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DETECTION OF FIELD NEWCASTLE DISEASE VIRUS BY AN INDIRECT IMMUNOPEROXIDASE ASSAY

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SUMMARY: In adopting indirect immunoperoxidase (IIP) assay for diagnosing animal diseases, we compared the performance of an IIP assay with a modified virus isolation-haemagglutination and haemagglutination-inhibition (HI) assay using Newcastle disease (ND) as a model. Both assays detected Newcastle disease virus (NDV) in 25 of the 38 tissue specimens from chickens showing signs and lesions of ND. However, the IIP assay could detect NDV in the filtrates of the specimens within 40 hours, at a much faster speed than by the virus-isolation-haemagglutination and HI assay which took 2 to 28 days.

Keywords: Newcastle disease virus, indirect immunoperoxidase assay, virus-isolation-haemagglutination and haemagglutination-inhibition assay

INTRODUCTION

The most commonly used technique to detect Newcastle disease virus (NDV) is the inoculation of tissue homogenates into embryonated chicken eggs. The harvested allantoic fluid is tested for haemagglutination activity (HA) with chicken erythrocytes. Confirmation of NDV in the fluid by haemagglutination-inhibition (HI) test is done by using NDV-antiserum. The isolation of NDV in embryonated eggs and the detection by HA and HI tests altogether usually take one to four weeks to produce results. Detection of other egg-grown viruses such as infectious bronchitis virus and influenza viruses also take a long time. We thus adopted a more rapid diagnostic assay such as the indirect immunoperoxidase (IIP) assay of Russell *et al.*, (1983) in our laboratory. In the present study, the IIP assay was performed on filtrates prepared from tissues of chickens showing clinical signs and lesions of Newcastle disease (ND), taking ND as a model disease. The objective of the study was to compare results of the IIP assay in bovine kidney cells with those of the HA and HI assay *via* virus isolation in embryonated chicken, eggs in order to determine the reliability and speed of the IIP assay before it could be accepted for detecting NDV and other viruses or bacteria. The results of the preliminary study are reported in this paper.

MATERIALS AND METHODS

Tissue specimens from chickens showing signs of ND were submitted to our Veterinary Virology Laboratory at different times. They were ground and processed into 10% (w/v) homogenates in phosphate buffered saline (PBSA) at pH 7.2. The homogenates were filtered through 0.45 µm cellulose nitrate filters (Sartorius, Germany). Each filtrate in 100 µl volume was inoculated into the allantoic cavity of 10-day-old embryonated chicken egg. Three eggs were used for each specimen. Embryos that died within 24 hours post-inoculation were discarded. Allantoic fluid harvested from embryos that died on the second and third days and from embryos that were killed by chilling for three hours at 4°C on day four post-inoculation were tested for HA activity. Allantoic fluid that did not show any HA activity were blind-passaged into other embryonated eggs up to three passages within 28 days.

A combination of HA and HI tests (Subcommittee on Avian Diseases, Committee on Animal Health, Agricultural Board and National Research Council, 1971) was used to detect NDV with some modifications. We used allantoic fluids instead of tissue extracts and white tiles instead of tubes. Each of the allantoic fluids in 25 µl volume was placed on the tile in two locations. The same volume of 1% (v/v) chicken erythrocytes suspension in PBSA was mixed with the allantoic fluid at one location (spot 1) on the tile. The same volume of undiluted reference NDV-antiserum (kindly supplied by Dr. Aini Ideris of The Australian Centre for International Agricultural Research (ACIAR) at Universiti Pertanian Malaysia) was mixed with allantoic fluid in the other location (spot 2). After incubation for 10 minutes at room temperature, 50 µl of 1% (v/v) chicken erythrocyte suspension was mixed at spot 2. The HA or HI activity was observed at spots 1 and 2, respectively, within 40 minutes.

The IIP assay was performed according to the method of Russell *et al.*, (1983). Undiluted and serially 10-fold diluted tissue filtrates in 100 µl volumes of Eagles' minimal essential medium (MEM) without foetal calf serum were added to confluent monolayers of Madin Darby bovine kidney (MDBK) cells in microwells. Confluent monolayers were obtained at 20 hours after cell preparation. After 18 hours incubation at 37°C, the cells were fixed with 200 µl volumes of 10% formol-saline for 10 minutes at room temperature and then washed with distilled water. At each washing, the wells went through three washing cycles filled with 200 µl volumes of distilled water and then emptied by flicking off the fluid and then blotting with a paper towel.

The reference NDV-antiserum as used in the HI test was diluted at 1:500 in PBSA and then added to the fixed cells in 100 µl volumes and incubated for 40 minutes at 37°C. The cells were then washed and reacted with 1:500 diluted rabbit-anti-chicken IgG (H+L) conjugated to horseradish peroxidase (Nordic Immunology) at 100 µl per well. After incubation for 30 minutes at 37°C, the cells were washed.. The binding between antibody and NDV antigens in the cells was visualised by treatment of cell monolayer with PBSA containing a substrate of 0.006% (w/v) of hydrogen peroxide and 2% (v/v) ethanol saturated with orthodianisidine (Sigma) for 10 minutes at room temperature.

Infected cells were seen with brown membranes and/or particulate antigen in the cytoplasm under an inverted light microscope (Leitz, Germany). NDV was considered as present in specimens that resulted in the brown staining of the cells in any of the wells.

Similar virus isolation-haemagglutination and HI and IIP procedures were performed on reference NDV-V4 as a positive control and on PBSA as a negative control.

RESULTS

The IIP assay detected NDV in 25 of the 38 tissue filtrates in single passages on average in 40 hours post-cell preparation. The virus-isolation-haemagglutination and HI assay also detected NDV in the same positive specimens but at various egg-passages between day 2 and day 28 post-inoculation (Table 1). Both assays produced the same results in the controls.

Table 1. Comparison of results of the IIP assay and virus-isolation HA and HI assay for the detection of NDV in the chicken tissue specimens.

No.	Specimen (Tissue)	IIP	HA	HI	Time (days)*
1.	Brain	+	+	+	18
2.	Brain	+	+	+	3
3.	Epithelium of turbinate bone	+	+	+	2
4.	Trachea	+	+	+	2
5.	Trachea	+	+	+	4
6.	Trachea	+	+	+	18
7.	Trachea	+	+	+	11
8.	Trachea	+	+	+	16
9.	Trachea	O	O	O	26
10.	Trachea	O	O	O	28
11.	Lung	+	+	+	2
12.	Lung	+	+	+	18
13.	Lung	O	O	O	21
14.	Lung	O	O	O	28
15.	Proventriculus	+	+	+	2
16.	Proventriculus	+	+	+	2
17.	Proventriculus	+	+	+	18
18.	Proventriculus	+	+	+	18
19.	Airsac	+	+	+	21
20.	Airsac	+	+	+	21
21	Caecal tonsil	+	+	+	11
22.	Caecal tonsil	+	+	+	3
23.	Caecal tonsil	O	O	O	21
24.	Caecum	+	+	+	18
25.	Lower intestine	+	+	+	3
26.	Egg yolk	O	O	O	21
27.	Egg yolk	O	O	O	21
28.	Pooled trachea and lung	+	+	+	16
29.	Pooled trachea and lung	+	+	+	3
30.	Pooled trachea and lung	+	+	+	4
31.	Pooled trachea and lung	O	O	O	28
32.	Pooled trachea, lung and proventriculus	+	+	+	3
33.	Pooled trachea, lung and proventriculus	+	+	+	2
34.	Pooled trachea, lung and proventriculus	+	+	+	2
35.	Pooled trachea and kidney	O	O	O	21
36.	Pooled trachea and kidney	O	O	O	21
37.	Pooled trachea and kidney	O	O	O	21
38.	Pooled egg yolk and liver	O	O	O	11
Positive control NDV-V4		+	+	+	4
Negative control PBSA		O	O	O	Not done

*Time (days) taken to complete virus-isolation HA and HI assays.
+ Presence of NDV as indicated by stained MDBK cells in IIP assay or by haemagglutination and inhibition of haemagglutination in HA and HI tests.
O Absence of NDV.

DISCUSSION

The results of the present testing of tissue specimens from field chickens for NDV by IIP assay correlated with those of the virus-isolation-haemagglutination and HI assay. This implies that the IIP assay is as reliable as virus-isolation-haemagglutination and HI assay.

The importance is the speed taken by IIP assay to produce results. By using the IIP assay, the results were known within 40 hours, including the time for the preparation of cell monolayers, whereas by virus isolation-haemagglutination and HI assay the results were known between two or 28 days post-inoculation. Results of the preliminary trial using the IIP assay indicates that the assay can be adopted in our laboratory and further developed for diagnosing ND as well as other diseases of the livestock.

REFERENCES

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RINGKASAN

PENGESANAN VIRUS PENYAKIT NEWCASTLE LUAR MENERUSI ASAI IMUNOPEROKSIDASE TAK LANGSUNG

Dalam usaha mengguna asai imunoperoksidase tak langsung (IIP) untuk mendiagnosis penyakit haiwan, kami telah membandingkan prestasi suatu asai IIP dengan asai pengasingan virus-penghemaglutinatan dan penghemaglutinatan-perencatan (HI) dengan mengguna penyakit Newcastle (ND) sebagai model. Kedua-dua jenis asai itu telah dapat mengesan virus Newcastle (NDV) dalam 25 daripada 38 spesimen tisu daripada ayam yang menunjukkan petanda dan lesi ND. Namun demikian, asai IPP telah dapat mengesan NDV dalam turasan spesimen tersebut dalam masa 40 jam, iaitu lebih cepat daripada asai pengasingan virus-penghemaglutinatan dan HI yang mengambil masa 2 hingga 28 hari.