J. Vet. Malaysia (1992) 4(1): 19-23

DETECTION OF MATERNAL ANTIBODY AGAINST NEWCASTLE DISEASE VIRUS IN CHICKS USING AN INDIRECT IMMUNOPEROXIDASE TEST

Awang, I.P.R., Wan-Ahmad-Kusairy, W.S. and Abdul-Razak, J.

Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine and Animal Science
Universiti Pertanian Malaysia,
43400 UPM Serdang, Selangor, Malaysia

SUMMARY: An indirect immunoperoxidase test (IIPT) was used to detect maternal antibody against Newcastle disease virus in one to 21-day-old Indian River chicks. The results of the IIPT were simultaneously compared with those of the microtitre version of the haemagglutination-inhibition test (HIT). The IIPT could uniformly detect maternal antibody in 100% of the test sera collected at two-day intervals between day 1 to day 17; the HIT could only detect varying percentages (5% to 90%) of the maternal antibody-positive sera. The IIPT could even detect the antibody until the later age (day 19) of the chicks, whereas the HIT could only detect the antibody until day 17.

The findings of this study supports the current practice of not vaccinating one-day-old chicks with Newcastle disease virus vaccine, as the maternal antibody at high levels may neutralize the introduced vaccine antigens to render the vaccine ineffective.

Keywords: indirect immunoperoxidase test, maternal antibody, Newcastle disease virus.

INTRODUCTION

Allan *et al.* (1973) had reported the detection of maternal antibody against Newcastle disease virus (NDV) in the chicks using a haemagglutination-inhibition test (HIT) but there has been no report on using an indirect immunoperoxidase test (IIPT) to detect the maternal antibody. Russell *et al.* (1983) indicated that the IIPT is 100 times more sensitive than a HIT in detecting NDV. Awang (1988) had used the IIPT to detect antibody in young chicks and poults that had been infected with avian turkey paramyxovirus type 3.

The objective of the present study was to determine the feasibility of using IIPT for detecting maternal antibody against NDV in young chicks from NDV-vaccinated parent flocks.

MATERIALS AND METHODS

Chickens

The breed of chicken flocks used in the present study was Indian River. The birds were kept under standard farm conditions at The Commercial Poultry Unit at Universiti Pertanian Malaysia (UPM), Selangor. They were vaccinated with NDV-F strain at the ages of one and three weeks and with NDV-S strain at the ages of nine and 21 weeks. The parents were 59 weeks old during the collection of experimental eggs. The eggs were incubated and hatched at the UPM Hatchery Unit. Two hundred and twenty healthy chicks were selected for the study. They were not vaccinated against Newcastle disease (ND) or any other diseases during the course of the study.

Test Sera

Test sera were first collected from one-day-old chicks. About 1.0 ml of heart blood was collected from each chick. Twenty blood specimens from 20 different birds were taken at each collection. The collections were done at two-day intervals until the chicks were 21 days old. The chicks whose blood had been taken were painted blue to prevent repetitive collection of blood from the same birds.

The serum was aliquoted into two vials; one meant for the IIPT and the other for the HIT. The vials were stored at -20°C. Before being tested the serum was warmed at 56°C for 30 minutes in a waterbath.

Reference Antiserum

The reference NDV-V4 antiserum used as positive controls in the IIPT and HIT was kindly supplied by The Australian Centre for International Agricultural Research at UPM. Phosphate-buffered saline (PBS) was used as negative controls.

Virus

The stock of allantoic NDV-F strain used in this study was prepared at the Department's Virology Laboratory.

Haemagglutination-Inhibition Tests

The procedure of the HIT as described by Anon *et al.* (1971) was modified into a microtitre technique in this study. Four haemagglutinating units of NDV-F antigen, 0.05% chicken erythrocyte suspension in 0.025 ml volumes were used in the HIT.

Indirect Immunoperoxidase Tests

IIPTs were performed according to the method of Russell *et al.* (1983). The presence of NDV-antibody in the serum was seen as a brown staining of the infected cell membranes or inclusions in the cytoplasm under an inverted light microscope. The reciprocal of the highest dilution of each of the sera was considered as the IIP titre of the test or control serum.

RESULTS

The results of the IIPTs showed that all test sera collected at the two-day intervals between day 1 to day 17 contained maternal antibody against NDV (Fig. 1). The percentages of test sera containing the maternal antibody varied from 5% to 90% when

duplicates of the same IIPT-tested sera were tested with HITs. The IIPTs could still detect maternal antibody in the test sera until day 19, but the HITs could only detect the antibody until day 17.

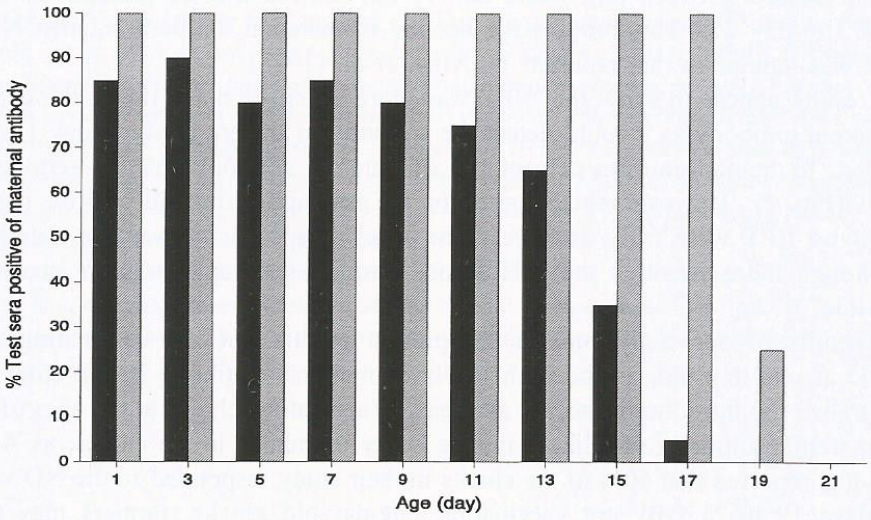

Fig. 1. Percentages of test sera positive of maternal antibody against Newcastle disease virus as detected by indirect immunoperoxidase test (▨) and haemagglutination-inhibition test (■). At each collection, different chicks (n=20) were tested.

The IIPT and HIT gave positive results upon using the reference NDV-antiserum as positive controls and negative results upon using PBS as negative controls.

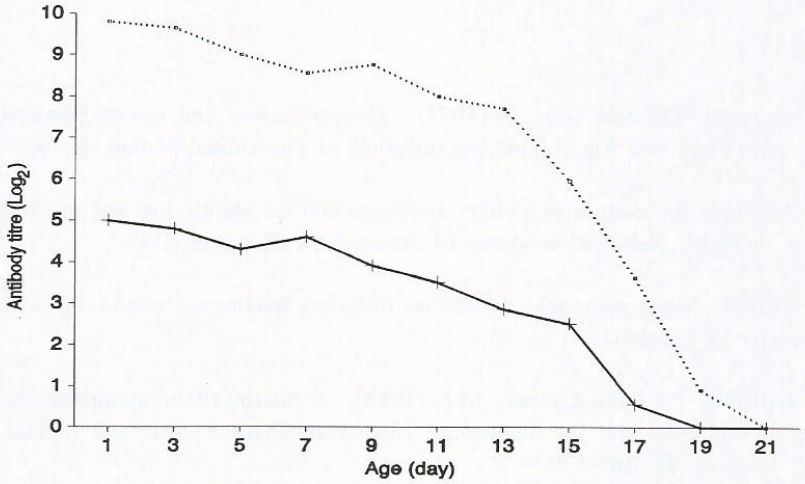

Fig. 2. Mean titres of maternal antibody against Newcastle disease virus in chicks as detected by indirect immunoperoxidase test (·····▪····) and haemagglutination-inhibition test (—+—). At each collection, different chicks (n=20) were tested.

Figure 2 shows that the mean HI and IIP titres of the maternal antibody decreased with the ages of the chicks.

DISCUSSION

The results of the present study showed that both IIPT and HIT could detect maternal antibody in the chicks on day 1 to day 17. The titres of the maternal antibody gradually decreased between day 1 and day 13 but showed a more precipitous decline from day 13 to day 21. This progressive decline of maternal antibody against NDV in the chicks was similar to that reported by Allan *et al.* (1973).

The results appear to show that IIPT was more sensitive than HIT in the detection of the maternal antibody as it could detect the antibody in all sera between day 1 and day 17, compared to the non-uniform percentages of maternal antibody-positive sera detected by the HIT (Fig. 1). However, this is based on the assumption that all positive reactions as detected by IIPT were truly positive. The level of specificity was not determined. Hence although more sensitive the IIPT is not simpler, less rapid or more economical than the HIT.

The results of our study support the present practice of not vaccinating chicks against ND at one day old, as the high levels of maternal antibody in the chicks may likely neutralize the introduced vaccine antigens. Vaccinating chicks at the ages of lower levels of maternal antibody will likely induce better immunity in the chicks, as Allan *et al.* (1973) had reported that 60% of the chicks in their study responded to the ND vaccine given at days 19 to 21. By not vaccinating one-day-old chicks, farmers may reduce vaccination cost and not incur stress on the one-day-old birds.

ACKNOWLEDGEMENTS

The authors wish to thank Rodiah-Husin, A.I. Kamaruddin, O. Abdul-Rahim, O. Zuhairi and J. Low for their assistance.

REFERENCES

Allan, W.H., Lancaster, J.E. and Toth, B. (1973). The production and use of Newcastle disease vaccine. p65, Food and Agriculture Organization of The United Nation, Rome.

Anon (1971). Methods for examining poultry biologics and for identifying and quantifying avian pathogens. p79-87, National Academy of Sciences, Washington, D.C.

Awang, I.P.R. (1988). Some pathogenicity studies of turkey paramyxovirus-3. Ph.D. thesis, p38-45, University of London.

Russell, P.H., Griffiths, P.C. and Cannon, M.J. (1983). A microwell immunoperoxidase test for screening hybridomas and for diagnosing Newcastle Disease virus and Sendai virus. *J. Immunol. Methods* **61**: 165-170.

RINGKASAN

PENGESANAN ANTIBODI MATERNAL TERHADAP VIRUS PENYAKIT NEWCASTLE DALAM ANAK AYAM DENGAN MENGGUNA UJIAN IMUNOPEROKSIDASE TAK LANGSUNG

Ujian imunoperoksidase tak langsung (IIPT) telah diguna untuk mengesan antibodi maternal pada anak ayam baka Indian River yang berumur 1 hingga 21 hari. Hasil IIPT telah secara serentak dibanding dengan hasil ujian penghemaglutinatan-perencatan (HIT) bentuk mikrotiter. IIPT boleh secara seragam mengesan antibodi maternal dalam 100% serum ujian yang telah dikumpul pada selang dua hari, dari hari 1 hingga 17; HIT hanya boleh mengesan serum antibodi-positif maternal pada berbagai peratusan (5% hingga 90%). Malah IIPT dapat mengesan antibodi maternal pada anak ayam yang lebih tua (hari 19), manakala HIT boleh mengesan antibodi tersebut pada anak ayam setakat umur 17 hari sahaja.

Penemuan dalam kajian ini menyokong amalan kini yang tidak memvaksin anak ayam pada umur satu hari dengan vaksin virus penyakit Newcastle, kerana wujudnya antibodi maternal pada aras tinggi mungkin meneutral antigen vaksin yang diperkenalkan itu, untuk menjadikan vaksin tersebut tak berkesan.