Experimental infection of quail by NDV and its immune response to vaccination.

Sharawi, S.[1]; El-Habbaa, A.S.[1], Heba, M. Zaied[1] and Khodeir, M.H.[2]

[1]Faculty of Veterinary Medicine, Benha University, Egypt. [2]Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

ABSTRACT

This work aimed to study quail susceptibility to Newcastle disease virus (NDV); their role in disease transmission and their immune response to ND vaccine. Forty percent (4/10) of quail were susceptible to experimental infection with virulent NDV with signs of loss of appetite, weakness, diarrhea and nervous symptoms then death. Chickens group housed in contact with infected quail and chickens group experimentally infected with NDV were suffering from typical NDV infection 15 days post contact infection and 3 days post experimental infection, respectively. Post mortem examination of dead birds revealed hemorrhagic lesions of the intestinal tracts and proventriculus; and NDV was recovered from tracheal and intestinal samples and identified by HI test using NDV-Specific antiserum. Birds vaccinated with inactivated NDV vaccine exhibited detectable antibody titers (2 log2) by the 1st week post vaccination (WPV) to reach their peaks by the 3rd WPV (6 log2 in quails and 7 log2 in chickens). These antibody titers were able to protect quail and chickens against challenge with virulent NDV recording 100% protection rates in comparison to non-vaccinated birds showing 70% and 100% mortality in quails and chickens, respectively. Being susceptible for NDV, quail have a role in transmission of NDV to chickens, so they should be vaccinated for their protection and prevent their role in NDV transmission to chickens.

Keywords: NDV; Quail; Chickens; HA; HI; Vaccine.

(http://www.bvmj.bu.edu.eg) **(BVMJ-29(2): 218-224, 2015)**

1. INTRODUCTION

Newcastle disease (ND) is a viral disease of chicken causing huge economic losses among poultry industries. It is one of the oldest infectious diseases affecting many domestic and wild avian species but it is most notable in domestic poultry due to their high susceptibility and the potential for sever impacts of an endemic on poultry industries (Alexander, 1993). The disease was caused by Newcastle Disease Virus (NDV) classified in genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae (Lamb et al., 2005). NDV was categorized on the bases of its pathogenicity and virulence into lentogenic, mesogenic and velogenic (Beard and Hanson, 1984). Production of Japanese quails (Coturnix coturnix japonica) is extensively distributed in several countries around the world (Murakami, 1991). However, there is little information available on health control programs in this species, intensive quail farming resembling broilers and turkey may cause dissemination of infectious diseases (Paulillo, 1989). Although Japanese quail (Coturnix coturnix japonica) are hardy birds that thrive in concrete and small cages; they are affected by common poultry diseases, but fairly resistant (Bakoji et al., 2013). Studies were carried out to clarify the real role played by quail in the epidemiological plan, under the perspective as infectious source for NDV. Quail challenged with NDV did not show clinical signs of ND, although the virus circulated in their body

systems (Lima et al., 2004). It was concluded that pathogenicity of NDV in quail depend chiefly on the strain of the virus, its dose and route of administration (Oladele et al., 2008). The present study was planned to investigate the susceptibility of quails to NDV infections; their role in disease transmission and their immune response to ND vaccine.

2. MATERIAL AND METHODS

2.1. Virulent Newcastle Disease Virus (NDV)

An Egg adapted Velogenic viscerotropic NDV strain had a titer of 6 $\log_{10}$ EID_{50}/ml was supplied by Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo. NDV was used for experimental infection of quail and chicken and challenge of vaccinated birds.

2.2. Newcastle Disease virus (NDV) vaccine:

An inactivated NDV vaccine, Nobilis® ND Vaccine, produced by Intervet International Company-Boxmeer 5831 AN- Holland was used for vaccination of experimental quails and chickens. The vaccine was produced as bottle contains 500 ml (1000 doses). Each dose contains ND clone-30 ≥ 50 PD_{50}/dose to be inoculated by the subcutaneous route.

2.3. Experimental birds:

2.3.1. Quail:

Thirty quail 45 days old were obtained from a private farm.

2.3.2. Chickens:

Forty specific pathogen free (SPF) 45 days old chickens; without history of vaccination against NDV; were reared and supplied by VSVRI.

These birds were found to be free from antibodies against NDV as screened by serum neutralization test. They were subjected for experimental infection and vaccination with inactivated NDV vaccine as shown in the experimental design.

2.4. Specific Pathogen Free-Embryonated chicken eggs (SPF-ECE):

SPF embryonated chicken eggs 9-11 day old were supplied by Koom Osheim SPF farm; El-Fayoum Governorate. SPF-ECEs were used for isolation of NDV from quail and chickens after experimental infection and after challenge of vaccinated birds.

2.5. Samples:

2.5.1. Tissue samples:

Samples were collected from the trachea and intestine from affected and dead birds with NDV infection in order to recover the causative virus as a confirmatory test.

2.5.2. Serum Samples:

Blood samples were obtained from the experimental birds and allowed to form clots at 4°C overnight. Sera were separated and kept in sterile screw capped vials at – 20°C till subjected for serological examination (Lennette, 1964). Serum samples were collected on week intervals post vaccination.

2.6. Newcastle disease antiserum:

It was obtained from VSVRI (Amer et al., 2013) and used for identification of NDV re-isolated from infected quail and chickens using Haemagglutination inhibition (HI) test.

2.7. Haemagglutination (HA) and Haemagglutination inhibition (HI) tests:

HA test was carried out to detect NDV reisolated from organ samples obtained from experimentally infected quail and chickens and to determine the HA titer of NDV used in HI test, while HI test (constant virus plus diluted serum) was carried out for titration of ND antibodies in sera from vaccinated birds. Both HA and HI tests were carried out according to the standard method of examining poultry biologics (Anon, 1971).

2.8.Experimental design:

Each bird species was divided into groups (10 birds/ group) as follow: Quail group-1 was infected intramuscularly with the virulent ND virus using a dose was 6 $\log_{10}$ EID_{50}/ bird. Quail group-2 was inoculated subcutaneously with inactivated ND vaccine in a dose of 0.5 ml/bird according to the manufacturer directions. Quail group-3 was kept without vaccination and used in challenge test against NDV. Chicken group-1 was kept in contact with experimentally infected quails with NDV. Chicken group-2 was infected intramuscularly with virulent ND virus using a dose was 6 $\log_{10}$ EID_{50}/ bird. Chicken group-3 was inoculated subcutaneously with inactivated ND vaccine in a dose of 0.5 ml/bird according to the manufacturer directions. Chicken group-4 was kept without vaccination and used in challenge test against NDV. Each bird group was housed under hygienic measures receiving balanced ration and adequate water in separate isolates and subjected for daily clinical observation where disease signs and mortalities were recorded.

3. RESULTS

3.1. Experimental infection of quail with NDV:

Only 4 out of 10 birds (40%) of quail infected with NDV were affected, showing loss of appetite, weakness, diarrhea, and nervous symptoms then death. These results were shown in table (1) and photos (1&2). Post mortem (PM) examination of dead quail revealed hemorrhagic lesions of the intestinal tracts and proventriculus. The virus was recovered from tracheal and intestinal samples and identified by HI test using NDV-Specific antiserum.

3.2. Evaluation of immune response of quail and pigeon to NDV vaccine:

Birds inoculated with the inactivated NDV vaccine showed no clinical abnormalities indicating safety of the vaccine for both quail and chickens. Follow up of the induced ND antibodies in vaccinated birds using HI test, showed that all birds exhibited detectable antibody titers (2 log2) by the 1st week post vaccination (WPV) to reach their peaks by the 3rd WPV (6 log2 in quails and 7 log2 in chickens) as shown in table (3). Such antibody titers were able to protect quail and chickens against challenge with virulent NDV recording 100% protection rates in both of them. Non-vaccinated birds could not withstand the challenge virus showing 70% and 100% mortality in quails and chickens, respectively as demonstrated in table (4).

3.3. Investigation of the role of quail in transmission of NDV to chickens:

Chicken group-1 housed in contact with NDV infected quail and chicken group-1 infected with NDV were suffering from infection, 15 days post contact infection and 3 days post infection, respectively. Typical signs of ND were shown including restlessness, increased respiration, weakness and green diarrhea then muscular tremors, paralysis of legs and wings ending by death. These results were shown in table (1) and photo (3). PM findings of died chickens showed catarrhal exudates in nasal passages, hemorrhagic necrotic lesions in the trachea and digestive tract. Recovery of NDV from tracheal and intestinal samples of dead chickens were done by inoculation of embryonated chicken eggs and identified by HI test.

4. DISCUSSION

ND can affect several birds' species worldwide (Kaleta and Baldauf, 1988). The present work spots the light on quail's susceptibility to NDV infection and their possible role in transmission of such diseases in addition to evaluate the immune response of such birds to NDV vaccine. Forty percent (4/10) of quail group-1 experimentally infected with NDV showed loss of appetite, weakness, diarrhea and nervous symptoms. PM examination of

Table (1): Experimental infection of quail and chicken with NDV.

Bird Group	Number of birds	Number of affected birds	Susceptibility %
Quail group-1	10	4	40
Chickens group-1	10	10	100
Chickens group-2	10	10	100

Quail group-1: Experimentally infected quail. Chickens group-1: Chickens in contact with experimentally infected quail. Chickens group-2: Experimentally infected chicken. Recovery of NDV was positive from tracheal and intestinal samples of infected birds.

Table (2): Mean ND hemagglutination inhibition antibody titer in quails and chickens vaccinated with inactivated NDV vaccine.

Bird Groups	Mean ND-HI antibody titer (log_2/ml)/ WPV*				
	Prevaccination	1WPV	2WPV	3WPV	4WPV
Quail group-2	0	2	4	6	6
Quail group-3	0	0	0	0	0
Chickens group-3	0	3	5	7	7
Chickens group-4	0	0	0	0	0

WPI: Weeks Post Vaccination. Quail group-2: vaccinated with inactivated NDV vaccine. Quail group-3: Non-vaccinated control group. Chickens group-3: vaccinated with inactivated NDV vaccine. Chickens group-4: Non-vaccinated control group.

Table (3): Survival rates in challenged birds against virulent NDV

Bird groups	Number of birds		Protection percentage
	Challenged	survived	
Quail group-2	10	10	100
Quail group-3	10	7	30
Chickens group-3	10	9	90
Chickens group-4	10	0	0

Quail group-2: vaccinated with inactivated NDV vaccine. Quail group-3: Non-vaccinated control group. Chickens group-3: vaccinated with inactivated NDV vaccine. Chickens group-4: Non-vaccinated control group.

Photo (1): Infected quail with NDV showing paralysis of the legs.

Photo (2): Died quail affected with NDV.

Photo (3): Infected chickens with NDV.

dead quail revealed hemorrhagic lesions in the intestinal tract and proventriculus. NDV was recovered from tracheal and intestinal samples and identified by HI test using specific NDV antiserum. These results come in agreement with that of Momayez et al., (2007) who recorded loss of appetite, weakness, decreased egg production, diarrhea and nervous symptoms in quail infected with NDV. Hemorrhagic lesions of the intestinal tract and proventriculus were recorded at PM but NDV was isolated only from brain samples and identified by HI test using NDV-Specific antiserum. Chickens in group-1 (kept in contact with infected quail) showed signs of ND 15-day post contact infection and chickens in group-2 (experimentally infected with NDV) showed signs of ND 3 days post infection (PI). Signs of NDV infection in chickens were represented by restlessness, increased respiration, and weakness, green diarrhea in birds that not die early in infection, and prior to death, muscular tremors, paralysis of legs and wings ending by death. PM findings of dead chickens showed dark red purplish hemorrhagic and necrotic lesions in the digestive tract, catarrhal exudates in nasal passages and hemorrhage in trachea. Also NDV was recovered from brain, tracheal and intestinal samples of dead chickens through inoculation of SPF-ECE and identified by HI test. These results agreed with Mcferran and McKracken (1988) who recorded restlessness, increased respiration, and weakness ending by prostration and death; and Oladele et al., (2008) who

recorded signs of ND in infected chickens 3 days PI. The major clinical signs were depression, greenish diarrhea, paralysis of legs and wings, opisthotonus and torticollis and mortality was 100%. There were hemorrhagic lesions in the wall of the intestine, proventricular mucosa and caecal tonsils. Role of quail in transmission of NDV to chickens was showed by Lima et al., (2004) who recorded that quail experimentally infected with NDV did not show any clinical sign of ND, although contact infected SPF broilers showed clinical signs of ND with 100% mortality suggesting that quails can be NDV carriers. In the vaccinated groups, NDV was not re-isolated, demonstrating the importance of vaccination to control virus dissemination by quails infected with NDV. Quail and chickens inoculated with inactivated ND vaccine showed no clinical abnormalities denoting that the inactivated NDV vaccine was safe for both of them. Vaccinated birds exhibited detectable antiboy titers (2 $\log_2$) by the 1st week post vaccination (WPV) using HI test, to reach their peaks by the 3rd WPV (6 $\log_2$ in quails and 7 $\log_2$ in chickens). These antibody titers were protective to quail and chickens against challeneg with virulent NDV giving 100% protection rates in both of them while non-vaccinated birds could not withstand the challenege virus showing 70% and 100% mortality in quail and chickens, respectively. These results agreed with findings of Danchev et al., (1978) who recorded that HI titers of 1/40 and higher values were protective to birds challenged with a velogenic NDV and Raghul et al., (2006) who found that chickens vaccinated with a live lentogenic vaccine on day 14 of age along with it, an inactivated vaccine at day 36 of age, have HI antibody titer of 128 and above was protective against direct damage of the reproductive tract, while the 32-64 titer range was protective when derived through secondary vaccination only. In conclusion, quail could be considered as a susceptible host for NDV and have a non-neglectable role in transmission of NDV to chickens, so they should be vaccinated against NDV infection to protect them against virus infection and prevent its transmission to chickens.

5. REFERENCES

Alexander, D.J. 1993. Paramyxovirus infections. In viral infections of vertebrates, Vol. 3. Viral infections of birds (M.C. Horzinek, ed.). Elsevier Science Publishers Co., Amsterdam, pp. 321-340.

Amer, M.I.S., El-Bagoury, G.F., Khodeir, M.H. 2013. Studies on experimental infection of pigeons with Newcastle disease virus. Benha Veterinary Medical Journal, 25(1):143-147.

Anon, 1971. Methods for examination of poultry biologics and for identifying avian pathogens. National Academy of Science, Washington D.C., pp. 83 – 87.

Bakoji, I., Aliyu, M. K., Haruna, U., Jibril, S. A., Sani, R. M., Danwnka, H. 2013. Economic analysis of Quail bird production in Bauchi local government area, Bauchi State Nigeria. Research Journal of Agriculture and Environment Management, pp. 420-425.

Beard, C.W., Hanson, A. 1984. Newcastle disease. Disease of poultry 8th ed. Iowa State University Press, Ames, pp. 452- 47

Danchev, P., Semov, P., Danchev, I. 1978. Evaluation of the immune state of poultry against Newcastle virus via serological reactions. Vet. Med. Nauki, 15(4):86-92

Kaleta, E.F., Baldauf, C. 1998. Newcastle disease in free-living and pet birds In: D.J. Alexander (Ed.), Newcastle Disease. pp. 197-246. Kluwer Academia Publishers, Boston, USA.

Lamb, R.A., Collins, P.L., Kolakofsky, D., Melero, J.A., Nagai, Y., Oldstone, B.A., Pringle, C.R., Rima, K. 2005. Family paramyxoviridae. In Fauquet, C.M.; Mayo, M.A.; Maniloff, J.;

Desselbreger, U., Ball, L.A. virus Taxonomy, Eighth report of the International committee on Taxonomy of viruses, Elsevier Academic press: San Diego, pp. 655-668.

Lennete, E.H. 1964. Diagnostic procedures for viral and ricketsial diseases 3rd Ed A public health Ass. Inc.; Broadway.

Lima, F.S., Santin, E., Paulillo, A.C., Doretto, J.L. 2004. Evaluation of different programs of Newcastle disease vaccination in Japanese quail (Coturnix coturnix japonica). International Journal of Poultry Science 3:354–356.

McFerran, J.B., McCracken, R.M. 1988. Newcastle disease. In Newcastle disease, ed. Alexander DJ, pp. 161–183. Kluwer Academic Publishers, Boston, MA.

Momayez, R., Gharahkhani, P., Pourbakhsh, S.A., Toroghi, R., Shoushtari, A.H., Banani M. 2007. Isolation and pathogenicity identification of avian paramyxovirus serotype 1 (Newcastle disease) virus from a Japanese quail flock in Iran. Archives of Razi Institute, 62(1):39-44.

Murakami, A.E. 1991. Níveis de proteínae energia para codornas japonesas (Coturnix coturnix japonica) nas fases de crescimentoe postura Jaboticabal, 92p. Tese (Doutorado em Zootecnia) – Faculdade de Ciências Agrárias e Veterinárias Campus de Jaboticabal, Universidade Estadual Paulista.

Oladele, S.B., Enoch, I., Lawal, S., Ibu, O.J. 2008. Clinico-Pathological Features of Newcastle Disease in Japanese Quails (Coturnix coturnix japonica) Infected with Newcastle Disease Virus Kudu 113 Strain. International Journal of Poultry Science 7(2):165-168.

Paulillo, A.C. 1989. Avaliação da resposta imune e da performance zootécnica de poedeiras vacinadas experimental mente contra a doença de Newcastle. Jaboticabal, 1989. 116p. Tese (Livre Docência em Ornitopatologia)- Faculdade de Ciências Agrárias e Veterinárias Campus de Jaboticabal, Universidade Estadual Paulista.

Raghul, J., Raj, G.D., Manohar, B.M., Balachandran, C. 2006. Protection of the reproductive tract of young chicks by Newcastle disease virus-induced haemagglutination inhibition antibodies. Vet. Res. Commun.; 30(1):95-102.