



The Susceptibility of Japanese Quails to the Infection with Chicken Originated Newcastle Disease Virus

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ABSTRACT

Newcastle disease virus (NDV) caused an outbreak among commercial broiler chickens in the educational farm of Faculty of Agriculture, Assiut University-Egypt. Its virulence was characterized by intracerebral pathogenicity index (ICPI) and its score was 1.65. This outbreak raised questionable concerns regarding the role of quails as a neighboring bird to the broiler chickens in the affected farm in transmission of this virus to chickens. Ninety 35- days-old quails were reared and divided into two categories one vaccinated against ND and the other is not vaccinated then infected oculonasal with virulent strain of Newcastle disease virus both categories accompanied with chickens were in contact with these infected quails to determine the role of quails in the epidemiology of ND infection. Hemagglutination inhibition (HI) antibodies were measured for assessment of antibody response as well as oropharynx swabs were used for detection of the virus shedding. The results revealed the susceptibility of quails to NDV infection was lesser than that were observed in chickens. 6.6-13.3% of challenged non-vaccinated quails were died in contrast to 80-100% mortality in experimentally non vaccinated infected chickens till the end of experiment 3 weeks after challenge. Up to 20% of infected non-vaccinated quails exhibited general clinical signs in contrast to 100% of non vaccinated chickens shown clinical signs were primarily respiratory. Infected quails excreted infectious virus from the oropharynx for a shorter period than that observed with infected in-contact chickens in special to the vaccinated groups the shedding was reduced significantly either in quails and chickens. The results confirmed that some of the naturally occurring NDV virulent strains can cause the disease in quails but in a mild form, and that quails play an important role in the epidemiology of ND and its transmission to chickens causing heavy economic losses. These results underscore the need to develop new vaccine strategies for use in quails to protect birds from both disease and infection to reduce virus shedding and its spreading

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Introduction

The Newcastle disease (NDV) is one of the severe viral diseases affecting birds that could cause huge losses for the poultry industry (Bank, 2011). NDV is infecting a broad range of avian species, Approximately 241 species of birds have been found to be vulnerable to infections with NDV (Lima *et al.*, 2004). This disease has been broadly

studied in commercial poultry as chickens and turkeys but only a small number of researches have been done to judge the role of other bird species on NDV epidemiology and disease transmission.

The Japanese quails are widely distributed in many countries around the world for several reasons; firstly, good performance in meat and egg production reaching 300 eggs per hen per year (Banks, 1979), secondly, it is an ideal and suitable model for poultry research due to their small size that require little cage space for rearing and a short reproductive cycle approximately eight weeks are

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needed to eggs for hatching and rearing the birds to sexual maturity age that be suitable for genetic studies which required rapid sexual maturity (Murakami, 1991; Ruskin, 1991). They are also high, disease resistant and can without difficulty get familiar to diverse environments (Lombin, 2007).

In Egypt, some commercial and scientific sectors have gone into production of quails for their demands as a food for human consumption or for carrying out several investigations on their disease affections. Lima *et al.* (2004) found quails as important carriers for ND virus. On the other hand, Higgins and Wong (1968) and Higgins (1971) affirmed that quails are somewhat resistant to NDV infection; nevertheless they may be infected under stressful circumstances. In contrast to other studies, quails were found to be susceptible to infection with ND virus of velogenic pathotype (Czirjak *et al.*, 2007; Lima *et al.*, 2004; Sa'idu *et al.*, 2004).

A number of epidemics for Newcastle disease were observed in Japanese quails (*Coturnix coturnix japonica*) (Chandrasekaran and Aziz, 1989; Islam *et al.*, 1994; Merino *et al.*, 2009). Challenged Quails with Newcastle disease virus at 20 day old showed mortality rate ranged from 20-100% according to the route of infection and more lower rates in older age 40 days which ranged from 20-60%. In Egypt, Assiut province El-Zanaty and Abd El-Motelib (1993) isolated viscerotropic velogenic ND in quails (*Coturnix coturnix japonica*).

Czirjak *et al.* (2007) showed 100% morbidity and mortality in ND outbreak in quails with harsh clinical signs and P/M lesions in digestive and nervous systems. Momayez *et al.* (2007) succeeded in isolation of velogenic strain of NDV with Intracerebral pathogenicity index (ICPI) of 1.62 from two out of five diseased quails. Also, Oladele *et al.* (2008) recorded histopathological and Hemagglutination inhibition (HI) antibody titer in Japanese quails infected experimentally with NDV which developed focal necrosis in most of the organs, mononuclear cells infiltration and diminution of lymphoid tissues as well as arise in HI titer from zero to maximum mean antibody titer of $10 \log_2$. Sa'idu *et al.* (2004) studied the serological response of ND in quails and detected infection in 12% of the examined birds with low mean HI titre ($0.4 \log_2$).

Ucan and Catatoluk (2002) affirmed that the raising of quails with chickens could be a route of

infection of ND virus for chickens causing outbreaks having virulent character.

Despite that, the importance of that species in the epidemiology of NDV is very poor. However, a study of Lima *et al.* (2004) showed that Japanese quail could be carrier of NDV by around 14 days after the experimental infection.

Until now the discussion still running, if NDV can cause the disease in quails and if it is sharing in the transmission of the NDV to chickens. This study was done to elucidate the role of the quail (*Coturnix coturnix japonica*) in the disease transmission as a source of infection for the Newcastle disease virus (NDV).

Materials and methods

Experimental birds and management

A total number of ninety 35-days-old Japanese quails were randomly separated into 6 groups. Three groups (1a, 1b and 4c) of 15 birds each were vaccinated against ND with HB1 and LaSota at 7 and 18 days respectively. The remained (45 quails) were separated into three groups (2a, 2b and 3c) and were not vaccinated against ND as shown in Table 1. Each group was kept in different cages and was provided feed and water ad libitum.

All birds, in groups (1a, 1b, 2a, 2b) were challenged at 35-day-old via oculonasal with a 50% embryo infecting dose titer of $8 \log_{10}/0.1$ ml velogenic NDV. Phosphate buffered saline (PBS) was used as diluent for the inoculum that was instilled by oculo-nasal route. There were two control groups; group 3c was not challenged, not vaccinated and group 4c was vaccinated not challenged.

In order to evaluate the virus shedding and pathogenicity of NDV that may be transmitted from quails to chickens, ninety 35-days-old chickens were located with every previously mentioned groups of quails in the manners as shown in Table 1. The observation will be carried every day for de-tetection of NDV clinical signs and mortality in birds

The dead birds were examined for gross lesions. Blood sampling started a day before infection to days 7, 14 and 21 pi. The blood samples were collected through wing vein puncture, then the collected sera kept at -20°C until they were used for HI analysis for the quails and broiler chickens groups.

Virological examinations

Oropharyngeal swabs were collected at zero day before challenge, 7, 14 and 21 days after infection from chickens and quails then placed in PBS having penicillin and streptomycin and kept at -70°C until virus isolation was done.

Amount of 150 μl of transport medium from the tracheal swabs homogenate was inoculated into the allantoic cavity of 11-day-old embryonated chicken eggs. After 2 days of incubation at 37°C , the eggs were chilled at 4°C for 12 hours, then the allantoic fluids were collected and examined for hemagglutinating character. The recovered hemagglutinating allantoic fluids were identified as NDV challenge using the hemagglutination inhibition (HI).

Results

No obvious clinical signs of ND were observed in any birds before challenge as well as in control groups 3c and 4c after challenge. None of the vaccinated quail birds in groups 1a and 1b as well as chickens in groups 1b and 2b showed clinical signs after challenge.

Depression, anorexia, ruffled feathers, mild dys-pnea and nasal discharge were noted in 2 out of 15 of infected quails in group 2a and 3 out of 15 the infected quails in group 2b. The affected quails started to die after 8 days from start of challenging. Mortality rate was 2/15 and 1/15 with a percentage of 13.3% and 6.6% in the groups 2a and 2b respectively. However the chickens that housed with the infected quails the clinical signs start after 6 days from their being contact with quails. The mortality rates were 80%-100% in non vaccinated chicken groups 1a and 2a in contrast to zero percent mortality in groups 1b and 2b (Table 1).

At necropsy, the dead quails showed no obvious gross lesions except some non specific lesions as generalized congestion of the internal organs (liver and trachea) as well as hemorrhages on the thigh muscles (Plate 2). In contrast to 100% of the infected chickens showed petechial hemorrhages on the serosal surface of digestive tract, particularly proventriculus, with dark red pneumonic areas (Plate 1) and enlargement of liver, spleen as well as kidney lesions identified included swelling, pale coloration, parenchymal urates (Urate Diathesis) (Plate1).

Seroconversions were detected in all surviving quails; on day 0 before challenge the mean titers of \log_2 were detectable and continued to raise at 7th day after infection then start to decline at the day 14th and continued in lowering till the end of observation in the day 21st after infection. In-contrast, the chicken groups showed antibody response with the same pattern as in quails but in higher titers than that observed in inoculated quails but the mean titers continued to raise till the day 14th after contact then start to decrease at the day 21st (Table 2). Control birds remained negative for HI antibodies. In group 4c, quails had a rapid decrease in the antibody titer in contrast to chicken birds that were in the same group (Table 2).

The shedding patterns in quails start to increase from the first day post infection then increased slowly till the fifth day after infection then start to decrease at the 7th day after challenge which is clear cut noticed in vaccinated group that diminished shedding of virus in the 14th day post infection in contrast to the non vaccinated group which diminished shedding at 21 days post challenge. On the other hand the chicken groups delayed its shedding to the 3rd after contact especially the vaccinated ones have low shedding rate in comparison

Table 1. Protection percentages of quails and chickens against ND virus challenge

Group	ND vaccine		Inoculation dose	Mortality	
	Quails	Chickens		Quails	Chickens
1a	-	-	$10^3/0.1$	0%	(12/15) 80%
1b	-	-	$10^3/0.1$	0%	0%
2a	-	-	$10^3/0.1$	(2/15) 13.3%	(15/15) 100%
2b	-	-	$10^3/0.1$	(1/15) 6.6%	0%
3C	-	-	Not challenged	0%	0%
4C	-	-	Not challenged	0%	0%

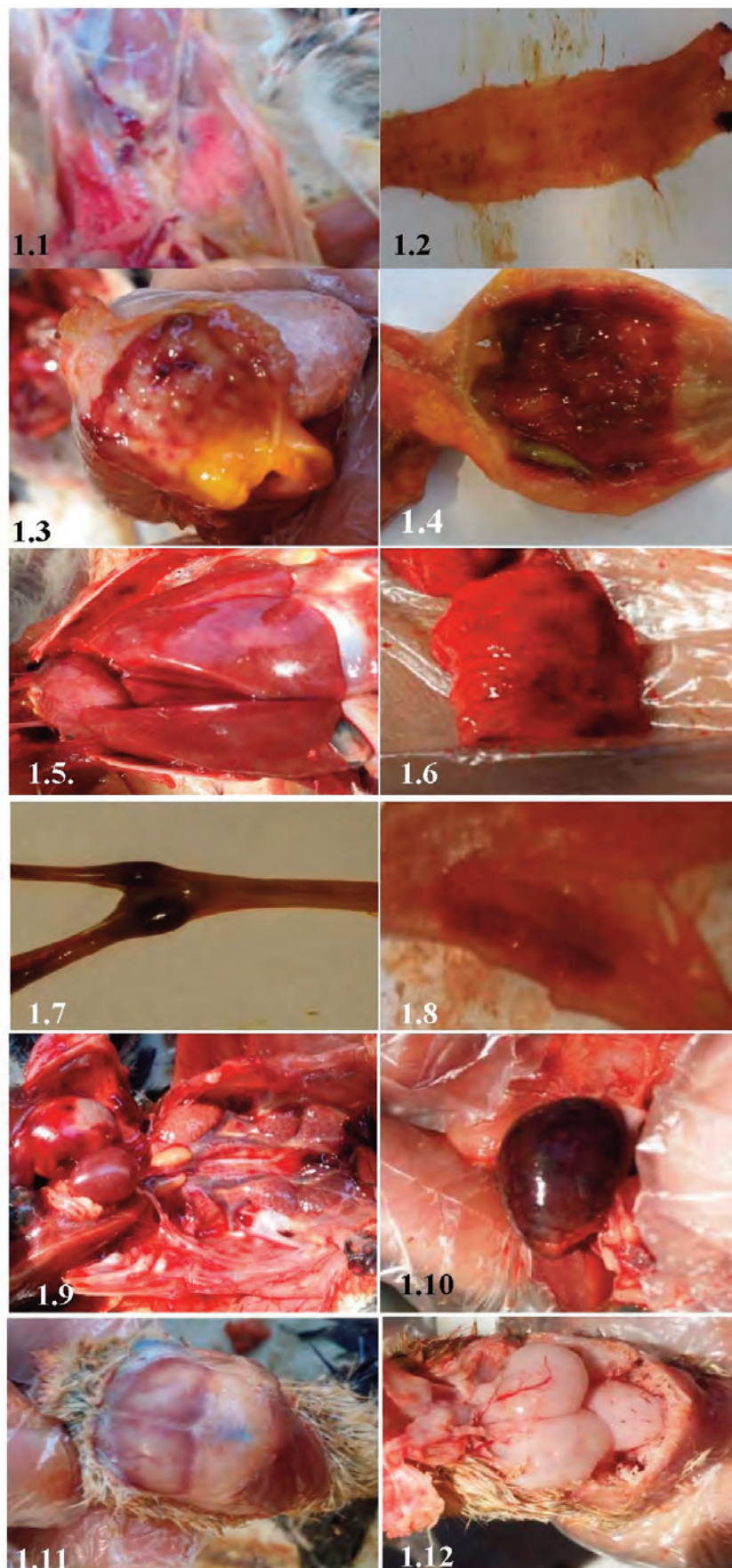


Plate 1. Pathomorphological changes in the internal organs of infected chickens by contact with challenged quails. 1.1) Congestion of lungs with air sacculitis. 1.2) Linear hemorrhages in the rectum. 1.3) Petechial hemorrhages on proventriculus. 1.4) Severe echymotic hemorrhages on proventriculus. 1.5) Congestion of liver with pale necrotic areas. 1.6) Congestion of lung with dark red area. 1.7) Swelling of cecal tonsils. 1.8) Hemorrhages on the mucosa of cecal tonsils. 1.9) Severe nephritis with prominent tubules. 1.10) Swelling and congestion of spleen with dark and pale areas (marbling appearance. 1.11) Congestion and redness of Skull. 1.12) Congestion of cerebral blood vessels.



Plate 2. Lesions found in the internal organs after challenging quails with NDV. 2.1) Slight congestion on the mucosal surface of proventriculus. 2.2) Congestion of the liver especially on the boundaries. 2.3) Congestion of pancreas. 2.4) hemorrhages on the thigh muscles.

Table 2. Serologic response before and after challenge in vaccinated and non vaccinated quails and chickens.

Groups	Mean HI titer (log ₂) ± SD							
	Quails				Chickens			
	0 dpi	7 dpi	14 dpi	21 dpi	0 dpc	7 dpc	14 dpc	21 dpc
1a	2.6±0.27	4.1±0.43 ^a	3.4±0.63 ^b	2.9±0.23 ^c	0	4.5±0.67	7.7±0.85 ^a	8.6±0.80 ^a
1b	2.3±0.46	4.5±0.32 ^a	3.1±0.78 ^b	2.7±0.44 ^b	4.2±1.3	7.8±0.81 ^b	9.1±0.67 ^a	10.1±1.0 ^a
2a	0	2.2±0.21	3.1±0.27 ^c	2.8±0.43 ^c	0	4.1±0.27	5.3±0.27 ^a	0*
2b	0	2.6±0.74	3.9±0.66 ^b	3.1±0.43 ^a	3.7±1.0	7.3±1.81 ^b	8.9±0.97 ^a	9.1±1.4 ^a
3c	0	0	0	0	0	0	0	0
4c	2.8±0.63	2.5±0.21	1.8±1.81	1.1±1.27	4.4±0.35	4.1±0.57	3.6±0.89	2.9±0.97

dpi: days post challenge dpc: day post contact

a, b, c Within a column values with different superscripts differ significantly (p < 0.05) with the values of 4c (Control vaccinated-not challenged group)

*no measures could be taken due to all of chickens were died

to the non vaccinated group which have a higher shed- ding rate that may be due to the titer of humoral antibodies in the vaccinated group (Table 3).

Discussion

In this study, different biological properties with respect to pathogenicity and transmission in chickens and quails were noticed and the results revealed the highly pathogenic nature for the infected chickens through contact with challenged quails with the percentage of mortality reached 100% which was in consistent with those previously observed in

field outbreaks due to velogenic NDV (Otim *et al.*, 2004). In contrast to the high pathogenic nature in chickens, there was a low pathogenic effect was observed in infected quails with mortality ranged from 6.6-13.3% which agrees with the previous reports of Higgins and Wong (1968); Higgins (1971); Sa'idu *et al.* (2004) and Tawfik *et al.* (2004).

The positive HI test results obtained from the quails 7 days post infection documented seroconversion higher than that vaccinated only which proved that chicken originated NDV could cause viraemia in the infected quails. The second group of in-contact chicks also tested positive for ND an-

Table 3. Virus isolations from Tracheal swab samples of chicken and quails

Type of birds	Days post challenge							
	1	2	3	5	7	14	21	
Quails (Vaccinated) ^{(1a)*}	1/15	2/15	5/15	3/15	1/15	0/15	0/15	
Quails (Not vaccinated) ^{(2a)*}	2/15	3/15	7/15	9/15	7/15	2/13 ⁱ	0/13 ⁱ	
	Days post contact							
	1	2	3	5	7	14	21	
Chickens (Vaccinated) ^{(2b)*}	0/15	0/15	1/15	1/15	0/15	0/15	0/15	
Chickens (Not vaccinated) ^{(2a)*}	0/15	0/15	3/15	8/15	10/13	4/7	0/0 ⁱⁱ	

*the number of group that exposed to examining the virus shedding

i The decreasing manner in the denominator number due to the mortality that happened from experimental infection

ii no number in the denominator due to the mortality that happened from experimental infection

tibodies, showing that quails excreted NDV and were capable to transmit NDV to chickens. So, quails in this study played an important role in the spreading of NDV virus (Spadbarrow, 1999).

In our study, there were weak or no overt clinical signs or gross lesions mentioned on the infected quails. We hypothesized that NDV might experience partial replication and continued for a short time in tissues, or it multiplied at low level which mirrored on the short time of shedding were observed in the infected quails either vaccinated or not vaccinated as shown in Table 3. Also the limited mortality in the infected quails may be due to “viral toxicity” as mentioned by Dai *et al.* (2014) rather than the result of a classical infectious process which the toxic act is result from an incomplete cycle of multiplication that conduct the infected cell to death, but not to the assembly of infective progeny (Friend and Trainer, 1972).

The ND viruses exhibited highly pathogenic for chickens infected by contact with challenged quails. Therefore, outbreaks by highly pathogenic NDVs can occur by the introduction of viruses into quails or other resistant species followed by serial passages under natural conditions, highlighting the significance of the host surroundings in the process of NDV selection and can make difficult and increase the charge of trials to prevent the infection.

So, it is an urgent to take strict biosecurity measures in poultry management, in particular to stay away from mixing diverse types of poultry raising to reduce cross-species spread. Moreover, we also fortify on regular check of species other than chickens that highlight the significance of supporting surveys in developing nations for transboundary chicken diseases.

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