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Susceptibility of Japanese Quail and Chickens to Infection with Newcastle disease Virus Genotype VIId

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A B S T R A C T

The susceptibility of Japanese quails to infection with Newcastle disease (NDV) virus was evaluated through an experimental study. A total of 25 quails (36 days old) and 25 chickens (28 day old) were divided into 4 groups, G1 (non-vaccinated, non-challenged quails), G2 (non-vaccinated challenged quails by genotype VIId isolate), G3 (non-vaccinated, non-challenged chickens), and G4 (non-vaccinated challenged chickens by genotype VIId isolate). Hemagglutination inhibition (HI) assay for NDV was conducted pre and post-challenge. Clinical signs, gross and histopathological examinations were performed and lesions were recorded. NDV genotype VIId (NDV-VIId) results in 33% mortality in quails and produced 100% mortality in chickens. From that study, we found that quails are resistant to NDV-VIId infection, however this virus was a highly virulent (velogenic) NDV strain according to intracerebral pathogenicity index test (ICPT), and caused highly contagious disease in chickens than quails. NDV resulted in respiratory and nervous signs in chickens and quails with a higher severity of lesion scores in chickens. Non suppurative encephalitis, trachitis pneumonia and enteritis were the main histopathological features that were recorded in infected birds.

Keywords: Newcastle disease, chickens, quails, pathology, Genotype VIId

1. Introduction

Newcastle disease (ND), is a highly contagious disease due to the high morbidity and mortality in poultry (Miller and Koch, 2013). The disease is caused by NDV of Avian Paramyxoviridae, Orthoavulavirus genus, and family (Mayo, 2002). Two major classes of NDV were recorded; classes I and II (Miller et al., 2013). Strains of Class I, are avirulent in chickens, whereas strains of Class II being further divided into sixteen genotypes. Although all NDV are members of APMV-1 and are of one serotype, genetic diversity is observed between the different genotypes (Miller et al., 2013). All NDV isolates are categorized into five pathotypes based on severity of the disease in chickens; viscerotropic velogenic, neurotropic velogenic, pneumotropic mesogenic, lentogenic and asymptomatic enteric type (Alexander et al., 1998).

Chickens, turkeys and other gallinaceous species are mostly affected (Aldous et al., 2007; Cattoli et al., 2011), whereas the ducks are highly resistant although they are susceptible to NDV infection (Cattoli et al., 2011; Nishizawa et al., 2007).

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After challenged with NDV, quails did not show clinical signs of ND, even though the virus circulated in the body systems (Higgins, 1971; Higgins and Wong, 1968; Lima et al., 2004) as the pathogenicity and clinical signs development of ND in quails depends mainly on the virus strain, infection dose, and route of infection.

Although there are several studies investigating infection and susceptibility of ND in chickens, the susceptibility of quails to the field isolates of NDV infection is poorly understood. To the extent of our knowledge, there is little data describing ND in Japanese quails (Coturnixcoturnix japonica). Furthermore, there is a lack in sequential description of histopathological alterations in various systems.

Therefore, to clarify full details about the susceptibility and pathogenesis of NDV in Japanese quail, we conducted this experiment via inoculation of Japanese quails and chickens by NDV genotype VIId (as it is the predominant virus circulating in Egypt) (Mazumder et al., 2012) to evaluate the pathogenecity of NDV-VIId and to compare its pathological features in the 2 examined species.

2. Material and methods

2.1. Virus

NDV genotype VIId, isolated from broiler chicken flock in 2016 at Gharbia, Egypt (accession number, KX686728) was used in this study. It was classified as velogenic NDV strain (Selim et al., 2018). The virus was obtained from the National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt. Viral purification and propagation were done by specific dilutions and passage in specific pathogen-free (SPF) embryonating chicken eggs (10 day-old) according to standard procedure (McGinnes et al., 2006).

2.2 .Intracerebral Pathogenicity Index(ICPI Test)

ICPI test was done using standard protocols (Pandarangga et al., 2016). As the SPF chickens (1-day-old), inoculated by a specific concentration of the virus, they were kept under daily observations for 8 days for scoring of clinical signs and no of dead birds (OIE, 2008).

2.3. Bird management:

A total no of 25 Japanese quails (Coturnix coturnix japonica), (36 days old mixed-sex, unvaccinated), and 25 broiler chickens (28 days old) were used in this study. Chickens and quails were obtained from commercial farms, caged under standardized environmental conditions. Birds were acclimated 5 days before the start of the experiment with access to food and water Ad libitum. The birds were grouped and housed in separate, well ventilated cages.

2.4. Experimental Design

Experimental design was designed and performed as follows:

Group 1: quails control -ve group: included 10 quails injected with 0.5 ml saline/ bird.

Group 2: challenged quail group: included 15 quails were challenged with Newcastle disease virus NDV-VIId 106 EID50 at dose of 100µ/bird / occulonasal route.

Group 3: chickens control -ve group: included 10 chickens injected with 0.5 ml saline/bird.

Group 4: challenged chicken group: included 15 chickens challenged with Newcastle disease virus NDV-VIId106 EID50 at a dose of 100µ/bird/occulonasal route.

All experimented birds were monitored daily for clinical signs observations. Experimental protocols were approved and performed by Animal Experiment Committee of the Faculty of veterinary medicine at Damanhour University.

2.5. Serology

Serum samples were obtained randomly from all birds at 28 days old and tested by hemagglutination inhibition (HI) assay.

Hemagglutination inhibition (HI) assay for NDV was conducted pre and post-challenge for all birds to ensure that these birds were free from NDV antibodies. HI was done according to standard procedures of ((OIE, 2012)). Serum titers of 1:2 (21) or 1:8 (23) were considered negative for antibodies against NDV (Alexander, 2000; McGinnes et al., 2006).

2.6. Histopathology

Post mortem examinations were done at 7 day post infection (dpi) from 3 birds from each group in addition to the dead birds. Birds were fully necropsied immediately after euthanasia. Tissue specimens were collected from the liver, heart, brain, lung, testis and intestine. The tissue specimens then preserved in neutral buffer formalin solution (10%) for at least 24 hours before being processed for histopathological examination according to (Bancroft and Gamble, 2008).

3. Results:

3.1. Pathogenicity Index—ICPI

The ICPI value for this virus strain (NDV-VIId) was 1.73. Therefore, this virus could be considered highly virulent (velogenic) according to OIE standards.

3.2. HI results

The HI antibody levels were negative in all birds prior to challenge, indicating freeness of quails from NDV infection. Whereas the HI titer at 4th dpi were 3 and 4 to the log2 in chicken and quail respectively, meaning viral replication in infected birds.

3.3. Clinical signs

A summary of clinical signs, gross findings and mortality among chickens and quails were reported in Table 1.

At4th dpi, quails challenged with NDV-VIId at a dose 106 EID50 (G2) suffered from depression, off food, ruffled feathers, increased body temperature, greenish watery diarrhea and nervous signs. Signs progressed at 10 dpi. However, chickens in (G3), challenged by the same virus, showed severe nervous signs in the form of head tremors, incoordination, and ataxia, respiratory distress, diarrhea, lameness and anorexia which appeared at the 2nd dpi and progressed at 3rd to 6th dpi (Table 1).

No clinical signs were observed in (G1, and G3).

3.4. Gross pathological lesions;

Birds were examined carefully for recording post-mortem (PM) changes.

No PM lesions were recorded neither in G1, nor G3.

Mild to severe congestion in the brain, lung, intestine with petechial hemorrhages on the proventriculus were recorded in the inoculated quails.

On the same time, the same PM lesions were recorded in the chickens with a higher degree of severity (Table 1).

3.5. Mortality rate

The highest mortality rate was seen in G4 of the NDV-VIId inoculated chickens which displayed 100% mortality while the quail in group 2 expressed a low mortality rate of 33%. In chickens; 3 birds died at 3rd dpi, 6 birds died at 4thdpi, 3 birds died at 4thdpi. Fnally, all the remaining of the infected chickens died at 6th dpi. However, in quails challenged group; the 1st bird died at 7dpi, 2nd birds died at 9 dpi and another 2 birds died at 10 dpi with a total number of 5 deaths out of 15 inoculated quails (Table.1).

No mortalities were seen neither in G1 nor G3.

3.6. *Histopathology*

A summary of histopathological findings and lesion scores in chickens and quails were recorded in Table 2.

Brain; non-suppurative encephalitis, in the form of perivascular cuffing, focal or diffuse gliosis and neuronal necrosis were seen in inoculated quail and chickens (Fig.2 a, d). Affected areas were randomly distributed to the forebrain, brain stem, and cerebellum, without obvious predilection for specific site.

Lesions were characterized by randomly distributed, multifocal perivascular cuffing of inflammatory cells composed mainly of lymphocytes with fewer macrophages. The inflammatory cells spilled into the adjacent tissue. The severely affected areas showed neuronal necrosis, multifocal gliosis, and vacuolation of the neuropil (Fig. 2 a, d).

In the cerebellum, there was segmental loss of Purkinje cells, perivascular accumulation of inflammatory cells that infiltrated the adjacent tissue, and multifocal gliosis of the molecular layer.

Trachea; Catarrhal tracheitis, desquamation of the epithelial lining, edema in the sub mucosal membrane, con¬gestion of blood vessels, and inflammatory cells infiltration of the lamina propria were also observed (Fig.1D, H).

Lung; hemorrhagic and lymphocytic interstitial pneumonia were detected. The alveoli were filled with inflammatory exudate with lymphocytes and RBCs (Fig.1C, G). .

Proventriculus; catarrhal and hemorrhagic proven¬triculitis were seen with degeneration, desquamation and necrosis of epithelial of proventriculus epithelial surface and epithelial gland.

Intestine; des¬quamation and necrosis of epithelial mucosal cell, hemorrhage, and catarrhal enteritis with inflammatory cell infiltration into submucosal layer (Fig.2 c, f).

Liver; mild to moderate lesions as hepatocyte degenerations, fatty degeneration, mild leukocytic cell infiltrations with mild congestion of blood sinusoids (Fig.2 b, e).

Heart; myocarditis marked by myocardium degeneration, edema, with mononuclear cell infiltration.

No pathological alterations were detected in control –ve group (G1, G3).

Histopathological alterations and lesions scoring of the examined tissues in quail and chickens are summarized in (Table.2). Briefly, non-vaccinated challenged chickens with NDV-VIId, showed severe lesions in all examined organs in comparisons to the challenged quails.

4.Discussion

In the present study, Japanese quails susceptibility to NDV-VIId infection was conducted by assessing certain parameters such as clinical signs, gross lesions, and histopathological lesion scores. We conducted this experiment via inoculation of Japanese quails and chickens by NDV-VIId (as this genotype is consider one of the predominant viruses circulating in various countries in Asia, Europe, and South Africa including Egypt) (Mazumder et al., 2012).

From our results, we found that, quails were less susceptible to NDV infection than chickens. As all inoculated chickens died within 6 days post infection (100%) while the inoculated quails expressed only 33% mortality within the experiment. This finding was in agreement with (Ellakany et al., 2019; Nakamura et al., 2013; Susta et al., 2018). Moreover; these findings are also in parallel with some previous studies, which reported moderate mortality (28%) in Japanese quail infected with virulent NDV strains, indicating that quails are resistant to infection in comparison to chickens. This resistance could be attributed to the low level of viral replication in quails (Sedeik et al., 2019; Susta et al., 2018).

The infected chickens and quails developed neurological clinical signs in the form of depression, weakness, ataxia, incoordination, tremor, torticollis, and paralysis at 2nd dpi and 4th dpi in inoculated chickens and quails respectively. The neurological clinical signs as a result of tropism of virulent NDV strains to the nervous tissues was previously reported (Ecco et al., 2011; Oladele et al., 2008; Susta et al., 2011; Susta et al., 2018). However, the same signs were not seen in quails by (Lima et al., 2004) as the experimented quails failed to develop any clinical signs.

PM lesions represented by widespread severe congestion in intestine, brain, liver, and trachea, together with greenish secretion in the gizzard, redness of the eye lid, and petechial hemorrhages and thickening of the wall of the proventriculus, were all observed in the chickens infected with NDV, similar findings were well documented (Ellakany et al., 2019; Gowthaman et al., 2013). Meanwhile, this lesions were observed in quails but in a mild to medium degree (El-Tarabili et al., 2009; Sharawi et al., 2015)

In the present study, non-suppurative encephalitis, gliosis, neuronal degeneration, and necrosis were consistently observed in the infected chickens and quails which were initially detected at 2nd and 7th dpi and progressed in intensity and prevalence by 6th and 10th dpi in chickens and quails respectively. This findings were in consistent with NDV

histopathology in brain tissues of chickens and quails infected with NDV (Cattoli et al., 2011; Ecco et al., 2011; Susta et al., 2018).

Association of low mortality with sporadic involvement of the nervous system and absence of lymphoid necrosis has been not uncommonly reported in avian species that are partially resistant to NDV development such as pigeons (Wakamatsu et al., 2006), cormorants (Heckert et al., 1996), and ducks (Lee et al., 2009) upon infection with highly virulent NDV strains.

Myocarditis, tracheitis, pneumonia, and enteritis were sometimes observed in inoculated quails and chickens. Similar findings were observed in quails infected intra- peritoneally by NDV (El-Tarabili et al., 2009), (Alexander and Senne, 2003; Ellakany et al., 2019; Susta et al., 2011). Other study reported neither morbidity nor mortality (Lima et al., 2004) in 17-week-old Japanese quails infected via oculo-nasal route by a velogenic NDV strain. Additionally, the NDV-VIId used in the present study was highly virulent according to standard pathogenicity tests (ICPI>1.73) (Pandarangga et al., 2016).

Lastly, the Japanese quails inoculated by NDV-VIId, failed to develop a severe systemic infection in comparison to the chickens however using the same dose (106 EID50 units/bird) (Sedeik et al., 2019) that was used in our study or a higher dose (108 EID50 units/bird) than it was used in chickens (105 EID50 units/bird) (Pandarangga et al., 2016; Susta et al., 2011). These findings referring to the low susceptibility and resistance of quails to NDV infection.

5. Conclusion;

NDV-VIId induced mild to moderate disease condition with low mortality in Japanese quail however it was a highly pathogenic contagious disease inducing severe mortality and losses in poultry farms reached 100% mortality. Japanese quails are susceptible to NDV-VIId and the susceptibility of the quails to the infection based upon the strain, and the host itself. Quails can resist NDV infection due to the limited replicative efficacy of NDV in inoculated birds.

Conflict of interest statement

No conflicts of interest.

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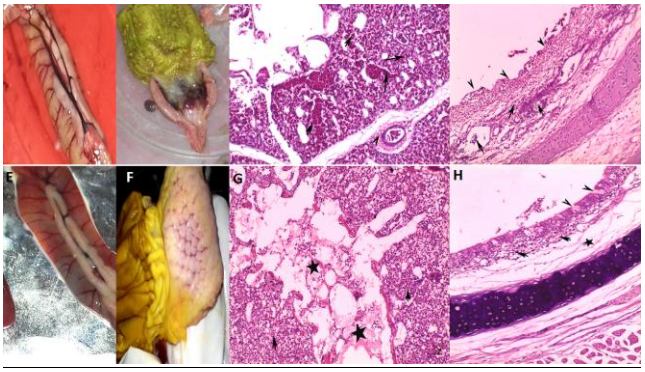

Fig.1. (A) Congestion of intestine of chicken inoculated with NDV-VIId. (B). Severe hemorrhages on proventriculus of infected chickens.(C) Lung of chicken showing hemorrhagic lymphocytic pneumonia (arrows) with peivascular edema of blood vessel (arrowhead);Hematoxylin and eosin (HE). (D) Trachaea of chicken showing tracheitis (arrows) with severe desquamation of epithilial mucous membrane (arrowheads). (HE). (E)Congestion of intestine of quail inoculated with NDV-VIId. (F) Petechial hemorrhages on proventriculus of infected quail.(G) Lung of infected quail showing lymphocytic pneumonia (arrows) with accumlation of inflammatory exudates (black star) in the bronchioles. (HE). (H) Trachaea of chicken showing deciliation of epithelial mucous membrane (arrowhead) with infiltration of mucosa with leukocytic inflammatory cells (arrows) and edema (black star of submucosa). (HE).

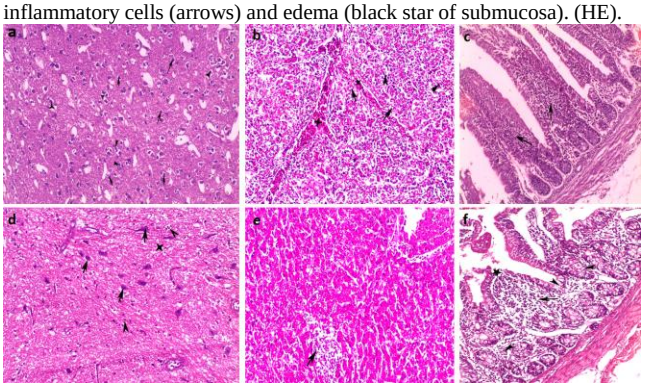

Fig.2. (a) brain of chicken infected with NDV-VIId showing severe neuronal degeneration and necrosis (arrows) with microglia cell infiltration of the brain (arrowheads) with vacuolation of the neuropil (black star). (HE). (b) Liver of infected chicken showing fatty change (arrows) with inflammatory cell infiltration (arrowhead) of hepatic lobules and congestion of blood vessels with RBCs (black star). (HE). (c) Intestine of infected chicken showing lymphocytic enteritis (arrows). (d) Brain of quail infected with NDV-VIId showing neuronal degeneration and necrosis (arrows), microglia cell infiltration of the brain (arrowheads), vacuolation of the neuropil (black star). (HE). (e) Liver of infected quail showing mild focal of inflammatory cells (arrows). (HE). (f) Intestine of infected quail showing lymphocytic enteritis (arrows), degeneration of columnar epithelial cell of villi (arrowheads), and desquamation of epithelial mucosa (black star). (HE).

Table (1): Summary of clinical signs and PM lesions in chickens and Japanese quails experimentally infected with NDV genotype VII.d

Items	Chickens	Quail
Clinical signs	at 2nd dpi	at 4th dpi
Signs progressed	at 3rd - 6th dpi	at 7th - 10th dpi
Depression	+++	+
Off food	+	++
Ruffled feathers	++	+
Increased body temperature	++	+
Greenish watery diarrhea	++	++
Ataxia, tremors, torticollis	+++	++
Incoordination	++	++
Mortality %	100% 3 bird died at 3rd dpi, 6 birds died at 4th dpi, 3 birds died at 4th dpi., All the remaining of the infected birds died at 6th dpi	33% 1 bird died at 7dpi, 2 birds died at 9 dpi , and 2 birds died at 10th dpi
PM		
Brain:	congestion/ (9b/15b)/ ++	congestion/ (6b/8b)/ ++
Intestine	congestion/ (11b/15b)/ ++ to +++	congestion/ (4b/8b)/ ++
Proventriculus	petechial hemorrhages /(8b/15b)/ ++ to +++	petechial hemorrhages /(4b/8b)/ ++
Gizzard:	Greenish contents, thickening of the wall / (12b/15b)/++ to +++	greenish contents, (3b/8b)/ ++
Eye	Swelling, redness of the eye/ (15b/15b)/ ++ to +++.	swelling, redness of the eye/ (6b/8b)/ ++
b/b= no of affected birds/ total sampled birds; += mild; ++= moderate; +++= severe		

Table (2): Summary of histopathological findings referring to the degree of severity of lesions in chickens and Japanese quail Infected with NDV VII.d

Organ affected	Lesions	Degree of severity/Chickens	Degree of severity/Quail
Brain	Non-suppurative -encephalitis perivascular cuffing gliosis neuronal necrosis Purkinje-cell loss	+++ +++ ++ +++ ++	++ ++ ++ ++ +
Trachea	catarrhal tracheitis deciliation of MM Edema	+++ +++ ++	++ ++ ++
Lung	Hemorrhagic pneumonia. Lymphocytic pneumonia	+++ +++	++ +++
liver	Fatty change Inflammation Hepatic necrosis Hepatic degeneration	+++ + + ++	+ + - +
Proventriculus	Haemorrhage with congestion proventriculitis	++ +++	++ +++
Intestine	Hemorrhagic-enteritis Lymphocytic-enteritis Necrotic enteritis	+++ ++ ++	++ +++ ++
Heart	myocarditis	++	**+ to ++**
Pancreas	necrosis	++	+
+ = Mild; **++ = Moderate;** **+++ = Severe**			