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Comparative Efficiency of Two Oil Emulsion Hydropericardium/Hepatitis Syndrome Vaccines

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ABSTRACT

This study was aimed to compare the efficacy of the experimental oil emulsion (OE) vaccine with the commercial oil based vaccine and existing formalin killed vaccine against Hydropericardium (HPS) or Hepatitis syndrome. Modified, OE vaccine @ 0.1mL s/c with (LD50: 106.08) showed better and long lasting humoral antibody response as compared to commercial oil based vaccine. The challenge experiment (with 20% infected liver homogenate) gave 100% protection in case of OEHPS (modified) vaccinates. In contrast, there was a sudden decrease in the antibody titre after three weeks of vaccination and there was 40 and 20% mortality recorded (post challenge) in the birds vaccinated with commercial oil based and formalin killed vaccines, respectively. Modified OEHPS vaccine proved effective in respect to better antibody titre and protection offered in the broiler chicks.

Key Words: Hydropericardium; Hepatitis; Oil-emulsion; Vaccine; Broiler

INTRODUCTION

Hydropericardium Syndrome (HPS) or Hydropericardium-Hepatitis syndrome (HHS) (Ahmed, 1999), for the first time was observed in an intensively broiler growing area in Karachi (Angara Goth) in August, 1987 (Jaffery, 1988) and was initially referred to as "Angara disease". The disease is characterised by the accumulation of clear, watery, jelly like fluid in the pericardium sac, mis-shapened and flabby heart, congested and enlarged liver with messave necrosis and congestion and oedema of the lungs (Cheema *et al*., 1988). Initially, the disease was of obscure aetiology and later it was declared that adenovirus is the main etiological agent (Cheema *et al.,* 1989).

The disease is now wide spread all over the country, mainly effects broiler of 3-5 weeks of age and young broiler breeders (Ahmad *et al*., 1989). The mortality rates are usually high during 4-5 weeks of age and in some flocks it may reach even upto 60-70% (Irfan, 1988). The out breaks of HHS have also been recorded in Mexico in the high density poultry producing states in 1989 (Borrego & Soto, 1995). Various vaccine formulations are being used in the field but none of them fulfil the criteria of eliciting a prompt and long lasting immune response against the natural outbreaks of the HHS.

The present project was designed to develop and modify the already reported oil-emulsion vaccine (Hussain *et al.,* 1996) against HPS and determination of comparative efficiency of two different oil-emulsion vaccines with the existing killed vaccine.

MATERIALS AND METHODS

Hydropericardium/Hepatitis virus (Avi-adeno virus) was isolated from and outbreak in Faisalabad. The infected liver suspension was processed and further formulated for the preparation of the oil-emulsion HPS vaccines.

The above material was processed for the qualitative and quantitative estimation of the virus on the basis of Direct hemagglutination test (Khawaja *et al.,* 1995), passive hemagglutination test (Rahman *et al.,* 1994) and Agar gel precipitation test (Tariq, 1988).

Preparation of Vaccines

Oil emulsion vaccine (Hussain *et al.,* 1996). The OVE was formulated by homogenising one part of antigen F-HPS (Formalised HPS) and three parts of adjuvant consisting of nine parts of mineral oil and one part of Tween 80.

Modified OE vaccine. The modified oil-emulsion (OE) vaccine was prepared by (F-HPS) using 1.8 parts of antigen (HPS virus filtered through 0.22u APD filter) with 1.2 parts of oil adjuvant consisting of (9-parts mineral oil + 1 part Tween 80) as detailed in Table I.

Table I. Comparative formulations of oil emulsion vaccine against HHS/HPS virus

Oil emulsion vaccine	Modified oil emulsion vaccine
1-vol. HPS (formaline killed Ag).	1.8 vol. HPS (Formaline killed Ag.)
3-vol. Oil adjuvant (9 parts mineral oil + 1 part Tween 80).	1.2 vol. Oil adjuvant (9 parts mineral oil + 1 part Tween 80 and Span 80).

The LD50 of the HHS / HPS suspension was determined through the courtesy of Sanna Labs. Faisalabad, using 200-day-old broiler chicks reared in the Department of Vet. Microbiology, University of Agriculture Faisalabad. At the age of 15 days, the chicks were vaccinated routinely for NDV and IBD virus at day 7th and 10th, respectively.

- Group A was vaccinated with commercially available formalin killed HPS vaccine (0.3 mL s/c) at the age of 15 days (single dose).
- Group B was inoculated with modified oil-emulsion vaccine (0.1 mL s/c) at the age of 15 days (single dose).

- Group C was inoculated with a commercially available oil-emulsion vaccine (0.1 mL s/c) at the age of 15 days (single dose).
- Group D was treated as unvaccinated control group.

Serum samples were collected from the experimental birds at 15, 22, 29 and 36th day of age. The efficiency of vaccines was determined on the basis of humoral antibody response (Rahman *et al.,* 1994) and protection against hydropericardium syndrome agent. The geometric mean titre (GMT) was calculated by Burgh method (1978).

All birds from each group were challenged with virulent HHS / HPS virus at 36th day of age. Mortality / morbidity data were also recorded.

Both the vaccines were also evaluated on the basis of cost effectiveness ratio, which includes the total cost of vaccine production and their significance with respect to efficacy.

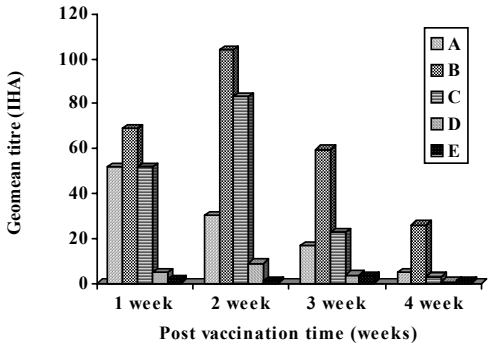
RESULTS AND DISCUSSION

In the prevaccination stage, all the experimental chicks were found to possess minimum antibody titre against HPS ranging from 1:4-1:8 the GMT was 4.3. At pre-vaccination stage, the IHA antibody titre against IBD was 97.0 (GMT). The highest antibody titre was due to the fact that the birds were routinely vaccinated against IBD at (10 day of age) and NDV (at 7 days of age).

Indirect hemagglutination antibody titre against HPS in group B that was vaccinated with modified oil emulsion vaccine showed higher titres particularly when compared with group B and C (Fig. 1).

The IHA antibody titre against HPS in group B increased significantly at 2nd week post vaccination (GMT, 104.0), it was much higher titre recorded at 1st week post vaccination (Table II). It may be due to deport theory of adjuvant action, which has been proposed for water in oil emulsion (Freund *et al*., 1937). The depository adjuvant hold the antigen at the deposition site, delay its absorption and subsequently released antigen which behaves as a

Fig. 1. IHA Ab titre in different groups of broiler chicks vaccinated against HPS/HHS



secondary stimulates to the sensitising action of the antigen released either (Frost & Lance, 1978). In this way, there is a marked and persistent induction of lymphocyte resulting in an enhanced and prolonged immunity.

The IHA antibody titre against HPS in groups A and C were significantly low as compared to group B at 2nd week post vaccination. At 3rd week post vaccination, the IHA antibody titre in all groups were decreased considerably but significantly higher GMT was recorded in group B as compared to other groups. There was very low GMT in group A (4.9) and group C (2.8) at 4th week post vaccination. But there was a high IHA antibody titre recorded in group B (GMT, 26.0) as compared to group A and group C.

The control / unvaccinated (group D) chicks did not show any significant IHA antibody titre against HPS during the whole period of study. Taking IHA titre of less than 1:8 as negative (Afzal & Ahmad, 1990), the control birds were considered as negative for HPS antibody throughout the entire experimental period.

After challenge / protection experiment, 100% of the control birds died at different days post challenge. The birds died of challenge infection showed typical HPS lesions. Most of the deaths were recorded on 2nd and 3rd day post

Table II. Indirect hemagglutination Antibody titre against different HPS / HHS vaccines in broiler chicks

Sample No.	IHA Ab. Titre against different HPS vaccines at weekly intervals															
	Group A (formaline killed vaccine)				Group B (Modified OE-HPS)				Group C (commercial OE-HPS)				Group D (control / unvaccinated)			
	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
1	32	64	32	4	256	64	32	16	16	256	64	4	4	-	4	4
2	32	32	16	4	256	256	32	16	1238	128	16	8	4	-	64	2
3	64	128	16	8	64	256	64	64	64	256	8	4	-	-	-	-
4	128	128	16	8	128	256	128	32	32	128	8	2	4	-	-	-
5	64	64	16	4	32	256	32	32	256	64	8	4	4	4	-	4
6	32	8	8	16	64	128	32	8	64	64	64	-	-	2	32	2
7	16	8	16	8	64	64	64	8	32	32	16	4	2	2	4	-
8	256	16	32	8	32	64	256	32	32	64	32	-	-	4	-	-
9	128	32	32	-	69	32	128	64	128	16	64	2	2	-	4	-
10	16	8	8	2	16	32	32	16	16	32	32	4	-	-	2	-
GMT	52.0	29.9	16.9	4.9	64.0	104.0	59.7	26.0	52.0	73.3	22.6	2.8	2.0	0.8	3.5	0.8

challenge as three out of five challenged birds in the unvaccinated / control (group D) died on these two days.

Vaccination with formalin killed, (group A) HPS vaccine showed 40% mortality and in group C, there was 20% mortality recorded upto day 7 post challenge. In contrast, group B vaccinated birds survived challenge infection throughout the whole challenge experiment and the protection in this group was 100%. This was highly significant from other groups. Over all, antibody titre in group B and C were much better than in group A, which was vaccinated with simple formalin inactivated vaccine.

While in the oil emulsified vaccinated groups (B and C), there was comparatively much better antibody response observed in broiler chicks vaccinated with modified oil emulsified vaccine (group B). This may be due to the fact that our modified oil emulsion vaccine consisted of field outbreak causing HPS virus and the concentration was (LD50 = 106.08 / mL) which was reasonably high as compared to commercially available oil emulsion vaccine. So, oil emulsion vaccine (modified) had shown better and long lasting protective effects than rest of the vaccines. Therefore, it was concluded that commercial oil based vaccine under study and formalin killed vaccine gave almost equal immune response and their titres became low after some days within the most susceptible age of the disease (3-5 weeks). Whereas, the modified oil emulsion vaccine gave protective levels for longer period of the time. The dose of OE-HPS vaccine (modified) was 0.1 mL in contrast to already suggested vaccine (Hussain *et al*., 1996) which was 0.5 mL.

CONCLUSION

The oil emulsion HPS / HHS vaccine in the present study was found much economical in terms of per dose cost of vaccine as well as it helps to promote better humoral antibody response in broiler chicks. Hence, it is concluded that the present modified OE-HPS / HHS vaccine may be recommended in the field trails with all the above factors which are related to the quality of an effective and successful vaccine against HPS / HHS in broilers and broilers breeders.

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