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EFFECTIVENESS OF SEROLOGICAL ASSAYS TO DETECT NEW CASTLE DISEASE VIRAL ANTIBODIES IN CHICKEN

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Abstract: Newcastle disease (ND) is a contagious disease affecting many domestic and wild avian species. A study was conducted to compare serological tests for the detection of ND in chicken. Fifty sera samples were collected from University Poultry Duck Farm, Mannuthy, Thrissur, Kerala, two weeks after vaccination. Out of this, 45 samples were positive by Haemagglutination Inhibition test (HI), eight samples were positive by Agar Gel Immuno Diffusion test (AGID). Counter Immuno Electrophoresis (CIE) detected NDV antibodies from 10 samples and 40 samples were positive by Dot- Enzyme Linked Immunosorbent Assay (Dot-ELISA). When comparing the efficacy of the tests the sensitivity, specificity and overall agreement obtained was 15.6 per cent, 100 per cent and 24 per cent between AGID and HI; 17.78 per cent, 100 per cent, 26 per cent between CIE and HI and 88.89 per cent,100 per cent and 90 per cent between Dot-ELISA and HI, respectively. The study showed that the highest rate of agreement between the results of Dot-ELISA and HI and therefore it was concluded that Dot-ELISA is more sensitive than other two tests.

Keywords: Newcastle disease, HI test, AGID, CIE, Dot-ELISA, chicken.

INTRODUCTION

Newcastle disease (ND) is considered as one of the most important viral disease that affects poultry and causes severe economic losses (Alexander *et al*., 1996). There were 464 reported outbreaks of ND in India during the year 2000 resulting in the death of 10,635 birds (Sadana and Sarkar, 2003). Frequent outbreaks of ND are reported in chicken and the virus has been repeatedly isolated from the birds brought for disease investigation to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences (CVAS) Mannuthy, Thrissur. Control of ND virus depends on regular vaccination followed by sero monitoring. Under field conditions, serological diagnosis by HI test is routinely used in Kerala. Other serological tests like AGPT, CIE and dot- ELISA were also been used for this purpose. The low cost and simplicity of AGPT and CIE; high specificity and earlier detection levels of ELISA are the main advantages. Keeping these facts, the study is aimed to compare the

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efficacy of the above mentioned serological tests to find out a reliable test with high sensitivity and specificity.

MATERIALS AND METHODS

Raising of hyperimmune sera:

Animal experimentation procedures were carried outin accordance with the guidelines of Institutional animal ethics committee. The ND virus maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala was passaged in embryonated chicken eggs by allantoic route. It was used as antigen to raise antisera in rabbits as per Polly (1977).

Collection of serum samples:

Serum samples were collected from 50 layers maintained in University Poultry and Duck Farm, Mannuthy, Thrissur. The wing vein of the bird was punctured with hypodermic needle and the blood was collected. The samples were brought to the laboratory and serum was separated. All sera samples were heat inactivated at 56^{o}C for 30 min and were stored at -20^{o}C until used.

Serological assays:

Serum samples were assayed for the presence of NDV antibodies by HI test as per Sulochana and Mathew (1991); AGID (Arun, 2004) and CIE (Bansal *et al*., 1981).Dot- ELISA was performed as described by Alam *et al* (2012) with minor modifications.

Statistical analysis:

To test the agreement of the findings obtained by the four methods, namely HI, AGID, CIE and Dot-ELISA, Kendall's 'W' was calculated with corresponding Chi-Square statistics. If the Chi-Square statistic was found to be significant, the agreement between two methods pair wise was done by using Mc Nemar's test. To test the significance, extent of agreement and the strength of association between the tests, Mc Nemar's test for HI and AGID , HI and CIE and between HI and Dot-ELISA were done and the p-values were examined at critical probability of $p<0.05$. Receiver operating characteristic (ROC) curve was plotted to calculate the agreement between the tests. The ROC curve was a plot of sensitivity as a function of specificity. The area under the curve with standard line indicated the agreement with standard. Area equal to one indicated perfect agreement and near to 0.5 indicated no agreement.

RESULTS AND DISCUSSION

In the present study, antibodies against NDV were detected by HI, AGID, CIE and Dot-ELISA. Serological studies could be helpful in monitoring the prevalence of pathogen since the presence of antibodies are highly specific, which may indicate an exposure to pathogen (Alam *et al*., 2012).

Rabbits were used to raise hyper immune serum as this species are convenient in size, easy to bleed relatively long life span and produce adequate quantities of antisera. Moreover they are diverged significantly from avian species and hence considered the best choice (Leeuw de and de Greeve, 1996; Samiullah *et al*., 2006). Similar method of hyper immune serum preparation for the detection of ND was successfully done by Roy and Venugopalan (1999); Roy *et al*. (2000); Arun, (2004); Ibu *et al*. (2008) and Arora *et al*. (2010).

Heat inactivation is done for the removal of complements from the serum samples and helps to improve storage time. Hemmatzadesh and Sharifzadesh, (2006) reported that heat inactivated serum could be stored up to eight months. These findings were similar to Tadesse *et al*. (2005); Numan *et al*. (2005); Nithinraj *et al*. (2009); Touko *et al*. (2013) and Jerabkova *et al*. (2014).

Haemagglutination inhibition test:

Haemagglutinationtitre, following HA test was obtained as 1:512. Out of a total of 50 chicken, 45 (90 percent) showed presence of anti NDV antibody by HI test. The findings were in agreement with Jerabkova *et al* (2014), who reported that100 percent of vaccinated chicken had anti NDV antibodies in Czech Republic. Similar results were also reported by Tadesse *et al* (2005), who found 58 out of 180 randomly selected free ranging scavenging chicken to possess anti NDV antibody following HI test. Nithinraj *et al.* (2009) who reported an average $\log_2$ HI titre of 4.5 in chicken sera and 5.6 in chicken yolk among 24 serum and 29 egg yolk samples collected from University Poultry and Duck Farm, Pookode, Wayanad.

Agar gel immuno diffusion test:

In the present study, eight (16 per cent) samples were positive out of 50 samples subjected to AGID. Similar seroprevalence study by AGID was reported by Buru *et al*. (2013). On comparison of results of HI test, it was noted that only the serum sample with an HI titre of 1:8 or above gave a positive AGID results. This is in accordance with the findings of Raj *et al.* (1995) who stated that titre of 1:8 or above is required to detect NDV antibodies by AGID.

Counter immuno electrophoresis:

Out of 50 samples screened, 10 (20 per cent) were positive by CIE. Roy and Venugopalan (1999) used AGID and CIE for the diagnosis of ND and found that out of 51 samples tested 27 were positive by AGID and 36 were positive by CIE indicating that CIE is more sensitive than AGID in detecting ND antibodies.

Dot-ELISA:

Out of 50 chicken sera samples, 40 (90 per cent) showed the presence of NDV antibodies. Roy and Venugopalan (1999) used Dot- ELISA for the detection of ND antigen; they tested 60 sera samples and found that 50 of them were positive. Alam *et al*. (2012) reported that 37 percent were positive when 504 sera samples were tested.

Comparison between four tests:

Kendall's 'W' was calculated as 0.635. Significance of this was tested by using Chi-square test. Chi-square statistics was found to be significant ($p<0.001$) indicating that there exits significant difference in the findings of the four method.

Mc Nemar's test revealed that p- value is less than critical probability for the comparison of findings obtained by AGID and HI ($p=0.000$, $p<0.05$), CIE and HI, ($p=0.000$, $p<0.05$) where as in the case of Dot ELISA and HI ($p=0.063$) p value was greater than 0.05 ($p>0.05$). Results showed that there exists significant difference in the findings of AGID and HI and also in the findings of CIE and HI. Non significant p-value in the Mc Nemar's test in the case of Dot-ELISA and HI indicated agreement in the findings of these two methods (Table 1).

Comparison between HI test and AGID:

Out of 50 samples tested for anti NDV antibodies by HI test, 45 samples were found to be positive and five samples were negative.

Eight samples out of 50 tested were positive for anti NDV antibodies by AGID. All the eight samples detected positive by AGID were also positive by HI test, whereas, 37 samples that were positive for HI showed negative in AGID. Five samples were negative for both AGID and HI test. Statistical analysis revealed a sensitivity of 15.6 per cent, specificity of 100 per cent and overall agreement was calculated to be 24 per cent. Details are provided in Table 2. Area under the ROC curve is 0.578 which shows an agreement of 24 per cent.

Comparison between HI test and CIE

Out of 50 samples tested for anti NDV antibodies by HI test, 45 samples were found to be positive where as five samples tested negative.

A total of 10 samples tested positive for anti NDV antibody out of 50 sera samples subjected by CIE. All the 10 samples detected positive by CIE were also detected as positive by HI test where as35 samples that were positive for HI was detected as negative in CIE. Five samples were negative for both CIE and HI test. None of the samples that were negative for HI tested positive on performing CIE. Statistical analysis revealed a sensitivity of 17.78 per cent, specificity of 100 per cent and overall agreement was calculated to be 26 per cent. Details are provided in Table 2. Area under the ROC curve is 0.589 which shows 26 per cent agreement.

Comparison between HI test and Dot-ELISA

Out of 50 samples tested for anti NDV antibodies by HI test, 45 samples were found to be positive where as five samples tested negative.

A total of 40 samples tested positive for anti NDV antibodies out of 50 sera samples subjected by Dot-ELISA. All the 40 samples detected positive by HI were detected as positive in Dot-ELISA test where as five samples that were negative in Dot-ELISA were also detected as negative in HI. Five samples were negative for both HI and Dot-ELISA. Statistical analysis revealed a sensitivity of 88.89 per cent, specificity of 100 per cent and overall agreement was calculated to be 90 per cent details are provided in Table 2. Area under the ROC curve is 0.944 per cent which shows an agreement of 70 per cent.

CONCLUSION

In the present study the sensitivity of AGID, CIE, and Dot-ELISA are 15.6 per cent, 17.78 per cent and 88.86 per cent respectively and specificity of 100 per cent for AGID, CIE and Dot-ELISA compared to HI test. When compared with HI, Dot- ELISA is more sensitive than AGID and CIE. There were no reports of Newcastle disease during the period of sample collection. In this study it was observed that standard vaccination was carried out since there was a sufficient time interval between vaccination and sample collection, the antibodies detected is to be considered as the effect of vaccination.

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List of Tables

Table 1. Comparison of four serological tests by Kendall's 'W'

Total number of samples (N)	50
Kendall's 'W'	0.635
Chi-Square	95.231
Degree of freedom	3
p- value	<0.001

Table 2. Comparison between HI test and AGID, HI and CIE, HI and Dot- ELISA

	HI				HI				HI				
		Positive	**Negative**	**Total**			**Positive**	**Negative**	**Total**			**Positive**	**Negative**	**Total**
AGID	**Positive**	8	0	8	**CIE**	**Positive**	10	0	10	**Dot-ELISA**	**Positive**	40	0	40
	Negative	37	5	42		**Negative**	35	5	40		**Negative**	5	5	10
	Total	45	5	50		**Total**	45	5	50		**Total**	45	5	50
Sensitivity = 15.5, Specificity = 100 & Overall agreement = 24					Sensitivity = 17.78, Specificity = 100 & Overall agreement = 26					Sensitivity = 88.89, Specificity = 100 & Overall agreement = 90				