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STUDIES ON A LOCALLY PREPARED, TEMPERATURE SENSITIVE MUTANT MYCOPLASMA GALLISEPTICUM VACCINE IN COMPARISON WITH A COMMERCIALLY PREPARED VACCINE

By

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INTRODUCTION

Mycoplasma gallisepticum (M.G) is considered as one of the most important pathogens affecting poultry causing severe economic losses (Carpenter et al., 1980). Current measures to combat Mycoplasma gallisepticum infection have included flock testing, eradication programmes, use of antibiotics in both prophylactic and therapeutic regimes as well as vaccination (Adler, 1975, Aghakhan et al., 1976; Jordan et al.,1981 and Chnabra and Goel, 1982).

Vaccination has been considered as a method of control for many years (Jordan et al., 1981). Both live and inactivated vaccines have been shown to stimulate a protective response. Many authors pointed out that vaccination of fowls with live organisms of M.G. protect them against mycoplasmosis (McMartin, 1967; Jordan, 1975; Rodriquez and Kleven, 1980 and Glisson and Kleven, 1983), while others used attenguated vaccine by culture passages (Adler et al., 1960; Papageorgiou, 1970; Carpenter et al., 1980; Rodriguez and Kleven, 1980; Lin and Kleven, 1982 and 1983), or by mutation (Nonomura and Imada 1982; Lam and Lin, 1983 and Lam et al., 1984).

Thus the aim of this investigation was to compare and evaluate vaccine prepared locally from temperature-sensitive mutant (TSM) and commercially live lyophilized vaccine provided by a private company (Gallivac).

MATERIAL AND METHODS

Five hundred and sixty chicks at different ages were used. These chicks were obtained from the General poultry [illegible] Company, Cairo. They were supposed to be free from mycoplasmas as evidenced by serological tests and supplied from serologically negative flock. The chicks were divided into 6 subgroups (40 each) according to age (1, 7, 14, 28, 42 days) and control group. All chicks in the first 5 subgroups were vaccinated intranasally with 0.2 ml/chick temperature-sensitive mutant vaccine which was prepared according to Nonomura and Imada (1982) from the M.G. PG 31 strain (kindly supplied by Dr. Freundt, FAO/WHO Mycoplasma Reference Laboratory, Aarhus, Denmark). One week post vaccination the chicks including the control group were challenged by intranasal instillation with 0.2 ml of M.G. S_6 strain ($2x10^8$ CFU/ml). All birds were examined for clinical signs, sera were collected at the end of the experiment and tested by slide agglutination (Adler et al., 1958); haemagglutination inhibition (Menzaros, 1964), growth-inhibition (Clyde, 1964) and metabolic inhibition (Pureaud et al., 1966) tests. Also the birds were examined for P.M. lesions.

The second group: 320 chicks (160 one-day old chicks and 160 2-week old chicks) were vaccinated with Gallivac vaccine (living attenuated vaccine provided by a private company. Twenty chicks were kept as infected non-vaccinated control. The vaccine was applied to one half of chicks by spray and the other half was by drinking water. Two weeks after vaccination, 40 chicks of each group were challenged with virulent M.G.S_6 strain by intranasal instillation in a dose of $2x10^8$ CFU/ml. All chicks were observed for clinical signs and P.M. lesions. Sera were collected and tested serologically by SAT, HI, GIT, and MIT.

The gallivac vaccine was subcultured and subjected to identification as unknown mycoplasma according to the methods described by Adler et al. (1958).

RESULTS

The results of serological tests and the percentage of protection of chickens vaccinated with the locally prepared or the commercial vaccines are demonstrated in (Tables 1 and 2).

From these tables it is clear that the humoral immune response was low in all birds vaccinated with the temperature-sensitive mutant vaccine except those of the age group 14 days. Also the best rate of protection (72%) was obtained in this age group. The commercial live lyophilized vaccine (Gallivac) was applied by spray and in drinking water. The application of Gallivac vaccine gave undetectable antibodies as well as low protection rate.

Trials to identify the commercial strain (Gallivac) indicated that this vaccine was not antigenically related to Mycoplasma gallisepticum.

Table (1) : Results of serological tests among chicken sera vaccinated with temperature-sensitive mutant M.G. vaccine and the protection rate after challenge with M.G.S_6 (2 x 10^8 CFU/ml)

Groups of chicks (age)	Serological tests				% of Protection			
					Live evaluation		P.M. examination	
	SAT	MIT	GIT	HIT titre	NO.of C.S.F.Total	M..W. at 8 weeks	No.of P. M free/ total	% of protect
1 day	0/40	2/40	3/40	1/32	15/25	1300	8/25	32 %
7 days	1/40	3/40	5/40	1/16	16/25	1310	10/25	40 %
14 days	4/40	5/40	7/40	1/64	20/25	1400	18/25	7 %
28 days	1/40	3/40	3/40	1/32	15/25	1350	12/25	48 %
42 days	0/40	2/40	3/40	1/16	14/25	1330	12/25	48 %
Control	0/40	0/40	0/40	1/8	2/25	1100	0/25	0.0 %

SAT = Slide agglutination test.
MIT = Metabolic inhibition test.
GIT = Growth-inhibition test
HIT = haemagglutination-inhibition test.
* Positive/total tested

C.S.F. = No of clinical signs free

M. W = Mean weigt in gm

Table 2 : The serological tests among chicken sera vaccinated with commercial live lyophilized M.G. vaccine and protection rate after challenge with 2 x 10^8 (CFU/Ml) S_6.

	Serological tests								Percent of protection			
	SAT		MIT		GIT		HIT titre		Live evaluation No. of C.S.F /total examined		P.M. examination No. of IM lesion free/total	
	S	D	S	D	S	D	S	D	S	D	S	D
. day	0/160	0/160	0/160	0/160	0/160	0/160	1/160	1/160	40/160	30/160	24/160	20/160
4 days	0/160	0/160	0/160	0/160	0/160	0/160	1/160	1/160	35/160	25/160	25/160	30/160
Control	0/20	0/20	0/20	0/20	0/20	0/20	1/4	1/4	0/20	0/20	0/20	0/20

S = Vaccination by spray.

D = Vaccination by drinking water.

DISCUSSION

It is interesting to note that no or little circulating antibodies were detected in birds vaccinated with the temperature-sensitive mutant vaccine also the protection rate varied from 32-72%. It seems that there are some factors other than the circulating antibodies that played a role in protection of birds vaccinated with T.S.M. vaccine. The data obtained in the present work confirm the regarding the T.S.M. M.G. published by Nonomura and Imada (1982) and Lam and Lin (1983) who reported that I/N immunized chickens by the mutant strain of M.G. were protected against air sacculitis for at least 21 weeks. Thus the mutant vaccine was needed for inducing protection in 3 week old chicks.

The testing of the commercially available live attenuated lyophilized vaccine (Gallivac) revealed that the vaccine did not protect the birds against challenge with the virulent M. G. . The vaccine as mentioned in the instructions of the producer was to be used against fowl mycoplasmosis without specification of species of Mycoplasma to be considered. This vaccinal strain was found to be antigenically not related to M. gallisepticum, as it was found to split glucose but serologically non-identical with M. gallisepticum reference antisera, though the trade name Gallivac would give the impression that this vaccine is intended for M.G. control. It is therefore recommended to test any vaccine experimentally for specificity, immunogenicity and purity before use. Also, further studies are necessary to evaluate the mutant vaccine with regard to the dose, the frequency of vaccination, cell mediated immunity and other factors.

SUMMARY

A temperature sensitive mutant Mycoplasma gallisepticum vaccine was prepared by addition of N-Methyl-N-Nitro-N-nitrosoguaridine. This vaccine was applied by intranasal route in

chicks at different ages. The humoral response was much less in all subgroups except those in 14 days. The protection rate ranged from 32 to 72% and the best protection rate was obtained in the age of 14 days.

A commercial live lyophilized vaccine (Gallivac) was tried in chicks by spray and in drinking water. The application of Gallivac vaccine gave undetectable antibodies as well as low protection rate.

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