COMPARISON OF ANTIBODY TITRES OF NEWCASTLE DISEASE VIRUS IN RANDOMLY COLLECTED SERA AND EGG YOLK OF LAYERS

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Abstract

A study was conducted to compare the Newcastle Disease (ND) virus antibody titre in randomly collected sera and egg yolk of layers using haemagglutination inhibition (HI) test. The mean log_2 HI titre values detected in sera and egg yolk were 4.50 and 5.68 respectively. Statistically there was significant difference between the two means ($p < 0.03$). Egg yolk samples may be used as a test material for detection of titre of ND virus antibodies in layers. But when egg yolk is the test material, the HI titre detected tends to be significantly higher.

Key words *Newcastle Disease, haemagglutination inhibition test, serum, egg yolk.*

Newcastle disease (ND) is a viral disease of birds caused by ND virus (NDV) of Genus *Avulavirus* of Family *Paramyxoviridae*. Antibody titre against this virus is commonly assessed in bird sera by haemagglutination inhibition (HI) test. Though studies to compare the HI titres of NDV antibodies in birds and their corresponding eggs have been conducted, those on randomly collected bird sera and eggs are scarce. If the levels of the antibodies in randomly collected sera and eggs are comparable, then eggs can be preferred over sera for assessment of the antibody titre especially in farms. Hence a study was conducted to compare the NDV antibody titres in sera and egg yolk of layers collected at random from an organised farm.

Materials and Methods

A total of 24 blood samples and 29 egg samples were collected at random from birds maintained at the University Poultry Farm, College of Veterinary and Animal Sciences, Pookode. All the birds had been vaccinated against ND using a commercial vaccine five months back. Blood was collected aseptically from the wing vein using sterile technique, allowed to clot and serum separated by centrifugation and stored at -20°C until tested. Eggs were collected on the same day and stored at room temperature. The eggs were broken and the contents gently transferred to separate filter papers. One ml of the yolk was collected and diluted in nine ml of sterile normal saline (1:10 dilution), mixed well and centrifuged at 2000 x *g* for 20 min. From the supernatant, 0.2 ml was collected and used in the HI test. Sera (0.2 ml) were used without any dilution.

A field isolate of NDV obtained from the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy was used in the study. The isolate was passaged in 9 to 11 day old embryonated chicken eggs by the allantoic route of inoculation. The allantoic fluid was collected from the inoculated eggs and used as source of virus in the HI test. The HI test (â method) was performed as described by Allan and Gough (1974). Briefly the HI test was conducted as follows. Initially a haemagglutination (HA) test was performed by making serial two fold dilutions of the virus

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(allantoic fluid) in a perspex HA plate and adding a fixed quantity of 0.5 per cent chicken RBC (cRBC) suspension to all the wells and incubating at room temperature for 30 to 45 min. A c RBC control without any virus was also kept. The HA titre was recorded as the reciprocal of the highest dilution of the virus showing complete HA. After the HA test was conducted, the HI test was performed by making serial two fold dilutions of the sera or diluted yolk and adding 4HA units of the virus to all the wells and incubating for 30 minutes for neutralisation to occur. Then a fixed quantity of 0.5 percent cRBC was added to all the wells and incubated at room temperature for 30 to 45 min. Suitable virus, serum and cRBC controls were also included in the test. The HI titre was recorded as the reciprocal of the highest dilution of sera or yolk showing complete inhibition of HA. The HI titre obtained was expressed as $\log_2$ values. In case of egg yolk, the HI titre obtained was multiplied by 10 and then converted to log values to account for the dilution (for example a HI titre of four in the test was converted to 40 and a log value of five; a titre of eight was converted to 80 and log value of six etc.). The mean $\log_2$ HI titre values in sera and egg yolk were calculated and compared using t test (Zar, 2003).

Results and Discussion

The results of the study are given in the table . The mean $\log_2$ HI titre values in sera and egg yolk were 4.50 and 5.68 respectively. Statistical analysis of the data showed significant difference between the two means ($P < 0.03$). This indicates that the antibody titre detected in egg yolk is significantly higher than that in sera. Reports on comparison of HI titre in randomly collected sera and egg yolk are limited. Yeo and Choi (1979) and Yeo *et al.* (2003) compared the NDV HI titres in hens and their corresponding eggs and reported that HI titres in egg yolk tend to be slightly higher than that in sera which is in accordance with our findings.

Table . Results of comparison of $\log_2$ HI titres from egg yolk and sera

	Serum	Egg
Sample size	24	29
Minimum	0	0
Maximum	8	8
Mean	4.50	5.68
Standard deviation	2.10	1.41
Standard error	0.43	0.26
Confidence interval (95%)	(3.61, 5.39)	(5.15, 6.21)
t value	2.26	
Probability (p)	< 0.03	

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References

Allan, W. H. and Gough, R. E. 1974. A standard hemagglutination inhibition test for Newcastle disease. A comparison of macro and micro methods. *Vet. Rec.,* **95:** 120-123

Yeo, S. G. and Choi, W.P. 1979. Immune status of breeding hens against Newcastle disease. *Korean J. Vet. Res.*, **19:** 45–51

Yeo, S. G., Nagy, E. and Krell, P. J. 2003. Indirect method for prediction of hemagglutination inhibition antibody titers to Newcastle disease virus in chickens by titration of antibodies in egg yolk. *J. Vet. Diagn. Invest.,* **15:** 184–187

Zar, J. H. 2003. *Biostatistical Analysis*. Pearson Education, Singapore.

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