UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY OF NEWCASTLE DISEASE IN CHICKEN IN THE SULTANATE OF OMAN

AL SAHAMI ALI ABDULLAH MOHAMMED

FPV 2018 20

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 2018

DEDICATION

In the name of Allah, my Creator and my Master, my great teacher and messenger, Mohammed (Peace be Upon Him), who taught us the purpose of life. I am dedicating this thesis to beloved people who have meant and continue to mean so much to me– my late Father and Mother. Although they are no longer in of this world, their memories continue to regulate my life. My dearest wife, who leads me through the valley of darkness with light of hope and support, my beloved kids: whom I can't force myself to stop loving. To all my family, the symbol of love and giving, my friends who encourage and support me, and all the people in my life who touch my heart.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EPIDEMIOLOGY OF NEWCASTLE DISEASE IN CHICKEN IN THE SULTANATE OF OMAN

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June 2018

Chairman : Professor Aini Bt. Ideris, PhD
Faculty : Veterinary Medicine

Newcastle disease (ND) remains as one of the major disease in poultry production worldwide. In the Middle East nations including the Sultanate of Oman specifically, the Newcastle disease virus (NDV) is known to have been circulating for the past two decades. Nevertheless, there is little information on the seroprevalence of the disease among chickens as well as the nature of the NDV causing outbreaks in various poultry management systems in Oman. To date, there is a lack of specific study on the epidemiology of ND in Oman. Hence, the objectives of this study were: were to: 1) determine the proportion of ND in the small holder and commercial farms in Oman from 2011-2015; 2) determine the seroprevalence of ND in backyard poultry; 3) determine the risk factors associated with the occurrence of ND and the efficacy of reporting and notification system in Oman; 4) isolate and characterize the NDV from the outbreaks of ND in Oman occurred in 2017; and, 5) assess the economic impact on the poultry sector of Oman due to ND outbreaks. In a retrospective study, the 5-year records of ND outbreaks between 2011 and 2015 were analyzed. In total, number of 1223 farms (mean ± SD, 256 ± 37) and 90,959 birds (mean ± SD, 23,400 ± 452) have been affected with ND between 2011 and 2015. The yearly proportion of ND at the farm level was the highest in 2011, 68% (95%Confidence interval, CI 66-71%) and the lowest was in 2015, 2% (95%CI 1-3%). At bird level, 2014 recorded the highest level of ND cases, 31% (95%CI 30-31%) and 2015 was the lowest (1.2%, 95%CI 1.1-1.2%). Majority of the farm (61%, 95%CI 58-64%) and bird (47%, 95%CI 46.4-47%) cases occurred in Al-Batinah governorate. The highest proportion of ND was in January (21%, 95%CI 19-24%) and lowest in July (0.16%, 95%CI 0-0.4%) with steady increase from August to December. There was a significant difference ($P<0.05$) in the reported ND cases between years, months and governorates. The backyard

poultry had the highest reported outbreaks of ND (70%, 95%CI 68-73%) and lowest in the closed system (5%, 95%CI 4-6%). Farms in backyard system was 47 times more likely to report ND cases (95%CI Odds ratio, OR 35-62) and open system (OR=7, 95%CI 5-9) compared to the close system. Contrarily, birds in backyard system were 1.8 (95%CI 1.7-1.8) times less likely to report ND cases compared to open and close systems.

The seroprevalence of NDV in backyard chickens was also determined through a cross-sectional study conducted from June to August 2016. Based on ELISA results, the average seroprevalence of NDV at bird level was 33.8% (95%CI 31-36) and at flock level, 57.1% (95%CI 44-60%). However, there was no statistical difference in the NDV seroprevalence at flock and bird ($P>0.05$) levels among regions of Oman. The study findings indicate that ND is endemic in Oman.

A questionnaire-based survey was used to investigate the risk factors for ND in the various poultry management systems in Oman. A response rate of 58% (n=857) was obtained. Thirty-eight percent of the respondents practiced the backyard system, whereas 54% and 28% of them were using open and close systems, respectively. The proportions of respondents with or without recorded ND outbreaks in their farms were 40% and 27%, respectively. However, 32% had no such information at their disposal. The backyard system had a significant association (OR=1.81, 95%CI 1.1-4.35) with the farms reporting ND compared to the close system. Farms lacking the service of a veterinarian (OR=5, 95%CI 1.98-14.5); the usage of dead vaccine (OR=2.3, 95%CI 1.2-4.2); farms not restricting visitors' entry (OR=6.4, 95%CI 2.0-20.3) and usage of temporary staff (OR=3.9, 95%CI 1.5-10.6) were significantly associated with ND outbreaks reporting. Only 16% of the farmers reported ND outbreaks in their farms to the appropriate authority. A higher proportion (57%) of the farmers stop the sales of chickens while only 27.8% and 13.9% restrict production or submit samples to veterinary clinics during outbreak. There was a significant positive weak correlation between farms with recorded ND outbreaks and those that stop sales of chicken products ($r=0.12$, $P=0.03$) and restricting of production ($r=0.17$, $P=0.002$) during outbreaks.

Newcastle disease virus isolated during this study characterized the NDV causing the outbreaks in Oman in 2017 which involved three poultry farms in Al-Dakhiliyah and Al-Batinah. Haemagglutination inhibition (HI) test and reverse transcription-polymerase chain reaction (RT-PCR) assay were used. The findings revealed that the Oman isolates showed high homology (98%) with other reported NDV isolates genotype VIIi which was previously isolated in Pakistan and considered to be highly pathogenic NDV.

The results from the economic impact assessment study on the poultry industry in Oman due to ND outbreaks using three scenarios estimated the direct impact at about 2, 11 and 21 million Omani Rial, respectively, at mortality rate of 10% due to lentogenic, 50% mesogenic and 90% velogenic strains of NDV, respectively.

In conclusion, ND and NDV are endemic in the poultry industry in the Sultanate of Oman. Backyard poultry is the most affected management system. Measures to reduce the occurrence of ND in backyard chickens could help prevent the spread to commercial flocks. Also, improving the management of chickens based on the factors associated with ND might contribute to effective preventive and control programme. More efforts by the authority in veterinary services are required to reduce the impact of the disease thus minimize the economic losses to the poultry industry of Oman.

Keywords: Newcastle disease, endemic, Oman, seroprevalence, backyard system, HI, RT-PCR

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EPIDEMIOLOGI PENYAKIT NEWCASTLE PADA AYAM DI KESULTANAN OMAN

Oleh

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Penyakit Newcastle (ND) kekal sebagai salah satu penyakit utama yang menghadkan pengeluaran ternakan poltri di seluruh dunia. Di negara Timur Tengah termasuk Kesultanan Oman khususnya, virus ND (NDV) diketahui beredar sejak dua dekad yang lalu. Walau bagaimanapun, tidak banyak maklumat mengenai seroprevalens penyakit tersebut pada ayam dan juga hakikat semulajadi NDV penyebab wabak pada sistem pengurusan poltri di Oman. Sehingga kini, kajian khusus mengenai epidemiologi ND di Oman adalah kurang. Oleh itu, objektif kajian ini ialah untuk: 1) menentukan perkadaran ND di ladang pekebun kecil dan komersil di Oman dari 2011 hingga 2015; 2) menentukan seroprevalens penyakit ND pada poltri sistem kecilan; 3) menentukan faktor risiko terkait dengan wabak ND dan keberkesanan sistem pelaporan dan notifikasi di Oman; 4) memencilkan dan menggambarkan sifat NDV dari wabak yang terjadi di Oman pada 2017; dan, 5) menilai impak ekonomi pada sektor ternakan poltri di Oman yang disebabkan oleh wabak ND.

Dalam satu kajian retrospektif, rekod lima tahun wabak ND yang berlaku antara 2011 dan 2015 telah dianalisis. Sejumlah 1,223 ladang (purata ± sisihan piawai (SD): 245 ± 333) dan 90,959 ekor ayam (30,320 ± 11,220) telah dijangkiti ND antara 2011 dan 2015. Perkadaran tahunan ND pada peringkat ladang adalah tertinggi pada 2011, 68% (95% Julat Keyakinan, CI 66-71%) dan terendah pada 2015, 2% (95%CI 1-3%). Pada peringkat ayam, 2014 telah merekodkan kes ND tertinggi, 31% (95%CI 30-31%) dan 2015 terendah (1.2%, 95%CI 1.1-1.2%). Majoriti kes ladang (61%, 95%CI 58-64%) dan ayam (47%, 95%CI 46.4-47%) berlaku di Wilayah Al-Batinah. Perkadaran ND tertinggi adalah tertinggi di bulan Januari (21%, 95%CI 19-

24%) dan terendah di bulan Julai (0.16%, 95%CI 0-0.4%) dengan peningkatan kukuh dari Ogos hingga Disember. Terdapat perbezaan signifikan (P<0.05) antara perkadaran kes ND dan tahun, bulan dan wilayah. Sistem poltri kecilan adalah 47 kali lebih mungkin melaporkan kes ND (95%CI nisbah mungkin,OR 35-62), sistem terbuka (OR=7, 95%CI 5-9) berbanding sistem tertutup. Sebaliknya, ayam dari sistem kecilan adalah 1.8 (95%CI 1.7-1.8) kali lebih kurang mungkin melaporkan kes ND berbanding sistem terbuka atau tertutup.

Seroprevalens NDV pada ayam sistem kecilan telah ditentukan melalui kajian keratan rentas yang dijalankan dari Jun hingga Ogos 2016. Berdasarkan keputusan ELISA, purata seroprevalens NDV peringkat ayam adalah 33.8% (95%CI 31-36) dan pada peringkat flok, 57.1% (95%CI 44-60%). Walau bagai mana pun, perbezaan seroprevalens ND peringkat ayam dan flok antara wilayah di Oman adalah tidak signifikan secara statistiknya (P>0.05). Dapatan kajian menunjukan ND adalah endemik di Oman.

Sebuah survei kaji selidik telah digunakan untuk menyiasat faktor risiko wabak ND di Oman. Kadar respons 58% (n=857) telah diterima. Tiga puluh lapan peratus responden mengamalkan sistem kecilan, 54% sistem terbuka dan 28% menggunakan sistem tertutup. Perkadaran sebanyak 40% responden ada kejadian wabak dan 27% tiada wabak ND di ladang manakala 32% responden tiada maklumat tersebut. Sistem kecilan adalah signifikan (OR=1.81, 95%CI 1.1-4.35) dengan ladang yang ada ND berbanding sistem terbuka. Ladang yang kurang perkhidmatan veterinawan (OR=5, 95%CI 1.98-14.5); penggunaan vaksin mati (OR=2.3, 95%CI 1.2-4.2); ladang yang tidak menghadkan kemasukan pelawat (OR=6.4, 95%CI 2.0-20.3) dan penggunaan staf sementara (OR=3.9, 95%CI 1.5-10.6) adalah berkait secara signifikan dengan pelaporan wabak ND. Hanya 16% penternak melaporkan wabak ND berlaku di ladang mereka kepada pihak berwajib. Perkadaran yang lebih tinggi (57%) penternak memberhentikan penjualan ayam, sementara 27% dan 13.9% menghadkan pengeluaran atau menghantar sampel kepada klinik veterinar semasa terjadinya wabak. Terdapat korelasi lemah positif yang signifikan antara ladang dengan perekodan wabak ND dan mereka yang menghentikan jualan hasil ayam (r=0.12, P=0.03) dan menghadkan pengeluaran (r=0.17, P=0.002) semasa wabak.

Kajian ini juga telah menggambarkan sifat NDV penyebab wabak di Oman pada tahun 2017 yang melibatkan tiga ladang poltri di Al-Dakhiliyah dan Al-Batinah. Ujian penghemaglutinatan-perencatan (HI) dan cerakin transkripsi berbalik-tindak balas berantai polimerase (RT-PCR) telah digunakan. Didapati, isolat mempunyai homologi yang tinggi (98%) dengan genotip isolat NDV lain yang telah dilaporkan iaitu isolat genotip VIIi yang sebelum

ini telah dipencilkan di Pakistan dan dipertimbangkan sebagai virus ND yang sangat patogenik.

Keputusan dari kajian penilaian impak ekonomi pada industri poltri Oman disebabkan oleh wabak ND menggunakan tiga senario menganggarkan nilai impak terus lebih kurang 2, 11 dan 21 juta Rial Oman, pada kadar mortaliti 10% disebabkan oleh NDV jenis lentogenik, 50% mesogenik dan 90% velogenik.

Kesimpulannya, ND dan NDV adalah endemik dalam industri poltri di Kesultanan Oman. Poltri kecilan adalah sistem pengurusan yang paling terkesan. Langkah mengurangkan kejadian ND pada ayam dari sistem kecilan boleh membantu mencegah penyebaran kepada flok komersil. Juga, penambah-baikan pengurusan ayam berdasarkan faktor terkait dengan ND boleh menyumbang kepada program pencegahan dan kawalan yang efektif. Lebih banyak usaha oleh pihak berkuasa dalam perkhidmatan veterinar diperlukan untuk mengurangkan impak penyakit seterusnya meminimakan kerugian ekonomi pada industri poltri Oman.

Kata kunci: Penyakit Newcastle, endemik, Oman, seroprevalens, sistem kecilan, HI, RT-PCR

ACKNOWLEDGEMENTS

In the name of Allah, the most beneficent, most gracious, and most merciful. First and foremost, I thank Allah (SWT) for letting me live to see this thesis through. I would like to express my deepest gratitude and appreciation to Professor Datin Paduka Dr. Aini Ideris, the chairperson of my supervisory committee for her guidance, encouragement, and support throughout my study in Malaysia. I also appreciate diligent efforts of my advisory committee members Professor Dr. Abdul Rahman Omar and Dr. Siti Zubaidah Ramanoon for their invaluable contributions and continuous support throughout my research study. I appreciate all of you for your supervision, advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the work. My sincere thanks are further extended to the Institute of Bioscience, UPM for providing research facilities and technical assistance during my graduate study. I would also like to acknowledge the financial support provided by Agriculture Development Funds and Ministry of Agriculture and Fisheries in the Sultanate of Oman. In my daily work, I have been blessed with a friendly group of fellow students and my special appreciation is extended to all of them especially Dr. Norhaiani, Dr. Oday, Dr. Bashir, Mr. Humam, Dr. Sadiq, Dr. Salim, and Dr. Salah. Thanks also go to the members of Reference Laboratory for Animal health, Animal Health Research Center, Ministry of Agriculture and Fisheries in Oman especially to Dr. Yousuf, Dr. Safwat, Dr. Mansoor, Dr Abdulmajeed, and all the staffs in the Directorate of Animal Health, Oman. Last but not least, I owe so much to my whole family for their undying support, their unwavering belief that I can achieve so much. Unfortunately, I cannot thank everyone by name because it would take a lifetime, but I just want you all to know that you count so much. Had it not been for all your prayers and benedictions, were it not for your sincere love and help, I would never have completed this thesis.
So, thank you all.

I certify that a Thesis Examination Committee has met on 25 June 2018 to conduct the final examination of Al Sahami Ali Abdullah Mohammed on his thesis entitled "Epidemiology of Newcastle Disease in Chicken in the Sultanate of Oman" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

Aa	Amino Acid
APMV	Avian Paramyxovirus
ASR	Annual Specific Rate
BLAST	Basic Local Alignment Search Tool
BSO	Black Seed Oil
CI	Confidence Interval
DNA	Deoxyribonucleic acid
DsRNA	Double strand Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assays
EDTA	Ethylenediaminetetraacetic acid
F	Fusion Protein
F0	Fusion Protein 0
GIT	Gastrointestinal Tract
HI	Hemagglutination Inhibition
HN	Hemagglutinin-Neuraminidase
IBD	Infectious Bursal Disease
ICPI	Intra Cerebral Pathogenicity Index
IVPI	Intra Venous Pathogenicity Index
L	Large Polymerase Protein
M	Matrix Protein
MSR	Monthly Specific Rate
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Centre for Biotechnology Information
ND	Newcastle Disease

NDV	Newcastle Disease Virus
vNDV	Velogenic Newcastle Disease Virus
NP	Nucleocapsid Protein
NV-ND	Neurotropic Velogenic Newcastle Disease
OR	Odds Ratio
OIE	World Animal Health Organization
P	Phosphoprotein Protein
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
Rpm	Revolutions Per Minute
RT	Reverse Transcription
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SPF	Specific-Pathogen-Free

CHAPTER 1

INTRODUCTION

The poultry industry in the Sultanate of Oman is one of the most important industries contributing to the provision of animal protein. There are three major types of management poultry production system in Oman namely open, close and backyard systems spreading across the country. However, the production level is yet to meet the increasing local demand. In the close and open systems, the production level has improved from average flock size of 1,000 birds to 20 thousand birds per cycle. Conversely, in the backyard system, flock size is still at the maximum of 500 birds. The Ministry of Agriculture and Fisheries provides technical support to improve the production systems with the aim to transform them into investment projects. Current information depict that an estimated number of farms that operate under this system is around 2500 according to the 2012/2013 census (Ministry of agriculture and fisheries, 2014).

In poultry production, infectious diseases are of the major constraints in achieving optimal yield. Newcastle disease (ND) is one of the most significant poultry diseases worldwide characterized by high mortality and morbidity. Newcastle disease is an endemic poultry disease and a leading cause of economic loss in the industry (Ashraf and Shah, 2014). Economic losses are attributed to the characteristic high morbidity and mortality caused by the disease in infected flock (Alexander, 2001). ND is caused by the Newcastle disease virus (NDV) which was firstly identified by Doyle in 1927 as a virus belonging to the family Paramyxovirus (Alexander, 2001).

The virus family Paramyxoviridae is a large group of virus that infects animals and humans causing diseases such as measles, mumps and respiratory syncytial viruses in the latter. In animals, Paramyxovirus causes diseases such as NDV, canine distemper and rinderpest viruses (Lamb et al., 2005). Similarly, the Avian Paramyxovirus-1 (APMV-1) is related to the NDV and driven from the genus Avulavirus, within the family Paramyxoviridae (Lamb et al., 2005).

The three widely known strains of NDV include the lentogenic, mesogenic and velogenic strains with each initiating specific manifestations when present in birds (Hassan et al., 2010; Haryanto et al., 2015). Lentogenic strains cause mild or subclinical respiratory infection while the mesogenic strains cause clinical disease but low mortality (Haryanto et al., 2015). Velogenic strains can show sudden death and increase the mortality rate in flocks along with respiratory signs, swelling of the head and neck, and neurological symptoms (Hasan et al., 2010).

Despite the classification of the virus according to severity, the disease does not always behave accordingly. The manifestation of clinical signs depends on several host and pathogen factors such as the ND strain, host immune status, species of birds the avian, host immune status and concurrent infections. Nevertheless, ND has been reported to induce greatest impact on poultry with high mortality especially in village and smallholder poultry (Hines and Miller, 2012). Such high mortality are attributed to the infection by the virulent strain of NDV. Virulent NDV strains have been shown to be endemic in poultry in most of the Asia, Africa, and some countries of North and South America. However, some countries are free from this strain like the USA and Canada. Cormorants, pigeons, and imported psittacine species are more commonly infected with velogenic NDV (vNDV) and have also been sources of vNDV infections of poultry. NDV strains of low virulence are prevalent in poultry and wild birds, especially waterfowl. Infection of domestic poultry with low NDV contributes to lower productivity (Lamb et al., 2005).

Vaccination with the low pathogenic strain of NDV remains the major preventive measure against ND, suggested to be achievable due to the similarity in the genotypic composition (Miller et al., 2007). However, the high prevalence of ND continues to be a cause of limited production. Despite the vaccination of birds, outbreak of ND has been reported in different occasions due to one or combination of factors such as vaccine failure, methods of vaccination and inadequate schedules (Numan et al., 2005); Van Brown et al., 2008). The reason for the widespread of ND has also been linked to several risk factors in the poultry industry ranging from birds to farm level. There are suggestions that migratory birds play a crucial role in the transmission of ND between different countries. However, in less developed nations where backyard poultry practice is dominant, their presence was linked to the increased prevalence of ND amongst commercial flocks (Njagi et al., 2010). In addition, the poor biosecurity and general low vaccination of birds were shown to be responsible for the rise in ND (Swayne et al., 2003).

ND is an important disease that affects the poultry sector in the Sultanate of Oman. The disease is considered the most important viral disease causing high mortality in poultry birds and economic losses to the farmers. The first outbreak of ND in the Sultanate Oman was reported in 1981 (Ministry of Agriculture and Fisheries in Oman, 2014) However, there was no detail information about the outbreak.

The major management practice in Oman entails backyard and smallholder systems with relatively small farm size. Recently, a high seroprevalence of 42% of NDV was reported in backyard poultry in Oman with indications of the system being a source of infection to commercial flocks (Shekaili et al., 2015). Nevertheless, there is no information on the seroprevalence of ND in poultry birds in the country. The fact that various risk factors have been identified in several studies worldwide in the transmission and management of the ND, such knowledge is still lacking in Oman chicken industry.

NDV has been circulating the Middle East nations for many decades. ND has been reported as an important disease in the poultry in Sultanate of Oman. Recently, a study by Shekaili et al. (2015) reported bird-level seroprevalence of antibody to Avian Influenza and NDV of 37.5% and 42% respectively in backyard poultry comprising of geese, turkeys, chickens, guinea fowl and geese (Shekaili et al., 2015). Backyard poultry was identified as a potential risk factor for the infection of commercial flocks (Shekaili et al. 2015). However, there is no current information on the seroprevalence of NDV in birds raised in backyard system in the country. Furthermore, vaccination of poultry chickens is only done in commercial flocks in Oman and seldom practiced in small holder farms. The increasing reports of rising genetic drift of the NDV with the emergence of new sub-linkages which also mean vaccination with the current available products might not provide the required protection (Snoeck et al., 2013). Hence, it is pertinent to carry out proper investigation entailing the isolation and characterization of NDV in cases of outbreaks.

Another important aspect is the risk factors associated with the transmission of NDV in various management systems in Oman. In order to curtail such potential issues and control the occurrence of NDV, obtaining information related to the risk factors associated with the outbreaks of ND is plausible. Also, whereas farms might be having adequate preventive measures, a well and functioning reporting system is important to educate farmers on how to prevent the disease and appropriate measure required during outbreaks. No study has been attempted to elucidate the reported cases of ND outbreak in Oman, as well as pattern of the disease occurrence. With the growing endemicity of the disease, despite the presence of 65 government clinics, there are indications that an effective reporting system is lacking. The findings from this study are expected to current knowledge on ND and guidelines for the control and prevention of ND in Oman.

Research objectives

The overall objective of this research is to study the epidemiology of Newcastle disease virus in Sultanate of Oman.

The specific objectives of this study were:

1. To determine the prevalence of ND in the small holder and commercial farms in Oman from 2011-2015.
2. To determine the prevalence of NDV in non-vaccinated village chickens in 2016.
3. To isolate and characterize the NDV from the outbreaks of ND in Oman in 2017.
4. To determine the risk factors associated with the occurrence of ND in various management systems in Oman.
5. To assess the economic impact of ND based on management system and scenario of ND outbreaks in Oman.

Hypothesis

The specific hypothesis that were explored in this study include the following:

Objective 1

- H_0 = there is no significant difference in the prevalence of reported ND cases from various governorates and chicken management system in Oman
- H_i = there is a significant difference in the prevalence of reported ND cases from various governorates and chicken management system in Oman

Objective 2

- H_0 = there is no association between management factors and the prevelence of ND in chicken in Oman
- H_i = there is an association between management factors and the prevelence of ND in chicken in Oman

Objective 3

- H_0 = there is no significant difference in the seroprevalence of NDV in apparently healthy village chicken from various governorates of Oman
- H_i = there is significant difference in the seroprevalence of NDV in apparently healthy village chicken from various governorates of Oman

Objective 4

- H_0 = NDV strains from outbreaks of ND in Oman is similar to that from other neighbouring countries
- H_i = NDV strains from outbreaks of ND in Oman is different from that isolated from other neighbouring countries.

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