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Kinetics of serum antibody response to Newcastle disease vaccine in Aseel, Kadaknath and White Leghorn chicken using ELISA*

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Newcastle disease (ND) is one of the most highly contagious viral diseases affecting avian species throughout the world (Al-Garib *et al*. 2003). Morbidity and mortality being so high in the ND affected birds, vaccination is recommended as routine practice in many countries where virulent strain of Newcastle disease virus (NDV) is endemic, and the vaccine immunization is reported to yield good effect (Van Boven *et al*. 2008). However, there are still immunity failures which have become the major problem in prevention of the ND. It is generally acknowledged that humoral immunity is the main immunity to NDV and is commonly evaluated by measuring antibody titres in the immune sera by haemagglutination inhibition (HI) test and enzyme-linked immunosorbent assay (ELISA). High titre of antibodies is generally accepted as a reliable indicator of flock immunity (Beard and Hanson 2003). However, ELISA proved more accurate, sensitive and rapid but less economic than HI test when used for detection of antibody titres against ND vaccine (Tabidi *et al.* 2004). ELISA may be regarded as the present recommended method to conduct sero-surveillance for ND exposure and in evaluation of efficacies of vaccine protocols (Cadman *et al*. 2011), when elucidating immune response to ND vaccine remains a top priority for the development of better control strategies in the face of reoccurring outbreaks (Kapczynski *et al.* 2013). Hence, the study was undertaken to evaluate kinetics of serum antibody response to ND vaccine in Aseel, Kadaknath and White Leghorn chicken using ELISA.

The day-old chicks (120) of Aseel, Kadaknath and White Leghorn maintained at this institute were wing-banded and subjected to standard litter brooding, housing and feeding with optimum management (Das *et al.* 2014). Birds were fed on the institute-formulated (Das *et al.* 2014) chick mash *ad lib.* at 0–6 weeks of age. The day-old chicks were vaccinated with a dose of $10^{6.5}$ EID_{50} of live attenuated ND vaccine F_1 strain through occulo-nasal route followed by a booster dose on 28th day of age. The immune sera were collected on 7, 14, 21, 28, 35 and 42 days post-immunization (dpi) and stored at –20°C until use.

The commercial ELISA kit, IDEXX NDV Ab test for chickens was used for titration of the NDV antibodies in the immune sera. The NDV antigen coated ELISA plate contained 100 µl of undiluted sera in negative and positive control wells and the wells for samples contained 100 µl of diluted sera (500 fold diluted with PBS-Tween20 sample diluent) was incubated for 30 min at room temperature (20–25°C). Aspirating liquid content, each well was washed thrice with distilled water (350 µl) following its complete aspiration. Then, dispensing 100 µl of (goat) antichicken immunoglobulin horseradish peroxidase (HRPO) conjugate into each well, the plate was kept for the incubation for 30 min at room temperature. After washing each well with distilled (deionized) water following complete aspiration, 100 µl of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was dispensed into each well and incubated for 15 min at room temperature. Stopping the reaction with 100 µl stopping solution dispensed into each well, the plate was subjected to spectrophotometric reading of absorbance at 650 nm. The titre related S/P ratio of the absorbance values (sample to positive control mean) for the diluted sera to an endpoint titer was calculated as $\log_{10}$ titre = 1.09 ($\log_{10}$ S/P) + 3.36 as instructed by the manufacturer.

The data of the antibody titres ($\log_{10}$) against ND vaccine in different chicken genotypes were analyzed by analysis of variance using SPSS 16.0 statistical software program and the significant differences among different means were determined by DMRT at $P<0.05$.

The estimated mean antibody titres ($\log_{10}$) against ND vaccine measured using ELISA in Aseel, Kadaknath and White Leghorn (WLH) chicken are presented in Table 1.

The analysis of variance revealed that the immune

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response to ND vaccine significantly varied among different dpi in Aseel (P<0.01), Kadaknath (P<0.01) and WLH (P<0.05) chicken. Significantly varied ELISA antibody titres among different dpi were reported earlier in different genotypes of chicken (Sivaraman and Kumar 2010, Al-Zubeedy 2009) and duck (Hauslaigner *et al.* 2009). There was declining trend till 14 dpi and thereafter an increasing trend in antibody response was observed in Aseel, the highest titre being found at 42 dpi in accordance to the earlier findings in high IC index line of SDL broilers (Sivaraman and Kumar 2010), though their low and high IC index lines demonstrated an increasing trend till 14 dpi at first, thereafter followed by the present decreasing (till 28 dpi) and increasing (till 42 dpi) pattern in antibody response. Similar pattern of antibody response to ND vaccine was reported with highest titre at 21 dpi in broiler chicks when live attenuated ND vaccine was inoculated on day 1 with boosters on 7, 14 and 21th day of age (Al-Zubeedy 2009). The Kadaknath also demonstrated the highest antibody titre at 42 dpi in consistence to the earlier report (Sivaraman and Kumar 2010) but showed an increasing trend in the vaccine response from 7 dpi onwards in accordance to the reports in broilers chicks (Al-Zubeedy 2009). Whereas, the present WLH chicken demonstrated a declining trend until 28 dpi and then an inclining trend in the vaccine response might be due to the effect of the booster dose inoculated at 28th day. The highest antibody titre recorded at 7 dpi (in WLH) might be due to maternal derived antibody response as evidenced earlier in unvaccinated broiler chicks also (Al-Zubeedy 2009). The results revealed that the vaccination of day-old chicks is very important to enhance the maternal derived antibody response.

Analysis of variance also revealed that the present chicken genotypes significantly (P<0.01) varied in antibody titres (log_{10}) against ND vaccine at 7, 14 and 42 dpi (Table 1). At 7 and 14 dpi, WLH chicken demonstrated the highest (P<0.05) antibody titres in its immune sera followed by Aseel and Kadaknath, respectively, wherein the titre-difference between Aseel and Kadaknath being non-significant (P>0.05). At 42 dpi, Aseel showed the highest titre (P<0.05) in its immune sera followed by WLH and Kadaknath, wherein the titre-difference between WLH and Kadaknath being nonsignificant (P>0.05). The attributed differences might be due to the different breeds and their serological functionary to raise the antibody response. Earlier workers also (Tabidi *et al.* 2004, Al-Zubeedy 2009, Sivaraman and Kumar 2010) achieved different antibody titres in different genotypes.

The investigation concluded that the vaccination of day-old chicks against Newcastle disease is very important to enhance the maternal derived antibody response and the study also speaks about much better antibody response to NDV in Aseel and Kadaknath native fowl.

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SUMMARY

The study aimed to evaluate kinetics of serum antibody response to Newcastle disease (ND) vaccine investigating 120 chicks from Aseel, Kadaknath and White Leghorn (WLH) maintained at this institute. The day-old chicks were vaccinated with a dose of $10^{6.5}$ EID_{50} of live attenuated ND vaccine F_1 strain through occulo-nasal route followed by a booster dose on 28th day. The antibody titre means were estimated and varied significantly among different dpi in Aseel, Kadaknath and WLH. The immune sera in Aseel and Kadaknath had gradual inclining antibody titre levels for longer dpi with the highest titre means at 42 dpi, whereas the sera in WLH had an irregular trend in antibody levels with the highest titre mean at 7 dpi. Aseel, Kadaknath and WLH also varied in mean antibody titres at 7, 14 and 42 dpi. The highest titre means at each 7 and 14 dpi were in WLH followed by Aseel and Kadaknath, respectively, whereas at 42 dpi was in Aseel followed by WLH and Kadaknath. The results indicated importance of the vaccination of day-old chicks against ND to enhance maternal derived antibody response and also speak about much better antibody response to NDV in Aseel and Kadaknath native fowl.

Table 1. The estimated mean ELISA antibody titres (log_{10}) against Newcastle disease vaccine in different chicken genotypes in different days post-immunization (dpi)

Chicken genotype	Antibody titre means ± standard errors					
	7 dpi	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi
Aseel	2.20±0.11^{bB} (39)	2.12±0.16^{bB} (37)	2.43±0.16^{bA} (34)	2.80±0.09^{aA} (31)	2.82±0.09^{aA} (29)	3.04±0.08^{aA} (28)
Kadaknath	2.08±0.16^{cB} (38)	2.16±0.14^{bcB} (37)	2.38±0.06^{abA} (31)	2.50±0.12^{aA} (28)	2.60±0.08^{aA} (27)	2.66±0.12^{aB} (27)
WLH	3.20±0.07^{aA} (39)	2.93±0.09^{bA} (38)	2.60±0.12^{cdA} (35)	2.43±0.12^{dA} (35)	2.60±0.08^{cdA} (33)	2.79±0.09^{bcAB} (29)

Values within a row having different small letters in the superscript differ significantly (P≤0.05); Values within a column having different capital letters in the superscript differ significantly (P≤0.05); Values within parenthesis denote numbers of observations.

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