



## Estimating serological immune response against Newcastle disease vaccine in Aseel, Kadaknath and White Leghorn chicken by haemagglutination inhibition test

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### ABSTRACT

The present study aimed to estimate serological immune response against Newcastle disease vaccine investigating 70 Aseel, 75 Kadaknath and 85 White Leghorn chicks. The day-old chicks were vaccinated with a dose of  $10^{6.5}$  EID<sub>50</sub> of RDF<sub>1</sub> strain through occulo-nasal route followed by a booster dose on 28<sup>th</sup> day. The sera collected on 7, 14, 21, 28, 35 and 42 days post-immunization (dpi) were used to measure antibody titres through haemagglutination inhibition test. The data were analyzed by analysis of variance using SPSS 16.0 statistical software. The corresponding mean antibody titre ( $\log_2$ ) estimates were  $8.10 \pm 0.22$ ,  $7.89 \pm 0.18$ ,  $7.92 \pm 0.16$ ,  $8.29 \pm 0.14$ ,  $8.40 \pm 0.16$  and  $8.94 \pm 0.19$  in Aseel,  $7.32 \pm 0.16$ ,  $7.74 \pm 0.13$ ,  $7.56 \pm 0.13$ ,  $7.86 \pm 0.17$ ,  $8.43 \pm 0.17$  and  $9.14 \pm 0.16$  in Kadaknath, and  $8.48 \pm 0.28$ ,  $8.02 \pm 0.31$ ,  $8.29 \pm 0.33$ ,  $8.14 \pm 0.30$ ,  $7.68 \pm 0.29$  and  $7.73 \pm 0.29$  in White Leghorn chicken. The estimates significantly varied among different dpi in Aseel and Kadaknath chicken except White Leghorn. Aseel and Kadaknath chicken demonstrated gradual increasing trend and higher means of antibody titres for longer periods of dpi and achieved the highest at 42 dpi, whereas White Leghorn chicken showed an irregular trend, the highest titre being observed at 7 dpi. Again the 3 chicken genotypes significantly varied in antibody titres at 7, 35 and 42 dpi; White Leghorn chicken demonstrated the highest antibody titre at 7 dpi, while Kadaknath chicken showed the highest titre at 35 and 42 dpi. The higher and longer immune responsive Aseel and Kadaknath chicken might be utilized for selective introgression of their candidate genes in high productive chicken germplasm with less NDV response.

**Key words:** Aseel, HI antibody titre, Kadaknath, NDV vaccine response, White Leghorn chicken

Newcastle disease is one of the most important and highly contagious viral diseases affecting wild and domestic avian species throughout the world (Al-Garib *et al.* 2003). The impact of the Newcastle disease is most notable in domestic poultry birds due to their high susceptibility to its virulent viral strains and severe consequences of outbreaks on the poultry industries. Because of high morbidity and mortality in the Newcastle disease affected birds, vaccination is recommended as routine practice in many countries where virulent strain of Newcastle diseases virus is endemic. Moreover, to prevent this viral disease, immunization is the major measure and yields good effect (Van Boven *et al.* 2008). However, there are still immunity failures which have become the major problem in prevention of this disease. It is generally acknowledged that

humoral immunity is the main immunity to Newcastle disease virus (NDV) and is commonly evaluated by measuring antibody titres in the sera by haemagglutination inhibition (HI) test and enzyme-linked immunosorbant assay (ELISA). High titre of antibodies is generally accepted as a reliable indicator of flock immunity (Beard and Hanson 2003). However, HI test seems to be cheaper than ELISA kit for detection of antibody levels against NDV vaccine (Bozorghmehrfard and Mayahi 2000). Hence, the present study was undertaken to estimate serological immune response against Newcastle disease vaccine in Aseel, Kadaknath and White Leghorn chicken by haemagglutination inhibition test.

### MATERIALS AND METHODS

*Experimental birds, husbandry and immunization:* The day-old Aseel (70), Kadaknath (75) and White Leghorn (85) chicks hatched out and maintained at this institute were wing-banded and standard litter brooding, housing and feeding were provided with optimum management (Das *et al.* 2014). The birds were fed *ad lib.* on the institute-formulated chick mash at 0–8 weeks of age followed by grower mash at 9–20 weeks and layer mash at 20 weeks

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onwards (Das *et al.* 2014). The day-old chicks were vaccinated with a dose of  $10^{6.5}$  EID<sub>50</sub> of RDF<sub>1</sub> strain through occulo-nasal route followed by a booster dose on 28<sup>th</sup> day of age. As a routine vaccination schedule being followed at this institute, fowl pox vaccine was administered through wing prick on 42-day of age after collecting venous blood for the NDV immunized sera collection. The RD R<sub>2</sub>B vaccine was inoculated on 56-day of age followed by its booster doses at 3–4 months' interval.

*Harvesting of immune sera and haemagglutination inhibition (HI) test:* The NDV immune sera were collected on 7, 14, 21, 28, 35 and 42 days post immunization (dpi) and stored at  $-20^{\circ}\text{C}$  until use. The sera were subjected to HI test performed as per Allan and Gough (1974). Twenty five microlitres sterile normal saline (0.85%) was loaded in each well on to a 'V' bottom microtitre plate, first row of which was then loaded with 25  $\mu\text{l}$  of each test serum and subjected to two-fold serial dilution. Thereafter, 25  $\mu\text{l}$  of 4HA NDV suspension in PBS (definite quantity of HA titre specific allantonic fluid from NDV infected embryonated eggs) was added up to 11<sup>th</sup> well in each row and kept at room temperature for 25–30 min. Twenty five microlitres of chicken erythrocytes suspension (1% v/v) in PBS was added into each well, and 12<sup>th</sup> well acted as the erythrocytes control. The plate was gently shaken and kept at  $37^{\circ}\text{C}$  for 45 min. The samples showing peculiar central button shaped settling of erythrocytes were recorded as positive and the maximum dilution of each serum sample causing 100% inhibition of haemagglutination was the endpoint. The antibody titre of HI test for each serum sample was expressed as reciprocal of the serum dilution ( $\log_2$ ).

*Statistical analysis:* The data on the antibody titres ( $\log_2$ ) 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  $P < 0.05$ .

## RESULTS AND DISCUSSION

The estimated mean antibody titres ( $\log_2$ ) against Newcastle disease vaccine measured using haemagglutination inhibition test in Aseel, Kadaknath and White Leghorn (WLH) chicken are presented in Table 1. The analysis of variance revealed that the immune response to Newcastle disease vaccine significantly ( $P < 0.01$ ) varied

among different dpi in Aseel and Kadaknath chicken except in WLH chicken and this significantly varied immune response among different dpi was also reported in different chicken genotypes (Nasser *et al.* 2000, Shuaib *et al.* 2003, Kafi *et al.* 2003, Sasipreeyajan 2005, Jalil *et al.* 2009, Yan *et al.* 2011). Aseel chicken demonstrated increasing trend of mean antibody titre from 14 dpi onwards and the highest ( $P < 0.05$ ) mean titre at 42 dpi; though the mean titre at 7 dpi was higher ( $P > 0.05$ ) than at 14 and 21 dpi which might be due to some maternally derived immune response. The estimated mean titre at 35 dpi significantly ( $P < 0.05$ ) differed from the mean titres at 14 dpi, 21 dpi and 42 dpi. Kadaknath chicken demonstrated increasing trend ( $P < 0.05$ ) of mean antibody titre from 7 dpi to 14 dpi, then being slightly decreased ( $P > 0.05$ ) at 21 dpi and slightly increased ( $P > 0.05$ ) at 28 dpi and got continuously increased ( $P < 0.05$ ) thereafter. The highest antibody titre was achieved at 42 dpi followed by at 35 dpi, both titres being significantly ( $P < 0.05$ ) higher than the titre estimates at other dpi. The mean titre at 28 dpi was also higher than the titre estimates at 7 dpi, 14 dpi and 21 dpi, wherein titre at 21 dpi was higher ( $P > 0.05$ ) than at 7 dpi. In WLH chicken, the highest mean antibody titre was observed at 7 dpi followed by at 21 dpi and thereafter it revealed decreasing trend at 28 and 35 dpi, then again increased at 42 dpi; thus could not follow any specific trend. The higher response at 7 dpi could reveal that the vaccination of day-old chicks might be important to enhance the maternal derived antibody response. Previously, Nasser *et al.* (2000) recorded significant rise of HI antibody titre against NDV vaccine following booster dose on 21-day in broiler chicks. Sasipreeyajan (2005) observed the highest immune response (HI titre) against NDV vaccine at 14 dpi being declined at 21 dpi in accordance to the present findings in Kadaknath chicken. Shivaraman and Kumar (2010) also observed the corresponding primary and secondary vaccination responses being the highest at 14 dpi and 42 dpi when studied HI antibody response to NDV vaccine in G2 generation of SDL broilers divergently selected for immunocompetence index (high and low line). Yan *et al.* (2011) also reported a gradual increasing trend of HI antibody titres to NDV vaccine from 7 dpi to 21 dpi in WLH chicken like achieved in the present Kadaknath chicken, though Aseel and WLH presently demonstrated the first increasing trend from 14 dpi being

Table 1. Estimated mean ( $\pm$ SE) antibody titres ( $\log_2$ ) against Newcastle disease vaccine in different chicken genotypes by haemagglutination inhibition (HI) test

Genotype	7 dpi	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi
Aseel chicken	8.10 $\pm$ 0.22 <sup>cdA</sup> (64)	7.89 $\pm$ 0.18 <sup>d</sup> (64)	7.92 $\pm$ 0.16 <sup>d</sup> (63)	8.29 $\pm$ 0.14 <sup>bce</sup> (63)	8.40 $\pm$ 0.16 <sup>bA</sup> (63)	8.94 $\pm$ 0.19 <sup>aA</sup> (62)
Kadaknath chicken	7.32 $\pm$ 0.16 <sup>dB</sup> (72)	7.74 $\pm$ 0.13 <sup>c</sup> (72)	7.56 $\pm$ 0.13 <sup>cd</sup> (71)	7.86 $\pm$ 0.17 <sup>c</sup> (71)	8.43 $\pm$ 0.17 <sup>bA</sup> (70)	9.14 $\pm$ 0.16 <sup>aA</sup> (70)
WLH chicken	8.48 $\pm$ 0.28 <sup>aA</sup> (82)	8.02 $\pm$ 0.31 <sup>ab</sup> (81)	8.29 $\pm$ 0.33 <sup>a</sup> (80)	8.14 $\pm$ 0.30 <sup>a</sup> (80)	7.68 $\pm$ 0.29 <sup>bB</sup> (79)	7.73 $\pm$ 0.29 <sup>bB</sup> (78)

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

lesser than at 7 dpi. Analysis of variance also revealed that the 3 present chicken genotypes significantly varied in HI antibody titres against NDV vaccine at 7 dpi ( $P < 0.01$ ), 35 dpi ( $P < 0.05$ ) and 42 dpi ( $P < 0.01$ ) (Table 1). At 7 dpi, WLH chicken demonstrated the highest antibody titre followed by Aseel and Kadaknath chicken while at 35 dpi and 42 dpi, the Kadaknath chicken showed the highest titre followed by Aseel and WLH chicken. The attributed differences might be due to the different breeds and their serological functionality to raise the antibody response. Earlier workers also (Nasser *et al.* 2000, Kafi *et al.* 2003, Shuaib *et al.* 2003, Sasipreeyajan 2005, Jalil *et al.* 2009, Shivaraman and Kumar 2010, Yan *et al.* 2011) reported different antibody titres in different genotypes.

It is concluded that the attributed higher and longer NDV vaccine response in Aseel and Kadaknath native fowls might be exploited by their selective introgression in high yielding chicken germplasm with less NDV vaccine response.

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