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Screening HN Epitopes Polypeptide of NDV and Establish Polypeptide ELISA for Detecting Antibody

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Abstract

Inspection of local mainly testing NDV antibody in chicken serum by enzyme linked immunosorbent assay (ELISA) and judging the immunity of chickens and whether there is wild virus infection, at present the envelope antigen of ELISA kit which testing NDV antibody mainly include NDV stain and purified HN protein, and these ELISA kits are mainly imported and the price is high and not conducive to the monitoring of the disease. In this study, the specific epitope polypeptides of HN protein in NDV were screened and used as the coating antigen of ELISA test to establish an ELISA test method for specific detection of NDV antibody, can clinical samples were tested. The result showed that epitope polypeptide P25 was successfully screened. The P25 epitope polypeptide was detected in the anti-chicken serum of AIV and IBDV respectively, and it was found that they did not react with them, and the coincidence rate between the test results of ELISA was 97.22%. It is expected to provide matching reagents for antibody detection of live vector vaccine and nucleic acid vaccine and play an import role in the detection of Newcastle disease.

Keywords: NDV; HN protein; P25 polypeptide; ELISA.

1. Introduction

Newcastle disease (ND) is a highly contagious disease caused by Newcastle disease virus (NDV) in chicken. The main manifestations of ND are seromucosal hemorrhage, dyspnea dysentery and nervous disorders [1-2]. ND spread rapidly, with high lethal rate, strong contact infectivity and wide distribution, which resulted in huge economic losses have been listed as a Class A epidemic by the world organization for animal health (OIE).

NDV is belongs to the Avualavirus genus in the Paramyxoviridae family [3]. Similar to other paramyxoviruses in its family, the 15186 nucleotide negative ssRNA genome of NDV encodes six genes, including the nucleocapsid protein(NP), phosphoprotein(P), matrix protein(M), fusion protein(F), hemagglutinin-neuraminidase(HN) and RNA-dependent RNA polymerase(L) [4-5].

The HN protein is one of the major structural proteins of NDV, which mediates receptor recognition of sialic acid at the end of host-cell surface proteins and possesses neuraminidase activity, necessary to prevent virus progeny self-aggregation during budding [6], and can induce the organism to produce protective immune antibody [7]. It can interact with F protein to promote the cell fusion. Further studies have been performed to elucidate the molecular determinants for viral pathogenicity.

In China, Vaccination effectively control ND transmission, but long-term use of inactivated vaccine and attenuated vaccine is prone to the variation of virus strains, and cannot distinguish vaccine immunity

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and natural infection [8], which brings difficulties to NDV detection. At present, the methods of hemagglutinin (HA), hemagglutinin inhibition(HI), enzyme-linked immunosorbent and real-time PCR are commonly used to detect the NDV [9-11]. However, these methods are time-consuming and laborious, also need special equipment in lab, and it cans not diagnose the spread of NDV in time.

In this essay, according the surface antigen structure of HN protein of NDV, 13 peptide sequences of HN protein were synthesized and coupled with the carrier protein. Then, the reaction of the peptides and antiserum of NDV was tested by Elisa. The reaction of the epitope P25 of NDV with the clinical antibody of NDV showed high sensitivity and specificity, no cross reactivity with other family disease such as IBDV and AIV antibody.

2. Material and Methods

2.1. Antibody and Serum

The clinical antibody of NDV was isolated from a chicken farm in Henan Province. The antibody of avian influenza virus (AIV) and infectious bursal disease virus (IBDV) were preserved in our lab. HRP-conjugated Rabbit anti-chicken IgG was purchased from Sigma Company.

2.2. Virus and Cell

The recombinant NDV were kept in our lab. Primary chicken embryo fibroblasts were prepared from 9-to-11-day-old SPF embryos, and maintained in DMEM (GIBCO, USA) supplemented with 10% FCS (GIBCO, USA) and 1% penicillin/streptomycin at 37℃ under 5%CO_2 atmosphere.

2.3. Preparation of High Immune Serum of NDV

NDV was inoculated into chicken fibroblasts and harvested after 72h. NDV was purified by sucrose density gradient centrifugation and intramuscular injected into chickens of 2 weeks old at a concentration of 1mg/ml. Serum was collected when the clinical symptoms of chickens appeared. Then, the collected serum was inactivated at 56℃ for 30min and stored at -70℃ for use in subsequent experiment.

Table.1 polypeptide sequence

Name	Amino acid sequences(N C) →	Length
P21	CRYNDTCPDEQDYQIRMA	18
P22	CYNDTCPDEQDYQIRMA	17
P23	CNDTCPDEQDYQIRMA	16
P24	CDTCPDEQDYQIRMA	15
P25	CTCPDEQDYQIRMA	14
P26	CPDEQDYQIRMA	12
P27	CDEQDYQIRMA	11
P28	CEQDYQIRMA	10
P29	CQDYQIRMA	9
P210	CDYQIRMA	8
P211	CYQIRMA	7
P212	CQIRMA	6
P213	CIRMA	5

2.4. Syntheses of HN Protein Epitope Polypeptides of NDV

According to the surface antigen structure of HN protein of NDV, 13 polypeptide sequences (as shown in Table.1) were synthesized by section. In order to meet the needs of the subsequent coupling experiment, a flexible amino acid Cys was added to the N-terminal of the HN epitope peptide. All the peptides were completed by Shanghai Sango bioengineering Co., Ltd. The lyophilized polypeptide was dissolved at the concentration of 1mg/ml in sterile distilled water or deionized water of anaerobic water. For neutral polypeptides with highly hydrophobic, adding a certain amount of DMF or DMSO is helpful to the dissolution of polypeptides. The polypeptide solution was filter with 0.2μm pore diameter filter membrane to prevent bacterial degradation. The amino acids of Cys, Met, Trp, Glu and Asp were contained in the polypeptide, which is easy to oxidize, so it must be kept in the environment without oxidant. When the polypeptide was preservation, repeated freezing and thawing must be avoided.

2.5. Connection Between Synthetic Polypeptide and Carrier Protein

The synthesized polypeptide was coupled to BSA carrier protein to facilitate the detection of antigenicity and polypeptide immunity. The heteroduplex reagent sulfo-SMCC (MW: 436.37; Spacer Arm Length:11.6 Å, Pierce) was used to connect the $–NH_2$ on the carrier protein BSA or KLH and –SH of Cys on the C-terminal of the peptide, the artitifically synthesized binding antigen peptide was formed and named BSA-Pep. The details are as follows:

(1) 4mg BSA was weigh and dissolve in 500μl coupling buffer (0.1mol/l phosphate, 0.15mol/l NaCl, 1μm/l EDTA, pH7.2); (2) 1mg Sulfo-SMCC was added to the carrier protein solution; (3) the obtained solution was incubated for 60min at room temperature or 30min at 37℃, and then mixed evenly; (4) the solution was dialyzed sufficiently to remove the excess coupling agent, then the carrier protein solution activated by Sulfo-SMCC is obtained and adjusted to 5mg/ml with the coupling buffer; (5) 10μl (50μg) of the activated carrier protein was mixed with 50μg dissolved polypeptide containing Cys at N-terminal, and incubated at 4℃ for 4h or overnight. When the coupling reaction complete, the coupling solutions are store at 4℃ for standby.

2.6. Peptide-ELISA Detection

The ELISA method was used to detect the reaction between the coupling peptide and the antiserum of NDV. The procedures are as follows: The BSA coupling peptide was diluted with coated buffer (0.1mol/l carbonate buffer, pH9.6) to 1μg/ml. The 96 –well plate was coated with the solution at 50μl per well, and then washed with PBST for six times. The plates were blocked with 5% pig serum in PBST and probed with the following antibodies overnight at room temperature for 30-60min: anti-NDV antibody (1:1000), then followed by a HRP-conjugated anti-chiken IgG secondary antibody (1:1000) 1h at room temperature. Then, the substrate solution and chromogenic agent was added and the reaction was terminated with 2mol/l sulfuric acid solution. The result of ELISA was read the absorption value of 450nm on the enzyme labelling instrument.

2.6.1. Sensitivity Analysis of ELISA

The NDV standard serum was diluted by double ratio dilution method such as 1:200, 1:400, 1:800, …., 1:102400. The synthesized epitope polypeptide was used to detect the NDV standard serum by ELISA method. Meanwhile, the NDV serum was detected by commercial ELISA kit (TSZ, American) as control.

2.6.2. Specificity Analysis of ELISA

The specificity of NDV HN polypeptide was tested by ELISA method. NDV positive antibodies (10 samples), NDV negative antibodies (5 samples), AIV antibody (1 sample) and IBDV antibody (1 sample) was diluted with PBS at the ratio of 1:100 and used to detected the selected epitope peptides.

2.7. Clinical Sample Test

36 serum samples of chicken from chickens suspected to be infected with NDV in different areas were collected, diluted with PBS at the ratio of 1:100 and detected by ELISA methods. At the same time, the commercial ELISA kit (TSZ, American) was used as control.

3. Results

3.1. Peptide-ELISA Detection

When the synthesized peptides were conjugated to BSA, the results of ELISA showed that only polypeptides P24 and P25 were positive for ND positive serum reaction.

3.1.1. Sensitivy of P24 and P25 Epitope Peptides Against NDV Antibody

The sensitivity of synthetic peptide was detected by NDV positive serum with double ratio dilution method, and NDV commercial ELISA kit was used as control. The results demonstrated that the P25 peptide still detected the positive result, when the positive serum was diluted to 1:51200. However, the result detected by the commercial ELISA kit was suspicious. The P24 peptide could only detect the serum dilution ratio of 1:1600.

3.1.2. Specificity analysis of P25 epitope peptide against NDV antibody

The NDV positive and negative serum and different avian virus antibody was used to tested specificity. The results of P25 polypeptide showed that it could react with NDV positive serum, not with NDV negative serum and other virus infected positive serum.

3.2. Results of clinical samples detection

36 samples of chicken serum were collected from chicken farms in Henan Province and used to test P25 polypeptide. The results suggested that the P25 polypeptide has a high coincidence rate (97.22%, 35/36) with ELISA (shown in Table.2)

Table.2 Clinical test results

P25	ELISA	
	Positive	Negative
Positive	30	1
Negative	0	5

4. Discussion

Newcastle disease (ND) is one of the most serious diseases that ham the poultry industry in China. It is one of the most important poultry disease recognized by the world as well as highly pathogenic avian influenza. The disease is a kind of animal disease that must be reported according to OIE, and it is also a

kind of animal disease regulated by our country. HN protein is an important multifunctional surface glycoprotein of NDV. It consists of cytoplasmic domain, transmembrane region, stem region and globular head. There are receptor binding epitopes, neuraminidase active sites and antigen epitopes. The preliminary tests showed that the P25 peptide (TCPDEQDYQIRMA) of HN protein could react with NDV hyperimmune serum.

5. Conclusion

The amino acid sequence of HN protein of NDV was used as template to synthesize HN antigen epitope polypeptides, one P25 epitope polypeptide (TCPDEQDYQIRMA) of HN antigen were obtained. The ELISA method for the specific detection of NDV antibody was established with the P25 polypeptide as the enveloped antigen. The method was proved to be free from the interference of antibodies produced by other avian viruses by the specificity test. The sensitivity tests proved that the method could detect the serum samples of 1:51200 dilution.

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