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AN ENZYME LINKED IMMUNOASSAY FOR ESTIMATION OF ANTIBODIES TO NEWCASTLE DISEASE VIRUS STRAINS

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Summary. — The sensitivity of enzyme linked immunoassay (ELISA) was compared to that of haemagglutination inhibition (HI) test in detection of humoral antibodies in birds vaccinated with Newcastle disease virus (NDV). The highest antibody titre at day 21 post-vaccination as detected by ELISA in the sera of vaccinated birds was 2560 by all the strains in contrast to the HI titres of 640 to 1280. The antibody titre was higher in all pre- and post-vaccination sera as determined by ELISA than detected by HI test.

Key words: ELISA; Newcastle disease virus; antibody detection; vaccination

ELISA has been used for antibody detection to several viruses. However, there are only few reports with avian viruses. Slaught *et al.* (1978) employed ELISA to detect reovirus antibody in chickens, Smith *et al.* (1979) used it to detect avian leucosis sarcoma virus and Mockett and Darbyshire (1981) described its application for the assay of antibodies to avian infectious bronchitis virus. The present paper describes the application of ELISA for the assay of antibodies to different strains of NDV.

Birds at 6 to 8 weeks of age were vaccinated with the Mukteswar vaccine strain. They were bled at 0, 7, 14, 21, 28, 35, 42 and 70 days post-vaccination. Alternatively, 1-day-old chicken were immunized with F and CDF-66 vaccines and bled at intervals described above. The anti-chicken globulin was prepared by the method of Rai and Venkataramanan (1982). The protein content of the globulin was adjusted to 10 mg/ml. Equal quantity of globulin was mixed with the Freund's complete adjuvant. Rabbits were inoculated intramuscularly with 0.4 ml of the globulin (0.1 ml in each leg). Fifteen days after the first inoculation, rabbits were again given 0.4 ml of equal quantity of globulin thoroughly mixed with Freund's incomplete adjuvant; this was repeated again after 15 days. Ten days after the last injection, the rabbits were bled, sera were separated and precipitated with 18% sodium sulphate. The antichicken globulin horseradish peroxidase conjugate was prepared as described by Avrameas (1969) (horseradish peroxidase RZ 3.0, Sigma). The coupled globulin-peroxidase fraction was separated from the free unattached peroxidase by precipitation with 50 percent saturated ammonium sulphate, dialyzed and kept at 4 °C. The 1:100 dilution of the conjugate gave satisfactory reaction and was used throughout.

The ELISA method described by Rai and Venkataramanan (1983) was followed. Microtitre plates were coated with viral antigens in carbonate-bicarbonate buffer (pH 9.6). The antigen-coated plates were incubated at 37 °C overnight and dried. The antigen was then fixed by pouring 50 µl of chilled absolute methanol into each well. The air dried antigen was washed twice with

Table 1. Antibody levels in sera of birds immunized with Mukteswar, F and CDF-66 strain vaccines

Vaccine	Dose	Age	Route of immunization	Method of antibody detection	Antibody titres on days post-vaccination							
					0	7	14	21	28	35	42	70
Strain F	$10^{9.24}$ EID_{50}/ml (in 0.2 ml)	1-day-old	IN	EIA	160	640	2560	2560	1280	1280	320	80
				HI	40	320	640	640	640	160	80	40
CDF-66 strain	$10^{8.75}$ EID_{50}/ml (in 0.2 ml)	1-day-old	IN	EIA	160	640	1280	1560	1280	640	320	80
				HI	40	160	320	640	320	160	80	20
Mukteswar strain(R_2B)	$10^{9.5}$ EID_{50}/ml (in 1 ml)	6-week-old	SC	EIA	0	640	2560	2560	2560	1280	640	80
				HI	0	320	640	1280	640	320	160	40

IN = intranasal; SC = subcutaneous.

PBS-Tween 20 buffer. Two-fold dilutions of antisera were prepared and 50 μl was added to each coated well. Incubation at 37 °C proceeded for 2 hr. After washing with PBS-Tween 20, 50 μl of peroxidase labelled rabbit anti-chicken globulin in PBS-Tween 20 was added to each well and incubated for 2 hr at 30 °C. The wells were washed twice with PBS-Tween 20. The attached enzyme was detected by adding 50 μl freshly prepared substrate solution (5 mg of orthophenylenediamine Sigma, dissolved in 10 ml of phosphate-citrate buffer pH 5.0 and 25 μl of 30% hydrogen peroxide). The plates were covered immediately with carbon paper and incubated for 30 min at 37 °C. The reaction was stopped with 0.2 mol/l H_2SO_4 and the results were recorded visually. The wells showing a dark yellow colour were regarded as positive while colourless or very slight yellow colour was considered to be negative.

The HI test was performed according to the beta-procedure described by Cunningham (1966).

The results of ELISA and HI tests for detection of antibody level in group sera of birds immunized with Mukteswar, F and CDF-66 strains are presented in Table 1. In groups of chicken vaccinated with different strains of NDV, a maximum antibody titre of 2560 was detected by ELISA on 14th and 21st day post-vaccination. A decrease in the antibody titre on day 35 and 42 was observed. On 70th day post vaccination the titre decreased to 80. The maximum HI titre detected was 640 to 1280 which decreased to 40 and 20 at 70 days post-vaccination.

The antibody response to the immunogen is considered to be one of the indicators for assessing the level of protection in bacterial and viral diseases. Newcastle disease vaccines advocated by various workers induced good HI antibody response and it was considered that the greater the HI antibody response, the better would be the immunity.

Recently the ELISA has turned out promising for the assay of various antigens and antibodies meeting the requirements of objectivity, simplicity and sensitivity. It has been employed to detect antibodies in chicken with high degree of sensitivity (Slaught *et al.*, 1978; Smith *et al.*, 1979; Mockett and Darbyshire, 1981). Our observations clearly showed that ELISA detected higher antibody levels than HI test in all pre- and post-vaccination sera. This was similar to the findings of various other workers (Mockett and Darbyshire, 1981; Dumanova *et al.*, 1982; Charan *et al.*, 1983) who compared the sensitivity of ELISA technique to that of HI test in various viral antigen-antibody systems and reported ELISA to be more sensitive than HI test. Further, while studying the ELISA and HI antibody titres by visual observation, it was found that the ELISA antibody titres were 2 to 4 times, in one case even 8 times higher than the HI titre. This findings confirmed the observations of Charan *et al.* (1981) and Snyder *et al.* (1983) who found ELISA 2 to 8 times more sensitive than HI by visual observation, while using an ELISA reader, the sensitivity was even much higher. The present investigation has confirmed that ELISA is the method of choice and may be advantageously used for the estimation of antibody level against NDV strains in bird sera.

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