EVALUATION OF RAPID QUANTITATIVE LATEX ASSAY TO DETECT NEWCASTLE DISEASE VIRUS IN CHICKEN

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Abstract

The present study was undertaken to evaluatethe efficacy of a rapid quantitative latex assay to detect Newcastle disease virus (NDV) in birds. Out of 28 clinical samples collected from disease suspected birds, haemagglutination test detected 12 samples as positive and were confirmed as NDV by HI test. The latex agglutination test (LAT) was standardized and only 6 samples were found to be positive. The HA titre ranged from 1:8 to 1:128 and LAT titres were 1:2 in all LAT positive samples. Based on the statistical analysis, there exists a significant difference ($p<0.05$) between the two tests employed and HA-HI tests was found to be having higher sensitivity than LAT.

Key words: *Newcastle disease virus, haemagglutination, haemagglutination inhibition, latex agglutination test, Kerala.*

Development of poultry sector is constrained by many factors and the most devastating is Newcastle disease (ND), which causes losses both in egg and meat production as a result of high morbidity and mortality. According to Waheed *et al*. (2013), ND alone is responsible for at least 40-60 per cent of total mortality of poultry populations. Therefore, when there is any outbreak of ND, rapid and confirmatory detection of the virus is crucial for an effective control of the disease. At present, diagnosis of ND being practiced at field level in Kerala are limited within the recording of the clinical signs and post-mortem findings. Though several serological and molecular assays are considered as the standard methods for the confirmation of the disease (OIE, 2012), these are always considered as time consuming and expensive. Hence, a sensitive, inexpensive and field specific test for rapid and accurate diagnosis is necessary to control the disease and to avoid further dissemination. Latex agglutination test (LAT) is a serological test of choice for the rapid detection of ND in birds.

Materials and methods

Revival of stock virus isolates and preparation of hyper immune serum (HIS): The NDV isolate preserved in the Department of Veterinary Microbiology, College of Veterinary Microbiology, Mannuthy, Thrissur, Kerala was revived by inoculation into allantoic cavity of nine-day-old embryonated chicken eggs (Arun, 2004). The collected allanto-amniotic fluids were clarified and purified by ultracentrifugation in a 30 per cent and 60 per cent discontinuous sucrose gradient at 1, 00,000 x g for one hour. This purified virus was used for haemagglutination (HA) and haemagglutination inhibition (HI) test. Hyper immune sera were prepared as per Polly (1977).

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Collection and processing of clinical samples:

The cloacal swab and tissue samples like visceral organs, spleen, brain, intestine and liver were collected from 28 birds suspected for ND, presented to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy and stored at - 20°C until used. The swabs were then squeezed against the wall of the test tube by sterile pipette and the separated fluid material was mixed with the tissue samples, homogenized and a 10 per cent suspension (w/v) was prepared in sterile PBS. Then, centrifuged at 1000 rpm for 10 min. and collected the supernatant. Antibiotic solution was incubated at 37°C for one hour and stored at - 20°C until used.

Screening of clinical samples by HA- HI test:

The HA activity of the samples were assessed as per OIE (2012) with few modifications. Two fold serial dilutions of 50µL of sample suspension were made in PBS in a microtitre plate. To each well 50µL of 0.5 per cent chicken RBC was added, mixed gently and allowed to react for 30 min. The titre was read as the reciprocal of the highest dilution showing complete HA and this represents one haemagglutinating unit (1HAU). To confirm it by HI, each well was added with 25µL of PBS and two fold dilutions of the serum were made across the plate. Twenty five microlitre of four-haemagglutinating unit (4HAU) virus suspension was added to each plate and incubated at room temperature for 30 min. following which, 0.5 per cent RBC suspension was added to each well at the rate of 50µL / well and was mixed gently and left at room temperature for 15 min. The HI titre was read as the reciprocal of the highest dilution of serum causing complete inhibition of 4HAU of antigen.

Standardisation and screening by LAT:

The test was standardized as described by Thirumurugan *et al*. (1997). One per cent suspension of latex beads (0.8 µm, diameter) was prepared with carbonate-bicarbonate buffer and mixed with an equal volume of hyperimmune serum and kept at 4°C overnight. The coated beads were centrifuged at 1000 rpm for five minutes and the non-specific sites on the beads were blocked with BSA in PBS (pH 7.2) at 37°C for two hours. The beads were washed in carbonate-bicarbonate buffer and finally made as a 0.6 per cent suspension. Twenty microlitre of coated bead suspension was added to five wells of a latex agglutination (LA) plate. Using each sample, two fold serial dilutions were prepared in carbonate-bicarbonate buffer in five wells of a microtitre plate. They were transferred to corresponding wells of the LA plate with latex beads and was kept at room temperature (RT) for two minutes. Positive and negative controls were also included. Latex agglutination titre was read as the highest dilution showing complete agglutination.

***Statistical analysis*:**

The results of combined HA and HI tests and LAT were done by using McNemar test.

Results and discussion

On examining the birds during necropsy, most of them revealed atleast one of the typical gross lesions of ND like hemorrhages of caecal tonsils, spleenomegaly, hemorrhage throughout the intestinal tract and proventicular gland. All the cloacal and tissue samples collected were first screened for the presence of NDV by HA. Out of 28 samples tested, 12 (42.85 per cent) were positive by HA test which agrees with Nabila *et al*. (2014), who observed 37.7 per cent positivity among clinically suspected birds to NDV. The HA titres of the samples ranged between 1:8 and 1:128. Titres of 1:4 and below were considered as negative to NDV infection, as suggested by Tewari *et al*. (1992). Many workers used HA test for the same purpose (Thirumurugan *et al*., 1997; Alexander, 2000 and Chakraborty *et al*., 2014). In the present study, PBS (pH 7.2) was taken as the standard diluent for performing HA test, as suggested by Kim *et al*. (2008), Guktur *et al*. (2013) and Nabila *et al*. (2014). The study used 0.5 per cent chicken erythrocyte suspension and the incubation was carried out at RT. Guktur *et al.*

Fig. 1. Screening of clinical samples by LAT
1- Positive
2- Negative

(2013) and Ezibe *et al*. (2014) reported that NDV strongly agglutinated the erythrocytes of chicken at RT or 4°C. Alsever's solution used for the collection of chicken erythrocytes, sediment at 1000 rpm for ten minutes and washed three times in PBS gave good result as reported by Chowdhury *et al.* (1982). Haemagglutination is determined by observing the presence or absence of tear-shaped streaming of the red blood cells. The titration is read as the highest dilution giving complete HA, this represents one HA unit (Alexander, 2000).

The HI test was employed to confirm the presence of NDV in those clinical samples which were found positive by HA test. Many workers confirm the NDV in suspected samples by using HI test (Mohammed *et al.*, 2013; Bukhari *et al*., 2014 and Mulisa *et al*., 2014).

Table 1. Comparison of HA and LAT titers in detecting NDV

Sample	HA titre	LAT titre
2	1:64	1:2
5	1:32	1:2
7	1:64	1:2
9	1:128	1:2
15	1:64	1:2
21	1:128	1:2

The samples showing peculiar central button shaped settling of RBCs were recorded as positive and the highest dilution of each sample which inhibits HA was considered as the end point. All the 12 samples which tested positive by HA test could be specifically inhibited in the HI test. The observations found in this study was correlated with the results of Thirumurugan *et al*. (1997) who observed that all HA positive samples were also positive by HI test.

Samples were subjected to LAT using antibody coated latex beads. Among 28 clinically suspected birds, six samples were positive in LAT (Fig. 1). Details are presented in Table 1 and Table 2. Application of LAT for the detection of viral antigens in suspected samples were successfully standardized by Thirumurugan *et al*. (1997); Xu *et al*. (2005) and Chen *et al*. (2007). Titre of 1:2 was obtained in all the six samples that were tested positive by LAT. It was further observed that only the samples with an HA of 1:32 and above gave a positive LAT.

Based on statistical analysis (using McNemar test) there was a significant difference between the results of combined HA-HI and LAT ($P=0.03<0.05$). This indicates the efficiency of LAT to detect NDV in suspected avian cases is less compared to combined HA-HI test. Latex

Table 2. Results of LAT and HA-HI tests

Sl. No	Tests	Total no. samples tested	*No. of samples positive*	No. of samples negative	% of positivity
1	HA-HI	28	*12*	16	42.85
2	LAT	28	*6*	22	21.42

agglutination test was able to diagnose only six of 12 HI positive samples, which shows 50 per cent sensitivity compared to HA-HI tests. The positive predictive value of the LAT was 100 per cent and the negative predictive value was 70 per cent. Specificity and accuracy were 100 per cent and 76.92 per cent respectively. The sensitivity of LAT to detect viral antigen was found to be very low in this study compared to the results of other workers (Thirumurugan *et al*., 1997; Xu *et al*., 2005 and Chen *et al*., 2007). The size of the latex beads used for LAT affected the sensitivity of the test. Kasempimolporn *et al*. (2000) reported small sized (0.25µm diameter) bead particles specifically agglutinate the virus and gave a better sensitivity. The present study used hyper immune sera for coating of latex beads. The sensitivity and accuracy of the LAT could be significantly improved by coating of monoclonal antibodies rather than hyper immune sera as opined by Kasempimolporn *et al*. (2000) and Chen *et al*. (2007).

On the basis of observations made from the present study, standard HA and HI test could be recommended in field condition for diagnosis of NDV infections in chicken.

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