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INACTIVATED VACCINES AGAINST NEWCASTLE DISEASE 2. HUMORAL IMMUNE RESPONSE

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The transformation of poultry husbandry from backyard avocation to industry is phenomenal. The development in the germplasm of poultry by introduction of foreign genome and upgradation of the existing variety resulted in decrease in the resistance of birds to viral infections. Besides, the production loss due to reduction in egg yield also warrants newer methods to sustain high level of immunity for a longer period to minimise the loss. The present concept in modern vaccinology is to prime the immune system with live vaccines and induce high level of immunity by giving inactivated vaccines with adjuvants. The cell mediated and humoral immunity play a role in immunity against Newcastle disease (ND) in birds (Timms and Alexander, 1977 and Chandrasekhar *et al.*, 1989); Besides these, local immune response mediated by IgA and IgG in respiratory and digestive tract also plays a role in protection (Parry and Aitken, 1977; Powell *et al.*, 1979). the presence of IgG in tracheal fluid could be due to transfusion of serum as a result of damage to tracheal mucosa (Ewert *et al.*, 1977). Humoral immune response plays a major role in immunity against ND. Two isotypes of antibodies viz IgM and IgG are involved; IgG appears between days 7 and 8 whereas IgM appears on day 6 itself (Muelmans *et al.*, 1984). The protective titre of antibodies gradually declined after 3 weeks (Alexander, 1991). It is with this idea, an attempt was made to study the humoral immune response by haemagglutination inhibition test (HI), serum neutralisation test (SN) and enzyme linked immunosorbant assay (ELISA) with six types of inactivated vaccines developed earlier using formalin and BPL with aluminium hydroxide as adjuvant.

Materials and Methods

Vaccination schedule : Each experimental group comprised of fifteen birds. They were vaccinated with RDVF and RDVK. Six experimental groups were maintained and one group was used for one vaccine. The experimental groups were vaccinated with one ml of the inactivated vaccine intra muscularly. The control group comprised of five birds which were vaccinated with RDVF and RDVK and not with inactivated vaccines. The experimental and control groups were maintained at same conditions. Serum samples were collected from experimental and control groups on 0, 7, 14 and 21 days post - vaccination.

Micro haemagglutination inhibition test : The HI test was performed as per the method of Allan and Gough (1974). The assay was performed in 96 well 'V' bottomed

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Table - 1 POST VACCINAL IMMUNE RESPONSE - MEAN HI VALUES

Sl. No.	Vaccine	Days Post Vaccination				Days Post-Challenge	
		0	7	14	21	7	14
1.	F/Formalin/Al(OH)$_3$	1.264±0.08	1.626±0.04	2.047±0.06	2.468±0.06	2.106±0.04	2.468±0.04
2.	LaSota/Formalin/Al(OH)$_3$	1.455±0.04	1.806±0.04	2.197±0.04	2.559±0.05	2.258±0.05	2.558±0.05
3.	LaSota/BPL/Al(OH)$_3$	1.355±0.05	1.957±0.05	2.499±0.04	2.709±0.00	2.137±0.05	2.589±0.05
4.	Komarov/Formalin/Al(OH)$_3$	1.234±0.06	1.625±0.08	2.378±0.05	2.830±0.05	2.438±0.05	2.829±0.05
5.	Komarov/BPL/Al(OH)$_3$	1.745±0.07	2.228±0.06	2.649±0.04	2.107±0.06	2.258±0.05	2.679±0.03
6.	VP 9/Formalin/Al(OH)$_3$	1.415±0.04	2.348±0.04	2.769±0.06	2.914±0.08	2.569±0.06	2.981±0.05
7.	Control	1.325±0.07	1.324±0.07	1.325±0.07	1.385±0.07	0.903±0.10	1.355±0.07

Table - 2 POST VACCINAL IMMUNE RESPONSE - MEAN ELISA VALUES

Sl. No.	Vaccine	Days Post - Vaccination				Post-Challenge	
		0	7	14	21	7	14
1.	F/Formalin/Al(OH)$_3$	2.312±0.04	2.783±0.05	3.084±0.05	3.234±0.03	2.843±0.04	3.235±0.05
2.	LaSota/Formalin/Al(OH)$_3$	2.572±0.03	2.843±0.04	3.114±0.04	3.204±0.04	2.843±0.06	3.174±0.05
3.	LaSota BPL/Al(OH)$_3$	2.452±0.05	2.753±0.05	3.023±0.05	3.174±0.07	2.783±0.02	3.114±0.04
4.	Komarov/Formalin/Al(OH)$_3$	2.572±0.03	2.803±0.07	3.325±0.04	3.445±0.05	3.084±0.05	3.325±0.05
5.	Komarov /BPL/Al(OH)$_3$	2.542±0.04	2.933±0.05	3.234±0.05	3.325±0.05	2.903±0.04	3.234±0.02
6.	VP 9/Formalin/Al(OH)$_3$	2.542±0.04	2.813±0.04	3.294±0.04	3.415±0.04	3.023±0.05	3.325±0.05
7.	Control	2.257±0.06	2.542±0.05	2.361±0.05	2.481±0.07	2.031±0.00	2.602±0.00

Table - 3 POST VACCINAL IMMUNE RESPONSE - MEAN SN VALUES

Sl. No.	Vaccine	Days Post - Vaccination				Post - Challenge	
		0	7	14	21	7	14
1.	F/Formalin/Al(OH)$_3$	1.023±0.06	1.355±0.05	1.475±0.08	1.896±0.10	1.595±0.07	1.987±0.06
2.	LaSota/Formalin/Al(OH)$_3$	0.933±0.05	1.355±0.06	1.836±0.06	2.198±0.09	1.656±0.09	2.078±0.10
3.	LaSota/BPL/Al(OH)$_3$	0.933±0.05	1.325±0.05	1.716±0.06	2.047±0.06	1.655±0.09	2.137±0.07
4.	Komarov/Formalin/Al(OH)$_3$	1.054±0.05	1.414±0.04	1.927±0.05	2.499±0.04	2.107±0.07	2.589±0.05
5.	Komarov BPL/Al(OH)$_3$	1.054±0.05	1.415±0.04	1.927±0.00	2.499±0.04	2.077±0.08	2.589±0.05
6.	VP 9/Formalin/Al(OH)$_3$	1.054±0.05	1.595±0.04	1.927±0.05	2.468±0.06	1.957±0.05	2.438±0.05
7.	Control	0.903±0.09	1.023±0.06	1.023±0.06	1.084±0.07	0.527±0.03	1.129±0.06

* Litre values are expressed to log $_{10}$.

** Mean litre values are expressed as Mean Values ±S.E.

Table - 4 RANDOMISED BLOCK DESIGN - FOR POST - VACCINATION AND POST - CHALLENGE PERIODS

Tests	Days Post - Vaccination				Days Post - Challenge	
	0	7	14	21	7	14
HI	1.398^{a}	1.844^{b}	2.266^{c}	2.425^{cd}	2.098^{bc}	2.494^{d}
ELISA	2.531^{a}	2.781^{b}	3.062^{b}	3.183^{c}	2.826^{ab}	3.144^{bc}
SN	0.991^{a}	1.355^{ab}	1.690^{ab}	2.099^{abc}	1.653^{abcd}	2.134^{c}
QDIA	1.351^{a}	1.854^{b}	2.236^{c}	2.559^{cd}	2.176^{bc}	2.134^{d}
LMH	12.02^{a}	15.42^{bc}	16.96^{cd}	18.17^{e}	16.38^{c}	17.86^{d}

* Means having atleast one common superscript do not differ significantly.

* $P<0.01$.

plates. By performing standard HA test 4 HA_{50} unit was calculated. To all the wells 25µl PBS was added. The serum samples were diluted serially and 25 µl of antigen was added to all the wells except to one row which was left with 25µl of antigen and PBS alone. The plates were incubated 37°C for one hour. Chicken RBC at a concentration of 0.5 per cent was added to all the wells. The highest dilution showing complete button formation was taken as end point and the reciprocal was expressed as the HI titre of the serum sample.

Micro serum neutralisation test : The micro SN test was performed as per the method of Ashokkumar (1984) with slight modifications. Two - fold serial dilution of the test serum samples were performed in sterile PBS with 25ml of the test serum. To these, 25 µl of the virulent virus was added and the mixture was incubated at 37°C for one hour. 25µl of the virus-serum mixture was added to 96 well flat bottomed tissue culture plates with fully grown monolayer of vero cells. An adsorption time of thirty minutes was given. The virus serum mixture was then discarded and maintenance medium was added to all wells. The plates were observed for cytopathic effect regularly upto five days. The reciprocal of the highest dilution at which the production of CPE was inhibited was taken as SN titre.

Enzyme linked immunosorbant assay : The ELISA was performed as per the method of Theirman and Garret (1983) and Tatabai and Deyoe (1984) with modifications proposed by Padmanaban (1995).

Purification of antigen : Amnioallantoic fluid infected with ND virus was clarified by spinning at 5000rpm for 30 minutes. The supernatant was centrifuged at 55000rpm for six hours in a Beckman (L7-80) ultra centrifuge in 70 Ti rotor. The crude pellet was again centrifuged at 55000 rpm for six hours at 35 per cent sucrose solution in a 60Ti swingout rotor. The precipitate was again centrifuged at 55000rpm in 60Ti swingout rotor for six hours at sucrose gradients of 55 per cent and 35 per cent. The protein concentration was measured in a spectrophotometer at 280nm using standard curve prepared with bovine serum albumin and purified virus obtained at the interphase. This was used to coat the ELISA plates. The purified virus at a dilution of 1 in 200 was used to coat the plates. This dilution was arrived after checker board titration. To all the wells 100µl of virus was added and the plates were kept in humid chambers at 4°C over night. The plates were emptied next day and washed thrice in PBS with Tween 20 (PBST).

Test Proper : The unbound sites on plates were masked with one per cent bovine serum albumin (BSA). One in 100 dilution of the test serum was added to the first well and dilutions were carried out upto one in 12,800. Triplicates of 1 in 100 negative serum were also used in the assay. Positive serum control samples were also kept. After addition of these, the plates were incubated for one hour at 37°C and washed with PBST after incubation time. Rabbit anti-chicken IgG-HRP conjugate was added to all the wells at a concentration of 1 in 2000 leaving a control well and incubated as mentioned earlier for one hour. The plates were washed in PBST after incubation time. 2'-2 azino-di-ethylbenzthiazoline 6 sulfonic acid (ABTS) was used as a substrate. The substrate was prepared in sodium citrate buffer (pH 4.2) and to this 35 per cent hydrogen peroxide was added. The substrate was prepared just prior to use. To all the well 100µl of substrate was added keeping a substrate blank. The plates were incubated at room temperature for 30

minutes. At the end of the time period, 100µl of 0.1 per cent hydrofluoric acid was added to stop the color reaction. The plates were read in a multiscan ELISA reader at 405nm. The HRP control well was usually blanked. The optical density values (OD) twice and above the negative values were considered as positive readings.

Challenge experiment : The experimental and control groups were challenged with 10^6EID_{50} of virulent virus on 21 days post - vaccination. Serum samples were collected from vaccinated and control groups on the day of challenge, 7 and 14 days after challenge.

Statistical Analysis : The data available in the form of titre values were subjected to statistical analysis as per the methods of Snedecor and Cochran (1968). The mean values were analysed by randomised block design and the parameter interdependance was found out by correlation coefficient.

Results and Discussion

The mean HI, ELISA and SN titres on the day of vaccination and at various time points of serum collection are expressed in tables 1, 2, 3 respectively as log to base 10. The titre values increased gradually and reached a peak at 21 days post - vaccination. A fall in the mean HI, SN and ELISA titres was noticed on 7 days post - challenge but the mean titre increased again on day 14 post - challenge and the increase was maintained until the end of observation period. In unvaccinated control group, the HI titre remained at constant level with appreciable increase only on day 14 post - challenge. The development of antibodies in vaccinated birds was found to be significant on statistical analysis whereas it was not so in the case of controls. By statistical analysis with randomise block design (RBD), antibody levels significantly increased on day 7 post - vaccination and the fall in titre was statistically significant only when tested by ELISA (Table - 4). A positive correlation exisit between all these three tests at 1 per cent level indicating high interdependability between them. Such type of positive correlations were reported by earlier workers (Snyder *et al.*, 1983; Brown *et al.*, 1990; Srinivasan *et al.*, 1992). In the challenge studies, the vaccinated birds did not develop any systemic reaction; were normal and feeding was not affected. However, 20 per cent mortality was noticed in control group. Post-mortem findings revealed lesions suggestive of ND.

The results of three tests viz, HI, SN and ELISA along with results of challenge studies have clearly showed that high levels of humoral factors in birds vaccinated with inactivated vaccines are essential to prevent birds from risk of infection since 20 per cent mortality was reported in control group.

Summary

Humoral immune response against six different types of inactivated vaccines were studied in layer birds in age group of 18 weeks. The humoral immune response was assessed by HI, SN and ELISA methods. The results of all the three tests proved the role of humoral immunity against ND. In challenge experiment, 20 per cent mortality was noticed in birds that were not vaccinated with inactivated vaccines, whereas no mortality was reported in birds vaccinated with inactivated vaccines. The results were also statistically analysed.

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