



Short Communication

Seromonitoring of Newcastle Disease in Backyard Poultry by Indigenously Developed User–friendly tool: Dipstick ELISA

Nilofar Yeasmin¹, Siddhartha Narayan Joardar^{1*}, Pradip Kumar Das², Palas Das¹, Arkendu Halder¹, Sk. Sahanawaz Alam¹

¹Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, 68, K. B. Sarani, Post Office–Belgachia, Kolkata– 700037 , India; ²Department of Veterinary Physiology (Rashtriya Krishi Vikash Yojona laboratory), West Bengal University of Animal and Fishery Sciences, 68, K. B. Sarani, Post Office–Belgachia, Kolkata– 700037 , India

*Corresponding author: joardar69@gmail.com

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ABSTRACT

With the aim of assessing anti–Newcastle Disease Virus (NDV) antibodies in backyard poultry using indigenously developed dipstick–ELISA tool, blood samples (n= 140) were collected randomly from apparently healthy birds from different districts of West Bengal state and the serum samples were screened subsequently. When assessed by Haemagglutination inhibition (HI) test, 92.86% serum samples were found positive. However, laboratory developed dip–stick ELISA showed 71.43% positivity with the same samples. The indigenously developed test was found primarily useful, however needs validation using large number of samples.

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Conventionally, diagnosis of Newcastle disease (ND) is done by virus isolation in chick embryos followed by haemagglutination (HA) and a haemagglutination inhibition (HI) test. The haemagglutination inhibition test is used most widely in NDV serology (OIE, 2012). Enzyme linked immunosorbent assay (ELISA) has proved to be very useful for diagnosis and control of avian diseases, including ND (Botus and Oncesku, 2006). Dipstick–ELISA seems to be faster and more user–friendly method as it doesn't need instrument for interpretation of results. However, regular sero–monitoring of ND by dipstick–ELISA is not commonly practised in organized farms as well as backyard poultry system. The present study was designed to assess suitability of indigenously developed dipstick–ELISA tool in detecting anti–NDV antibodies in backyard poultry.

Blood samples (n= 140) were collected randomly from apparently healthy birds from different districts of eastern Indian state, West Bengal covered under the scheme–Rashtriya Krishi Vikas Yojona (RKVY), sponsored by Govt. of India. Sera were separated and stored at –20°C for further use.

Allantoic fluid obtained from specific pathogen free embryonated chicken eggs (ECE) of 9–11 days old, inoculated with R₂B live vaccine (Institute of Animal Health and Veterinary Biologicals, Govt. of West Bengal) material, was further filter sterilized and used as crude viral antigen preparation. Protein concentration of the viral antigen was measured as per Lowry's method (1951). The concentration was found to be 3.45 mg/ml.

Haemagglutination (HA) test was done to assess the titre of virus in the test antigen and haemagglutination inhibition (HI) test was performed to determine the titre of anti–NDV antibody in the test serum samples. The tests (HA and HI) were performed as per OIE Manual (2012).

Dip–stick ELISA was standardized in the laboratory using the principle of dot–ELISA (Swain et al., 1999). Standardization was made in the steps of coating (concentration of antigen), blocking (buffer composition and time), use of positive and negative sera (10 for each) and use of immunoconjugate (dilution and time). The known positive sera were collected from NDV infected (clinically positive) birds and known negative from unvaccinated normal (healthy) ones. At first, the dip–sticks were coated with 4µg viral antigen in coating buffer and kept at 4°C for overnight. The sticks were blocked by blocking buffer (3% skimmed milk and 2% gelatine in PBS) at 37°C for 2 hrs and washed thrice subsequently by Tris buffered saline–Tween 20 (TBST) solution. Then, the sticks were dipped separately into diluted sera (test, known positive and known negative) and were incubated at 37°C for an hour. In the next step, after washing with TBST solution, the sticks were dipped in the diluted anti–chicken horse radish peroxidase (Sigma, USA) immunoconjugate (1:1000) solution at 37°C for an hr. After washing, the dried sticks were finally dipped into substrate solution (Tetra–methyl benzidine, Genei, India) for colour development.

When the serum samples (n=140) were assessed for the presence of anti–NDV antibody by HI test, 130 (92.86%) serum samples were found positive and 10 (7.14%) samples

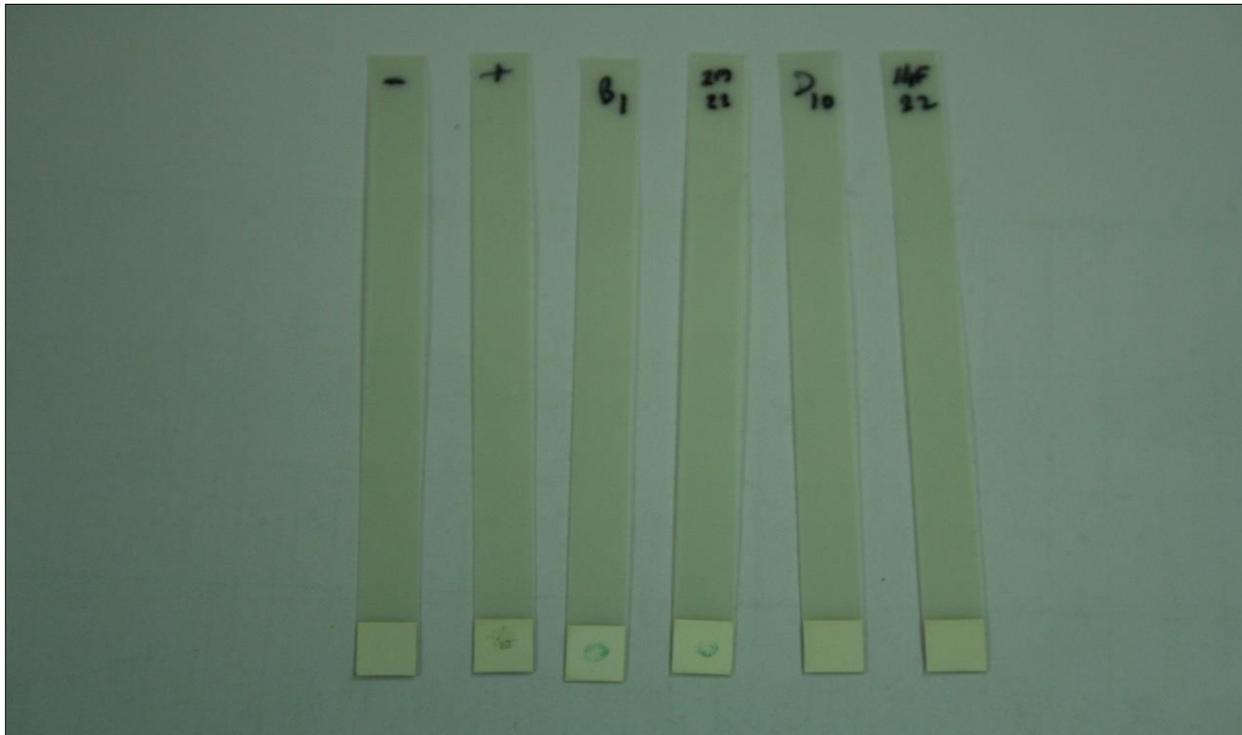


Figure 1: Assessment of anti-NDV antibodies by Dipstick-ELISA showing coloured dot in positive cases and no dot in negative ones (in representative samples); '-': Negative serum sample (control); '+': Positive serum sample (control); B₁ and M₂₁: test samples showing dot (positive); D₁₀ and 14F₂₂: test samples showing no dot (negative)

were negative. However, laboratory developed dip-stick ELISA showed 71.43% positivity of the same samples used. Results showing representative samples were depicted in Figure 1.

Newcastle disease is really a menace to poultry industry. The virus is wide spread in Asian continent and some parts of Africa and America. Despite of availability and regular use of vaccines, ND outbreaks are quite frequent in field conditions. To combat the disease problem only vaccination is not sufficient. It needs continuous sero-monitoring of the birds with the help of different diagnostic techniques to evaluate the presence of antibody against NDV. The present study was conducted to detect presence of antibodies against NDV in backyard poultry birds in West Bengal. In backyard rearing system, birds are led to move freely at premises during day time and housed at the evening with no rearing cost of the owner. In this study, an effort was made to detect presence of anti-NDV antibodies in backyard poultry birds using laboratory developed dipstick-ELISA. In this study, 140 serum samples were collected from backyard poultry birds of different districts of West Bengal, of which 92.86% were found positive and 7.14% were negative by HI test. Further, the same samples were used in dipstick-ELISA system, developed in the laboratory based on dot-ELISA principle. It is a solid phase immunoassay that can detect antibodies against specific antigen in a user friendly manner involving less time to perform. As for this test no instrument is required for interpretation of result (since it is a qualitative assay), it is considered as economical and best suited option for grass-

route level diagnostic laboratories in the field where large number of sera from backyard poultry are to be screened having little infrastructure. The dipstick-ELISA test of the present study estimated 71.43% of serum samples as positive, showing clear dot on nitrocellulose paper blocks and rest 28.57% were found negative. Earlier, dot/dipstick-ELISA was used by various workers for detection of antibody against various pathogen antigens (Rattan et al., 1993; Folitse et al, 1998; Roy and Venugopalan, 1999; Ghosh et al, 2005; Kumari et al., 2009). For the first time, dipstick-ELISA technique was used for sero-monitoring of ND in backyard poultry system. Earlier worker reported less percent sero-positivity than the present study. Alam *et al.* (2012) performed dot-ELISA and found that 9.5% serum samples from healthy birds and 37% samples from diseased birds were positive. The variation in the figures obtained in earlier studies and the present study might be attributed to breed, agro-climatic conditions and health status of the poultry birds used. All the birds in the present study were apparently healthy and most of the backyard poultry birds were vaccinated. However, anti-NDV antibodies couldn't be detected in all the serum samples. This might be due to various reasons, viz. vaccine efficacy, individual immune competence of birds etc.

In short, the results of the indigenously developed dipstick-ELISA were found satisfactory as compared to the standard test recommended by OIE (HI test). However, as the number of samples tested in the present investigation was less, the suitability of dipstick-ELISA test for field

diagnosis of ND should be substantiated by using large number of samples in future occasion.

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