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Experimental Vaccination against Challenge with Genotype VI, and VII Newcastle Disease Viral Strains in Quails: Clinical, Pathological and Molecular parameters.

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The inoculated birds exhibited low to moderate susceptability and had mild to severe neurological signs at 3rd day - 14th, and 4th day - 10th day post challenge, and the mortality rate was 46% and 33% for birds inoculated with NDV *Pigeon F-VI*, and NDV *GHB-328* strains, respectively. Briefly from 3rd up to 14th dpc, NDV *Pigeon F-VI*-inoculated quails (G4) were off food and exhibited ruffled feathers, great thrust, and greenish watery diarrhea together with redness and petechial hemorrhages of the eyelid, lacrimal discharges and nervous signs as well. The neurological signs were severe and in the form of depression, head tremors, ataxia, torticollis and paralysis. Similar clinical signs with lower degree of severity appeared in NDV *GHB-328*-inoculated quails of G5 at 4 dpc and continued up to 10 dpc. However, milder clinical signs represented by depression and conjunctivitis were displayed by the birds vaccinated by genotype II *LaSota* strain vaccine then challenged by *Pigeon F-VI* strain (G6) at 4 dpc. In contrast, moderate to severe clinical signs were exhibited by birds vaccinated by genotype VII *KBNP* strain vaccine then challenged by *Pigeon F-VI* strain (G7) at 3 dpc and continued up to 10 dpc. Moreover, almost other all vaccinated birds as G8 and G9, expressed milder clinical signs from 7-10 dpi. On the same side, the contact birds with G6 and G9, displayed mild clinical signs at 4 and 7 day post-in-contact, respectively.

Gross lesions varied from moderate to severe congestion were observed in the brain, crop, proventriculus, liver, intestine, and lung along with petechial hemorrhages in the eye, proventriculus and intestine in birds inoculated with *Pigeon F-VI* strain, G4. Presence of white secretions with offensive odour in the crop together with black to greenish content in the gizzard was also seen in G4. Similar gross pathological changes however, of severity degrees less than G4 were observed in G5 and G7. The mildest gross pathological changes were observed in G6, G8 and G9.

Features of non-suppurative encephalitis were marked in brains of birds in G4 than those in G5. Very mild to minimal microscopical lesions were detected in the vaccinated and challenged groups (G6, G8 and G9) however, the birds died in G7 showed mild to moderate lesions in the liver, trachea and lung with moderate to severe lesions in the intestine, proventriculus and brains. Moreover, contact birds showed mild lesions in brain, lung, trachea, proventriculus and intestine. Overall, the most severe and extensive nervous lesions were observed in G4; while the extensive enteric pathological lesions were observed in G5.

Considering viral shedding by infected birds, quails inoculated with NDV *Pigeon F-VI* strain (G4) followed by NDV-genotype VII 1.1-inoculated quails (*GHB-328* strain, G5) showed the highest titer for viral cloacal shedding at all tested days (3, 6 and 9 dpc) with the maximum level of viral shedding at 6 dpc. However, the lowest cloacal shedding was detected in vaccinated birds by NDV genotype VII *KBNP* strain vaccine and challenged by NDV-genotype VII 1.1, G9 at all tested days. These results were parallel to the decreased clinical signs and mortalities by that time after infection (9 dpi) and the enhanced immune response by infected birds through formation of antibodies to neutralize the virus after its replication in tissues. Furthermore, the vaccinated-challenged birds (G6, G7, G8 and G9) displayed lower viral shed at all tested days compared to the un-vaccinated challenged birds (G4 and G5). Among the vaccinated challenged groups they displayed the lowest cloacal viral shed titer at all tested days.

No mortalities, gross lesions, clinical signs, histopathological lesions or viral shedding were observed in G1 (control group), G2 and G3 vaccinated with NDV *LaSota* and *KBNP* strains, respectively.

In summary, strains of highly virulent NDV-genotype VI and VII 1.1 showed moderate to severe pathogenicity with mortality varied from 46-33% respectively in Japanese quails. Moreover, the efficacy of the two investigated vaccines was indicated whereas they significantly reduced viral shedding as well as mild exhibition of clinical signs, gross and histopathological lesions in vaccinated-challenged birds compared to unvaccinated-challenged one. Interestingly, vaccination with NDV vaccine formulated with antigens homologous to the challenge virus significantly reduced viral shedding in the cloacal swabs, decreased clinical signs, severity and incidence of pathological changes, and sustained elevated antibody titers in inoculated quails with no mortalities compared to heterologous antigen.

Conclusion

Comparison of the NDV-genotype VI and VII 1.1 viral Infections:

Although the basic features of NDV infection were common among quails infected with NDV *Pigeon F-VI* (NDV-genotype VI), and *GHB-328* strains (NDV-genotype VII 1.1), findings that were specific for each inoculated virus included the following;

- First, the onset of neurological signs following infection with NDV *Pigeon F-VI*, and NDV *GHB-328* strains occurred at 3 continued up to 14 dpc, and 4 continued up to 10dpc, respectively.
- Second, the mortality rates of birds inoculated with NDV *Pigeon F-VI*, and NDV *GHB-328* strains were 46%, and 33%, respectively.
- Third, clinical signs, gross and histopathological changes were of moderate to severe, and mild to moderate degree of severity in birds inoculated with NDV *Pigeon F-VI*, and NDV *GHB-328* strains, respectively.
- Fourth, NDV-genotype VI-inoculated birds (NDV *Pigeon F-VI* strain) showed higher titer for viral cloacal shedding compared to NDV-genotype VII 1.1- inoculated birds (NDV *GHB-328* strain).
- Fifth, Features of non-suppurative encephalitis were marked in brains of birds inoculated with NDV *Pigeon F-VI* than *GHB-328* strains.
- Sixth, extensive nervous lesions were observed in NDV-genotype VI-inoculated birds (NDV *Pigeon F-VI* strain), while the extensive enteric pathological lesions were observed in NDV-genotype VII 1.1- inoculated birds (NDV *GHB-328* strain).

Further studies in genetic engineering could be effective in producing poultry lines less susceptible/ resistant to NDV via reaching to the quail genes that make quails less susceptible to NDV infection.

Dalguban N+ vaccine induced delayed onset protective antibodies titer in quails after vaccination which still increase dramatically until reach its peak and persists for long duration. In contrast, Nobilis ® (Lasota) vaccine induced rapid onset protective antibodies which reaches its early but also decline more earlier than Dalguban N+ vaccine.

Finally, we recommended that the control of NDV must include strict biosecurity management via prevention of breeding of chickens with quails (as a reservoir of NDV) to

decrease cross species spread with proper administration of efficient vaccines. The best vaccine must focus not only on prevention of clinical disease and mortality, but also on prevention or at least decreasing virus shed amount from vaccinated birds especially in countries which endemic with NDV.