Vet.Med.J.,Giza. Vol.46, No.4B (1998): 867-875.

TRIALS FOR PREPARATION OF A COMBINED INACTIVATED VACCINE OF SALMONELLOSIS AND FOWL CHOLERA IN POULTRY

By

EMAN M. EL-RAWY; NAHED, I.M.M. KHAMIS; SAMIRA, A.A. SOLIMAN and GERGIS, S.M.

* Veterinary Serum And Vaccine Research Institute, Abbasia, Cairo, Egypt.

SUMMARY

In an attempt for preparation a combined inactivated vaccine containing *P. multocida, S. typhimurium* and *S. enteritidis*, this vacine was injected in a dose of 0.5ml S/C twice one month apart, in groups of chickens. The humoral immunity was measured by indirect haemagglutination (IHA) and enzyme linked immunosobent assay (ELISA) tests. The combined vaccine elicited high levels of antibody and showed protection rate of 96.5% against virulent challenge with these bacteria. The cellular immune response was measured by 3-(4,5 - dimethyl- thiazol - 2- yl) 2,5- diphenyl tetrazolium bromide (MTT) utilization test and heterophil/lymphocyte ratio. the conjugation of these organisms conferred T-dependent properties of their lipopolysaccharides. The combination had no mutual competitive effect but enchancing each other inducing improvement the immunogenicity of fowl cholera vaccine. The combined vaccine seems to be of a good economical value for poultry industry with less shedding of salmonellae after challenge with virulent strain.

INTRODUCTION

Poultry industry and human public health are of the novel Egyptian government interest. Diseases remain the greatest threat to the poultry industry. Among the major diseases encountered are salmonellosis and fowl cholera (Ibrahim and Seng, 1993) which exert a wide economic impact on poultry breeding *P. multocida* causes a highly contagious disease which infects birds and mammals where it produces the most serious causes of death losses in domesticated and wild fowls (Choi et al., 1989). *S. typhimurium* and *S. enteritidis* are food animal reservoirs and posses public health significance in poultry and man and they cause gastroenteritis, septicaemia with mortality up to 30% (Pritchard et al., 1978). These serovars of salmonellae involved invariably multi-resistance to up to 9 antimicrobial agents, also they possess a plasmid which is known as

CamScanner

virulence factor for some enteric pathogens and cause numerous epidemics for man and poultry by ingestion of contaminated food, water and eggs (Leslie et al, 1998). The antimicrobial resistance factors may be carried form poultry to man. The prophylactic vaccination against salmonellosis and fowl cholera is the only mean for controlling of these diseases to reduce the members of salmonella shedding in faeces, to reduce environmental contamination during both the production and procesing of poultry, it also abates the hazard of salmonellosis from poultry products, in the mean time it is inexpensive and easily administered (Jarolmen et al., 1976).The aim of this study was to prepare a combined oil adjuvant bacterin comprised of *S.typhimurium, S. enteritidis* and *P. multocisa* to be used in chicken.

MATERIAL AND METHODS

1. Experimental chicks:

A total of 100 Leghorn chicks, one day old, were purchased from the United company for Poultry Production and kept under strict hygienic measures or rearing and feeding. Faecal swabs were collected to confirm that they were salmonella free.

2. Mice:

A total of 100 Swiss albino mice about 18-20g weight were used for passage of the bacterial strains and for safety test of the prepared vaccines.

3. Bacterial strains:

Standard strains of *P. multocida* serotype"A" and "D", as well as local isolates of *S. enteritidis* and *S. typhimurium* were used. These strains and isolates were identified morphologically culturally, biochemically and serologically. They were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

4. Vaccines:

a. Formalized fowl cholera oil adjuvant vaccine:

It was prepared by Aerobic Bacterial Vaccine department., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

b. Formalized *S.typhimurium* oil adjuvant vaccine:

This vaccine was prepared according to Richard and Beard (1989).

c. Formalized *S. enteritidis* oil adjuvant vaccine:

The method described by Nagaraja et al. (1991) was followed for preparing the vaccine.

d. Combined oil adjuvant vaccine of *P.multocida, s. typhimurium* and *S. enteritidis*:

Equal amounts of the formalized cultures were mixed in waving blander with oil (Risela 17 oil, sorbitan moncleate (span) and emulsified, polyoxyethylene sorbitan (Tween 80) in a ratio of 500:486:14, respectively. Safety and purity tests were carried for all prepared vaccines.

Vaccines Potency:

A. Evaluation of the cell mediated immunity:

1- Blastogenesis of T and B lymphocytes and 3- (4,5 - dimethyl-thiazol-2-yl) 2,5 -

diphenyl tetrazolium bromide (MTT) utilization using the method of Lessard et al. (1994) was carried out.

2- Evaluation of the heterophil/lymphocytes ratio was conducted as described by Gross and Siegel (1983) was used.

3- Determination of lesion scores was recorded as recommended by Snedecor and Corchran (1967).

B. Evaluation of the humoral immunity:

The antibodies titres of the vaccinated chicken groups were monitored by:

1- Indirect (Passive) haemagglutination test:

Titres against *P. multocida* was determined according to Carter and Rappy (1962).

2- Microagglutionation test:

Antibody titres against *S. typhimurium* and *S. enteritidis* was estimated as described by williams and Whittemore (1973).

3- Enzyme linked immunosorbent assay (ELISA) test:

The antibody titres against *P. multocida, S. enteritidis* and *S. typhimurium* were estimated by the methods described by Gaunt et al. (1977), Pritchard et al. (1978) and Lessard et al. (1994), respectively.

Experimental design:

One hundred leghorn chicks were divided into 5 groups (20 chicks for each). Tabel (1) explains

Table (1): Scheme of Experimental Design.

Type of vaccine	Vaccinated chicks groups				
	Group (1) Fowl cholera	Group (21) *S.typhimurium*	Group (3) *S.tenteritidis*	Group (4) Combined	Group (5) Control
Dose of vaccine	0.5 ml	0.5 ml	0.5 ml	0.5 ml	None
Route of vaccination	S/C	S/C	S/C	S/C	None
Intervals of blood collection	Prevaccination 1 week, 2 weeks, 3 weeks post the first vaccination and 1 week, 2 weeks, 3 weeks, 4 weeks post booster vaccination				
Challenge dose	0.5 ml SC of 5 LD50 of 1.2 x105 CFU *P multocida*	1 ml oral of 3.8 x108 CFU *S.typhimurium*	1 ml oral of 107 CFU *S.tenteritidis*	0.5 ml oral of *S.tenteritidis* + *S.typhimurium* +*P multocida*	

S/C Subcutaneous. CFU Conlony Forming Unit.

the groups of chicks, schedule for vaccination, route of infection and the time of blood collection.

RESULTS AND DISCUSSION

New strategies are urgently required for development of new vaccine (Nagarija et al., 1991). Protection of poultry against more than one disease ate the same time is of a great importance to reduce labor, costs and stress on vaccinated birds. The use of salmonella vaccine strains would induce the development of a first line of defense against a diversity of pathogens, and this reduces contagious spread of many pathogen and induces long lasting immunity (Curtiss et al., 1987). This investigation was initiated to prepare a combined oil adjuvant bacterin against fowl cholera, *S. typhimurium* and *S. enteritidis* in chicken. The cell mediated immunity was evaluated as illustrated in Fig. (1) and (2). The mitogenic stimulation of T and B lymphocytes to concanavalin and Pokeweed mitogens showed higher in cellular immune

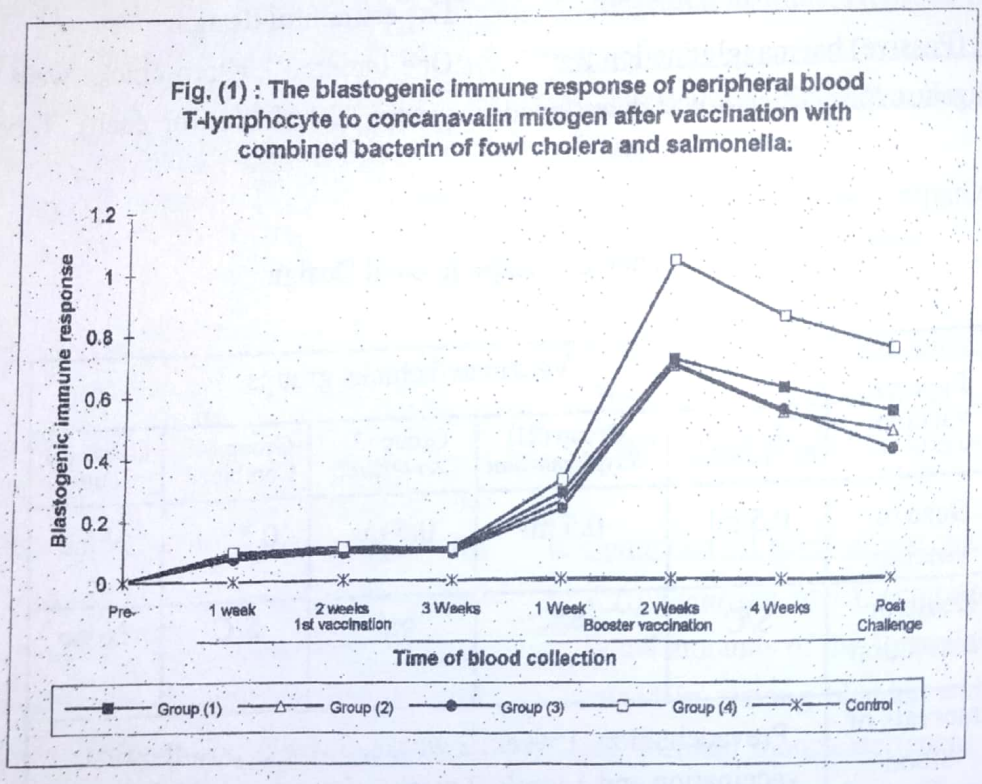
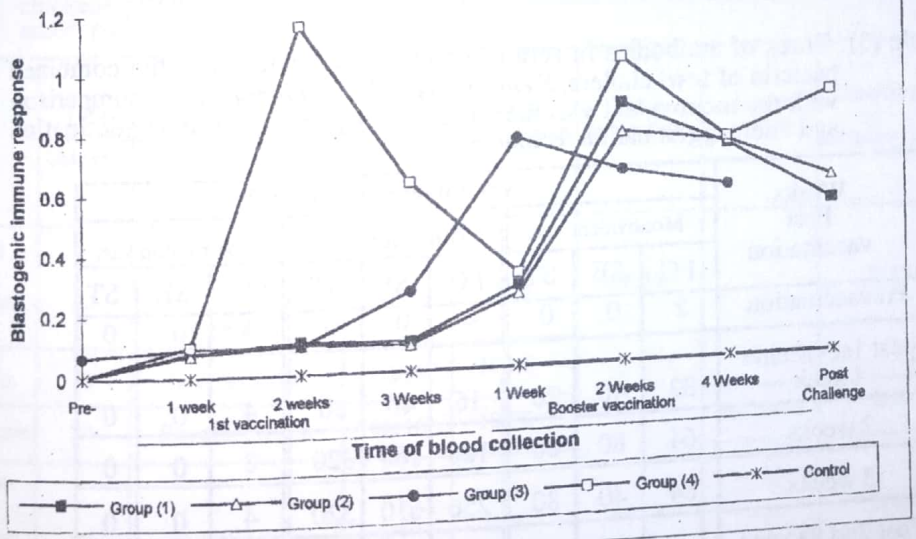


Fig. (1) : The blastogenic immune response of peripheral blood T-lymphocyte to concanavalin mitogen after vaccination with combined bacterin of fowl cholera and salmonella.

Mitogenic response of T lymphocytes	Overall mean of T lymphocytes blastogenesis in chicken groups				
	Fowl cholera	S. enteritidis	S. typhimurium	Combined	Control
Overall mean	2.458	2.256	2.168	3.27	0.035

response in the combined vaccinated gorup with salmonellae and *P. multocida* than the monovalent vaccinated chicken groups. These results are in agreement with Marshall and Zeigler (1991) who stated that the lipopolysaccharides of *S. typhimurium* and *S. enteritidis* enhance the potential activation of B and T lymphocytes. The immuncompetent cells activated the natural killer cells (NK) and induce production of interferon, interleukin 1 and interleukin 2 which might be important of the non specific immune responses to other pathogens and had stimulatory effects on the chicken immune system. The cellular immunity was also measured by estimation of heterophils/lymphocytes ratio (H/L ratio). Table (2) shows a significant decrease in H/L ratio of the combined vaccinated group than the monovalent vaccinated chicken gorups. These data were explained by Brandtzaeg et al. (1987) who stated hat salmonellae must retain its ability to colonize the intestine, gut associated lymphoid tissue(GALT) and spleen without impairing normal host physiology, growth and proliferation of GALT, liver and spleen. the

Fig. (2) : The blastogenic immune response of peripheral blood B-lymphocyte to pokeweed mitogen after vaccination with the combined bacterin of fowl cholera and salmonella vaccine.



Mitogenic response of B lymphocytes	Overall mean of T lymphocytes blastogenesis in chicken groups				
	Fowl cholera	S. enteritidis	S. typhimurium	Combined	Control
Overall mean	2.142	2.009	2.5	3.955	0.033

Table (2): Evaluation of the heterophils/lymphocytes ratio post vacciantion with combined fowl cholera and salmonella serovars.

Intervals post vaccination	Types of vaccinated gorups				
	Fowl cholera	S. enteritidis	S. typhimurium	Combined	Control
Prevaccination	0.90	1	0.95	0.99	0.9
post 1st vaccine 1week	0.84	0.93	0.84	0.76	1.6
2 weeks	0.86	0.83	0.74	0.62	1.4
3 weeks	0.72	0.68	0.61	0.62	1.4
Post 2nd vaccine 1week	0.68	0.56	0.52	0.41	1.82
2 weeks	0.40	0.34	0.37	0.37	2.1
4 weeks	0.65	0.40	0.375	0.32	2.0
Post challenge 2 weeks	1.1	0.96	1.17	0.76	1.82
4 weeks	1.7	0.65	0.88	0.60	1.70
Overall mean	0.87	0.61	0.71	0.60	1.65

Table (3): Titres of antibodies in sera of chicken vaccinated with the combined bacterin of fowl cholera, *S.enteritidis* and *S. typhimurium* in comparison with the monovalent vaccines measured by indirect haemagglutination and microagglutination techniques.

Weeks Post vaccination	Vaccinated gorups with:								
	Monovalent vac.			Combined vac.			Control non vac.		
	FC	SE	ST	FC	SE	ST	FC	SE	ST
Prevaccination	2	0	0	4	0	0	4	0	0
post 1st vaccine 1week	32	40	20	16	40	80	4	0	0
2 weeks	64	80	80	128	160	320	8	0	0
3 weeks	64	40	80	256	610	320	4	0	0
Post 2nd vaccine 1week	128	80	80	256	80	160	8	0	0
2 weeks	256	160	160	512	160	320	8	0	0
4 weeks	256	160	320	512	320	320	8	0	0
Post challenge 2 weeks	32	40	80	128	80	80	Died	40	80
4 weeks	64	80	80	256	80	160	Died	80	80
Overall mean	99.7	75.5	100	120	120	178	4.8	13.3	17.7

FC: Fowl Cholera. SE: *S.enteritidis.* ST:*S.typhimurium*

Table (4): Mean absorbance values as measured by ELISA in chicken sera vaccinated with combined bacterin of *P. multocida, S. enteritidis* and *S.typhimurium* in comparison with monovalent vaccines.

Weeks Post vaccination	Vaccinated gorups with:								
	Monovalent vac.			Combined vac.			Control non vac.		
	FC	SE	ST	FC	SE	ST	FC	SE	ST
Prevaccination	0.375	0.375	0.375	0.395	0.674	0.348	0.348	0.506	0.429
post 1st vaccine 1week	0.981	0.403	1.32	1.633	1.27	1.95	0.375	0.526	0.437
2 weeks	1.43	2.519	3.12	2.5	3.206	3.82	0.624	0.376	0.228
3 weeks	1.781	2.519	2.78	2.677	2.538	3.45	0.450	0.544	0.542
Post 2nd vaccine 1 week	1.47	1.53	2.20	1.56	2.06	2.126	0.302	0.580	0.526
2 weeks	2.39	3.3	3.4	1.75	3.277	3.56	0.341	0.472	0.549
4 weeks	2.158	2.03	2.34	2.350	2.51	2.98	0.302	0.351	0.532
Post challenge 2 weeks	1.66	1.48	2.29	1.757	2.002	1.95	Died	1.7	0.526
4 weeks	1.82	1.7	2.2	2.0	2.2	2.35	Died	1.9	2.2
Overall mean	1.56	2.00	2.22	1.80	2.19	2.5	0.304	2.77	2.66

FC: Fowl Cholera. SE: *S.enteritidis.* ST:*S.typhimurium*

Table (5): The survival rate, lesion score and shedding of salmonellae in the vaccinated chickens group after challenged with virulent strains of *P. multocida, S. enteritidis* and *S. typhimurium.*

Type of Vaccine	Total No. of chicken	Challenge strains	No. of survived chicken/ No. of total chicken	Protection percentage	Total percentage	Lesion score	Shedding of salmonellae
Combined Vaccine: Fowl cholera	10	P.multocida type A,D	9/10	90%	96.5%	+	0/10
S.enteritidis	10	S. enteritidis	10/10	100%		+	1/10
S.typhimurium	10	S.typhimurium	10/10	100%		No. lesion	1/10
Monovalent Vaccine: Fowl cholera	10	P.multocida	8/10	80%	86.5%	++	0/10
S. enteritidis	10	S. enteritidis	9/10	90%		++	3/10
S. typhimurium	10	S. typhimurium	9/10	90%		+	2/10
Control group:	40	P.multocida 20	All died	12.5%	12.5%	Died	No.
		S. enteritidis 10	2/10			+++	2/2
		S. typhimurium 10	3/10				3/3
			5/40				

+ : Low lesion score. ++: Mild lesion score. +++: Sever lesion score.

antibodies secreting cells (lymphocytes) peaked rapidly and multiplicated in mature broiler vaccinated birds with attenuated salmonellae, as described by Barrow et al. (1990). The overall mean of circulating antibodies of the combined oil adjuvant vaccine was significantly higher than the monovalent vaccinated chicken gorups. The antibody titres against *S. enteritidis* and *S. typhimurium* in the control gorup were increased over the vaccinated groups after 2 and 4 weeks post challenge as in (table 3 and 4). In this reapect, Lessard et al. (1994) stated that a lipopolysaccharide of salmonellae acted as a potent activator of plasma cells and increased the antibody response to oval albumin in rabbits. Marshall and Zeigler (1991) found that non specific activation of immunocompetent cells by *S. typhimurium* may have primed lymphocytes to enhance the immune response to NDV vaccine. Table (5) reveals that the overall mean of the protection rate of the combined vaccine was 96.5% and in the monovalent vaccine was 86.5% as compared with the control group which was 12.5%. The lesion scores varied from (+) in the combined vaccinated gorup to (++) in the monovalent vaccianted groups in comparison with (+++) in the control gorup. The highest shedding of salmonellae from the control group and was less in the monovalent vaccinated groups while; less shedding of salmonellae in the combined vaccinated gorup. These results coincide with Eisentein et al. (1988) who demonstrated non specific activation of salmonella infection in the form of transient cross-protection against *Listeria monocytogens* in mice. the vaccinated fowl cholera bacterin showed milder gross lesions in sacrificed chickens than in those of non vaccianted control which developed the disease and died. These results and in agreement with those of Gaunt et al. (1977). There was a considerable variation in the salmonella shedding pattern among chickens. Soerjadi et al. (1982) found a relationship between the clearance of salmonellae from the internal organs with the increase of circulating antibodies and age of chickens.

In conclusion, a combined bacterin against *P. multocida, S. enteritidis* and *S. typhimurium* was prepared which had a potent immungenic effect on the immune response of chicken due to the enhancing effect of the inactivated salmonellae on the chickens vaccinated with fowl cholera vaccine. The enhancing antibody secreting cells rises the level of serum antibodies and leads to increase of the maternal immunity of chickens. The clearance of the internal organs from salmonellae shedding was one of the most important economic value to obtain chicken meat and egg with less incidence of salmonellae. Thus, it is essential for human public health to avoid treatment of salmonellae and hence present transmission of the antimicrobial drug resistance strains.

REFERENCES

Barrow, P.A.; Hassan, J.O. and Berchieri, Jr. (1990): Reduction in faecal excretion of *S. typhimurium* strain F98 in chickens vaccinated with live and killed *S. typhimurium* organisms. Epidemiol. Infect., 104: 413-426.

CamScanner

Brandtzaeg, P.; Bjerke, K. and Kett, K. (1987): Production and secretion of immunoglobulins in the gastrointestinal tract. Ann. Allergy, 59: 21-26.

Carter, G.R. and Rappy, D.E. (1962): Formalized erthrocytes in the haemagglutination test tor typing *P. multocida*. Brit. Vet. J., 118: 289-292.

Choi, K.H.; Maheswaran, S.K. and Felice, L.J. (1989): Characterization of outer membrane protein enriched extracts from *P. multocida* isolated from turkeys. Am. J. Vet.Res., 50 (5): 676-683.

Curtiss, R.I.; Goldschmidt, R. and Kelly, S.M. (1987): Recombinant avirulent salmonella for immunization to induce mucosal immunity to bacterial pathogens. New concepts and Developments Proceedings of the 10th International Convocation on Immunol., pp. 261-262.

Eisenstein, T.K.; Dalal, N.; Killar, L.; Lee, J.G. and schafer, R. (1988): Paradoxes of immunity and immunosuppression in salmonella infection. Adv. Exp. Med. Biol., 239: 353-356.

Gaunt, G.; Moffat, R. and Mukkur, T.K.S. (1977): Immunization of chickens with potassium thiocyanate extract of *P.multocida* serotype 3 Avian Dis., 21 (4): 543-549.

Gross, W.B. and Seigel, H.S. (1983): Evaluation heterophil/lymphocyte ratio as a measure of stress in chicken. Avian Dis., 27(4): 972-979.

Ibrahim, A.L. and Seng, L.H. (1993): Profiles of Veterinary prodcution and quality control in developing and less advanced countries vaccines for poultry. Quality Control Vet. Vaccines in Developing Countries, FAO Animal production and Health, Paper No. 116, pp. 17-20.

Jarolmen, H.R.J.; Sairk, Langworth, B.F. (1976): Effect of cholotetracycline feeding on the salmonella reservoir in chickens. J. App. Bact., 40: 153-161.

Leslie, Collier; Albert, Balows and Max sussman (1998): Salmonella. Topley Wilsons's (Microbiology and Microbial Infections) 9th Ed., 2: 969-997.

Lessard, A.M.; Hutchings, B.D.L. and Spencer, B.J.L. (1994): Cell mediated and humoral immune responses in chickens infected with *S.typhimurium*. Avian Dis., 39: 230-238.

Marshall, N.e. and Ziegler, H.K. (1991): Lipopolysaccharide responsiveness is an important factor in the generation of optimal antigen specific T-cell response during infecition with Gram negative bacteria. J. Immunol., 147: 2333-2339.

Nagaraja, K>V.; Kumar, M.C. and Pomeroy, B.S. (1991): Is vaccination a feasible approach for the control of salmonella. Proceeding Conference Control of Human Bacterial Enteropathogen in Poultry, 243-256.

Pritchard, D.G.; Nivas, S.C.; york, M.D. and Pomeroy, B.S. (1978): Effects of gal-E mutant of *S. typhimurium*. avian Dis., 22 (4): 562-575.

Richard, K.; Gast and Beard, C.W. (1989): Age related changes in the persistence and pathogenicity of *S. typhimurium* in chicks. Poultry Sci., 68: 1454-1460.

Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods. 6th Ed. Iowa State Univ. Press, ?Ames Iowa, USA.

Soerjadi, A.S.; Rnfner, R.; Synoeyenbos, G.H. and weinack, OM. (1982): Adherence of salmonellae and native gut microflora to the gastrointestinal mucosa of chicks. Avian Dis., 26: 576-584.

Williams, J.E. and whittemore, A.D. (1973): Microantiglobulin test for detecting *S.typhimurium* agglutinins. appl. Microbiol., 26: 1-3.