EFFECT OF MAREK'S DISEASE VACCINATION IN DAY-OLD BROILERS

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ABSTRACT

A study was conducted to evaluate the utility of Marek's disease vaccination in day-old broilers reared under local conditions. Day-old broilers were vaccinated with Rispen strain vaccine, HVT or combination of HVT and Rispen and were observed for weight gain, mortality and humoral and cell mediated immune responses. Mortality rate did not differ among any of the vaccinated groups and unvaccinated controls. Marek's disease vaccination had no effect on the titre of the Newcastle disease vaccine. Similarly cell mediated immune response of different groups were also non-significant as measured by reaction to phytohaemagglutinin. The study shows that vaccination against Marek's disease in day-old broilers does not offer any advantage under local poultry farming practices.

Keywords: Marek's disease, day-old, broilers, vaccination effects

INTRODUCTION

Marek's disease is a lymphoproliferative infection of chicken caused by Herpes virus. The disease exists in all major poultry producing countries of the world and is associated with heavy economic losses (Calnek and Witter, 1997). Marek's disease has also been reported from Pakistan in breeders, layers and broilers (Siddique *et al.*, 1987; Anjum, 1997). Marek's disease is typically controlled by vaccination of day-old chick at hatchery and this practice has significantly reduced the economic losses caused by the disease (Okazaki *et al.*, 1970, Rispen *et al.*, 1972; Kreager, 2000).

Vaccines based on all three serotypes of the virus (individually or in combinations) and recombinant DNA vaccines have been developed. Rispens strain (CVI-988) represents serotype 1, SB-1 strain belongs to serotype 2 and HVT (FC-126) falls in serotype 3 group (Oei and de Boer, 1986, Payne, 1996). Recombinant vaccines using fowl pox virus and expressing gB gene of serotype 1 Marek's disease virus have been developed (Nazerian *et al.*, 1992; Liu *et al.*, 1999). However, field studies on comparison of these recombinant vaccines with already existing vaccines have still not been carried out.

Marek's disease vaccine is routinely used in breeders and commercial layers in all major poultry producing countries of the world (Kreager, 2000). However, utility of Marek's disease vaccination in broilers is equivocal. Some workers have supported the day-old vaccination of broilers against Marek's disease and have reported improved survival and growth rates (Oei and de Boer, 1986) while others did not find any advantage (Vielitz, 1987; Miles *et al.*, 1992; Sarma *et al.*, 1995). Marek's disease vaccination of day-old broilers is commonly practiced in USA and some other countries (Calnek *et al.*, 1983; Rosenberger *et al.*, 1999). Some commercial vaccine companies and poultry experts are propagating the day old vaccination in broilers in Pakistan also. The present study was carried out to determine if Marek's disease vaccination in day-old broilers could improve production performance and health status of chicken under local management conditions.

MATERIALS AND METHODS

Two hundred, day-old broiler chicks were randomly divided into four equal groups at a commercial hatchery (SB Poultry, Rawalpindi). At hatchery on day-1, group-A was vaccinated against Marek's Disease using Rispen strain, group-B with HVT (apathogenic strain of Herpes virus of Turkey) and group-C with both HVT and Rispen. Group-D was left as unvaccinated control.

Birds were brooded under electric brooders and reared in separate pens at laboratory animal facility of the institute. Commercial poultry feed i.e. broiler starter for first four weeks and finisher ration for rest of the period of the experiment was fed. Feed and water was given *ad libitum*.

Vaccination schedule for other diseases was uniform for all groups and included: Newcastle disease (ND) vaccine (TAD, Germany) via eye drop on day-7, Gumboro disease (IBD) vaccine (Bursine-2, Solvay) via drinking water on day-13 and hydro pericardium

syndrome (HPS) vaccine (Bio-Lab., Rawalpindi) subcutaneously at day-18. Booster vaccination was carried out at day-23 and 28 for IBD and ND via drinking water, respectively.

Five birds from each group were slaughtered on day-5 for serum collection to evaluate the maternal antibody titre. Later on blood samples from wing vein were collected from 15 randomly selected birds from each group at 20, 42 and 49 days of age, in sterile disposable syringes. Sera were separated and stored at -20°C till further use.

Ten chicks from each group were randomly selected and weighed at weekly intervals throughout the study period. Experiment was terminated at 49 days of age when all the remaining birds were weighed and slaughtered.

Antibody titre for Newcastle disease virus were carried out using haemagglutination inhibition test using 10 HA units in microtitre plates (Beard, 1989). Geo-mean titres (GMT) were calculated.

Cellular immune response was assessed twice during the course of the experiment by injecting 75 µg (0.1 ml) of phytohaemagglutinin-P (PHA-P, Sigma) in left wattle of seven birds from each group on day 40 and 47 of the experiment. Phosphate buffered saline solution (0.1 ml) was injected in right wattle as a control. Thickness of both the wattles were recorded at 0, 24 and 48 hours post inoculation with the help of Vernier callipers. The results were expressed in units; one unit being equal to 0.5 mm increase in thickness in PHA injected wattle as compared to control wattle. Statistical analysis was carried out using analysis of variance. Means were compared using Tukey's test.

RESULTS

Day-old broilers received from the hatchery were healthy with an average weight of 46.5 gm per bird. Only six out of 250 birds died during the experimental period of seven weeks. One bird from group C (Rispen + HVT vaccinated) died on 2nd day of experiment and had the lesions of septic yolk/omphalitis. Two more birds died from group C, one at day 11 with septicaemia lesions (*Staphylococcus aureus* and *Proteus spp.* were isolated from the liver) and the other bird died on day 33 with the lesions of HPS. Only one bird died from group A (Rispen vaccinated) with respiratory lesions on day 22 while moribund birds at day 42 was slaughtered and had lesions of Newcastle disease. A moribund bird from group B (HVT vaccinated) slaughtered at day 39 also had the lesions of Newcastle disease. All birds of the un-vaccinated control group remained healthy. Non-significant differences in mortality were seen among all vaccinated and unvaccinated controls suggesting no effect of Marek's disease vaccination in day-old broilers on livability of the chicken.

Weight of the birds of all groups during 7 weeks period is shown in Fig 1. Non-significant differences were seen in weight of birds among all four groups indicating no effect of Marek's disease vaccination on chicken weight gain.

Pre and post vaccination antibody titres against Newcastle disease virus did not differ significantly among different groups (Table 1). Low antibody titres were recorded in group A at day 20 compared with other groups. However, this variation in antibody titre was negligible at day 42. The GMT of 12 was recorded in group B at day 49 which was higher than the titres seen in other groups.

Table 1: Immune response of birds against Newcastle disease vaccine as determined by haemagglutination inhibition test (Geomean titres).

Group (vaccination)	Age of chicken (Days)			
	5	20	42	49
A (Rispen)	10	12	8	7
B (HVT)	15	37	9	12
C (Rispen & HVT)	17	30	10	6
D (Control)	15	25	9	6

The cell mediated immune (CMI) response of the birds as determined by increase in thickness after injecting PHA-P (75 µg/bird) in wattles at day 40 and 47 is presented in Table 2. The CMI response was less in group A (Rispen vaccinated group) at day 40. However, minor differences in CMI response were noted at day 47. The unit increase in thickness of wattles was non-significant for vaccinated and non-vaccinated groups indicating similar cellular immune response capability in all groups.

DISCUSSION

Protection of chicken against Marek's disease is of utmost importance for commercial poultry farming. The disease not only results in direct losses but can also be immunosuppressive making the birds susceptible to other infections. Protection against Marek's disease can be achieved by breeding chicken for genetic resistance, practising bio-security principles and vaccination (Calnek and Witter, 1997; Kreager, 2000). Although Marek's disease vaccination is routinely carried out in breeders and layers in Pakistan, this practice is not in

vogue in broilers. Use of Marek's disease vaccination in broiler in some countries, particularly USA (Rosenberger *et al.*, 1999), prompted us to see whether this practice will be useful under local conditions. We used four parameters to evaluate the usefulness of vaccination including body weight, overall mortality and humoral and cell medicated immune responses. Similar parameters have also been used by other workers to evaluate the usefulness of vaccination in a given situation (Bacon and Witter, 1982; Witter, 1987).

Marek's disease vaccination in broilers did not seem to offer any advantage under local managemental conditions. Vaccination in day-old broilers did not improve weight gain, reduce mortality or enhance humoral and cellular immune competence of the birds. These findings corroborate the results of Vieltiz (1987), Miles *et al.* (1992) and Sarma *et al.* (1995) who also showed that weight gain and mortality did not improve following Marek's disease vaccination. Marek's disease is occasionally reported in broilers which has a shorter life (6 to 8 weeks). Broilers from unvaccinated parents if experimentally inoculated on day 1 with virulent Marek's disease virus showed clinical signs and gross lesions in 3 or 4 weeks (Calnek, 1972) In fact, incubation period of Marek's disease depends upon virus strain, dosage, maternal antibody status, route of infection, age, genetic strain and sex of the host (Witter *et al.* 1980; Witter, 1982).

Thus Marek's disease is rarely seen in broiler birds which normally come from immune parents and are only raised for 6-8 weeks. Furthermore, establishment of solid immunity following vaccination requires at least 7 days (Basarab and Hall, 1976), so if an early heavy field virus exposure is present, vaccination may not be able to protect the birds. Marek's disease vaccination is usually practiced at farms with multi-age broiler operation and where new chicks are placed on old litter exposing the birds to early exposure (Calnek and Witter, 1997).

The present study shows that vaccination against Marek's disease in day-old broilers does not offer any advantage under local poultry farming practices. This is also supported from field observations in which more than 100,000 broiler birds were vaccinated with Marek's disease vaccine at day-old and no difference in mortality rate or weight gain was observed compared to unvaccinated birds raised simultaneously at the same premises.

However, effect of Marek's disease vaccination at farms with high previous incidence of the disease and multiple age groups needs to be studied.

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Table 2: Comparison of cell-mediated immune response following Marek's disease vaccination at day-1 in broilers

Age	Time post Inoculation	Unit increase in wattle thickness*			
		Group-A (Rispen)	Group-B (HVT)	Group-C (HVT+Rispen)	Group-D (unvaccinated)
Day-40	24 hours	5.4±2.1*	8.0±1.2	9.0±2.2	9.1±1.9
	48 hours	2.6±1.1*	5.1±1.4	4.7±2.6	4.7±1.6
Day-47	24 hours	5.1±1.1	5.6±1.7	3.4±0.5	4.9±1.5
	48 hours	2.1±1.5	2.0±1.2	1.6±1.0	1.6±1.0

* = CMI was determined by difference in increased thickness of wattle by injecting PHA-P compared with saline control. Each unit represents 0.5 mm increase in thickness over saline control.

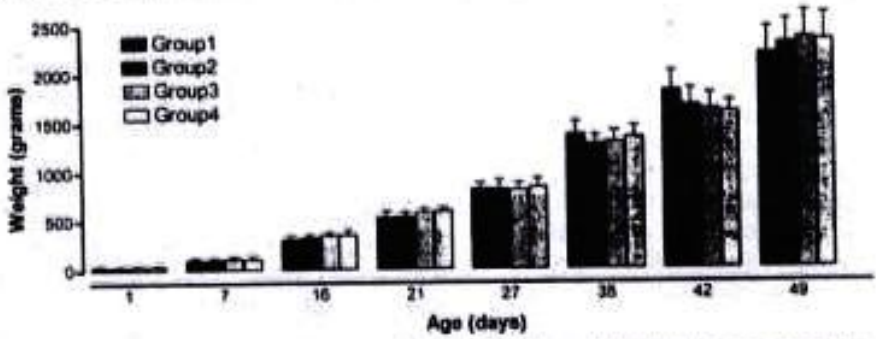

Fig. 1: Average weight of birds vaccinated with different Marek's disease vaccines.

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