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EFFICACY OF FORMALIN KILLED FOWL CHOLERA VACCINE IN EXPERIMENTALLY IMMUNIZED FAYOUMI CHICKENS

M. K. Rahman, M. B. Rahman, M. N. A. Siddiky, M. A. Kafi, M. A. Islam[1] and K. A. Choudhury

Department of Microbiology and Hygiene and Department of Medicine[1], Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh – 2202, Bangladesh

ABSTRACT

Efficacy of experimentally prepared formalin killed fowl cholera vaccine in Fayoumi chickens via different routes of vaccination was determined during the period from April 2002 to March 2003. *Pasteurella multocida* (PM-38) serotype 1 (X-73) was employed for vaccine preparation and antibody titres of the chicken sera were determined by passive haemagglutination (PHA) test. Vaccination was done either intramuscularly or subcutaneously. Each of the experimental chickens was challenged with a virulent isolate of *P. multocida* @ 3.8 x 10^8 CFU / ml per bird intramuscularly. The 100% vaccinated chickens protected against virulent *P. multocida* infection but all (100%) unvaccinated control birds died within 72 hours of challenge. Intramuscular (both primary and booster) route of vaccinations was found superior and more effective than subcutaneous route of inoculation. The higher PHA antibody titre was recorded with intramuscularly (222.86 ± 25.60) than subcutaneously (111.43 ± 12.80) vaccinated groups of birds. The result revealed the fact that intramuscular route followed by subcutaneous inoculation could be done for immunization against fowl cholera in chickens.

Key words : Efficacy, fowl cholera vaccine, formalin killed, Fayoumi chickens

INTRODUCTION

Fowl cholera is one of the most important contagious bacterial diseases of poultry caused by *Pasteurella multocida*. It occurs sporadically or enzootically in most countries of the world including Bangladesh. It causes mortality about 25 to 35% in chickens of Bangladesh (Choudhury *et al.*, 1985). Vaccination as a means of controlling infectious diseases of animals and birds is now a universal approach. Both humoral immunity (HI) and cell mediated immunity (CMI) are considered to be of primary importance in the protection of animals and birds against infectious diseases (Collins, 1977; Mondal *et al.*, 1988). The immune responses (Mondal *et al.*, 1988; Choudhury *et al.*, 1990), efficacy of oil adjuvanted broth culture (Choudhury *et al.*, 1987), and alum precipitated (Khan *et al.*, 1994; Islam *et al.*, 2004) and comparative efficacy of different fowl cholera vaccines (Choudhury *et al.*, 1988) have been evaluated under local conditions (Samad, 2000). However, a standard titre of fowl cholera vaccine per field dose of inoculum is important for obtaining dependable immunity against the disease. This paper describes the efficacy of a formalin killed fowl cholera vaccine in Fayoumi breed of chickens with their antibody responses.

MATERIALS AND METHODS

Ten weeks old (no. 25) Fayoumi chickens used for this study were purchased from the BAU Poultry Farm, Mymensingh on 10th April 2002. These birds were maintained in the poultry experimental house of the Department of Microbiology and Hygiene during the period from 10th April 2002 to 15th March 2003. *Pasteurella multocida* (PM-38) serotype 1 (X-73) was obtained from the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh, which was used for the preparation of formalin killed fowl cholera vaccine as well as challenge virulent organisms. These birds were divided into five experimental groups, (A, B, C, D & E) each consisting of 5 chickens, which were maintained in separate cages. Each of the birds of group A, B, C and D was vaccinated with 1.0 ml of experimentally prepared formalin killed vaccine, through IM, IM, SC, and SC route, respectively. Booster vaccination was done in group A, B, C and D with 1.0 ml of same vaccine through IM, SC, SC and IM route respectively. Birds of group E served as unvaccinated control throughout the experimental period.

The immune response was studied by using growth inhibition test (GIT), Passive haemagglutination assay (PHA) and protection test to determine the presence of antibody against *P. multocida* in the serum of chickens immunized with formalin killed fowl cholera vaccine. GIT and PHA were conducted according to the procedure described by Tripathy *et al.* (1970), Siddique *et al.* (1997) and Islam *et al.* (2004).

The protection test was conducted on both vaccinated and unvaccinated groups of chickens with individual dose rate of 1.0 ml of *P. multocida* bacteria (3.8×10^8 CFU / ml) through intramuscular route as described by Choudhury *et al.* (1985), Khan *et al.* (1994) and Islam *et al.* (2004). The chickens were observed for one month in every 12 hours interval.

RESULTS AND DISCUSSION

The antisera treated culture of virulent *P. multocida* was inoculated to blood agar and nutrient agar plates. No growth of bacteria on the plates after 24 hours incubation at 37^{0}C which indicated positive GIT for vaccinated chickens, but growth was observed in case of control sample that indicated negative GIT for unvaccinated chickens (Table 1).

Table 1. Growth inhibition test (GIT), antibody response and survivability of chickens immunized with formalin killed fowl cholera vaccine

Group	No. of birds used	Route of vaccination		Pre-vaccination	Post-vaccination after 2 weeks						Survivability
					Primary		Booster		Challenge		
		Primary	Booster	Ab. titre	GIT	Ab. titre*	GIT	Ab. titre*	GIT	Ab. titre*	No. (%)
A	5	IM	IM	4	+	32.00 ± 0.00	+	**222.86 ± 25.60	+	445.72 ± 51.20	5 (100)
B	5	IM	SC	4	+	32.00 ± 0.00	+	**168.89 ± 31.35	+	388.02 ± 62.70	5 (100)
C	5	SC	SC	4	+	27.86 ± 3.20	+	**111.43 ± 12.80	+	256.00 ± 00.00	5 (100)
D	5	SC	IM	4	+	27.86 ± 3.20	+	**128.00 ± 00.00	+	337.79 ± 62.70	5 (100)
E	5	–	–	4	–	–	+	–	+	–	0 (000)

IM = Intramuscular route, SC = Subcutaneous route, + = Unable to grow in culture media, – = Able to grow in culture media, *Mean ± SE, **Significant at $p < 0.01$.

The mean antibody titres of primary vaccination, booster vaccination and challenge exposure were 32.0 ± 0.0, 222.86 ± 25.6 and 445.72 ± 51.2 respectively, when the chickens of group A vaccinated through IM route in both primary and booster vaccination (Table. 1). Similarly the mean antibody titres of group B were 32 ± 0.0, 168.89 ± 31.35, and 388.02 ± 62.70 after primary, booster and challenge exposure through IM and SC respectively. The serum mean antibody titres of chickens of group C were 27.86 ± 3.2, 111.43 ± 12.80 and 256.0 ± 0.0 after primary vaccination, booster and challenge exposure through SC respectively. In the group D, chickens were vaccinated through SC at primary vaccination followed by IM route at booster vaccination. The post-vaccination serum mean antibody titres of group D after primary vaccination, booster vaccination and challenge exposure were 27.86 ± 3.20, 128 ± 0.0 and 337.79 ± 62.70 respectively (Table. 1).

The mean antibody titres of different routes of vaccination indicated that IM route of vaccination induced better results in respect of protection against experimental challenge infection and higher antibody titre than SC route of vaccination. All the four groups of vaccinated chickens induced significantly higher antibody titre in comparison with their pre-vaccination antibody titer. The findings of this experiment partly correlated with the results of Leonchuk and Tsimokh (1976) who reported that the immunogenicity of the vaccine depended on the method of vaccination and IM route gave stronger and long lasting immunity than SC route. The chickens received booster dose of vaccine induced significantly ($p < 0.01$) higher antibody titre than the chickens of primary vaccinated groups. The titre becomes peak level after two weeks of challenge exposure (Table. 1) Wu *et al.* (1986) observed that two inoculations provided better immunity that a single inoculation. The administration of booster dose of same vaccine induced a high level of antibody and protective immunity with no adverse reactions has been reported by Schlink *et al.* (1987) and Choudhury *et al.* (1987). The challenge dose used for protection test was 3.8×10^8 CFU / ml *P. multocida*. Chickens of all the four vaccinated groups were protected against virulent *P. multocida* challenge. But all the unvaccinated control chickens died within 72 hours after challenge which has shown in Table 1.

Therefore, it may be suggested that to prevent and to reduce the occurrence of fowl cholera, the formalin killed fowl cholera vaccine prepared with highly antigenic strain of *P. multocida* should be used to provide better protection against the epidemic of fowl cholera in poultry and it is also advised to practice IM route in both primary and secondary vaccination. However, further study with large number of chickens to determine the efficacy of routes of vaccination is necessary to conclude about the present study.

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