World's Veterinary Journal
World Vet J, 5(1): 07-12, March 30, 2015
ISSN 2322-4568

Comparison of Immunochromatographic Assay and Haemagglutination Inhibition Test in the Detection of the Presence of Newcastle Disease Virus Antibodies in Commercial Chickens

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ABSTRACT

This study was performed to ascertain the effectiveness of a technique known as "immunochromatographic assay, also called, Lateral Flow Technique (LFT)" in the detection of Newcastle Disease Virus (NDV) antibodies (Abs) in comparison with the Haemagglutination Inhibition (HI) test in commercial chickens in Jos South Local Government Area (LGA), Plateau State, Nigeria between February and April 2012. A total of 407 sera were collected. Out of this number, the LFT showed that a higher number (401) of the birds tested (98.5%) had NDV Abs, while HI test showed that 394 birds tested (96.6 %) had NDV Abs with a titre equal to and above the minimum protective titre of log2 3.0. Both the LFT and HI test detected absence of NDV Abs in a particular layer tested. It was concluded that the immunochromatographic assay can also detect presence of NDV Abs in commercial chicken and it can therefore complement HI for prompt and rapid detection of NDV Abs among flocks. It was recommended that farmers can be informed through awareness programmes, such as seminars, trainings and workshops that prompt, quick and rapid Sero-monitoring of NDV Abs is possible through the use of lateral flow technique in order to prevent sudden outbreak of ND in their farms.

Key words: Newcastle Disease Virus Antibodies, Immunochromatographic Assay, HI, Commercial Chickens.

ORIGINAL ARTICLE
pii: S232245681500002-5
Received: 25 Jan 2015
Accepted: 28 Feb 2015

INTRODUCTION

Newcastle disease is a highly contagious viral disease that affects many species of domestic and wild birds (Al-Garib et al., 2003). Newcastle disease has been reported to be the most important poultry disease, with variations in mortality rate and seasonal influence in Nigeria (Abdu et al., 1992; Sonaiya et al., 2000). The disease is a major disease problem of poultry in many countries of the world, especially in Africa and Asia (Spradbrow, 1992; Awan et al., 1994; Oladele et al., 2005). In a country like Nigeria, ND is enzootic (Fatumbi and Adene, 1979; Adu et al., 1986; Echeonwu et al., 1994; Sa'idu et al., 1994; Alders and Spradbrow, 2001). The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability (Alexander, 2000). The mortality rate could be up to 100% and it is considered to be among the most important viral diseases of poultry in the world (Heath et al., 1991; Alders and Spradbrow, 2001). Newcastle disease has a worldwide distribution (Alexander, 1997). Since 1953, when ND was first documented in Nigeria, in and around Ibadan, between December 1952 and February 1953 (Hill et al., 1953), the disease has become a threat to poultry industry in Nigeria, where it causes havoc to different species of birds (Adu et al., 1986). Since then, the disease has been reported to be endemic in both domestic and exotic birds with annual epidemics being recorded in highly susceptible poultry flocks (Adu et al., 1986; Sa'idu et al., 1994; Halle et al., 1999; Orajaka et al., 1999). In Africa and Asia, ND is a major constraint against the development of both industrial and village poultry production (Alders et al., 2001). A wide range of tests may be used to detect antibodies to NDV in poultry sera and tests based on neutralization or enzyme-linked immunosorbent assay (ELISA) reactions have been used to support the diagnosis of ND (OIE, 1996). However, currently, the haemagglutination inhibition (HI) test is the most widely used (Jordan, 1990). It is regarded as the gold test for the diagnosis of ND (OIE, 1996). Newcastle disease virus may be confirmed by HI test using specific NDV antiserum (OIE, 1996). Though, HI test is the well-known and the traditional test for diagnosis of NDV and determination of antibodies against NDV, there is a new technique designed for this purpose, this is called the immunochromatographic assay, also known as the lateral flow technique (Allan and Gouph, 1984; CELFD, 1999). The lateral flow technique is a membrane based assay that provides visual evidence of the presence of an analyte in a liquid sample (CELFD, 1999). It is also known as 'dip-stick' or immunochromatographic strip tests (CELFD, 1999). They are a popular platform for rapid tests since their introduction in the late 1980s and have been designed to diagnose disease and determine the immune status of farm animals and poultry (CELFD, 1999). It is

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Journal homepage: http://wvj.science-line.com/

sensitive and its operation is simple and quick (Martin et al., 2009). It can be used to screen out disease status on site (CELFD, 1999). This has necessitated the need to determine the presence of NDV antibodies in birds using a simple, quick and reliable technique. This study therefore, focuses on the detection of the presence of Newcastle disease antibodies in commercial chickens using the lateral flow technique in comparison with the conventional HI test.

MATERIAL AND METHODS

Sample size

The sample size was calculated from an expected prevalence of 51.9% based on the highest prevalence obtained from a previous study in Plateau State by Musa et al. (2009) at 5% accuracy and a total of 407 sera were collected from commercial chickens between February and April 2012, in Jos South LGA, Plateau State, Nigeria.

Sampling procedure

Convenience sampling procedure was used to select nine districts under Jos South LGA, Nigeria. These districts were; Federal low cost, Rantya, Bukuru, Rayfield, Da zarmangada, Dung village, Dadinkowa, Gyel and State low cost. Furthermore, 1- 3 farms (depending on the number of farms in each district) were also randomly selected and 10 to 20 birds were selected at random from each flock. Structured questionnaire was administered to farmers. The birds were categorized into chicks (0 to 6 weeks of age), pullets (6 to 18 weeks of age) and layers (more than 19 weeks of age)

Sample collection and processing

Two milliliters of blood was collected aseptically via the wing vein of each bird, using a 21–G sterile hypodermic needle and 5 ml syringe in adult birds (layers) and 23-G sterile hypodermic needle and 2 ml syringe in young birds (pullets). The samples were labelled with the name and location of the farm, type of bird and date of collection. The blood samples were kept in a slanting position at room temperature to allow for clotting and sera formation. The sera were separated by transferring into two types of labeled sterile bottles; one for lateral flow test which was carried out immediately and the other bottle for HI test which was stored frozen at -200C and sent in a cold pack to Regional Laboratory for Avian Influenza and other Transboundary Animal Diseases at the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Newcastle disease antigen and ND positive and negative serum were obtained from the National Veterinary Research Institute, Vom, Nigeria.

Test procedure for immunochromatographic assay

The test kit for immunochromatographic assay was obtained from Quicking Biotech Co. Ltd, Shanghai, in China. The test kit has a test/result window. On the window of the test kit are letters “T” and “C” which connotes the test line and the control line, respectively. These lines are not visible until the serum sample is applied. Sera were transferred into the centrifuge tubes. The cassette was removed from its foil pouch and placed horizontally. Three drops of sera was gradually dropped into the sample hole “S” on the sample pod using the disposable pipette provided. As the serum moved slowly on the strip, 1 – 2 drops of assay buffer was dropped into the sample hole “S” as well. Results were read and interpreted after 10-20 minutes. Results after 20 minutes were considered to be invalid because it could give a false positive or false negative results (CELFD, 1999).

Preparation of chicken red blood cells (C-RBCs) for HI test

A total of 4 ml of blood was collected aseptically from ND antibody- negative chicken in a disposable syringe containing 1 ml of Acid Citrate Dextrose (ACD) as anticoagulant. Cells were washed three times in Phosphate buffered saline (PBS) of pH 7.2 by centrifuging at 47.2 g for 5 minutes (Allan and Gouph, 1984). One percent RBC (packed cell V/V) suspension was prepared by adding 99 ml of phosphate buffered saline (PBS) to 1 ml of washed RBC.

Determination of titre of Newcastle disease antigen using HI test

Haemagglutination (HA) test was carried out according to the method described by OIE (2005). The HA titre was the highest dilution that caused agglutination of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming). This represents 1 HA Unit (HAU). The titre of the antigen was determined as: 1:256, i.e., 8.0 log2 or 4HA units and this were used in the haemagglutination inhibition test.

Determination of Newcastle disease virus antibody levels using the HI Test

The HI test was carried out by the method described by OIE (2005). The HI titre was considered to be the highest dilution of serum causing complete inhibition of 4 units of virus (4 HAU). The HI titre of each serum sample was determined and expressed in log 2.

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Interpretation of Results

For immunochromatographic assay, results were read and interpreted after 10-20 minutes. Results after 20 minutes were considered to be invalid because it could give a false positive or false negative result (CELFD, 1999). The appearance of purple colour on both the C (control) band and T (test) band/line was indicative of a positive result, no matter how clear or vague the purple colour was on the T-band/line. A negative result was indicated by only C-band appearing within the result window, while HI test results showing NDV Abs titre below the minimum protective titre of log2 3 were considered negative for NDV Abs while results showing NDV Abs titre of log2 3 and above were considered positive for NDV Abs (Allan and Gouph, 1974).

STATISTICAL ANALYSIS

Chi-square test followed by tukey's post hoc test were used to determine the association between various numbers of birds positive for NDV antibodies using both HI test and lateral flow tests. They were analyzed using Graphpad prism version 4.0 window. Values of $p \leq 0.05$ were considered significant for these two tests.

RESULTS AND DISCUSSION

Out of the total number (407) of birds tested, the LFT showed that 367 (98.66%) layers were positive for NDV Abs and 34 (97.14%) pullets were also positive for NDV Abs, while the HI test showed that 362 (97.31%) layers were positive for NDV Abs and 32 (91.42%) pullets were also positive for NDV Abs (Figure 1). In this study, the LFT detected the presence of ND antibodies in a higher number (98.5%) of birds tested than the HI test which detected presence of NDV antibodies in 96.6% of the birds tested. The LFT in this study can be said to have the capability to detect the presence of NDV antibodies as HI test. This result can be compared with the previous work carried out by Martin et al. (2009), on the evaluation of the immunochromatography assay in the serodiagnosis of tuberculosis, in comparison with X-ray diagnosis. The sensitivity, specificity and efficiency of the immunochromatography assay were 89.5%, 77.7% and 85.7%, while that of X-ray were 75%, 50% and 66.6%, respectively. They concluded that the immunochromatography assay gave better results in sensitivity and specificity (as well as in other performance indices) than X-ray diagnosis and it is rapid, reliable and field operable. This result is also in line with the previous work carried out by Bautista et al. (2011) on the detection of Salmonella sp. using LFT (immunochromatographic assay) in comparison with isolation of Salmonella sp. on Xylose- Lysine- Tergitol (XLT) - 4 agar. The LFT in that study, detected Salmonella sp. as early as 18–48 hours during pre-enrichment and enrichment with a sensitivity of 94.7 % and specificity of 96.8 % while isolation on XLT-4 agar, which required an overnight incubation step for the presumptive isolation and identification of Salmonella sp. had a sensitivity of 93.8 % and specificit y of 89%. Shangjin et al. (2008) in their research also detected the sensitivity and specificity of LFT as compared to HI test to be 88.8% and 97.6 % respectively in detecting H5N1 antibodies in 830 serum samples from vaccinated chickens. Dapeng et al. (2007) reported in their research that the LFT had many advantages over the 'gold standard', that is, HI and agar gel immunodiffusion (AGID) assays in terms of high sensitivity, high specificity, convenience and rapidity in the detection of antibodies against avian influenza virus. They concluded that the LFT provides a unique tool for the on-site surveillance and diagnosis of avian influenza. The result of the research conducted by Pablo et al. (2006) also showed that the diagnostic accuracy of the immunochromatographic assay was significantly better than that of the rapid plasma regain in the screening of antenatal syphilis in Mozambique. This present study can also be compared with the previous work carried out by Jamshidi et al. (2013) on comparison of immunochromatographic rapid test with molecular method in diagnosis of canine parvovirus (CPV); the result of that study showed that immunochromatography based office-use test kits are sensitive in comparison with molecular methods to diagnose CPV infections and they concluded that the LFT are reliable in daily practice.

In this study, the LFT and HI test showed no significant association ($p \geq 0.05$) between the presence of NDV Abs and the type of bird tested; although a higher percentage of the layers showed presence of NDV Abs than the pullets in these two tests (Figure 1). This may be due to the fact that continual boosting of immunity with ND vaccine over time in layers, as a result of their economic value, in terms of generation of income through egg production, may increase their ND antibody titre than the pullets. In this study, the LFT can be said to be a suitable alternative to the conventional HI test. This is in agreement with the work carried out by Rauw et al. (2009).

In this study, both the LFT and HI test showed that a particular layer tested had no NDV antibody in its sera. It can conveniently be said that the immunochromatographic assay is a suitable alternative to the traditionally used HI test. This is also in agreement with the work carried out by Rauw et al. (2009).

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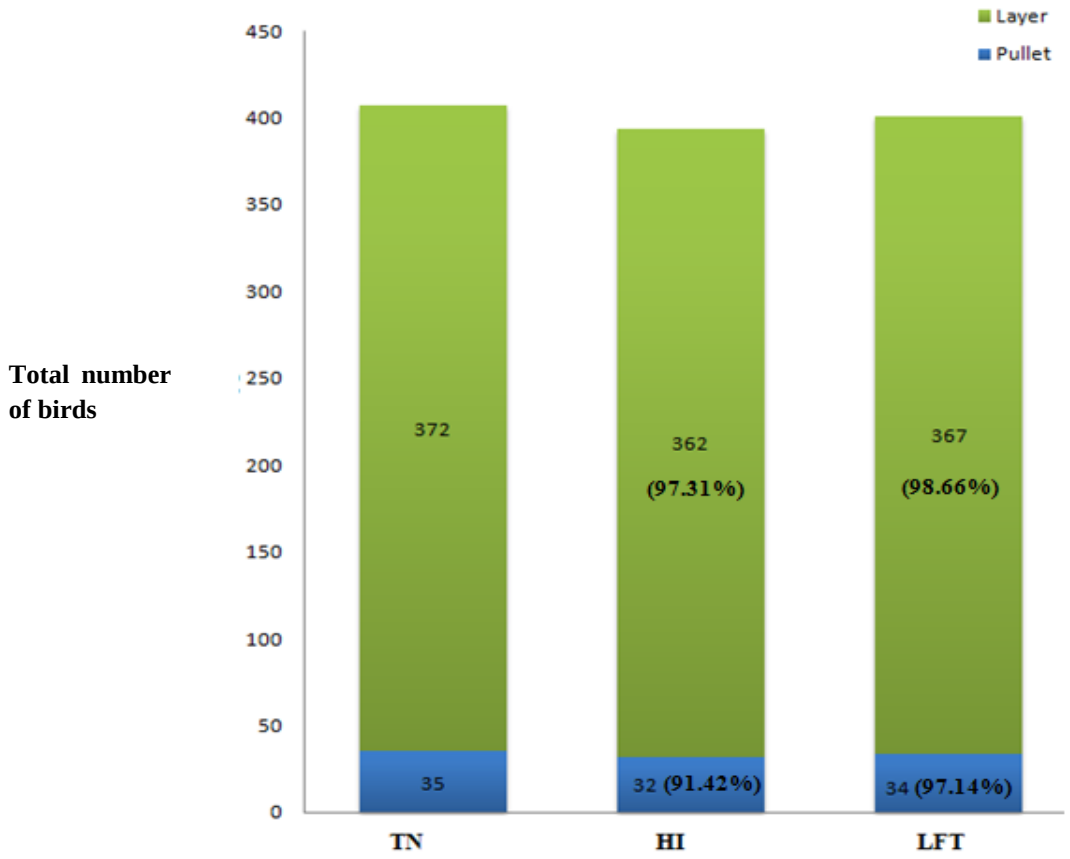

Figure 1. Comparison of the results of haemagglutination inhibition (HI) test and lateral flow test (LFT) in relation to number of samples positive for Newcastle disease virus antibodies based on type of birds (For HI, the statistical analysis showed that the P value = 0.0916, relative risk = 0.3519 and confidence interval = 0.1236 to 1.003 and for LFT, the statistical analysis showed that the P value = 0.3642, relative risk = 0.4239 and confidence interval = 0.07130 to 2.521. Key: TN- Total number of birds)

CONCLUSION

In conclusion, the LFT was simple and easy to perform, quick and sensitive. The LFT can complement HI test for rapid detection of NDV antibodies in commercial chickens. The LFT can be employed or recommended for rapid detection of NDV antibodies and for time to time sero-monitoring in farms and among flock (s).

ACKNOWLEDGEMENTS

The authors wish to thank all that have contributed to this work, especially farmers who granted us access to their various farms and making the sampling possible.

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