

Full Length Research Paper

Evaluation of serological response of chicks against angara disease agent through indirect haemagglutination test

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Indirect Haemagglutination test is a reliable quantitative serological test for measuring antibodies against angara disease. The etiological agent contained in the liver of affected chick did not directly haemagglutinate erythrocyte of chickens. The antibody titre measured through IHA varied from 1:8-1:128, in the sera (samples) of experimentally infected chickens at days 7, 14 and 21 post vaccination.

Key words: Haemagglutination, titre, sera, angara disease.

INTRODUCTION

Hydropericardium hepatitis syndrome (HHS) was first recognized in broiler flocks in Angara Goth (Goth means small town or village) near Karachi Metropolitan City of Pakistan, in the Province of Sindh (Jaffery, 1988). The disease was initially referred as "Angara Disease". The disease was then spread within six months in all densely populated broiler growing areas of the country. The outbreak of the disease was also recorded during 1989 in the highly poultry producing states of Mexico (Borrego and Soto, 1995).

In the light of present work on the serological response of broilers to vaccines against angara disease using indirect haemagglutination and adsorption of bacterial/viral sonicated material to the erythrocytes and subsequent haemagglutination of treated erythrocytes by specific antibody (Rahman et al., 1987) to identify the Angara Disease agent antibodies present in the convalescent serum samples of chicks. In this report indirect haemagglutination technique was adopted that produced highly encouraging results.

MATERIALS AND METHODS

Rearing of chicks

One hundred and fifty day old broiler chicks were reared at National Veterinary Laboratory (NVL), Islamabad under standard husbandry conditions. At the age of day 20 the chicks were divided into five groups K, L, M, N and O, each group having 30 chicks. On the same day, the chicks of groups K, L, M, and N were vaccinated with ADVac-I, ADVac-II, ADVac-III and ADVac-IV, respectively (Table 2). The chicks of group O were kept as non-vaccinated control. Route and dose of vaccination was adopted according to manufacturer's instructions (Table 2). The serum samples from chicks of groups K, L, M, N and O were also obtained at days 7, 14 and 21-post vaccination for serological investigations.

Preparation of antigen

A 40% suspension of affected frozen liver of chickens, infected with Angara Disease was prepared in phosphate buffered saline through use of ultrasound vibration for 7 min. The suspension was then centrifuged at 5000 rpm for 15 min. The supernatant was collected and filtered by 0.22-micron membrane filter.

Sensitization of erythrocytes

Human (Blood group O) and sheep erythrocyte were prepared

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Table 1. Composition of vernal buffer.

Sodium chloride	42.5 g
Barbituric acid	2.875 g
Sodium diethyl barbiturate	1.875 g
Magnesium sulphate	1.018 g
Calcium chloride	0.147 g
Distilled water	1 L

Table 2. Angara disease vaccines prepared from chicken's liver and used during present study.

Groups of chicks	Vaccines used	Age of vaccination (Days)	Dose of vaccination (ml)	Route of administration of vaccines	No. of chicks in each group
K	ADVac-I	20	0.20	S/C	30
L	ADVac-II	20	0.20	S/C	30
M	ADVac-III	20	0.20	S/C	30
N	ADVac-IV	20	0.20	S/C	30
O	Control (Unvaccinated)	-	-	S/C	30

separately by centrifugation of citrated blood taken from the donors. The erythrocytes were washed thrice with physiological saline solution and then sensitized as follows.

Human O erythrocytes

A 2 ml amount of antigen were mixed with 0.2 ml of packed human O erythrocytes. After thorough mixing the antigen and the erythrocytes were placed at 37°C for one hour. The erythrocytes were then separated by centrifugation and washed with 5 ml physiological saline solution. After which sufficient saline was added to give 1% suspension (Carter, 1995).

Sheep erythrocytes

A 0.75 ml amount of packed sheep erythrocytes were mixed with 1 ml of 1:400 dilution of chromium chloride solution (0.1 M, pH 6.8), furthermore, 2 ml of vernal buffer and 2 ml sonicated antigen was also added. The mixture was thoroughly mixed and then placed at room temperature for 10 min. The erythrocytes were then separated by centrifugation and washed for three times with vernal buffer to give 1% suspension (Bansal et al., 1986) (Table 1).

Indirect haemagglutination (IHA) test

The haemagglutination was carried out separately with human blood (O) and sheep erythrocytes. Two fold serial dilution of serum samples were prepared with physiological saline solution in microtitration plate (U-shaped). Routinely 0.05 ml of 1% sensitized erythrocytes suspension were added in each well containing 0.05 ml of serum dilution. Two control well contained 0.05 ml sensitized erythrocytes only. The plates were taped then left at room temperature for 30 to 60 min after that a reading was taken. The plates were then placed in the refrigerator overnight and a second reading was made. A positive reaction consisted of marked haemagglutination, while negative showed an evidence of button formation at the bottom of the wells.

RESULTS AND DISCUSSION

Direct haemagglutination of emulsified liver extract of chickens, guinea pigs and rats erythrocytes could not occur which indicated that material did not contain haemagglutinating agent. Actually, this observation prompted the author to perform IHA test. The presence of disease causing agent in liver of affected birds had been recorded by various workers (Cheema et al., 1988). The sera of various groups of chickens gave variable results with IHA test indicative of variation in antibody levels. The results of IHA regarding antibody level with both human and sheep erythrocytes were similar with all the serum samples. All the serum samples of chickens under test had indirect haemagglutination titres varying from 1:8-1:128.

Sensitization of sheep erythrocytes without coupling agent (chromium chloride) was found to be unsatisfactory because sheep erythrocytes contain heterophile antigens. IHA test has been found to be rapid and simple technique for measuring the antibody level against Angara Disease agent. This technique can be adopted for screening the large number of sera in epidemiological studies of this disease. Owing to its inexpensive procedure it can also be used to evaluate the immune status of the chickens of all kinds of (diseased /normal).

Serum samples were collected from chicks of sub group k, l, m, n and o, were subjected to indirect haemagglutination test for antibodies titration against Angara Disease virus. Serum samples were collected from chicks at 7, 14 and 21 days post vaccination. Seven days post vaccination GMT titre was 1.87, 1.37, 1.75, 1.25 and 0.5 in chicks of sub groups k, l, m, n and o, respectively (Table 3). Fourteen days post vaccination

Table 3. Anti HPS- IHA-Antibody response of broiler chicks 07 days post vaccination.

Subgroups	No. of wells												GMT (Log2)
	1	2	3	4	5	6	7	8	9	10	11	12	GMT
K	3	3	2										1.87
L	6	1	1										1.37
M	3	4	1										1.75
N	6	2	-										1.25
O	3	1	-										0.5

Table 4. Anti HPS- IHA-Antibody response of broiler chicks 14 days post vaccination.

Subgroups	No. of wells												GMT (Log2)
	1	2	3	4	5	6	7	8	9	10	11	12	GMT
K	3	2	1										2.25
L	2	2	4										2.25
M	1	-	5										3.12
N	4	1	1										2.25
O	4	2	2										1.75

Table 5. Anti HPS- IHA-Antibody response of broiler chicks 21 days post vaccination.

Subgroups	No. of wells												GMT (Log2)
	1	2	3	4	5	6	7	8	9	10	11	12	GMT
K	1	2	4	1	-	-							2.62
L	1	3	1	1	1	1							3.12
M	2	1	3	1	1	-							2.75
N	2	1	2	2	1	-							2.87
O	3	1	-	2	-	-							2.16

Table 6. Geometric mean titre of antibodies in the sera of chicks 7, 14 and 21 days post vaccination.

Experimental sub-groups	Vaccines administered	Mean antibody at day 07 post vaccination	Mean antibody at day 14 post vaccination	Mean antibody at day 21 post vaccination
K	AD Vac-I	1.87	2.25	2.62
L	AD Vac-II	1.37	2.25	3.12
M	AD Vac-III	1.75	3.12	2.75
N	AD Vac-IV	1.25	2.25	2.87
O	Unvaccinated	0.50	1.75	2.16

GMT titres were 2.25, 2.25, 3.12, 2.25 and 1.75 in chicks of sub-group k, l, m, n and o respectively (Table 4). Twenty-one days post vaccination GMT titres were 2.62, 3.12, 2.75, 2.87 and 2.11 in chicks of sub-group k, l, m, n and o (Table 5). The cumulative results of antibody profile at 7, 14 and 21 days post vaccination in chicks of sub-groups k, l, m, n and o are given in (Table 6).

Statistically, there was no significant differences among the titre after 7, 14 and 21 days of post vaccination. Also, there was no significant differences among the titres with

in the groups, however numerically, there was difference in the mean values within the titres where post vaccination gave increased antibody titres as compared to non-vaccinated control. Antibody titres in the sera of chicks are in close agreements with the results of previous study (Tizard et al., 1992) who determined antibody titres varied from 1:4 to 1:256. The results can be supported partially with Rahman et al. (1997), who titrated the IHA antibodies in the convalescent sera of chickens in the same age group.

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