Comparison of enzyme-linked immunosorbent assay and haemagglutination inhibition test for the detection of Newcastle disease virus antibodies in human sera

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SUMMARY A comparison of haemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) techniques for the detection of antibodies against Newcastle disease virus in sera from persons working in poultry farms and veterinary vaccine institutes and from the general population revealed that 22% more sera were positive by ELISA compared to HI. No samples were negative by ELISA but positive by HI. While HI titres of positive sera were found in the range 8-64, ELISA titres were between 16 and 512. It was interesting that though 78% sera had concordant results by the two tests, titres obtained by ELISA were nearly six times higher than those by HI.

Newcastle disease is primarily an infection of chickens and turkeys. In man it has been recognised chiefly as an acute and transient conjunctivitis with preauricular adenitis, fever, and chills. Generalisation of the infection with the presence of virus in blood, urine, and respiratory secretions has been encountered. Owing to frequent outbreaks of this disease in poultry farms and the extensive use of live vaccine, persons engaged in poultry farming and vaccine production units have been constantly at a much higher risk compared to the general population.

To study the prevalence of Newcastle disease virus (NDV) antibodies in the human population, neutralisation and haemagglutination inhibition (HI) tests have been used as serological tools.¹⁻³ Owing to the presence of non-specific neutralising substances and antihaemagglutinins against NDV in human sera, the formerly used procedures require some modifications. The conflicting results obtained by these tests⁴ demanded that a more sensitive and specific test be used. We compared HI and enzyme-linked immunosorbent assay (ELISA) tests for the detection of NDV antibodies in human sera. Previously, ELISA had been used for NDV antibodies only in chicken sera.⁵

Material and methods

ANTIGEN

Chick embryos of 9-11 days were inoculated into the allantoic cavity with F strain of NDV obtained from the Veterinary Vaccine Institute, Hissar (Haryana). After 72 hours' incubation, embryos were chilled and the allantoic fluid was pooled. The haemagglutination (HA) titre of the pooled allantoic fluid antigen was found to be 1024 using 0.5% chick erythrocytes.

SERA

A total of 213 human sera were tested for NDV antibodies, of which 104 were from subjects working in poultry farms and veterinary and vaccine institutes. The remaining 109 samples were obtained from the Clinical Chemistry Laboratory, New Delhi, and served as controls representing the normal population. All the sera were stored at -70°C till used.

HI TECHNIQUE

Non-specific reactions of NDV haemagglutination were removed by heat inactivation and kaolin treatment of the sera.³ A serum-antigen (4 HAU) mixture was incubated at 37°C for 1 hour before the addition of chick red blood cells. Appropriate controls were included during the procedure.

ELISA TECHNIQUE

This was the modified micromethod of Voller and coworkers.⁶ Polystyrene plates (Microtitre M 29 AR, Dynatech Laboratories, Sussex) were coated with 200 μ l of optimally diluted NDV antigen in 0.06 M carbonate-bicarbonate buffer, pH 9.6, after overnight incubation at 4°C. The plates were washed three times in phosphate-buffered saline (PBS), pH 7.2, containing 0.05% Tween-20 (PBS/T). Twofold serial dilutions of the test sera were made, and 200 μ l was added to each well of the plate in vertical rows, except the last row which was left as an antigen control. The plates were incubated at 37°C for 1 hour and washed three times conventionally with PBS/T; 200 μ l amounts of 1:8000 diluted anti-human IgG-peroxidase conjugate (Miles Laboratories, Slough, UK) were added to each well. Again the plates were incubated at 37°C for 1 hour. After further washing, *o*-phenylenediamine substrate (40 mg/100 ml phosphate-citrate buffer, pH 5.0, with 40 μ l of 30% H₂O₂) was added in equal amounts of 200 μ l to each well, and the reaction was allowed to proceed at room temperature for 20 $\pm$ 5 minutes. The reaction was stopped by adding 50 μ l of 5N H₂SO₄ to each well. Results were recorded on the basis of the intensity of colour developed.

Results

Of the total 213 sera, 167 (78%) gave concordant results, 44 (20%) being positive and 123 (58%) negative for NDV antibodies by both tests. Forty-six (22%) sera were found to be positive by ELISA but were negative by HI test. Of these 46 sera, 43 belonged to the high-risk population while only three were from the general population category. No serum was negative by ELISA but positive by HI (Table 1 and Figure).

The correlation between ELISA and HI titres among the high-risk population is shown in Table 2.

Table 1 Comparison of ELISA and HI tests for detection of NDV antibodies in high-risk population and general population

Type of population	Serum samples		NDV antibody by:	
	Number	%	ELISA	HI
High-risk 104 Sera	40	39	+	+
	21	20	-	-
	43	41	+	-
General 109 Sera	102	93.5	-	-
	4	3.7	+	+
	3	2.8	+	-
Combined 213 Sera	44	20	+	+
	123	58	-	-
	46	22	+	-

Comparison of antibody titres measured by HI and ELISA in 40 human sera positive by the two tests.

Table 2 Correlation of corresponding HI titres of NDV antibodies with that of ELISA in 104 sera from high-risk population

HI titre	Number of sera with ELISA titres:				Total
	<16	16-32	64-128	256-512	
<8	21	33	8	2	64
8	0	11	8	1	20
16	0	1	7	2	10
32	0	1	4	2	7
64	0	0	0	3	3
Total	21	46	27	10	104

ELISA titres of the 64 sera that were negative by HI were in the range <16-512; 21 sera had titres <16; 33 sera had titres of 16-32; while the remaining 10 sera had titres of 64-512. Moreover, of the 67 sera that had ELISA titres of up to only 32, 54 were negative by HI, 11 had HI titres of 8, and the remaining two were HI positive with titres of 16 and 32, respectively.

Discussion

The ELISA technique has been applied and compared

with other conventional tests in various conditions as a diagnostic and surveillance tool.⁷ By virtue of its sensitivity, specificity, and other advantages, it safely gets recommendations even for routine purposes. Earlier it had been used in studies on NDV infection in chickens. In our studies also, ELISA has been shown to be far more sensitive than HI. Only 20% of a total of 213 sera from a mixed population were positive for NDV antibodies by HI (39% from a high-risk population and 4% from the general population) with titres ranging between 8 and 64. ELISA could detect antibodies in 42% of sera (80% from a high-risk population and 7% in the general population) with titres up to 512. The additional advantage of ELISA over HI is that, while screening for NDV antibodies, the occurrence of non-specific antihaemagglutinin and inhibitors is not important. The confusion regarding its antigenic relation to mumps virus is overcome due to its high specificity. Since HI antibodies and neutralising antibodies are the functions of different components on the surface of the virus (haemagglutinin and neuraminidase receptors), the pattern of the two antibodies does not need to be parallel. The tests detecting either type of antibody will be a misrepresentation of the facts. Hence, the lack of correlation between HI and neutralising antibodies encountered by many workers^{1 3 4 8} can easily be answered. Because ELISA can detect both types of antibodies against NDV, a high positivity of sera by this technique can be justified. No serum sample was negative by ELISA but positive by the HI test.

Both the tests had 78% concordant results, and sera positive by the two tests have shown a good correlation in their titres. By ELISA, 22% more sera were positive than by HI, of which 41% sera

were from a high-risk population and only 3% from the general population, indicating that high positivity by ELISA was due to detection of the lowest levels of antibodies rather than to false-positive indications. On an average the titres obtained by ELISA were six times higher than by HI.

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