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EFFECTS OF LOCAL GAMETOCYTE AND LIVACOX VACCINES ON LIVE BODY WEIGHT GAIN AND LYMPHOID ORGANS IN CHICKENS

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ABSTRACT

Organ to body weight ratio in chickens is considered to be an important parameter to study the immune status due to any infection or vaccination. The present paper reports the effects of local gametocyte and Livacox vaccines on live weight gains and lymphoid organs in chickens. The mean body weight gains in chickens with local gametocytes vaccine were significantly better ($P<0.05$) than in chickens with Livacox vaccine. Higher organ to body weight ratio was recorded in vaccinated chickens compared to control, although difference was non significant. Organ to body weight ratio of the lymphoid organs had higher values in chickens vaccinated with Livacox compared to local gametocyte vaccinated group, the difference was also found to be non significant. It was concluded that local gametocyte vaccine significantly increased the body weights of chickens compared to Livacox and control groups.

Key words: Gametocyte vaccine, Livacox, chickens, lymphoid organs, body weight.

INTRODUCTION

Several commercial vaccines are being used to control coccidiosis in different countries of the world. There has been a general limitation to use these vaccines in broiler and high roster birds because of reduced weight gain and feed conversion ratios compared to those for prophylactically medicated chickens (Shapiro, 2001; Crouch *et al*., 2003). Moreover, there is a risk of introducing unwanted *Eimeria (E.)* species into the environment as there is a regional variation in the antigenicity of coccidial strains (Martin *et al*., 1997). Also, certain strains of *Eimeria* exhibit immunological variation (Lee, 1993) and the presence of such strains in the live vaccines can affect their efficacy in terms of cellular and humoral responses. It may also affect the lymphoid organs in the chickens. In our previous studies, local gametocyte (*Eimeria tenella*) vaccine produced significantly higher cellular, humoral and mucosal immune responses and protected the chickens against mixed species of coccidia (Hafeez *et al.*, 2006; Ayaz *et al*., 2008). The present paper reports the effects of local gametocyte and Livacox vaccines on the lymphoid organs in chickens.

MATERIALS AND METHODS

Isolation of parasite stages

Sporulated oocysts of *E. tenella* (local isolates) maintained in Immunoparasitology Laboratory, University of Agriculture, Faisalabad, Pakistan were processed for excystation followed by ex-sporocystation to release sporozoites (Speer *et al*., 1973). The sporozoites were given two washings with phosphate buffered saline (PBS), their concentration was adjusted to $1.8x10^3$- $2x10^3$ per 0.1 mL and stored at 4°C till further use.

Two hundred chicken embryos (9 days old) procured from local hatchery were maintained at 39°C and 70% humidity in an incubator. Candling was performed to confirm the viability of the embryos. At 12 days of age, 0.1 ml suspension of sporozoites was inoculated into the chicken embryos through chorio-allantoic membrane along with penicillin (2000 IU) and streptomycin (0.05 mg). The embryos were maintained at 39°C and 70% humidity for 5-7 days (Akhtar *et al*., 2002). On day 5^{th}-7^{th} post-inoculation, chorio-allantoic fluid was collected from dead embryos to harvest the gametocytes (Hafeez, 2005). Gametocytes were concentrated by centrifugation (14500 g for 5 minutes), washed twice with sterile PBS and stored at 4^oC till further use.

Vaccine formulation

Gametocytes vaccine was prepared following the method of Hafeez (2005). Briefly, gametocytes (*E. tenella*; local isolates) were homogenized by sonication (1x3 minutes; Nissei, Model US 330, Japan) in a jacketed vessel at 4-8^oC. Supernatant thus collected after centrifugation (9500g for 15 minutes) was used as vaccine; its protein concentration was measured (Bradford, 1976) and adjusted to 500 µg/0.2 ml with PBS; each vaccine dose contained 500 µg of protein (Ayaz, 2003).

Experimental chicks

A total of 200 chicks (Hubbard) purchased from local market were raised under standard management conditions (coccidian free environment) at the

Experimental Station, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. At 5th day of age, chickens were divided into three groups (A, B and C; 60 each). On 10th day of age, chickens in groups A and B were inoculated orally with gametocytes and Livacox vaccines (BioPharm, Czech Republic), respectively; while group C served as control (given PBS only)

On day 5th and 14th post vaccination, 10 chickens (each time) selected randomly from each group were weighed and slaughtered; lymphoid organs including spleen, caecal tonsils, bursa of fabricius and thymus were removed and weighed.

On 15th day post vaccination, the remaining chickens were given the booster dose of the respective vaccine and control chickens were given PBS. On day 21st post vaccination, 10 chickens selected randomly from each group were weighed and slaughtered; their lymphoid organs were removed and weighed.

The remaining chickens in all the groups were challenged with 65,000 sporulated oocysts of mixed species of genus *Eimeria* (mainly *E. tenella*, *E. acrervulina*, *E. maxima* and *E. necatrix*; local isolates). Lymphoid organs from survived and dead chickens were also collected.

Results were expressed as per cent organ weight relative to the body weight. Live weight gains in each group were also recorded from day first to 21 post vaccination, on every third day. Data thus collected was analyzed statistically by using MINITAB^R statistical package.

RESULTS AND DISCUSSION

In chickens, a variety of specialized lymphoid organs, including caecal tonsils, bursa of fabricius, thymus and spleen play significant role in immunity against harmful intestinal pathogens (Lillehoj and Lillehoj, 2000). Their organ to body weight ratio is considered to be an important parameter to study the immune status due to any infection or vaccination. Coccidiosis had adverse effects on the immunity of the host by somehow having a direct or indirect effect on the development of lymphoid organs in chickens (Akhtar and Zafar, 2003; Rashid, 2004). There are reports of either no effect on the weights of lymphoid organ (Augustine and Thomas, 1979) or an increased weight of lymphoid organs (Venkatratnam *et al*., 1985) due to coccidial infection/vaccination. Further, effects of anti-coccidian vaccine on live weight gains of commercial chickens are also considered an important papameter to recommend any vaccine for commercial use.

In the current study, effect of vaccines on live body weight gains was recoded, from day 1 to 21 post vaccination, on every third day. On day 1-21 post vaccination, mean body weight gains in chickens given local gametocytes vaccine were significantly better ($P<0.05$) than in chickens given the Livacox vaccine or control (Table 1). The lower weight gains in Livacox vaccinated chickens may be due to the presence of three different antigens that may impose a stress on the growing birds (Bedrnik and Kucera, 1988). Further, it has been observed that use of live oocyst vaccines resulted in some loss of body weight, and consequently reduced feed conversion (Kopko, 1998) that is undesirable in the broiler industry, limiting the use of these vaccines to replacement birds.

Results of the effects of local gametocytes and Livacox vaccines on lymphoid organs in comparison with the control are presented in Table 2. Apparently higher organ to body weight ratio was recorded in vaccinated chickens compared to control, although the difference was non significant ($P>0.05$). Further, organ to body weight ratio of the lymphoid organs had higher values in chickens vaccinated with Livacox compared to local gametocyte vaccinated group, the difference was also non significant ($P>0.05$).

Upon challenge with mixed species of genus *Eimeria*, a decrease in organ to body weight ratio in control group compared to local gametocyte and Livacox vaccinated chickens was observed. This might have been due to micro-necrosis in the lymphoid organs or cellular migration from lymphoid organs (Olariu-Jurca *et al*., 1994).

It was concluded that local gametocyte vaccine significantly increased the body weights of chickens compared to Livacox and control groups. There was a comparable effects of local gametocyte vaccine with that of Livacox on lymphoid organs in chickens.

Table 1: Weight gains (g ± SE) in chickens of three groups from day 1 to 21 post vaccination (PV)

Days (PV)	Group A (Gametocyte vaccine)	Group B (Livacox vaccine)	Group C (Control)
1	228.9 ± 8.16^a	212.6 ± 8.32^b	214.3 ± 6.46^b
3	369.3 ± 16.8^a	344.9 ± 13.9^b	341.6 ± 12.2^b
6	511.4 ± 10.2^a	473.0 ± 6.67^b	475.2 ± 6.17^b
9	605.0 ± 9.60^a	586.2 ± 10.6^b	584.2 ± 3.73^b
12	712.6 ± 6.88^a	667.2 ± 9.64^b	660.2 ± 7.12^b
15	791.6 ± 6.80^a	765.2 ± 12.8^b	702.8 ± 6.99^b
18	896.0 ± 7.72^a	865.4 ± 12.4^b	871.8 ± 9.13^b
21	1007.0 ± 9.04^a	973.2 ± 14.4^b	963.8 ± 10.3^b

Values with different superscripts in a row are significantly different from each other ($p<0.05$).

Table 2: Organ to body weight ratio on day 5th, 14th and 21st post vaccination (PV)

Days PV	Groups	Spleen	Ceacal tonsil	Bursa Fabricius	Thymus
5	A	0.109 ± 0.02	0.093 ± 0.02	0.121 ± 0.02	0.152 ± 0.01
	B	0.110 ± 0.04	0.102 ± 0.02	0.123 ± 0.02	0.156 ± 0.01
	C	0.108 ± 0.05	0.099 ± 0.07	0.121 ± 0.03	0.149 ± 0.01
14	A	0.121 ± 0.02	0.108 ± 0.02	0.129 ± 0.02	0.206 ± 0.01
	B	0.123 ± 0.03	0.109 ± 0.02	0.131 ± 0.01	0.208 ± 0.02
	C	0.120 ± 0.04	1.109 ± 0.05	0.130 ± 0.01	0.205 ± 0.02
21	A	0.128 ± 0.02	0.143 ± 0.02	0.145 ± 0.02	0.216 ± 0.01
	B	0.131 ± 0.01	0.149 ± 0.01	0.141 ± 0.03	0.218 ± 0.01
	C	0.129 ± 0.02	0.151 ± 0.02	0.139 ± 0.01	0.217 ± 0.02
Post challenge	A	0.176 ± 0.03[a]	0.173 ± 0.02[a]	0.184 ± 0.02[a]	0.274 ± 0.01[a]
	B	0.182 ± 0.02[a]	0.185 ± 0.01[a]	0.189 ± 0.02[a]	0.283 ± 0.01[a]
	C	0.143 ± 0.02[b]	0.154 ± 0.02[b]	0.156 ± 0.01[b]	0.243 ± 0.02[b]

Values with different superscripts in a column are significantly different from each other ($p<0.05$).

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