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PREPARATION OF CONJUGATE FOR USE IN AN ELISA FOR HUMORAL IMMUNE RESPONSE AGAINST EGG DROP SYNDROME VIRUS IN LAYER CHICKS

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ABSTRACT

An indirect enzyme-linked immunosorbent assay (ELISA) was performed for the detection of antibodies against Egg Drop Syndrome (EDS) virus. Virus identification was done through haemagglutination inhibition (HI) test using known antisera. Antichicken immunoglobulins were raised in goats and purified by ammonium sulphate precipitation technique. These goat-antichicken immunoglobulins were conjugated with horseradish peroxidase. Twenty-seven serum samples were collected from a layers flock vaccinated against EDS and specific antibodies were determined by using a horseradish conjugate.

Key words: Egg drop syndrome, ELISA, antichicken antibodies, humoral immune response.

INTRODUCTION

Egg Drop Syndrome (EDS) is a disease of chicken caused by haemagglutinating adenovirus (Regenmortel *et al*., 2000), which are DNA containing particles, and replicate in nucleus of the host cells. The prevalence of EDS in Pakistan was reported on many poultry breeding farms (Naeem, 1994). The incidence was recorded up to 30% in non-vaccinated commercial layer farms showing low egg production (Siddique and Haq, 1997). In order to control the EDS, serum antibody titration is very important to evaluate the immune status of the birds and thus to determine the exact time for vaccination. Present study was conducted to measure the antibodies against EDS virus in layer chicks using indirect ELISA.

MATERIALS AND METHODS

The EDS virus was obtained from Department of Veterinary Microbiology, University of Agriculture, Faisalabad. Hyperimmune serum raised in rabbits against commercial EDS vaccine (Izovac EDS) was used to identify and confirm the EDS virus using AGPT and HI tests (Buxton and Fraser, 1977).

A 25 ml serum obtained from adult chickens was processed for the separation of immunoglobulins through ammonium sulphate precipitation technique (Hudson and Hay, 1980; Zia *et al*., 2000). The solution containing chicken immunoglobulins was injected in three goats with increasing dosage on alternate days using Freund's complete adjuvant for first shot and incomplete adjuvant for further three shots. The goat blood was collected after14 days of 4th inoculation, serum was separated and stored at –20 ^{o}C for further use.

Goat serum was processed for purification of immunoglobulins through ammonium sulphate precipitation technique and the goat anti-chicken antibodies were confirmed by AGPT (Hudson and Hay, 1980). The total protein concentration in purified immunoglobulin of goat antichicken serum was determined by Biuret method (Gornall *et al*., 1949). Two-step gluteraldehyde method was adopted for conjugation of anti-antibodies with 10 mg horseradish peroxidase. A sterile charged, flat-bottom polystyrene microtitration plate with 96-wells (Titerteck, UK) was used for antigen coating. The EDS virus was prepared in coating buffer with 1:100 dilution. Then 100 µl of the diluted virus was poured in each well of microtitration plate except blank, incubated at 4^{o}C for 24 hours, and was washed three times with washing buffer. This plate was filled with blocking buffer by pouring 100 µl in each well, incubated at 4 ^{o}C for 24 hours, washed three times and stored at 4^{o}C.

Phosphate-citrate buffer (pH 5.0) was prepared (12.15 ml of 0.1 M citric acid, 12.85 ml of 0.2 M disodium hydrogen phosphate and 25 ml distilled water). A 20 mg of Orthophenylene diamine (OPD) was dissolved in 50 ml of substrate buffer solution and 20 µl of 30% H_2O_2 was added. Indirect ELISA was performed according to the method described by Kemeny and Challacombe (1989).

RESULTS AND DISCUSSION

In the present study, goat antichicken antibodies were raised by multiple shot regimens, which were isolated and partially purified through ammonium sulphate precipitation technique. Protein contents of the partially purified antibodies ranged from 0.139 to 2.957 mg/ml (Table 1).

In the present study, two-step gluteraldehyde method was adopted for conjugation. There are other methods like one-step gluteraldehyde method for alkaline phosphatase, periodate-oxidation method for horseradish peroxidase (HRP) and alkaline phosphatase (Nakane and Kawaoi, 1974) and maleimide method for β-D-galactosidase (Kato *et al*., 1976), but two-step glutaraldehyde method was proved to be more efficient than one-step gluteraldehyde method (Barker, 1989).

The anti-antibodies were raised to conjugate with HRP. Different enzymes like alkaline phosphatase, β-D-galactosidase and urease, can be used for conjugation, but peroxidase is one of the most thermostable enzymes, which can even withstand boiling for a few minutes (Reed, 1975). This enzyme has a high turnover rate, relatively cheaper and can readily be coupled to proteins. The β-D-galactosidase has slow turnover rate and alkaline phosphatase is expensive.

The serum samples were applied to micro-titration plate in duplicate and optical density (OD) values were recorded (Table 2). Mean of highest OD of negative control was 0.196 ± 0.02. All the test samples were compared with this highest negative mean. Lowest mean OD value was 0.226, while the highest mean OD value was 0.360. This study shows that improved experimental conditions and facilities can lead to the preparation of more sensitive conjugate and anti-species immunoglobulins coated plates in local conditions and can save a lot of foreign exchange consumed during import of ELISA kits.

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Table 1: Protein concentration of anti-chicken goat antibody, AGPT and HI results

Samples	OD	Standard factor	Protein conc. (mg/ml)	AGPT	HI titer
1	0.039	3.58	0.139	+ve	4096
2	0.825	3.58	2.957	+ve	4096
3	0.485	3.58	1.736	+ve	4096

Table 2: Mean OD values of standard test sera and samples (S_1 to S_{27})

+Ve control	S 4	S 10	S 16	S 22
0.482 ± 0.096	0.257 ± 0.112	0.275 ± 0.101	0.254 ± 0.139	0.323 ± 0.168
-Ve control	S 5	S 11	S 17	S 23
0.196 ± 0.020	0.265 ± 0.111	0.289 ± 0.147	0.235± 0.058	0.328 ± 0.152
Blank	S 6	S 12	S 18	S 24
0.057 ± 0.023	0.274 ± 0.113	0.278 ± 0.160	0.296 ± 0.160	0.279 ± 0.124
S 1	S 7	S 13	S 19	S 25
0.258 ± 0.119	0.276 ± 0.109	0.268 ± 0.143	0.293 ± 0.142	0.360 ± 0.153
S 2	S 8	S 14	S 20	S 26
0.256 ± 0.109	0.319 ± 0.079	0.266 ± 0.173	0.278 ± 0.111	0.226 ± 0.091
S 3	S 9	S 15	S 21	S 27
0.273 ± 0.098	0.263 ± 0.121	0.290 ± 0.125	0.358 ± 0.173	0.315 ± 0.147