UNIVERSITI PUTRA MALAYSIA

CLINICO-PATHOLOGICAL FEATURES OF NEWCASTLE DISEASE IN JAPANESE QUAILS (COTURNIX COTURNIX JAPONICA) INFECTED WITH NEWCASTLE DISEASE VIRUS AF2240 STRAIN

NUR ATIKAH BT HASHIM

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Selangor Darul Ehsan

It is hereby certified that we have read this project paper entitled "Clinico-pathological Features of Newcastle Disease in Japanese quails (*Coturnix coturnix japonica*) Infected with Newcastle Disease Virus AF2240 strain", by Nur Atikah Bt Hashim and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901- Project.

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DEDICATED TO:

My lovely mother

Asmat Bt Awang

My late father

Hashim B. Yusoff

My family

Hashimah Bt Hashim

Mohd Khailani B. Hashim

Mohd Fatihe B. Hashim

Fatonah Bt. Hashim

Zaleha Bt Hashim

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Mohd Zuriman B. Hashim

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Dr Mohd Hezmee B. Mohd Noor

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Dr Lokman Hakim B Idris

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CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

NDV	Newcastle Disease Virus
ml	millilitre
EID_{50}	Embyonic Infective $Dose_{50}$

ABSTRAK

Abkstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901- Projek

CIRI- CIRI KLINIKAL DAN PATOLOGIKAL PENYAKIT NEWCASTLE DALAM PUYUH JEPUN (COTURNIX COTURNIX JAPONICA) DIJANGKITI NEWCASTLE DISEASE VIRUS STRAIN AF2240.

Oleh

NUR ATIKAH BT HASHIM

2015

Penyelia: Dr. Mohd Hezmee bin Mohd Noor

Penyelia bersama: Dr. Lokman Hakim bin Idris

Pelbagai spesis burung terdedah kepada virus Newcastle Disease. Ia menjadi kebimbangan kepada industri ayam itik yang mana puyuh berupaya menjadi pembawa kepada penularan wabak penyakit ini seperti yang dilaporkan dalam sesetengah kajian. Kajian ini dijalankan bagi menentukan pendedahan puyuh jepun (*Coturnic corturnix japonica*) terhadap virus Newcastle Disease (NDV) strain AF2240. Strain AF2240 merupakan strain *Malaysian viscerotropic velogenic* yang diasingkan sewaktu penularan wabak dalam negara pada 1960an. Jadi melalui induksi NDV jenis ini, adalah menjadi jangkaan bahawa ia dapat menjelaskan pendedahan puyuh termpatan terhadap penyakit ini dalam negara. 24 ekor puyuh

yang berumur satu minggu telah dipilih secara rawak dan dibahagikan secara sama rata kepada empat kumpulan. Tiga kumpulan pertama (Kumpulan A, B, dan C) disuntik dengan tiga dos antigen (EID_{50} 7.3log10/0.1ml) yang berbeza bagi setiap kumpulan (0.1ml, 0.2ml, 0.3ml) secara *intramuscular* dan terdapat satu kumpulan kawalan negatif. Pada hari ke-lapan selepas infeksi, perubahan klinikal termasuk bulu tidak kemas, kemurungan, hilang daya penyelarasan, ketimpangan, anoreksia, cirit- birit, rebah, lumpuh kaki dan sayap dapat dilihat secara signifikan dalam sesetengah puyuh yang dijangkiti dalam setiap kumpulan. Tanda- tanda klinikal bagi semua puyuh adalah signifikan ($p<0.05$) bermula dari hari ke- 6 hingga hari ke- 9 selepas cabaran. Namun, tiada perbezaan yang signifikan ($p< 0.05$) antara ketiga-tiga kumpulan yang dijangkiti. Berdasarkan pemeriksaan serologi menggunakan ELISA, semua puyuh dalam setiap kumpulan tidak menunjukkan kenaikan titer antibodi sepanjang tempoh kajian. Lesi yang signifikan telah dilihat sewaktu pemeriksaan postmortem dalam puyuh yang dijangkiti termasuk congesi dan pendarahan dalam usus, congesi dalam hati, otak, otot pektoral, jantung dan paru- paru. Lesi patologi meningkat dengan signifikan ($p< 0.05$) sepanjang tempoh kajian. Terdapart perbezaan signifikan ($p<0.05$) antara kumpulan kawalan dang kumpulan yang dijangkiti. Namun, tiada perbezaan signifikan ($p<0.05$) antara ketiga-tiga kumpulan yang dijangkiti. Konklusinya, Japanese quail boleh terdedah kepada virus Newcastle Disease strain AFF2240 yang boleh memberi kesan klinikal dan menjurus kepada kematian. Namun, kajian ini turut mencadangkan bahawa dos yang tertinggi tidak semestinya menghasilkan kesan kilinikal dan pathologi mahupun titer antibodi yang paling tinggi.

Kata kunci: Newcastle Disease Virus strain AF2240, viscerotropic velogenic, congesi, antibodi NDV

ABSTRACT

An abstract of the project paper presented to Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901- Project

CLINICO-PATHOLOGICAL FEATURES OF NEWCASTLE DISEASE IN JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*) INFECTED WITH NEWCASTLE DISEASE VIRUS AF2240 STRAIN

By

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2015

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Co-supervisor: Dr. Lokman Hakim bin Idris

Various birds are susceptible to Newcastle disease viruses (NDV). This has become a concern to poultry industry as quails might become a potential carrier in disease outbreak as reported in some studies. This experiment was conducted to determine the susceptibility of Japanese quails (*Coturnic corturnix japonica*) towards Newcastle disease virus (NDV) AF2240 strain. The AF2240 strain is a Malaysian viscerotropic velogenic strain that was isolated during an outbreak in the country in the 1960s. The induction of NDV of this strain is expected to determine the susceptibility of the local domesticated quails towards NDV AF2240 strain. 24 quails of 2 weeks of age were randomly selected and equally divided into four

groups. The first three groups (Group A, B, and C) were administered with three different doses of antigen (EID_{50} 7.3log10/0.1ml) (respectively (0.1ml, 0.2ml, 0.3ml) intramuscularly with a negative control group. On day six of post infection, clinical changes including ruffled feathers, depression, incoordination, lameness, anorexia, diarrhoea, recumbent and paralysis of wings and legs were significantly observed in some of the infected quails in each groups. Clinical signs of all quails significantly shown ($p<0.05$) starting from day 6 to day 9 of post challenge. However, there were no significant differences ($p< 0.05$) between the three infected groups. Based on serological examination using ELISA, all quails in each group did not show increase in antibody titre during experimental period. Significant lesions were observed upon postmortem examination of infected quails of all groups including congestion and haemorrhages of intestine, congestion of liver, brain, pectoral muscles, heart and lungs. The pathological lesions significantly increased ($p< 0.05$) throughout the study. There were significant difference ($p<0.05$) between control and infected group. However there were no significant difference ($p<0.05$) between the three treatment groups. In conclusion, Japanese quails are susceptible towards Newcastle Disease Virus AFF2240 which may cause severe clinical impacts and even lead to fatality. However, this study also suggesting that highest dose of virus does not necessarily causing most severe clinico- pathological effects or highest antibody titre.

Keywords: Newcastle Disease Virus AF2240 strain, Viserotropic velogenic, Congestion, NDV Antibody

1. 0 INTRODUCTION

In Malaysia, Newcastle disease is an endemic disease that occurs throughout a year. This disease is a major threat in poultry industry, not only in Malaysia but also occur widely in Asian region. Newcastle Disease Virus is included in List A of the Office International des Epizooties which require report in case of an outbreak. The epidemiology of the disease involves the agent: Newcastle Disease virus of different strains, environment that as a predisposing factor especially during climatic stress and the host which may include varies species of birds. Prophylactic vaccination against this disease has been developed and practiced in countries with wide scale of commercial poultry production including Malaysia. There are several outbreaks of Newcastle disease in flocks of Japanese quails (*Coutrnix coturnix japonica*) that have been reported. However, there is lack of study being carried out on the quails in Malaysia and Newcastle disease is not widely diagnosed in many part of the country which may be due to small scale of quail industry.

Newcastle Disease Virus AF2240 strain is a Malaysian velogenic viscerotropic strain that was isolated during an outbreak in the country in the 1960s and used as a vaccine challenge virus in Malaysia.

Since there is lack of evidence regarding Newcastle disease infection in Japanese quails in Malaysia, therefore by using this strain of virus, it is expected that this strain can prove that quails in this country are susceptible towards the infection of Newcastle disease virus.

2. 0 LITERATURE REVIEW

2. 1 Newcastle Disease Virus AF2240 strain

According to Murulitharan et al. (2012), Newcstle Disease is caused by Avian Parainfluenzavirus serotype 1 (APMV-1) viruses, which is a member of the genus Avulavirus, belong to Paramyxoviridae family and it is classified into three pathotypes: the lentogenic strains are non-virulent, the mesogenic strains have intermediate virulence, and the velogenic which consist of velogenic neurotropic and velogenic viscerotropic strains are highly virulent and cause high mortality in infected animals. Different strains will show different clinical manifestation in infected birds. NDV AF2240 strain is a Malaysian velogenic viscerotropic strain that was isolated during an outbreak in the country in the 1960s and used as a vaccine challenge virus in Malaysia (Murulitharan et al., 2012). The clinical manifestations of velogenic viscerotropic strain include uncoordinated gait and movements, myoclonic jerking, abnormal positioning of the head and neck (opisthotonus), paralysis of legs and wings and hemorrhagic diarrhoea (Czirjak et al., 2007). Pathological signs that can be seen in infected birds are inflammation and petechial hemorrhages on brain, and proventriculus, while the small intestine and caecal tonsils had multifocal, necrotic and hemorrhagic areas (Rahman et al., 2014).

2. 2 Newcastle Disease threat in quails

Czirjak et al. (2007) reported that Newcastle disease virus isolate from natural Newcastle Disease outbreak in Japanese quail manifested clinically by central nervous system dysfunction with 100% morbidity and mortality. Based on the study done by Lima et al. (2004), when the quails infected with NDV velogenic viscerotropic strain were put in direct contact with SPF free chicken, the quails did not show clinical signs while at the same time it cause 100% mortality in the chickens with clinical signs and macroscopic lesions were indicative of NDV post infection. This showed that quails have potential to become important carrier of NDV to other poultry species and may harm the poultry industry as what stated by Lima et al. (2004).

2. 3 Serological Test

The purpose of serological test is to detect the presence of antibodies to Newcastle disease virus which can be used to assess the efficacy of Newcastle disease vaccine trial program, to detect and evaluate the Newcastle disease virus antibodies in the field from natural infection. According to FAO (2012), there are two assays commonly used to perform this test which is the Haemagglutination inhibition (HI) test and ELISA (Enzyme linked immunosorbent assay) using a commercialized ELISA kit for NDV. According to OIE (2012), HI test is considered as gold standard test; however the usefulness in diagnosis of ND is dependent on immune status of the birds and the current disease conditions.

2. 4 Economy impacts of Newcastle disease

The wide range of viral infectivity of Newcastle Disease Virus reach approximately 241 species of 27 orders of birds (Madadger et al., 2013) and Japanese quails is included (Nanthakumar et al., 2000). Newcastle disease cause different clinical impacts depending on the type of the virus and in case of outbreak of ND that belong to virulence strain, it lead to devastating effect on poultry due to high morbidity and mortality rates (Ashraf & Shah, 2014). In Southeast Asia, it is now endemic and cause massive economic losses to commercial poultry (Munir et al., 2012 b). If the chickens are not vaccinated with NDV, the mortality rate may reach up to 100% (Ashraf & Shah, 2014). Annually, millions of dollars were wasted from the clinical impacts of this disease (Waheed et al., 2013) and hence it is one of the top ranked poultry diseases (OIE, 2012).

2. 5 Japanese quails

The Japanese quails are included in order *Galiformes*, genus *Coturnix*, and species japonica and can be found in Japan, Korea, Mongolia and Eastern China (Mizutani, 2008). The domestication of Japanese quail started in Japan during the eighth century as a singing bird and later in 1907, selection of this type of quails were performed to increase the production of egg from poultry origin while European countries selected them due to their excellent body weight gain mostly for meat production (Mizutani, 2008). According to Mizutami (2008), the quail reach its sexual maturity at 38 to 42 days of age and they could live for 3 to 4 years.

3. 0 MATERIAL AND METHODS

3. 1 Experimental design

24 quails of one week of age were randomly selected and purchased from Puyumas Farm Best. The quails were acclimatized for a week at the Laboratory Animal House, UPM. The mice were divided into four groups: Group A, B, C, and a control group with each of the group containing six quails with mixed genders. Each group were placed in different cage and the control group were separated in different room with commercial feed and water *ad libitum*. All experimental procedures were carried out according to the UPM's Institutional Animal Care and Use Committee (IACUC) guidelines. Blood samples were taken in all quails for NDV antibody detection using enzyme linked immunosorbent assay (ELISA) as for screening purpose. The quails in the Group A were injected with 0.1 ml of NDVAF2240 strain ($10^{7.3}$ EID_{50} / 0.1ml) using intramuscular route (at pectoral muscle region), Group B injected with 0.2 ml and Group 3 were injected with 0.3 ml of the same virus intramuscularly. No virus was injected to quails in control group. Clinical changes in the quails after the virus challenge were recorded and blood samples were taken for NDV antibody detection using enzyme linked immunosorbent assay (ELISA) at day 5 and day 9 of post challenge. After second blood collection was performed, all quails in each group were euthanized. Necropsy was then conducted to examine the affected organs and gross lesions of the organs were recorded.

3. 2 Viral Antigen

NDV AF2240 strain was obtained from Laboratory of Vaccine and Immunotherapeutic, Institute of Bioscience, UPM in form of suspension. The EID50 of the virus is 7.3 log10/0.1ml.

3. 3 Antibody Titre

Serum samples collected from quails at day 5 and day 9 post challenge were tested for NDV antibodies using commercial enzyme linked immunosorbent assay (ELISA) kit (Cusabio Chicken NDV antibody ELISA Kit). Two blank wells were set with 100µl of sample diluents per well. Two negative control wells and two positive controls were set and 100µl of negative control or positive control were added to each well. 100µl of diluted sample was added into sample well. The wells were covered and incubated for 30 minutes at 37˚C. The wells were then washed 3 times with 250µl wash buffer per well. After washing, 50µl of HRP-conjugate was added to each well and incubated for 30 minutes at 37˚C. Then, the wells were washed 3 times. According to required amount, equal substrate A and substrate B were mixed; the 100µl of mixed substrate was added to each well. The wells were covered and incubated for 10 minutes at 37˚C. Stop solution to each well was added to stop the reaction. Microtitre plate reader was used to record the absorbance at 450nm and the NDV antibody titre was obtained using Infinite 200Pro software.

3. 4 Clinical Sign and Gross Pathological Lesion Scoring

As previously described by (Rahman et al., 2014) each clinical signs was graded on a scale of 0-3: 0 described as no clinical signs, followed by 1 was mild, 2 were moderate, and 3 were severe. Each mortality was graded as 9. The scores were added and the grand total was calculated at the end of the experiment. As for pathological scoring method, lesions in major organs including brain, intestine, liver, heart, proventriculus and muscles were scored as none (0), mild (1), and severe (2). Lesion scores per bird (0–12) were calculated by adding scores of all six organs.

3. 5 Statistical analysis

For clinical score analysis, the Kruskal-Wallis test was independently used to compare the median clinical scores for each day post challenge, and then multiple pair wise comparisons were performed for post hoc comparisons. One – way analysis of variance (ANOVA) statistical analysis followed by Tukey's post hoc test for comparison of postmortem lesions in each groups were performed. In this test, a value of $p< 0.05$ is considered significant. Correlation coefficient was performed to determine the correlation between clinical signs and postmortem changes. All data were analysed using GraphPad Prism 6.

4. 0 RESULT

4. 1 Clinical signs scoring

Clinical signs of all quails was significantly exhibited ($p<0.05$) starting from day 6 to day 9 of post challenge. However, there were no significant differences ($p< 0.05$) between the three infected groups.

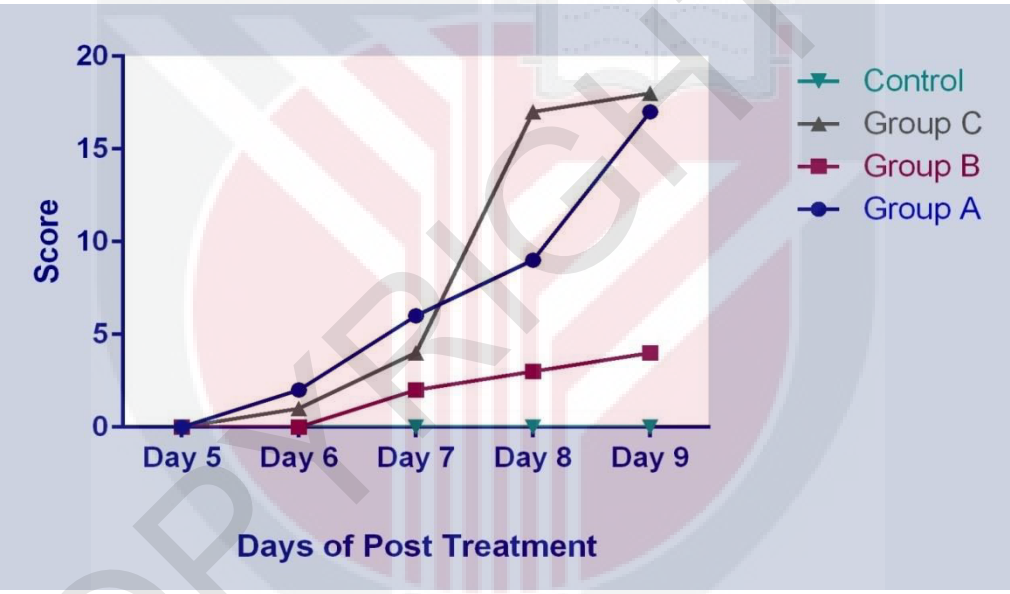

Figure 1: Clinical sign scoring throughout the study

Figure 2: The quail in control group after day 9 of post challenge with NDV virus (score 0).

Figure 3: The infected quail after day 9 of post challenge with NDV (score 3).

4. 2 Antibody titre

All quails in each group did not show increase in antibody titre during experimental period.

4. 4 Gross pathological lesion scorings

The pathological lesions significantly increased ($p < 0.05$) throughout the study. There were also significant difference ($p<0.05$) between control and infected group. However there were no significant differences ($p<0.05$) between the three infected groups.

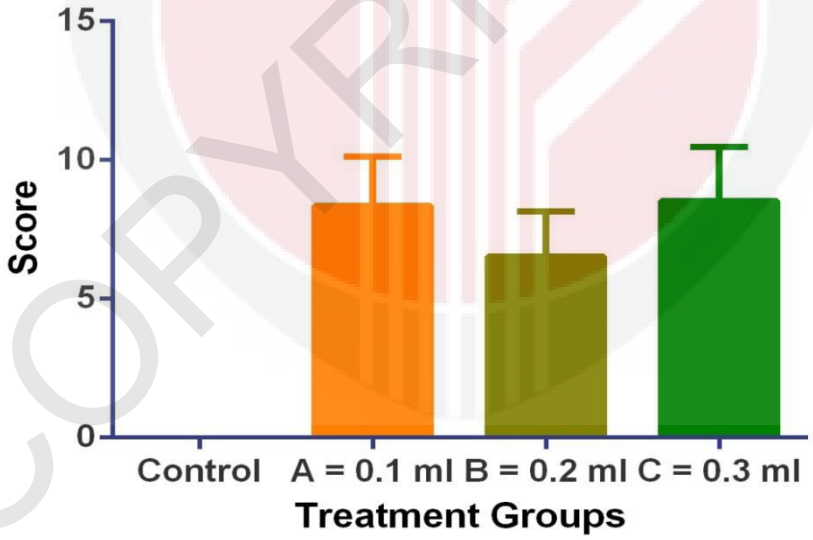

Figure 4: Gross pathological lesions scoring at the end of the study.

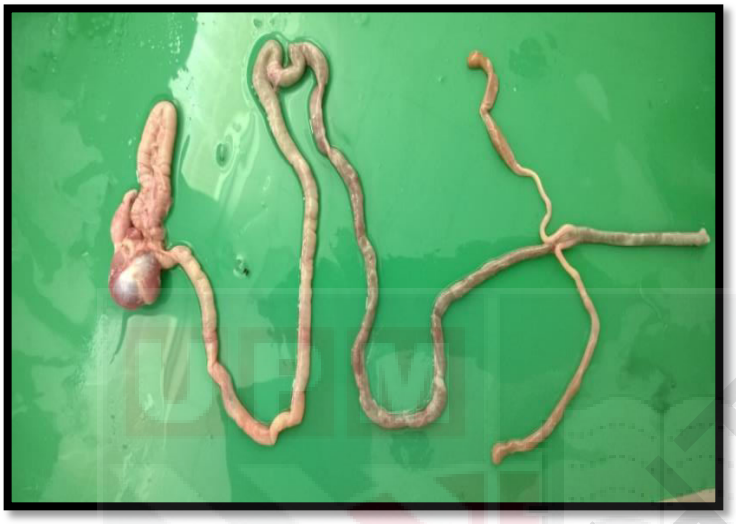

Figure 5: The intestine of a quail in the control group (score 0).

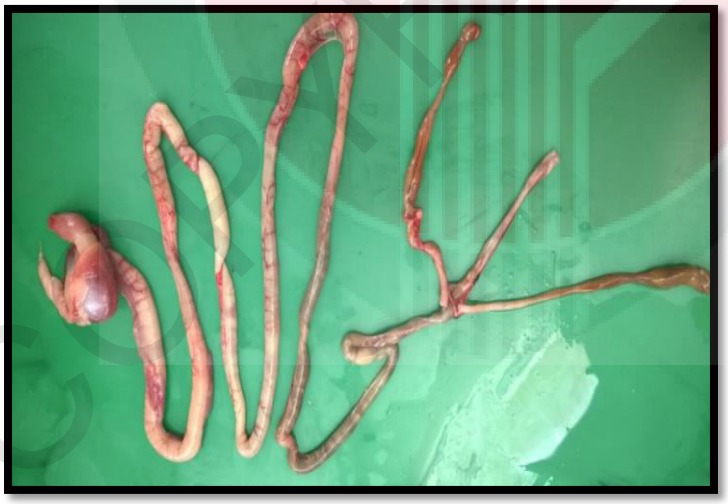

Figure 6: The congested intestine of a quail infected with NDV (score 2).

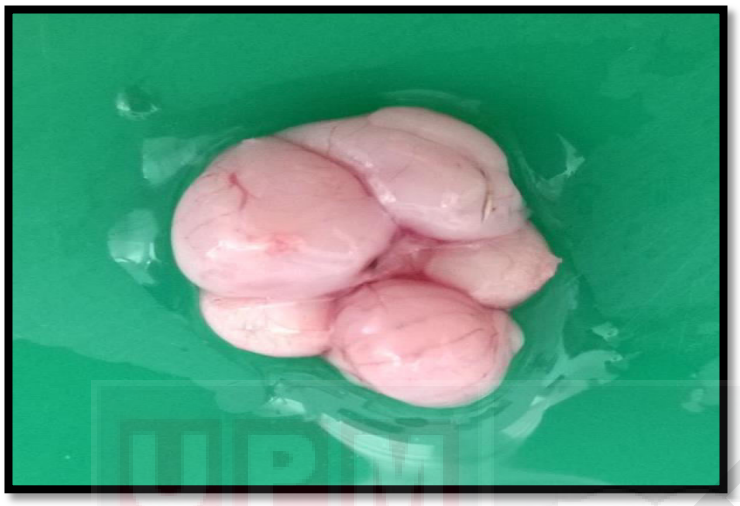

Figure 7: The brain of a quail in control group (score 0).

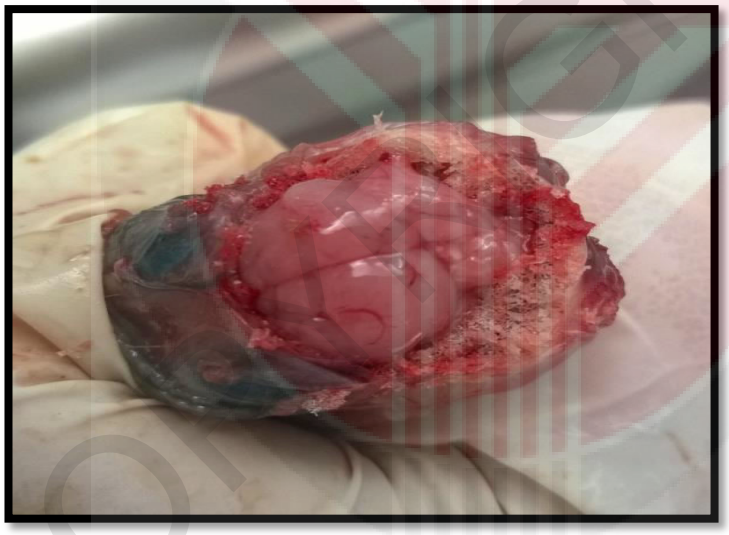

Figure 8: The congested brain of a quail infected with NDV (score 2).

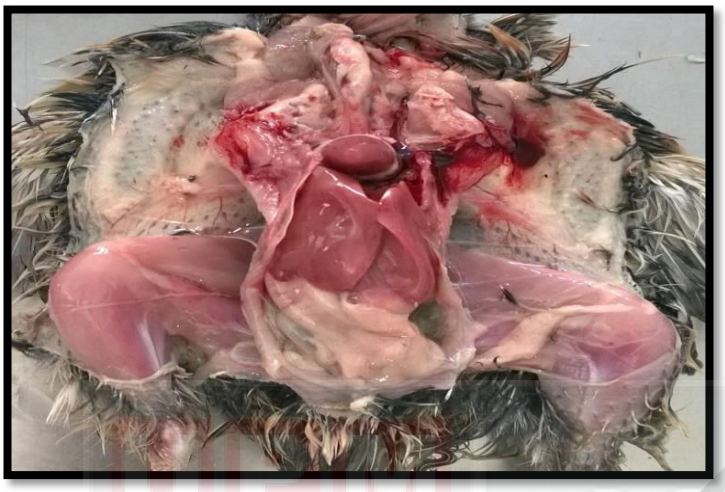

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Figure 9: The liver of a quail in control group (score 0).

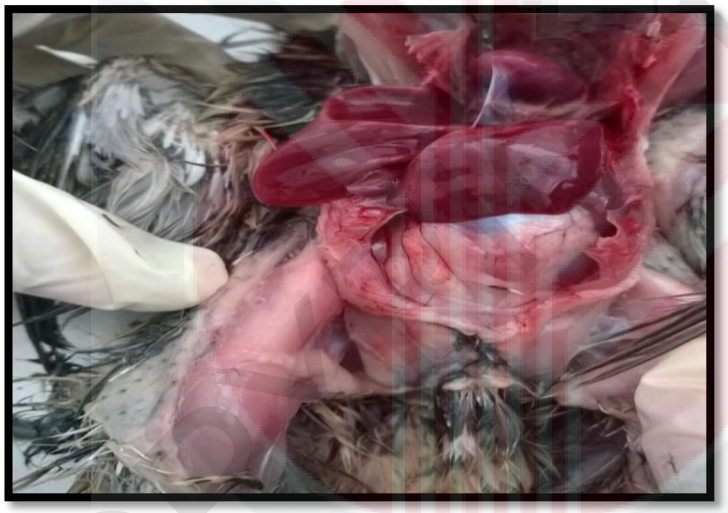

Figure 10: The congested liver of a quail infected with NDV (score 2)

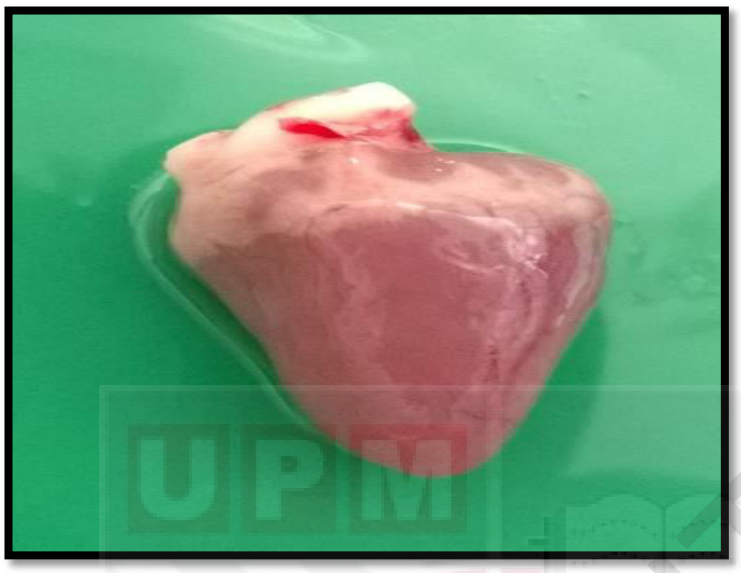

Figure 11: The heart of a quail in control group (score 0).

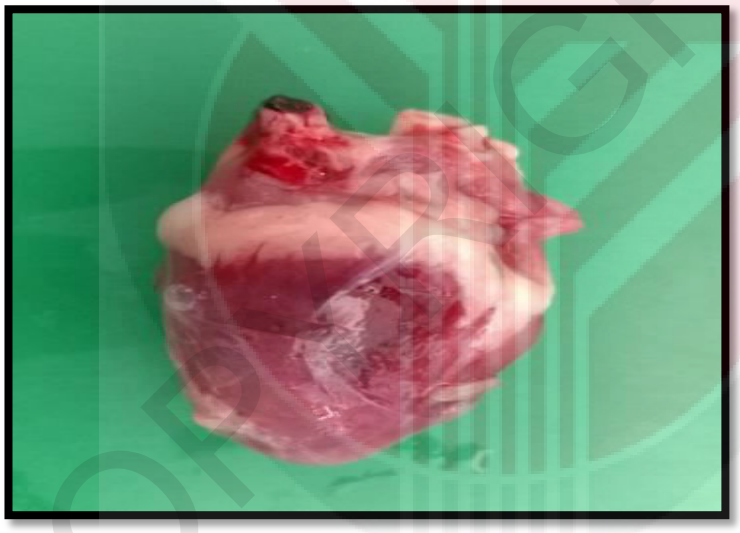

Figure 12: The congested heart of a quail infected with NDV (score 2).

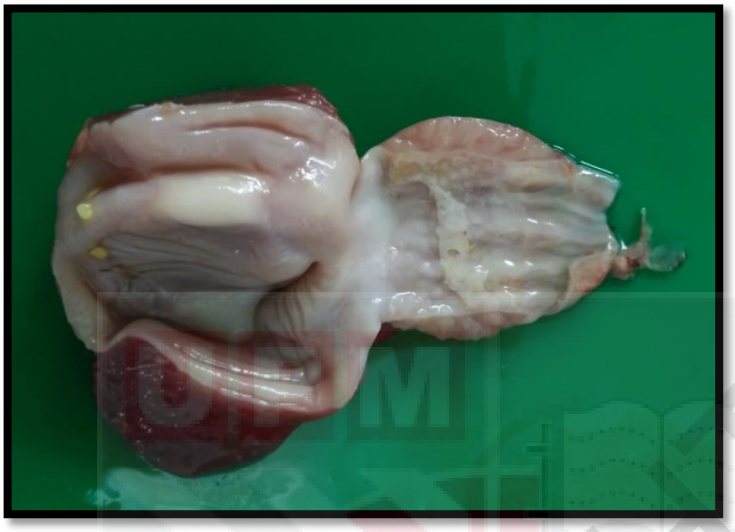

Figure 13: The proventriculus of a quail in control group (score 0).

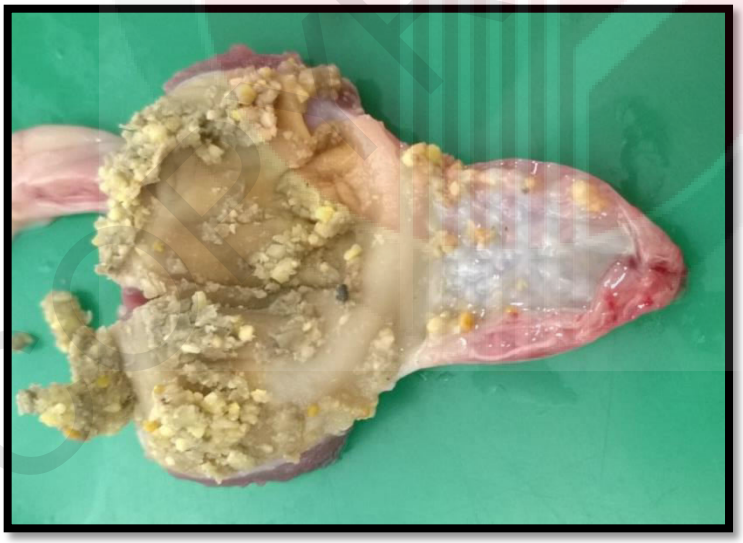

Figure 14: The haemorrhagic proventriculus of a quail infected with NDV (score 1).

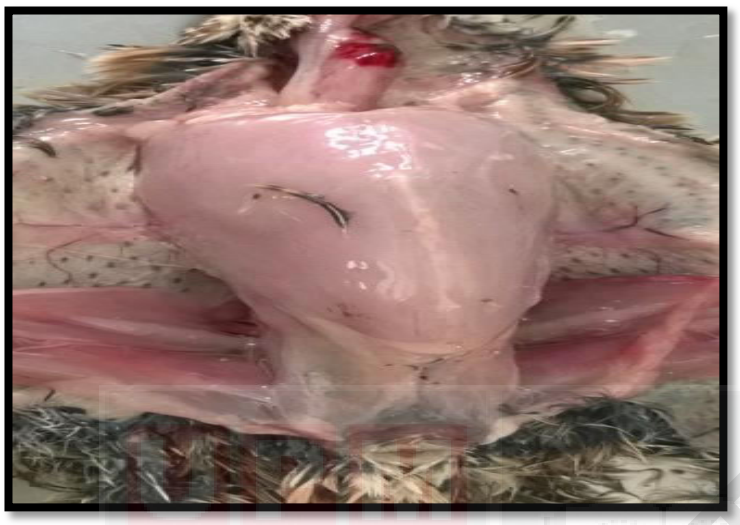

Figure 15: The pectoral muscle of a quail in control group (score 0).

Figure 16: The pectoral muscle of a quail infected with NDV (score 2).

4. 5 Correlation between clinical signs and postmortem lesions.

There was a strong positive correlation between clinical signs and postmortem lesions (r = 0.8556).

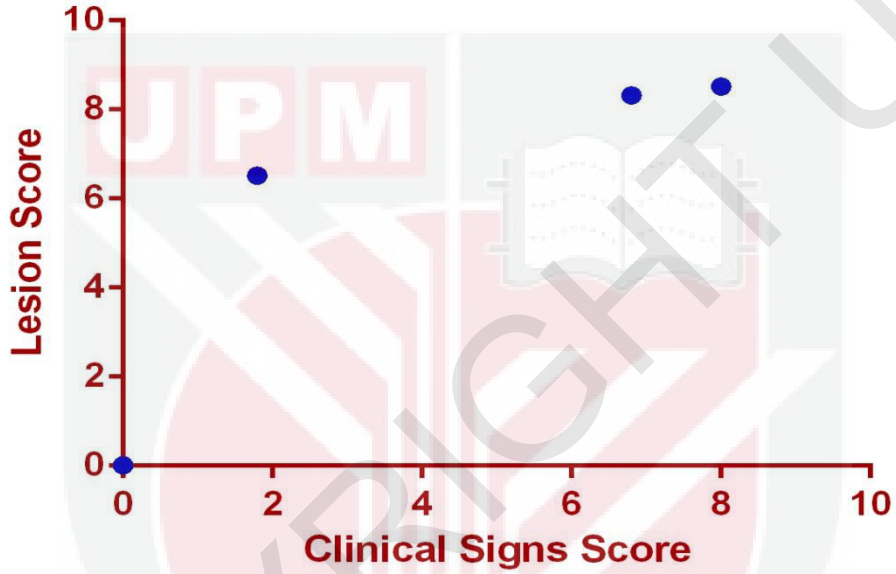

Figure 5: Correlation between clinical signs and postmortem lesions of quails infected with NDV in each group.

5. 0 DISCUSSION

The objective of the study was to determine the susceptibility of Japanese quails towards Newcastle Disease Virus AFF2240 strain. Clinical and postmortem changes and the expression and rise in the antibody titre were used as parameters to assess the susceptibility of the quails towards the Newcastle disease virus infection. Starting from day six of post infection, clinical changes including ruffled feathers, depression, incoordination, lameness, anorexia, diarrhoea, recumbent and paralysis of wings and legs were significantly seen in some of the infected quails in each groups. Group C showed most severe clinical changes with the earliest mortality at day eight of post infection. Oladele et al., (2008) mentions that major clinical signs that indicating Newcastle Disease including ruffled feathers, weakness, depression, anorexia and incoordination. Apart from that, Odalele et al., (2008) also mentioned about the wing and leg paralysis in the quails administered with 0.3ml of the virus when introduced intramuscularly. This finding is a variation to this study in which quails that were injected with 0.1ml of virus also develop severe neurological signs. This may be due to higher virulence of NDV AF2240 strain as compared to other strain even though they are all categorized under velogenic viscerotropic strain. Apart from that, even it is the similar virus, it is dependent on the age, health status of the host itself (Capua & Terregino, 2011).

Oladele et al. (2008) used oral and intramuscular route to administer the virus to see the comparison between these routes. However the study method was altered whereby only intramuscular route was used. The natural routes for Newcastle Disease virus infection are via nasal, oral and ocular route. However, this study use

only intramuscular route because it is expected to enhance the neurological signs, meanwhile natural route appears to display more respiratory signs (Oladele et al., 2008). It is in line with the result of this study where the neurological signs were observed in each group of infected quails.

After 9 days of post challenge with NDV via intramuscular route, there was no rise in antibody titre throughout the experimental study in all groups. This could be due to late antibody production following virus challenge. This finding was similar to those by El-Tarabili et al. (2009) where the mean antibody titres only appeared after the second week of post infection. However it is opposite with the findings by Oladele et al. (2008) who reported that the highest mean HI antibody titre on day seven of post infection. The variation between times of antibody production can be due to individual immune status of the quails and laboratory condition of the virus (Oladele et al., 2008). Although there was absence of HI antibody, the clinical changes were observed starting from day 6 of post challenge onwards. Therefore, it could be said that the virus caused clinical signs but at the same time it may not cause rise in antibody titre. Other problems that may be encountered leading to these conditions were the improper blood sampling, storage of serum samples or incorrect ELISA technique for determination of HI antibody.

Regarding the dose of NDV virus administered, this study showed that a higher dose of virus load may not cause more severe clinical signs or post-mortem lesions which contradicted to result found by Oladele et al. (2008) which only quails administered with highest dose showed neurological signs. Pathogenicity of NDV depends on the virus strain, dose and route of administration (Oladele et al., 2008).

This did not occur in this study which may be due to high virulence of the strain which lead to severe effect even when given at low dose.

Based on the pathological changes, the major lesions observed were similar to that of Oladele et al. (2008). Most of the infected quails from each group have congestion and haemorrhages of intestine, congestion of liver, brain, pectoral muscles, heart and lungs. Tropisms of NDV velogenic viscerotropic strain are the central nervous system and gastrointestinal tract (Capuo & Terregino, 2011) hence the clinical signs and post-mortem effect are more profound in these two systems. There was a strong correlation between clinical signs and postmortem changes. Therefore it could be said that more severe clinical signs will have more profound postmortem effects.

6. 0 CONCLUSION

From this study, it shows that Japanese quails are susceptible towards Newcastle Disease Virus AFF2240 which may cause severe clinical impacts and even lead to fatality. However, this study is also suggesting that highest dose of virus does not necessarily cause the most severe clinico-pathological effects or highest antibody titres.

7. 0 RECOMMENDATION

Intramuscular route is not the natural route of infection for Newcastle Disease Virus, hence further study on other routes of infection including nasal, ocular and oral routes which are more incline towards natural routes of NDV (Lima et al., 2004: El-Tarabili et al., 2009) to see the expected of impacts from each different routes. Apart from that, the duration of post NDV challenge should be prolonged as according to by Oladele et al. (2008) for more accurate antibody analysis since there are variation between time and amount of antibody titre following NDV infection.

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