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EVALUATION OF THE EFFICIENCY OF INACTIVATED MYCOPLASMA GALLISEPTICUM (FORMALINE KILIED ADJUVANT) VACCINE

BY

M. REFAI, A. EL-EBEEDY, A.G. HEGAZI, G.O. EDRIS, AIDA, M. ABDEL AZIZ AND NAWAL ALLAM

Faculty of Vet. Med. Cairo University Animal Health Research Institute, Dokki and National Research Centre, Egypt.

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INTRODUCTION

Mycoplasma gallisepticum (M.G.) infection in poultry can result in severe economic losses. During last few years many types of drugs and control measures were used for *M. gallisepticum* (Chalquest and Fabricant, 1959; Barnes et al., 1960; Levine and Fabricant,1962; Olson et al., 1962, while others used live vaccine (Luginbuhl et al., 1967, Zuzuki et al., 1971; Jordane et al., 1981; Chnabra and Goel, 1982 and Glisson and Kleven, 1983) as a method of control. Also many others as Adler et al. (1960); Adler and Da Silva (1970) ; Takemitsu et al. (1970), and Kuniyasu and Yoshida (1971) attempted to obtain protection by Killed M.G. either by plane vaccine or by vaccine with adjuvant. Most reports concerning M.G. vaccines are contradictory, thus the aim of this investigation was to evaluate the efficiency of inactivated *Mycoplasma gallisepticum* in protection of chickens at various ages against experimental infection with virulent M.G.S_6 strain in Egypt.

MATERIAL AND METHODS

M.gallisepticum (M.G.S_6) strain and antiserum were kindly supplied by Dr. Freundt, FAO/WHO Mycoplasma Reference Lab., Aarhus Denmark and Stipkovits , Animal Health Research Institute of Hungarian, Academy of Science. The M.G.S_6 strain was propagated in P.P.L.O. broth (Frey et al., 1968). The propagated M.G. S_6 was used for preparation of the formalin killed adjuvant (Aluminium potassium sulphate, Fischer Sci. Company. U.S.A. inactivated vaccine(Hayatsu et al., 1975).

A total of 540 birds of different ages, one day (160), one week (120), 2 weeks (100), 4 weeks (80) and 42 days (80) were used in this study. These chicks were supposed to be free from Mycoplasma as evidenced by serological tests. and supplied from serologically negative flocks. They were obtained from the poultry Organization Company, Cairo. Each group was divided into 4 subgroups. The first three subgroups were vaccinated with inactivated M.G. vaccine (Hayatsu et al., 1975) in a dose of 0.2 ml/chick intramuscularly (I/M) subcutaneous (S/C) and intranasally (I/N) while the fourth subgroup was left as non-vaccinated control. Two weeks after vaccination all chicks including the cotrol was challenged with virulent M.G.S_6 strain by intranasal instillation (2×10^8 C.F. U/ml). All birds were examined for signs, 6 birds were killed every week for 5 weeks and their sera were collected and the birds were examined for P.M. lesions. All serum samples were examined by slide agglutination test (Adler et al., 1958) and haemagglutination inhibition test (Meszaros, 1964). The HI titre was determined by geometric mean and it was considered as suspecious at 1:16 and positive at 1:32 and more.

Table (1) : Slide agglutination and HI titre among sera of one week old chickens vaccinated with inactivated M.G.

Serological test	Slide agglutination					Haemagglutination Inhibition				
	Weeks post vaccination					Weeks post vaccination				
Groups	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
I/M	3/6	4/6	4/6*	5/6	5/6	1/8	1/32	1/64	1/128	1/128**
S/C	0/6	2/6	2/6	3/6	2/6	1/4	1/4	1/64	1/64	1/64
I/M	0/6	0/6	1/6	0/6	1/6	1/4	1/4	1/8	1/8	1/8
Control	0/6	0/6	0/6	0/6	0/6	1/2	1/4	1/8	1/8	1/8

* Number of positive / total examined number.

** The highest HI titre after 5 weeks post vaccination

Table (2) : The protection rate of chicks vaccinated at one week old with inactivated M.G. vaccine and challenged 2 weeks post vaccination

Groups	Route of Vaccination.	Live evaluation of protection			Post martum examination	
		No of clinical healthy birds/ total	Weight means at 5 week / gm	% protection	NO. of birds PM free/ total	% protection
1	I/M	14/L5	1/50	93.3 %	14/I5	93.3 %
2	S/C	15/15	1/40	100 %	13/15	86.6 %
3	I/N	10/15	1050	66 %	9/15	60.0 %
4	Control	0/15	1000	0.0 %	2/15	13.3 %

RESULTS

All the serum samples were examined by slide agglutination and HI tests. The results revealed that the best results were obtained when the vaccine was injected intramuscularly at one week old, the agglutinin started to appear one week after vaccination in few number of birds, however, the number of positive birds increased in subsequent weeks. There was no significant difference among birds vacciantед at different age groups but at one week old was the best. The I/M route of vacciantion was the best followed by S/C and I/N **(Table, 1)**. The protection rate reached 93.3% based on the evaluation of P.M. lesion or clinical signs **(Table, 2)**.

DISCUSSION

The results obtained of the present study indicate that the inactivated vaccine gave good rate of protection of birds at almost all ages from 1 day to 6 weeks, particularly when the vaccine was given intramuscularly or subcutaneously. Better results was obtained when the vaccine was injected at one week old chicks. The available literature indicates the low protective activity of the inactivated vaccine. Alder et al. (1960) produced a vaccine of killed Mycoplasma organisms by ultrasonic disintegration. In addition an adjuvant vaccine was produced by adding formalin and aluminium hydroxide to the disintegrated organisms. Little or no protection was induced with these vaccines given to young Cockerels intavenously, intramucularly or into the bursa of Fabricius. Warren, et al. (1968), Adler et al. (1960), Adler and Desilva (1970), Takemitsu et al. (1970) and Kuniyasu and Yoshida (1971) attempted to obtain protection by killed M.G either by plane vaccine or as vaccine with adjuvant and found only little effects.

The controversy regarding the inactivated vaccine of M.G. strain may be due to variation in immunogenicity of the organism, the type of adjuvant, the dose and the route of injection of the vaccine.

The route of vaccination with inactivated vaccine seems to play a role in the rate of protection of the vaccinated birds. The I/M and S/C routes gave the best results in this study. The results obtained by other authors as Adler, et al. (1960), Warren et al. (1968). Hayatsu et al. (1974). Adler (1976) , Hildebrand et al.. (1983) who reproted that one dose of the bacterin was capable of protecting chicks of verious ages from clinical signs following challenge with a virulent strain.

The application of the inactivated vaccine at different ages in the present study showed insignificant differences in protection against challenge with the virulent M.G. strain but the best protection was obtained when the vaccine was injected at on week old therefore, it would be preferable to vaccinate one week old chicks in order to acquire a resistance against natural infection as early as possible.

SUMMARY

The inactivated Mycoplasma galliseplicum vaccine mixed with aluminium sulphate adjuvant was used for vaccination of 540 chicks at different ages and different route, (7, 14, 28 and 42 days). After 2 weeks all of these chicks were challenged with M.G.S_6 strain($2x10^8$ colony forming U/ml).There was no significant difference among birds vaccianted at different ages but at one week old is the best. The I/M route of vacciantion was the best followed by S/C and I/N. The protection rate reached up to 93.3% based on the evaluation of P.M. lesions or clincial signs, the birds were kept for 5 weeks.

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