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SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS (NDV) ANTIBODIES IN PIGEONS IN SELECTED AREAS IN KLANG VALLEY, MALAYSIA

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It is hereby certified that we have read this project paper entitled "Seroprevalence of Newcastle Disease Virus (NDV) Antibodies in Pigeons in Selected Areas in Klang Valley", by Nurfatin Shakira Binti Zaini and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 –Final Year Project.

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DEDICATION

This project paper is dedicated to

To my parents,

Zaini Ramli & Norliza Ahmad

Sisters,

for the love,

for always believe in me and for the endless

motivation and support.

To all dearest birds,

for allowing me to complete my project.

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LIST OF ABBREVIATIONS

NDV	Newcastle Disease Virus
ND	Newcastle Disease
HA	Hemagglutination test
HI	Hemagglutination inhibition test
IACUC	Institutional Animal Care and Use Committee
RBC	Red Blood Cell
Ag	Antigen
Ab	Antibody
°C	Degree Celcius
-ve	Negative
+ve	Positive
%	Percentage

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

PREVALEN ANTIBODI VIRUS PENYAKIT SAMPAR PADA BURUNG MERPATI DI KAWASAN TERPILIH DI LEMBAH KLANG, MALAYSIA

Oleh

NURFATIN SHAKIRA BINTI ZAINI

2017

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Penyakit sampar adalah penyakit ayam domestik dan spesies burung lain yang disebabkan oleh virus Newcastle Disease (NDV) yang berasal dari keluarga Paramyxoviridae. Tiada kajian yang telah dilakukan mengenai seroprevalen antibodi terhadap NDV dalam merpati berbanding dengan ayam komersial di Malaysia, keperluan untuk melakukan seroprevalence awal ini adalah untuk mengetahui

kehadiran tahap antibodi terhadap NDV dalam merpati dan penting untuk mengukur klinikal yang mereka boleh , menyumbang sebagai pembawa penyakit sampar bagi kumpulan Galliformes. Penyakit sampar boleh disebarkan oleh hubungan secara langsung dengan berpenyakit atau pembawa burung, fomites, penetasan anak ayam dari telur dijangkiti dan juga potensi penyakit zoonotik. Sebanyak 60 sampel sera merpati dikumpulkan dari dua kumpulan burung merpati; liar dan kurungan. Merpati telah disampel dari beberapa kawasan di Klang termasuk liar dan burung merpati kurungan. Sera itu telah dianalisis untuk kehadiran antibodi terhadap NDV menggunakan ujian HI. Daripada enam puluh sampel, 50.0% (30/60) sampel adalah seropositif untuk antibodi NDV. Merpati liar menunjukkan 41.7% (15/36) seroprevalen manakala merpati kurungan dengan 62.5% (15/24) seroprevalen. Ini bermakna bahawa burung merpati kurungan mempunyai seropositive lebih tinggi berbanding dengan merpati liar. Kesimpulannya, kajian ini membuktikan bahawa burung merpati terdedah kepada virus Newcastle Disease pada peringkat tertentu kehidupan mereka dan boleh menghasilkan antibodi terhadap NDV. Kajian lanjut perlu dilakukan dalam pengesanan virus dari najis, lendiran trakea dan hidung untuk menentukan sama ada burung merpati adalah takungan dan menyebarkan virus kepada persekitaran.

Kata kunci: Antibodi, haemagglutination perencatan, merpati, seroprevalen, virus Newcastle.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine as a partial requirement in the course VPD 4999- Final Year Project.

SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS (NDV) ANTIBODIES IN PIGEONS IN SELECTED AREAS IN KLANG VALLEY, MALAYSIA

By

NURFATIN SHAKIRA BINTI ZAINI

2017

Supervisor: Associate Professor Dr Jalila Abu

Co-Supervisor: Associate Professor Dr Siti Suri Arshad

Newcastle disease is a disease of domestic poultry and other bird species caused by Newcastle Disease virus from family of *Paramyxoviridae*. No study was done for NDV seroprevalence in pigeons compared to commercial chicken in Malaysia. Therefore, there is a need to do this preliminary seroprevalence study so that we able to know the prevalence of antibody level against NDV in pigeons and further, it is important for clinical measure such as they may serve as reservoirs of ND for galliformes group.

Newcastle Disease (ND) can be transmitted by direct contact with diseased or carrier birds, fomites, hatching chicks from infected egg and also potential zoonotic disease. A total of 60 sera samples of pigeons were collected from two group of pigeons; wild and captive. Pigeons were sampled from several areas in Klang Valley. The sera had been analyzed for presence of antibodies against NDV using haemagglutination inhibition (HI) test. Out of sixty samples, 50.0% (30/60) samples were seropositive for NDV antibodies. Wild pigeons showed 41.7% (15/36) seroprevalence while captive pigeons with 62.5% (15/24) seroprevalence. This means that captive pigeons has higher seropositive compared to wild. In conclusion, this study proved that pigeons are exposed to Newcastle Disease virus at certain stage of their life and can develop antibodies against NDV. Further study should be done on virus detection and isolation from faeces, tracheal and nasal swabs to determine whether pigeons are reservoir and shedding the virus to the environment.

Keywords: Antibodies, haemagglutination inhibition, Newcastle disease virus, pigeon, seroprevalence

1.0 INTRODUCTION

Newcastle disease is a disease of domestic poultry and other bird species caused by Newcastle disease virus (NDV). It is a worldwide problem that presents primarily as an acute respiratory disease, but depression, nervous manifestations, or diarrhea may be the predominant clinical form. Severity depends on the virulence of the infecting virus and host susceptibility. The disease is present worldwide and affects many species of birds causing severe losses in the poultry sector (Cattoli *et al*., 2011).

Occurrence of the disease is reportable and may result in trade restrictions. Newcastle Disease is on the A List of the World Organisation for Animal Health (OIE) which is notifiable disease, means need to be reported when the disease is diagnosed (OIE, 2012).

This disease can be transmitted by direct contact with secretions of infected birds via ingestion fecal-oral route and inhalation, fomites (e.g feed, water, human clothing) and also hatching chicks through egg (OIE, 2012). The incubation period is 2-15 days with an average of 5-6 days; some species may be over 20 days (OIE, 2012).

Birds in the *Columbiformes* order, which includes pigeons and doves, can be infected with NDV (Wakamatsu *et al.*, 2006). Pigeons are wild and freely to move which closely associated with human when they searching for food near to house compound which may cause zoonotic disease.

According to OIE (2012), humans may be infected with NDV by manifestation of unilateral or bilateral reddening, excessive lacrymation, oedema of the eyelids, conjunctivitis and sub-conjunctival haemorrhage.

In Malaysia, lack of knowledge whether pigeons are susceptible to natural infection with Newcastle Disease virus and potential for zoonotic transmission is difficult to explain for the awareness to the public.

The objective of this study includes:

To determine the seroprevalence of Newcastle Disease virus in pigeons in selected area in Klang Valley by detecting antibody titer using Haemagglutination Inhibition (HI) assay.

The hypothesis for this study is:

Pigeons in Klang valley, Malaysia are seropositive with NDV antibodies.

2.0 LITERATURE REVIEW

2.1 Pigeons in Malaysia

Columba livia or most commonly known as rock pigeons, also known as domestic pigeon can be seen anywhere in Malaysia where they are able to move freely in flock and sometimes they are seen associated with human for the food.

The rock pigeon has green and purple patch on the side of the neck and their life span is 3 – 5 years in the wild; and up to 15 years in captivity (S. Johnson, 2011). They often gather in flocks, when spooked, the flock may suddenly fly into the air and circle several times before coming down again (S. Johnson, 2011).

They have a wide range that includes western and southern Europe, North Africa, and southwest Asia. They were introduced to North America from Europe in 1606 at Port Royal, Acadia (now Nova Scotia) (S. Johnson, 2011). Its domesticated form (feral pigeon) has been introduced in other areas; and they are now very common over much of the world.

2.2 Diagnosis of Newcastle Disease

The clinical signs seen in birds infected with NDV vary widely and are dependent on factors such as the virus, host species, age of host, and infection with other organisms, environmental stress and immune status as stated by Alexander (2000).

Clinical signs in pigeons vary mainly according to age where in young animals, mortality can reach 100%, whereas in adults, mortality is minimal, and morbidity is approximately 10%. Clinical signs consist mainly of nervous signs (most prominent in young birds) and diarrhea.

The incubation period of paramyxovirosis in pigeons varies from 4-6 days to 3-4 weeks. Clinical signs of PPMV-1 infection are generally similar to the symptoms caused by viruses from the neurotrophic velogenic group of ND viruses (Pestka *et al*., 2014).

2.3 History of Newcastle Disease in Pigeons

In pigeons, Newcastle Disease is called paramyxovirosis and is caused by antigenic "pigeon variant" of the virus (pigeon paramyxovirus type 1, PPMV-1) (Pestka *et al*. , 2014). Alexander *et al*. (2004) stated that variant of NDV was discovered in the Middle East during the third panzootic in the 1970s. This disease has spread easily and now has a global distribution. In 1984 the virus spread from pigeons into domestic poultry in Great Britain. The outbreaks indicated the virus had the ability to replicate and cause infection in other avian species. The disease was no longer limited to feral pigeons and could be a source of economic loss.

Alexander *et al*. (2004) also mentioned that PPMV-1 disease in pigeons has been an ongoing panzootic since the 1980s. It remains an endemic disease in several countries due to lack of vaccination, housing methods, and the sport of pigeon racing (Hines *et al*., 2012).

2.4 Agent of the Disease

Newcastle Disease virus is from family of Paramyxoviridae. The paramyxovirus genome is a single-stranded RNA chain of negative polarity, consisting of approximately 15000 nucleotides, encoding at least seven proteins:RNA polymerase (L), haemagglutinin-neuraminidase(HN), fusion protein (F), matrix protein (M), phosphoprotein(P) and nucleocapsid protein (NP). During transcription of the gene encoding protein P, additional nonstructural protein V is produced by means of mRNA processing (Dortmans *et al*. 2010).

Dortmans *et al*. (2011) also stated that HN and F glycoproteins, forming two types of viral surface projection, play a special role in the course of infection and are the major antigens, which cause an immune response. HN glycoprotein has haemagglutinin activity, which allows the attachment of the virus-infected cells and the activity of neuraminidase, which enables a virus to leave the infected cell.

2.5 Virus Identification and Isolation

According to OIE, the supernatant fluids of faeces or tissue suspensions and swabs, obtained through clarification by centrifugation at 1000 *g* for about 10 minutes at a temperature not exceeding 25°C, are inoculated in 0.2 ml volumes into the allantoic cavity of each of at least five embryonated SPF fowl eggs of 9–11 days incubation. If SPF eggs are not available, at least NDV antibody negative eggs are required. After inoculation, these are incubated at 35–37°C for 4–7 days.

To accelerate the final isolation, it is possible to carry out two passages at a 3-day interval, obtaining results comparable to two passages at 4–7-day intervals (Alexander & Senne, 2008). Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the incubation period, should first be chilled to 4°C for 4 hours or overnight and the allantoic fluids tested for haemagglutination (HA) activity. NDV can be confirmed by the use of specific antiserum in a haemagglutination inhibition (HI) test. Usually chicken antiserum that has been prepared against one of the strains of NDV is used.

2.6 Epidemiology of Newcastle Disease

Chickens are highly susceptible to Newcastle Disease and many other species of birds both domestic and wild may also infected. This disease has been recorded in ostriches (order Struthioniformes) and pigeons (order Columbiformes) are known to be susceptible (OIE, 2012).

The morbidity and mortality rates vary among species, and with the strain of virus.

The sources of virus are from respiratory secretions/discharges and faeces of infected birds and all parts of the carcass (OIE, 2012).

Transmission can be through direct contact, fomites, hatching chicks from infected egg (OIE, 2012).

Virus is shed during the incubation period, during clinical stages and for a limited period during convalescence. Wild birds such as pigeon may act as reservoir hosts for lentogenic pathotypes of ND; subsequently, these viruses could become virulent following mutation upon establishment in domestic poultry (OIE, 2012).

3.0 MATERIALS AND METHOD

3.1 Animals

A total of 60 pigeons were sampled from several different locations in Klang valley. The birds were selected through random sampling. The wild pigeons were randomly trapped while giving feed near the jungle fowl farm located in Dengkil. Whereas the captive pigeons were sampled from two different owners in Shah Alam and Klang.

The birds were physically restrained by body grab, then placed the birds on dorsal recumbency and hold the feet using fingers in between two feet. Other hand hold supports the breast while another person that the blood.

Figure 3.1: Body grab technique

All the birds sampled were healthy. Sampling of the animals was approved by IACUC dated 30th December 2016 (AUP No: FYP.2016/FPV.41).

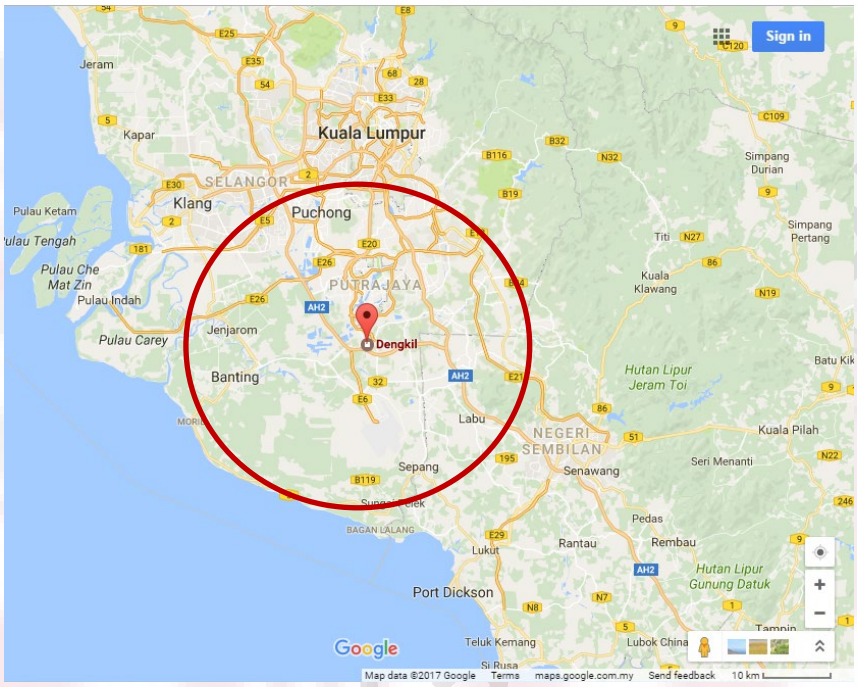

Figure 3.2: Wild pigeons were sampled from Dengkil area

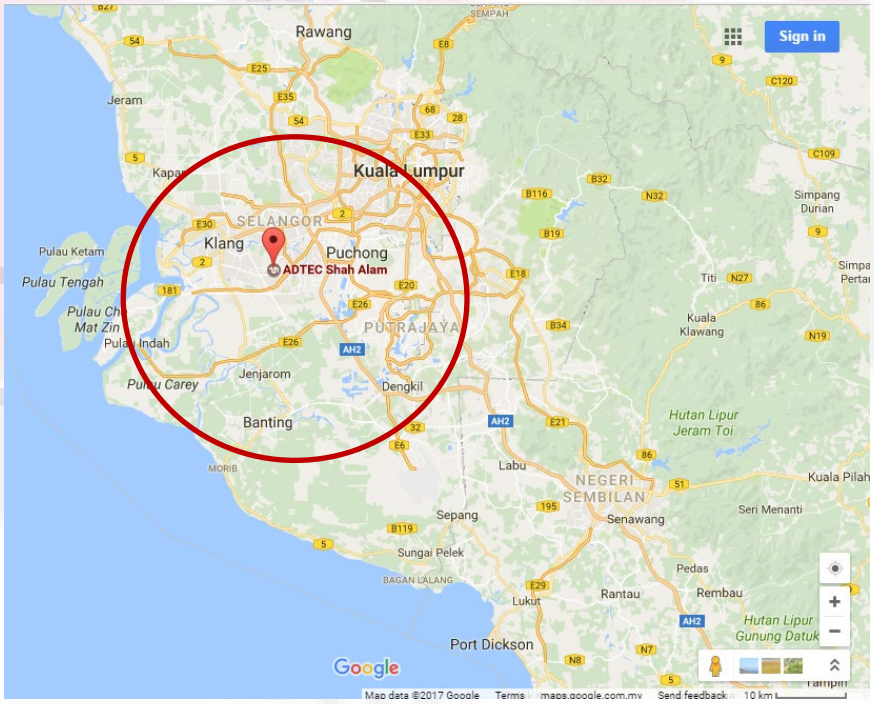

Figure 3.3: Captive pigeons were sampled from ADTEC Shah Alam and Klang area

3.2 Sample Collection

A total of 0.5 to 1.0 ml of blood was collected from brachial vein for each bird using 25G needles (B.Braun®, Germany) and a 3mL syringe (TERUMO syringe, Philippines). This wing vein was chose as it is easily accessible because the vein crosses the elbow medially.

Blood collected was then transferred into 3mL red top Plain vacutainer tubes (BD Vacutainer®, United States) and gently inverted to ensure thorough mixing of the blood with the clot activator and to prevent haemolysis. All tubes were labeled clearly prior to blood collection. The tubes were then transported back to Virology laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM).

3.3 Sample Processing

All blood samples in tubes were left standing for one to three hours in room temperature before centrifuged at speed of 2000 x g for 5 minutes (Hettich Universal 32R, United Kingdom). Each serum from each sample was extracted using a micropipette and placed into 1.5mL Eppendorf® tubes. Eppendorf® tubes were labeled accordingly and stored in -20°C (Sanyo, Japan) for further analysis.

3.4 Serological Test

Haemagglutination inhibition (HI) test that was performed in Virology Laboratory, Faculty of Veterinary Medicine was using a 4 HA unit antigen. Serial two-fold dilutions of each serum were prepared so that is diluted out through the wells. The no. 1 well in each well receives no antigen and serves as a control of non-specific agglutination. 0.025 ml of PBS from well no. 2 was placed through well no. 12. Then, 0.025 ml of serum was placed in well no. 1. After that, 0.025 ml of serum from well no. 1 was transferred and mixed to the second well, and continued through the 11^{th} well, using a 0.025 ml diluter, finally 0.025 ml discarded from the last well.

Next, 0.025 ml of virus dilution containing 4HA units was added to each serum dilution starting from well no. 2 through 11th. The 4HA unit's virus dilution was obtained by taking out 0.025 ml virus dilution from a 8HA unit virus stock.

After that, the dilution was mixed by hand shaking for about 10 seconds and maintain at room temperature for 20 minutes. 0.05 ml of 0.5% RBC suspension was added ineach well and maintain at room temperature.

Finally the result of the test is read after RBC has settled out approximately 30 minutes. The HI titre is the highest dilution of serum causing complete inhibition of antigen. In this picture (Figure 3.4), the highest dilution of complete inhibition is at 2^3 or at dilution of 8, which means negative. If the inhibition is at dilution of 16 or more, the result should be positive.

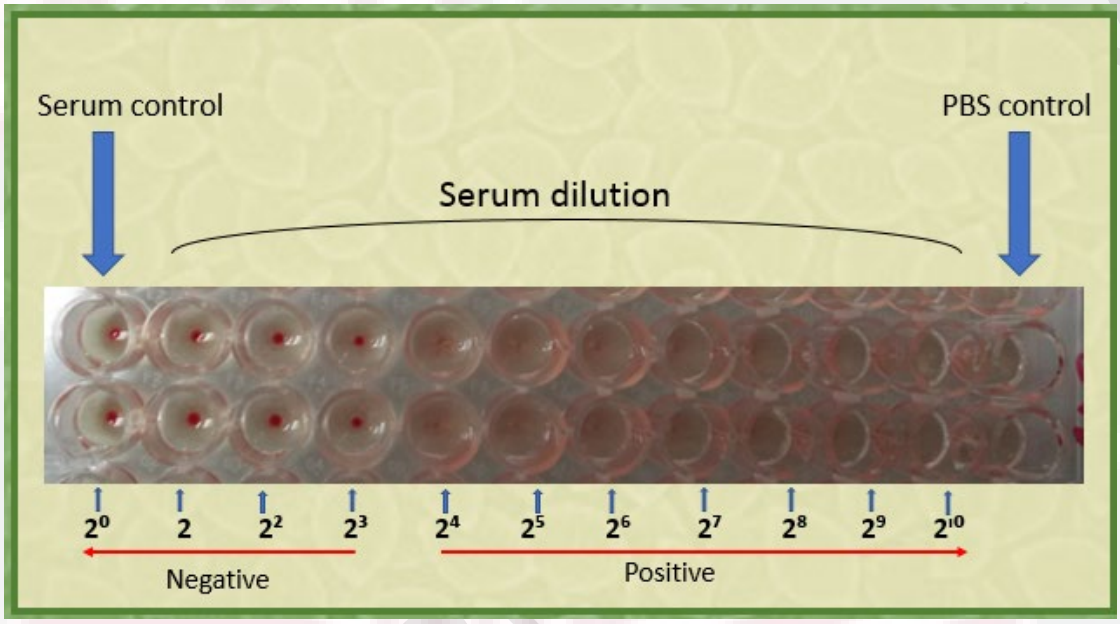

Figure 3.4: Indication for positive and negative haemagglutination inhibition (HI) test

4.0 RESULTS

A total of 60 sera samples of pigeons were collected from three different location in Klang valley and had been analyzed for presence of antibodies against Newcastle Disease virus using haemagglutination inhibition test. As the result, NDV antibodies have been detected in 50% (30/60) samples which the antibody titer at serum dilution of 16 or more. Among all positive samples, 17 samples have antibody titer at serum dilution of 16, 9 samples were at serum dilution of 32 and 4 samples were at dilution of 64.

From 60 samples, they were according to two groups, 24 were captive while another 36 were wild pigeons. The prevalence was high in captive pigeons 41.7% (15/24) compared to wild pigeons 62.5% (15/36). Chi-square test was performed and statistically there was no association between the group of pigeons and seropositivity since the P value was more than 0.05.

Seroprevalence was calculated using formula:

Seroprevalence = (Seropositive birds/Total birds) x 100%

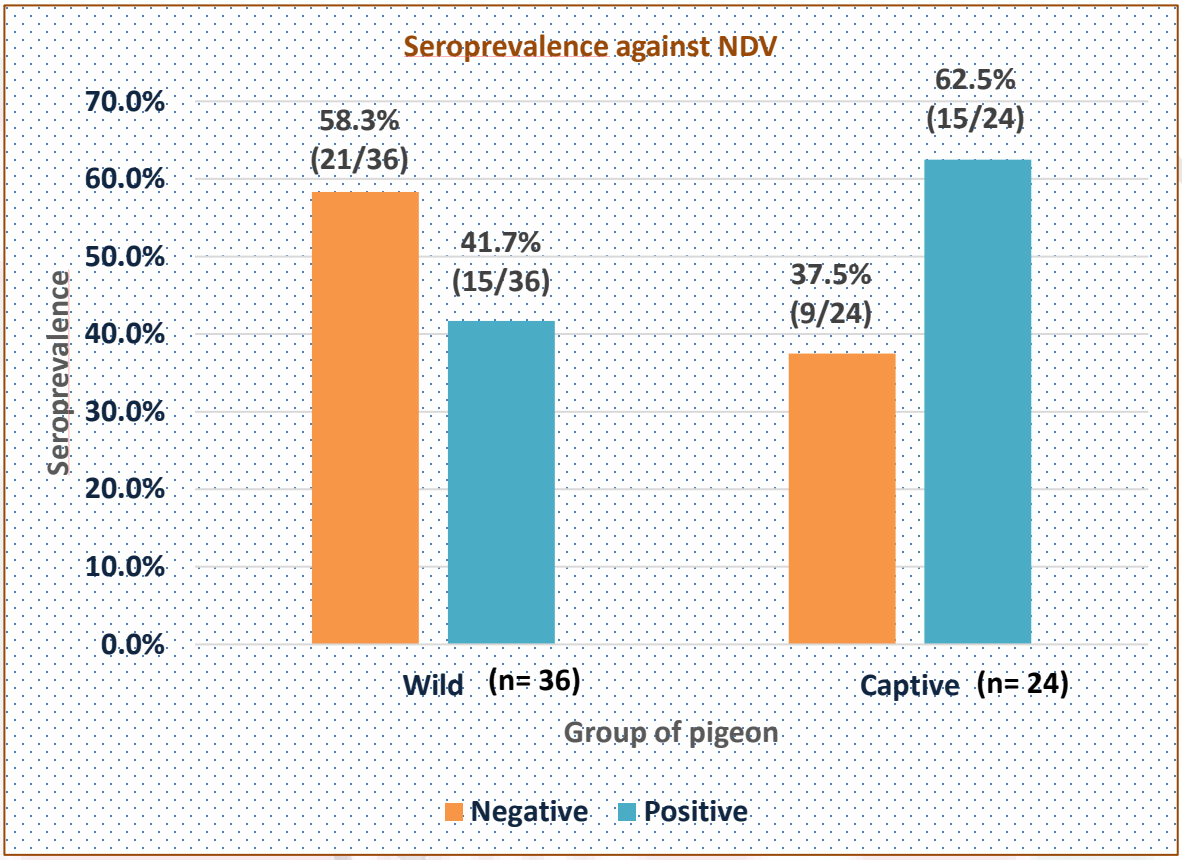

Figure 4.2: Seroprevalence against NDV in wild and captive pigeons

5.0 DISCUSSION

In this study, the seroprevalence of NDV in pigeons in selected areas in Klang valley was found to be 50% indicating that pigeons' are developing antibodies against NDV, with evidence the presence of antibodies against NDV in serological test. Means that, half of the pigeons sampled in Klang valley Malaysia known to have and develop antibodies against ND virus due to seropositive result. The prevalence can be considered high compared to study done by Sai'du *et al.* (2004) where seroprevalence study was conducted in Nigeria stated that the number positive for ND virus antibodies were 11 (44%), 5 (0.2%), 6 (0.24%) and 11 (44%) of 25 each of pigeons, laughing doves, mourning doves and turtle doves sampled.. While Wambura (2010) did study in Morogoro Municipality revealed that ND antibody prevalence in pigeons is 10%.

Onapa *et al*. (2006) mentioned that transmission of NDV can be by direct contact among ducks and chickens, as well as environmental contamination by viruses shed in the feces. This study supported my findings that captive pigeons showed high seropositive prevalence which is 62.5% (15/24) because the pigeons are located in same aviary.

Thus the virus circulating in the environment within the enclosure. Whereas among wild pigeons, 15 out of 36 (41.7%) pigeons are seropositive, which is lower compared to captive pigeons due to low possibility of circulating virus in one place and less contact with each other.

Furthermore, Alexander *et al.* (2008) stated that the presence of antibodies for NDV is due to as a result of vaccination programs or natural exposure to the antigen. In this study, all pigeons sampled were not vaccinated. Therefore, the presence of antibody against ND virus is due to natural exposure at some stage of life.

Haemagglutination Inhibition (HI) test was used for detection and quantification of antibodies against Newcastle Disease (ND) virus. (OIE, 2012). According to OIE**,** the haemagglutination inhibition (HI) test is used most widely in ND serology, its usefulness in diagnosis depends on the vaccinal immune status of the birds to be tested and on prevailing disease conditions Conventional serological test which are Haemagglutination (HA) and haemagglutination inhibition (HI) tests will be conducted by using technique that are available at Virology Laboratory, Faculty of Veterinary Medicine.

As summary of discussion, although ND virus is effectively controlled by vaccination, it is still endemic in Malaysia and outbreaks of the disease have contributed to major losses for the poultry industry (Yusoff K., 2008). Means that, pigeons may also have this disease as they had develop the antibodies against NDV but did not show any clinical signs.

6.0 CONCLUSION AND RECOMMENDATIONS

This study had revealed that there was presence of antibodies against Newcastle Disease virus. Pigeons in selected areas in Klang valley were exposed to Newcastle Disease virus in certain of their life and can develop antibodies against NDV.

The recommendations for further study are to continue further work on ND virus isolation and antigen detection via PCR from faeces, tracheal and nasal swabs to determine if pigeons are reservoir and shedding the virus. If they are known can be reservoir and can be infected with ND, the captive pigeons are recommended to be vaccinated with ND vaccine as they are precious and owner bought them with high price. Further study can be done also by detecting antibodies against NDV in other Columbiformes species such as doves. Besides that, determination of the strain can be performed by using full genome sequencing via next generation sequencing (NGS).

REFERENCES

Alexander, D. J. (2000). Newcastle disease and other avian paramyxoviruses. *Revue scientifique et technique (International Office of Epizootics)*, *19*(2), 443-462.

Alexander, D. J., Bell, J. G., & Alders, R. G. (2004). *A technology review: Newcastle disease, with special emphasis on its effect on village chickens* (No. 161). Food & Agriculture Org..

Cattoli G., Susta L., Terregino C., Brown C. (2011) 'Newcastle disease: a review offield recognition and current methods of laboratory detection'. *Journal of Veterinary Diagnostic Investigation 23(4)* 637– 656.

D. Pestka, T. Stenzel, A. Koncicki. (2014) 'Occurrence, characteristics and control of pigeon paramyxovirus type 1 in pigeons'. *Polish Journal of Veterinary Sciences Vol. 17*, No. 2

Dortmans, J. C., Koch, G., Rottier, P. J., & Peeters, B. P. (2011). Virulence of Newcastle disease virus: what is known so far?. *Veterinary research*, *42*(1), 122.

Hines, N. L., & Miller, C. L. (2012). Avian paramyxovirus serotype-1: a review of disease distribution, clinical symptoms, and laboratory diagnostics. *Veterinary medicine international*, *2012*.

Leow, B. L., Shajarutulwardah, M. Y., & Ramlan, M. (2011). Newcastle disease in Malaysia: diagnostic cases in veterinary research institute (VRI) IPOH from 2004-2009. *Malaysian Journal of Veterinary Research*, *2*(1), 45-51.

Sa'idu, L., L. B. Tekdek, P.A. Abdu. (2004) 'Prevalence of Newcastle disease antibodies in domestic and semi-domestic birds in Zaria, Nigeria'. *Vet. Arhiv 74*, 309-317.

Wakamatsu, N., King, D. J., Kapczynski, D. R., Seal, B. S., & Brown, C. C. (2006). Experimental pathogenesis for chickens, turkeys, and pigeons of exotic Newcastle disease virus from an outbreak in California during 2002-2003. *Veterinary Pathology*, *43*(6), 925-933.

Wambura, P. N. (2010). Detection of antibody to Newcastle disease virus in semi-domesticated free-range birds (Numida meleagris and Columba livia domestica) and the risk of transmission of Newcastle disease to village chickens-short communication. *Veterinarski Archives*, *80*, 129-134.

Yusoff, K. (2008). *Newcastle disease virus: a journey from poultry to cancer*. Universiti Putra Malaysia Press.

APPENDICES

Name /ID	Antibody titer	Status	Management	Area
Pigeon 1	2^3	Negative	Wild	Dengkil
Pigeon 2	2^3	Negative	Wild	Dengkil
Pigeon 3	2^3	Negative	Wild	Dengkil
Pigeon 4	2^2	Negative	Wild	Dengkil
Pigeon 5	2	Negative	Wild	Dengkil
Pigeon 6	2^3	Negative	Wild	Dengkil
Pigeon 7	2^2	Negative	Wild	Dengkil
Pigeon 8	2^3	Negative	Wild	Dengkil
Pigeon 9	2^2	Negative	Wild	Dengkil
Pigeon 10	2^3	Negative	Wild	Dengkil
Pigeon 11	2^2	Negative	Wild	Dengkil
Pigeon 12	2^3	Negative	Wild	Dengkil
Pigeon 13	2^0	Negative	Wild	Dengkil
Pigeon 14	2^0	Negative	Wild	Dengkil
Pigeon 15	2	Negative	Wild	Dengkil
Pigeon 16	2^4	Positive	Wild	Dengkil
Pigeon 17	2^2	Negative	Wild	Dengkil
Pigeon 18	2^4	Positive	Wild	Dengkil
Pigeon 19	2^6	Positive	Wild	Dengkil

Pigeon 20	2	Negative	Wild	Dengkil
Pigeon 21	2^3	Negative	Wild	Dengkil
Pigeon 22	2^3	Negative	Captive	Shah Alam
Pigeon 23	2^4	Positive	Captive	Shah Alam
Pigeon 24	2^3	Negative	Captive	Shah Alam
Pigeon 25	2^4	Positive	Captive	Shah Alam
Pigeon 26	2^5	Positive	Captive	Shah Alam
Pigeon 27	2^2	Negative	Captive	Shah Alam
Pigeon 28	2^2	Negative	Captive	Shah Alam
Pigeon 29	2^2	Negative	Captive	Shah Alam
Pigeon 30	2^3	Negative	Captive	Shah Alam
Pigeon 31	2^4	Positive	Captive	Shah Alam
Pigeon 32	2^5	Positive	Captive	Shah Alam
Pigeon 33	2^4	Positive	Captive	Shah Alam
Pigeon 34	2^3	Negative	Captive	Shah Alam
Pigeon 35	2^5	Positive	Captive	Shah Alam
Pigeon 36	2^6	Positive	Captive	Shah Alam
Pigeon 37	2^4	Positive	Captive	Shah Alam
Pigeon 38	2^5	Positive	Captive	Shah Alam
Pigeon 39	2^5	Positive	Wild	Dengkil
Pigeon 40	2^6	Positive	Wild	Dengkil
Pigeon 41	2^4	Positive	Wild	Dengkil

Pigeon 42	2^4	Positive	Wild	Dengkil
Pigeon 43	2^4	Positive	Wild	Dengkil
Pigeon 44	2^4	Positive	Wild	Dengkil
Pigeon 45	2^5	Positive	Wild	Dengkil
Pigeon 46	2^3	Negative	Wild	Dengkil
Pigeon 47	2^3	Negative	Wild	Dengkil
Pigeon 48	2^4	Positive	Wild	Dengkil
Pigeon 49	2^6	Positive	Wild	Dengkil
Pigeon 50	2^5	Positive	Wild	Dengkil
Pigeon 51	2^5	Positive	Wild	Dengkil
Pigeon 52	2^4	Positive	Wild	Dengkil
Pigeon 53	2^3	Negative	Wild	Dengkil
Pigeon 54	2^4	Positive	Captive	Klang
Pigeon 55	2^4	Positive	Captive	Klang
Pigeon 56	2^3	Negative	Captive	Klang
Pigeon 57	2^5	Positive	Captive	Klang
Pigeon 58	2^4	Positive	Captive	Klang
Pigeon 59	2^3	Negative	Captive	Klang
Pigeon 60	2^4	Positive	Captive	Klang

Appendix 1: Captive pigeons in an aviary

Appendix 2: Wild pigeons in a village chicken farm