0	chicken	2.332	1.429	0.075	0	45.62
<b>69</b>	Chicken	80	Y	2 / 5	793/B	0	chicken	3.687	1.333	0.18	4.11	40.28
<b>70</b>	Chicken	120	Y	3 / 5	0	0	chicken	5.844	0.777	0.535	6.23	38.87

Appendixes

<b>71</b>	Chicken	200	Y	0/3	0	0	chicken	8.705	1.64	0.051	5.85	49.65
<b>72</b>	Chicken	30	Y	3 from 5	0	0	chicken	11.14	0.945	0.006	13.23	56.39
<b>73</b>	Chicken	60	N	0	0	0	chicken	4.004	5.744	0.178	7.13	107.3
<b>74</b>	Chicken	100	N	0	Mass41	0	chicken	5.4	4.305	0.312	7.42	106.9
<b>75</b>	Chicken	80	Y	2/5	0	0	chicken	5.402	4.305	0.313	7.42	106.9
<b>76</b>	Chicken	50	Y	3/5	0	0	chicken	1.539	3.742	0.298	10.96	105.1
<b>77</b>	Ducks	80	N		793/B	0	Ducks	1.822	0.519	0.231	4.8	81.61
<b>78</b>	chicken	55	Y	1 from 5		0	chicken	2.464	0.138	0.596	3.5	84.76
<b>79</b>	Chicken	75	Y	0/5	793/B	0	chicken	4.581	1.009	0.859	4.4	89.5
<b>80</b>	Chicken	150	N	0	793/B	0	chicken	3.797	0.153	0.933	4.33	88.81
<b>81</b>	Chicken	300	N	0	0	0	chicken	0.432	0.657	0.152	4.75	90.28
<b>82</b>	Chicken	50	Y	0/3	0	0	chicken	3.727	1.368	0.028	8.68	89.23
<b>83</b>	Chicken	300	N	0	0	0	chicken	1.675	1.175	0.398	6.05	98.47
<b>84</b>	Chicken	500	Y	0/3	0	0	chicken	2.676	0.291	0.261	7.01	99.89
<b>85</b>	Chicken	40	N	0	0	0	chicken	12.032	0.799	0.68	38.09	76.73
<b>86</b>	Chicken	70	N	0	0	0	chicken	11.316	2.435	0.05	35.48	76.35
<b>87</b>	Chicken	200	N	0	0	0	chicken	11.769	1.96	0.072	36.17	76.03

Appendixes

<b>88</b>	Chicken	150	Y	3/ 5	0	0	chicken	12.961	1.197	0.08	38.66	76.04
<b>89</b>	Chicken	70	N	0	0	0	chicken	34.258	1.428	0.015	2.94	152
<b>90</b>	Chicken	30	N	0	0	0	Ducks	34.473	0.77	0.374	2.66	152.6
<b>91</b>	Chicken Ducks	70	N	0	0	0	chicken	40.843	2.812	0.269	0.27	154.9
<b>92</b>	Chicken	60	N	0	0	0	chicken	36.501	0.794	0.072	0.84	155.1
<b>93</b>	Chicken	120	Y	0/ 3	0	0	chicken	8.189	0.935	0.159	20.79	94.55
<b>94</b>	Chicken	200	Y	0/3	793/B	0	chicken	7.205	0.583	0.136	16.11	101.3
<b>95</b>	Chicken	400	Y	1/ 3	0	0	chicken	2.364	2.08	0.757	30.05	79.4
<b>96</b>	Chicken Ducks	120	N	0	0	0	Ducks	2.358	2.191	0.805	30.13	79.25
<b>97</b>	Chicken Ducks	40	Y	0/ 5	793/B	0	chicken	5.424	0.345	0.014	13.93	28.65
<b>98</b>	Chicken	50	Y	0/ 5	793/B	0	chicken	11.786	0.765	2.44	2.06	40.22
<b>99</b>	Chicken	40	Y	4/ 5	0	0	chicken	5.932	1.334	0.305	8.65	36.22
<b>100</b>	Chicken	200	Y	0/ 5	0	0	chicken	5.678	1.683	0.15	8.68	36.03
<b>101</b>	Chicken	50	Y	0/ 5	0	0	chicken	5.855	1.662	0.021	8.37	36.25
<b>102</b>	Chicken	300	Y	2/ 3	0	0	chicken	2.615	0.258	0.7	11.05	129.1
<b>103</b>	Chicken	80	N	0		aMPV B	chicken	9.685	1.206	0.18	11.45	134.8
<b>104</b>	Chicken	200	N	0	Mass41	0	chicken	0.344	2.118	0.022	9.44	127.5

Appendixes

<b>105</b>	Chicken	200	Y	0/3	0	0	chicken	0.814	2.705	0.536	8.92	164.1
<b>106</b>	Chicken	50	Y	0/5	0	0	chicken	5.087	0.869	0.212	3.05	104.8
<b>107</b>	Chicken	80	Y	0/5	D274	0	chicken	2.846	1.723	0.662	4.18	97.93
<b>108</b>	Chicken	100	Y	0/5	0	0	chicken	1.61	1.692	0.801	0.79	110.3
<b>109</b>	Chicken	20	N	0	0	0	chicken	1.876	2.347	0.132	0.33	67.35
<b>110</b>	Chicken	200	Y	0/3	793/B	0	chicken	4.579	1.084	0.243	5.65	33.87
<b>111</b>	Chicken	150	Y	0/3	0	0	chicken	4.888	1.997	0.16	4.82	33.03
<b>112</b>	Chicken	50	Y	0/3	0	0	chicken	5.547	1.967	0.365	4.32	32.55
<b>113</b>	Chicken	200	Y	0/3	0	0	chicken	6.002	1.952	0.511	4.1	32.3
<b>114</b>	Chicken	60	Y	0/3	0	0	chicken	1.621	0.137	0.05	12.09	40.1
<b>115</b>	Chicken	30	N	0	793/B	0	chicken	1.087	0.367	0.28	12.57	40.61
<b>116</b>	Chicken	40	N	0	0	0	chicken	7.981	14.57	8.609	16.64	53.68
<b>117</b>	Chicken	80	N	0	0	0	chicken	3.007	0.417	0.364	2.3	70.13
<b>118</b>	Chicken	80	Y	0/5	0	0	chicken	5.102	0.412	1.056	0.34	68.31
<b>119</b>	Chicken	200	N	0	0	0	chicken	6.986	0.708	0.105	2.87	71.91
<b>120</b>	Chicken	60	N	0	793/B	0	chicken	0.647	0.084	0.384	4.98	71.62
<b>121</b>	Chicken	200	N	0	0	0	chicken	1.074	2.576	0.888	1.18	64.4

Appendixes

122	Chicken	50	N	0	0	0	chicken	2.37	3.817	0.115	1.39	65.26
123	Chicken	40	N	0	0	0	chicken	5.967	0.765	0.336	1.43	64.87
124	Chicken	20	N	0	0	0	chicken	9.577	0.367	0.166	1.37	66.49
125	Chicken	200	N	0	0	0	chicken	1.259	1.061	0.014	6.93	50.45
126	Chicken	5	N	0	0	0	chicken	1.777	0.971	0.144	3.38	48.98
127	Chicken	60	N	0	0	0	chicken	3.46	1.286	0.164	2.72	48.86
128	Chicken	200	N	0	0	0	chicken	2.151	1.314	1.068	4.4	50.49
129	Chicken	90	N	0	0	0	chicken	3.567	0.233	0.098	6.11	87.98
130	Chicken Ducks + Gf	300	Y	1/3	0	0	Ducks	2.193	0.398	0.022	7.58	89.37
131	Chicken Ducks + Gf	301	Y	1/3	0	0	Guinea fowl	2.193	0.398	0.022	7.58	89.37
132	Chicken	100	N	0	0	0	chicken	1.862	3.373	0.023	0	80.88
133	Chicken	60	Y	1/5	0	0	chicken	1.263	1.059	0.018	6.93	50.45
134	Chicken	100	Y	1/3	0	0	chicken	3.138	0.882	0.313	33.74	4.177
135	Chicken	500	N	0	0	0	chicken	3.455	0.766	0.155	33.87	4.19
136	Chicken	40	N	0	0	0	chicken	2.516	0.002	0.034	11.53	30.04
137	Chicken	15	N	0	0	0	chicken	2.792	0.724	1.546	12.8	28.37

Appendixes

<b>138</b>	Chicken	20	N	0	0	0	chicken	1.097	0.203	1.24	3.42	5.436
<b>139</b>	Chicken Ducks	9	Y	2 / 4	0	0	Ducks	0.993	1.408	0.265	2.94	0.725
<b>140</b>	Chicken	30	N	0	0	0	chicken	1.005	1.573	0.075	2.9	0.572
<b>141</b>	Chicken	18	N	0	0	0	chicken	2.575	3.113	0.125	1.53	1.615
<b>142</b>	Chicken	200	N	0	0	0	chicken	1.009	1.625	0.007	2.91	0.512
<b>143</b>	Chicken	300	N	0	0	0	chicken	38.179	39.01	1.028	34.58	36.81
<b>144</b>	Chicken	20	N	0	0	0	chicken	41.724	42.55	0.803	33.56	33.28
<b>145</b>	Chicken	15	N	0	0	0	chicken	40.949	41.8	0.11	34.24	33.98
<b>146</b>	Chicken	3	N	0	0	0	chicken	24.698	6.729	0.079	1.54	10.18
<b>147</b>	Chicken	20	N	0	793/B	0	chicken	24.513	6.648	0.047	1.75	2.4
<b>148</b>	Chicken	12	N	0	0	0	chicken	25.257	4.666	1.054	4.27	6.736
<b>149</b>	Chicken	50	Y	0 / 3	0	0	chicken	8.49	0.288	0.144	0.82	6.236
<b>150</b>	Chicken	100	Y	0 / 3	0	0	chicken	8.478	0.276	0.157	0.81	5.076
<b>151</b>	Chicken	100	Y	1 / 3	0	0	chicken	18.669	0.239	0.266	0.09	3.924
<b>152</b>	Chicken	80	N	0	0	0	chicken	19.496	0.831	0.34	0.11	4.988
<b>153</b>	Chicken	300	N	0	0	0	chicken	7.115	0.572	0.54	4.43	3.477
<b>154</b>	Turkey	70	Y	1 / 4	0	0	chicken	16.425	1.419	0.001	1.21	39.63

Appendixes

<b>155</b>	Chicken	150	N	0	793/B	0	turkey	9.213	5.902	0.691	0.73	5.439
<b>156</b>	Chicken	155	N	0	0	0	chicken	1.698	0.065	0.003	5.69	5.436
<b>157</b>	Turkey	5	Y	1/5	0	0	Chicken + turkey	1.97	1.177	0.19	3.96	0.65
<b>158</b>	Geese	5	Y	0/3	0	0	Turkey	5.417	2.834	0.006	3.34	1.745
<b>159</b>	Ducks	30	N	0	0	0	geese	1.051	0.382	0.048	5.57	6.811
<b>160</b>	Chicken	45	N	0	0	0	chickens	1.733	0.14	0.004	3.65	7.704
<b>161</b>	Chicken	80	Y	2/3	793/B	0	Ducks	4.493	1.892	0.639	3.13	7.995
<b>162</b>	Turkey	50	Y	0/3	793/B	0	Ducks	3.756	0.034	0.034	0.034	15.29
<b>163</b>	Chicken	70	N	0	0	0	Chickens	4.349	0.034	0.034	0.034	15.29
<b>164</b>	Chicken	200	Y	2/5	0	0	chicken	7.033	2.91	0.783	3.96	5.229
<b>165</b>	Chicken	60	Y	1/5	0	0	chicken	7.037	0.173	0.223	11.58	3.498
<b>166</b>	Chicken	20	Y	3/5	0	0	chicken	7.742	0.181	0.229	11.59	0.205
<b>167</b>	Chicken	40	Y	1/3	0	0	chicken	10.703	1.267	1.135	13.12	2.413
<b>168</b>	Chicken	80	N	0	0	0	chicken	2.236	0.366	0.28	13.47	2.078
<b>169</b>	Chicken	200	Y	0/3	0	0	chicken	7.072	0.723	0.238	8.18	2.078
<b>170</b>	Chicken	80	N	0	0	0	chicken	8.179	0.376	0.004	11.25	9.828
<b>171</b>	Chicken	50	Y	0/3	0	0	chicken	2.024	0.656	0.068	12.14	7.735

Appendixes

<b>172</b>	Chicken	100	N	0	0	0	chicken	4.131	1.168	0.327	1.19	4.638
<b>173</b>	Chicken	80	N	0	0	0	chicken	4.038	0.124	0.005	0.81	4.642
<b>174</b>	Chicken	90	Y	0 / 5	0	0	chicken	4.376	1.359	0.126	2.54	23.49
<b>175</b>	Chicken	120	N	0	0	0	chicken	3.28	1.405	0.205	3.23	23.87
<b>176</b>	Chicken	1000	N	0	0	0	chicken	0.992	0.974	0.187	1.13	7.774
<b>177</b>	Chicken	100	Y	0 / 5	0	0	chicken	4.421	0.802	0.097	3.13	5.463
<b>178</b>	Chicken	350	N	0	0	0	chicken	3.768	0.961	0.93	0	7.636
<b>179</b>	Ducks	70	Y	2 / 3	0	0	Ducks	5.279	0.654	0.296	0	4.475
<b>180</b>	Ducks	90	N	0	0	0	Ducks	3.752	0.497	0.218	2.18	5.685
<b>181</b>	Ducks	50	Y	1 / 5	P	0	Ducks	1.633	1.611	0	1.55	5.164
<b>182</b>	Chicken	300	N	0	P	0	chickens	13.721	4.38	1.039	7.57	2.413
<b>183</b>	Chicken	90	Y	2 / 3	0	0	chickens	2.537	1.92	1.281	20.49	4.244
<b>184</b>	Chicken	200	N	0	0	0	chickens	4.967	0.116	0.289	4.43	6.978
<b>185</b>	Chicken	200	Y	1 / 3	0	0	chickens	2.34	3.45	0.18	2.12	3.922
<b>186</b>	Chicken	100	Y	1 / 3	793/B	0	chickens	2.718	0.488	0.044	6.32	4.208
<b>187</b>	Chicken	200	N	0	793/B	0	chickens	2.484	1.617	0.143	6.69	44.81
<b>188</b>	Ducks	60	N	0	0	0	Ducks	2.244	1.633	0.036	6.88	44.82

Appendixes

<b>189</b>	Chicken	5	N	0	0	0	chickens	2.325	2.014	0.23	4.98	45.53
<b>190</b>	Chicken	300	N	0	0	0	chickens	3.136	1.897	0.351	7.28	48.09
<b>191</b>	Chicken	60	Y	1 / 3	0	0	chickens	0.713	2.03	0.002	7.6	40.06
<b>192</b>	Chicken	50	Y	1 / 3	0	0	chickens	5.68	3.216	0.179	2.88	44.76
<b>193</b>	Chicken	200	Y	0 / 3	0	0	chickens	5.679	0.989	0.168	2.3	45.86
<b>194</b>	Chicken	24	N	0	0	0	chickens	10.915	1.089	0.064	2.5	106.5
<b>195</b>	Chicken	5	N	0	Mass41	0	chickens	1.574	1.599	0.003	0	2.039
<b>196</b>	Chicken	200	N	0	0	0	chickens	1.609	0.7	0.036	13.54	3.562
<b>197</b>	Chicken	100	N	0	0	0	chickens	1.424	0.879	0.084	13.74	0.634
<b>198</b>	Chicken	150	Y	1 / 3	0	0	chickens	0.05	0.371	0.007	13.37	0.929
<b>199</b>	Chicken	48	Y	0 / 3	0	0	chickens	2.502	0.274	0.111	14.25	1.191
<b>200</b>	Chicken	10	N	0	793/B	0	chickens	3.136	0.192	0.003	12.78	3.756
<b>201</b>	Chicken	5	N	0	0	0	chickens	0.355	2.03	0.002	7.6	2.652
<b>202</b>	Chicken	100	N	0	0	0	chickens	0.355	3.488	0.262	0.06	2.49
<b>203</b>	Ducks	100	Y	0 / 5	793/B	0	Ducks	0.467	3.488	0.262	0.06	1.497
<b>204</b>	Chicken	200	Y	0 / 3	0	0	chickens	0.644	4.285	0.21	0.21	4.054
<b>205</b>	Chicken	70	Y	0 / 3	0	0	chickens	2.018	4.756	0.03	0.41	45.59

Appendixes

<b>206</b>	Chicken	50	Y	0 / 3	793/B	0	chickens	4.136	7.806	0.002	0.28	3.918
<b>207</b>	Chicken	20	Y	0 / 3	0	0	chickens	5.68	8.596	0.127	0	5.496
<b>208</b>	Chicken	400	Y	0 / 3	0	0	chickens	3.729	0.989	0.168	2.3	2.627
<b>209</b>	Chicken	30	Y	0 / 3	793/B	0	chickens	0.219	3.861	0.667	1.54	1.285
<b>210</b>	Chicken	15	N	0	0	0	chickens	0.18	4.186	0.046	0.47	1.264
<b>211</b>	Chicken	40	N	0	0	0	chickens	0.14	6.233	0.35	1.11	1.919
<b>212</b>	Chicken	100	N	0	0	0	chickens	4.75	6.266	0.429	1.03	2.164
<b>213</b>	Chicken	50	N	0	0	0	chickens	3.923	6.052	0.113	0.01	0.233
<b>214</b>	Chicken	70	N	0	0	0	chickens	8.398	9.23	0.18	0.14	0.75
<b>215</b>	Chicken	50	N	0	0	0	chickens	8.447	8.816	0.107	0.83	2.952
<b>216</b>	Chicken	50	N	0	0	0	chickens	8.427	8.958	0.015	0.95	2.644
<b>217</b>	Chicken	54	N	0	0	0	chickens	5.965	8.034	0.431	0.21	18.98
<b>218</b>	Chicken	150	Y	0 / 5	0	0	chickens	8.848	1.513	0.119	2.14	36.36
<b>219</b>	Ducks	80	Y	2 / 5	0	0	Guinea fowl	8.631	7.796	0.701	0	36.57
<b>220</b>	Chicken GF	85	Y	0 / 5	0	0	Ducks	7.239	8.116	0.54	0.11	36.15
<b>221</b>	Chicken	250	N	0	0	0	chickens	6.745	8.479	0.212	0.66	2.4
<b>222</b>	Chicken	100	N	0	0	0	chickens	6.201	0.147	1.676	0.64	6.736

Appendixes

223	Chicken	150	N	0	0	0	chickens	9.369	0.588	1.336	0.38	6.236
224	Chicken	30	N	0	0	0	chickens	8.591	1.002	0.297	1.6	5.076
225	Chicken	200	N	0	0	0	chickens	1.035	1.202	0.022	2.41	3.924
226	Ducks	80	N	0	0	0	Ducks	2.388	0.181	0.866	3.08	4.988
227	Chicken	150	N	0	0	0	chickens	1.262	0.073	0.576	3.74	3.477
228	Chicken	70	N	0	0	0	chickens	1.1	1.458	0.37	4.14	39.63
229	Chicken Gf+ Ducks	88	N	0	0	0	chickens	1.097	0.2	1.236	3.42	5.439
230	Chicken	80	Y	0 / 3	0	0	chickens	5.717	0.203	1.24	3.42	5.436
231	Chicken	100	N	0	0	0	chickens	5.73	4.453	1.147	0.69	0.65
232	Chicken	80	N	0	0	0	chickens	1.208	3.845	1.756	0	1.745
233	Chicken	150	N	0	0	0	chickens	0.125	1.502	1.132	0.5	6.811
234	Chicken	200	Y	0 / 3	0	0	chickens	1.775	1.038	0.263	0.18	7.704
235	Chicken	300	Y	1 / 5	0	aMPV B	chickens	9.695	2.713	0.015	1.82	7.995
236	Chicken Ducks	93	Y	1 / 3	0	0	chickens	9.695	1.836	0.262	9.68	15.29
237	Chicken Ducks	93	Y	1 / 3	0	0	Ducks	4.166	1.836	0.262	9.68	15.29
238	Chicken	200	N	0	0	0	chickens	1.864	0.766	0.042	4.2	5.229
239	Chicken	150	N	0	0	0	chickens	0.874	0.764	0.246	8.34	3.498

Appendixes

<b>240</b>	Chicken	80	N	0	0	0	chickens	1.051	0.981	0.364	9.45	0.205
<b>241</b>	Chicken	200	N	0	0	0	chickens	7.387	0.382	0.048	5.57	2.413
<b>242</b>	Chicken Turkey	69	N	0	0	0	chickens	7.387	0.04	0.093	10.3	2.078
<b>243</b>	Chicken Turkey	69	N	0	0	0	turkey	7.387	0.04	0.093	10.3	2.078

## 11.4. Appendix 4: R codes for Risk factor analysis

- **First import the data file**

```
data <- read.csv("ChickenData1.csv")
```

- **Pull out the data for only chickens**

```
nrow(data)
```

```
length(unique(data$farm.number))
```

```
data$MultiSpecies<-rep(0,nrow(data))
```

```
data$MultiSpecies[which(apply(data[,15:18],1,sum)>0)]<-1
```

```
sum(data$MultiSpecies)
```

- **attach(data)**
- **fix(data)**
- **names(data)**
- **library(mgcv)**
- **NDV GAM testing**

```
gam1an<gam(cbind(NDVPos,NoSampledBirdsNDVPos)~Region2+s(AltitudeG)+s(
FlockSize)+MultiSpecies+R.fightingcock+s(NEAR_PFARMK)+s(NEAR_WLINEK
)+s(Near_WAK)+s(NEAR_ROADK)+s(Near_coastK)+s(AIPerc),family=quasibino
mial)
```

```
gam2an<gam(cbind(NDVPos,NoSampledBirdsNDVPos)~Region2+MultiSpecies+R
.fightingcock+s(AIPerc)+s(AltitudeG)+s(FlockSize)+s(NEAR_PFARMK)+s(NEAR
_WLINEK)+s(sqrt(Near_WAK))+s(sqrt(NEAR_ROADK))+s(Near_coastK),family
=quasibinomial)
```

```
summary(gam1an)
```

```
summary(gam2an)
```

Appendixes

```
plot(gam1an)
```

```
plot(gam2an)
```

- **GLM for NDV**

```
glmNDVdata<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+AltitudeG+FlockSize+MultiSpecies+AIPerc+R.fightingcock+N  
EAR_PFARMK+NEAR_WLINEK+sqrt(Near_WAK)+sqrt(NEAR_ROADK)+Near  
_coastK,family=quasibinomial,data=ChickenData)
```

```
summary(glmNDVdata)
```

```
anova(glmNDVdata,test="F")
```

- **Backward remove**

```
# remove Multispecies
```

```
glmNDVdata1<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock+NEAR_PFARM  
K+NEAR_WLINEK+sqrt(Near_WAK)+sqrt(NEAR_ROADK)+Near_coastK,famil  
y=quasibinomial,data=ChickenData)
```

```
summary(glmNDVdata1)
```

```
anova(glmNDVdata1,test="F")
```

```
# remove Near coast K
```

```
glmNDVdata2<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock+NEAR_PFARM  
K+NEAR_WLINEK+sqrt(Near_WAK)+sqrt(NEAR_ROADK),family=quasibinomi  
al,data=ChickenData)
```

Appendixes

```
summary(glmNDVdata2)
```

```
anova(glmNDVdata2,test="F")
```

```
#remove near production farm
```

```
glmNDVdata3<-glm(cbind(NDVPos,NoSampledBirds-
```

```
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock+NEAR_WLINE
```

```
K+sqrt(Near_WAK)+sqrt(NEAR_ROADK),family=quasibinomial,data=ChickenDat
```

```
a)
```

```
summary(glmNDVdata3)
```

```
anova(glmNDVdata3,test="F")
```

```
# remove near water line
```

```
glmNDVdata4<-glm(cbind(NDVPos,NoSampledBirds-
```

```
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock+sqrt(Near_WA
```

```
K)+sqrt(NEAR_ROADK),family=quasibinomial,data=ChickenData)
```

```
summary(glmNDVdata4)
```

```
anova(glmNDVdata4,test="F")
```

```
# remove near water area
```

```
glmNDVdata5<-glm(cbind(NDVPos,NoSampledBirds-
```

```
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock+sqrt(NEAR_RO
```

```
ADK),family=quasibinomial,data=ChickenData)
```

```
summary(glmNDVdata5)
```

```
anova(glmNDVdata5,test="F")
```

Appendixes

```
# remove near road
```

```
glmNDVdata6<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+AltitudeG+FlockSize+AIPerc+R.fightingcock,family=quasibin  
omial,data=ChickenData)
```

```
summary(glmNDVdata6)
```

```
anova(glmNDVdata6,test="F")
```

```
# remove altitude
```

```
glmNDVdata7<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+FlockSize+AIPerc+R.fightingcock,family=quasibinomial,data=  
ChickenData)
```

```
summary(glmNDVdata7)
```

```
anova(glmNDVdata7,test="F")
```

```
#remove Fighting cock
```

```
glmNDVdata8<-glm(cbind(NDVPos,NoSampledBirds-  
NDVPos)~Region2+FlockSize+AIPerc,family=quasibinomial,data=ChickenData)
```

```
summary(glmNDVdata8)
```

```
anova(glmNDVdata8,test="F")
```

- **AIV GAM plotting**

Appendixes

```
gam1<-gam(cbind(AIPos,NoSampledBirds)~Region2+MultiSpecies+R.fighting  
cock+s(AltitudeG)+s(FlockSize)+s(NEAR_PFA  
RMK)+s(NEAR_WLINEK)+s(Near_WAK)+s(NEAR_ROADK)+s(Near_coastK),family=quasibinomial)
```

```
summary(gam1)
```

```
plot(gam1)
```

- **GLM modelling of AIV**

```
glmAIData<-glm(cbind(AIPos,NoSampledBirds-  
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG+FlockSize+NEAR_PFA  
RMK+Near_WAK+NEAR_ROADK+Near_coastK,family=quasibinomial)
```

```
summary(glmAIData)
```

```
anova(glmAIData,test="F")
```

Backward removal

```
#remove near water areas
```

```
glmAIData1<-glm(cbind(AIPos,NoSampledBirds-  
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG+FlockSize+NEAR_PFA  
RMK+NEAR_ROADK+Near_coastK,family=quasibinomial)
```

```
summary(glmAIData1)
```

```
anova(glmAIData1,test="F")
```

```
#remove flock size
```

## Appendixes

```
glmAIdata2<-glm(cbind(AIPos,NoSampledBirds-
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG+NEAR_PFARMK+NE
AR_ROADK+Near_coastK,family=quasibinomial)

summary(glmAIdata2)

anova(glmAIdata2,test="F")

#remove near road

glmAIdata3<-glm(cbind(AIPos,NoSampledBirds-
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG+NEAR_PFARMK+Near
_coastK,family=quasibinomial)

summary(glmAIdata3)

anova(glmAIdata3,test="F")

#remove near coast

glmAIdata4<-glm(cbind(AIPos,NoSampledBirds-
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG+NEAR_PFARMK,famil
y=quasibinomial)

summary(glmAIdata4)

anova(glmAIdata4,test="F")

#remove production farm

glmAIdata5<-glm(cbind(AIPos,NoSampledBirds-
AIPos)~Region2+MultiSpecies+R.fightingcock+AltitudeG,family=quasibinomial)

summary(glmAIdata5)
```

Appendixes

```
anova(glmAIdata5,test="F")
```

```
#remove Multispeaceis
```

```
glmAIdata6<-glm(cbind(AIPos,NoSampledBirds-  
AIPos)~Region2+R.fightingcock+AltitudeG,family=quasibinomial)
```

```
summary(glmAIdata6)
```

```
anova(glmAIdata6,test="F")
```

```
#remove R fighting cocks
```

```
glmAIdata7<-glm(cbind(AIPos,NoSampledBirds-  
AIPos)~Region2+AltitudeG,family=quasibinomial)
```

```
summary(glmAIdata7)
```

```
anova(glmAIdata7,test="F")
```

## **11.5. Appendix 5: Publications**

Molecular detection of infectious bronchitis and avian metapneumoviruses  
in Oman backyard poultry

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## Abstract

Infectious bronchitis virus (IBV) and avian metapneumovirus (aMPV) are economically important viral pathogens infecting chickens globally. Identification of endemic IBV and aMPV strains is central in controlling disease and production losses. Oropharyngeal swab samples were taken from 2317 birds within 243 different backyard flocks. Swabs from each flock were examined by RT-PCR using part-S1 and G gene primers for IBV and aMPV respectively. Thirty-nine flocks were positive for IBV and five flocks for aMPV. Five IBV genotypes were identified whereas all aMPV isolates belonged to subtype B. Data presented here demonstrates that both viruses, including recent variant IBVs, are endemic in Oman backyard poultry. Furthermore, 793/B-like IBV genotype was not limited to chicken flocks, but also found in one turkey and one duck flock. Though no disease was witnessed at the time of sampling, identified viruses may still pose a viable threat for both backyard and commercial poultry in Oman.

**Keywords:** infectious bronchitis virus; avian metapneumovirus; backyard poultry; surveillance; Oman

## **Preventive Veterinary Medicine**