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IMMUNOLOGICAL EVALUATION OF RABBITS AND CHICKEN VACCINATED WITH PASTEURELLA MULTOCIDA VACCINE PREPARED BY SINGLE AND MULTIPLE EMULSION METHOD

(With 2 Tables and 6 Figures)

By

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التقييم المناعي للقاحات الباستريلا ملتوسيدا المحضرة بطريقة المستحلب الأحادي والمستحلب المتعدد فى كل من الأرانب والدجاج

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في هذه الدراسة تم تحضير لقاح مضاد لعدوى الباستريلا في الدجاج والأرانب وذلك بطريقة جديدة تسمى طريقة المستحلب المتعدد. وقد تم مقارنة الصفات الطبيعية والبيولوجية لهذا اللقاح مع مثيلاتها من اللقاح الزيتي التقليدي حيث ثبت الأتي: أولا: اللقاح الجديد يتميز بثباتة وسهولة حقنه وأنه آمن تماماً حيث لا يسبب أى تفاعلات موضعية أوعامة على الدجاج أو الأرانب المحصنة به. ثانيا: أدى هذا اللقاح إلى التنبيه المبكر لجهاز المناعة وذلك عقب أسبوعين فقط من الجرعة الأولى من هذا اللقاح وذلك من خلال تكوين مستوى متوسط من الأجسام المضادة فى الكتاكيت والأرانب المحصنة والتي أعطت بدورها نسبة حماية جزئية (عقب إختبار التحدي) بلغت ٥٣% و ٥٠% على التوالي مقارنة بنسبة ٢٧% و ١٢,٥% على التوالي في الحيوانات المحصنة باللقاح التقليدي الزيتي. لهذا فإننا نوصي بإستخدام هذا اللقاح الجديد خاصة فى المناطق الموبؤة بمرض الباستريلا.

SUMMARY

Adifferently formulated pasteurellosis vaccines containing multiple-emulsion (ME) adjuvant system were experimentally produced. Such vaccines were fully evaluated in vitro as well as in vivo (chicken and rabbits) versus the classical oil emulsified (OE) ones. The ME vaccines

present several advantages over the OE vaccines. Minor local reactions were detected at ME injection sites compared to severe ones produced by OE vaccines. Moreover, ME vaccines are stable, less viscous, easily injected and stimulated an early production of protective antibodies. Challenged vaccinated chickens and rabbits receiving ME pasteurellosis vaccines were protected at a level of 53% and 50%, respectively. Protection in corresponding vaccinates receiving OE vaccines were 27% and 12.5%, respectively. The ME vaccines should be recommended in pasteurellosis endemic area to offer an early partial immunity for chickens and rabbits.

Key words: *Rabbits, Chicken, Pasteurella Multocida, Vaccome*

INTRODUCTION

Pasteurellosis in avian species (fowl cholera) and rabbits is a commonly occurring and worldwide distributed disease. It usually occurs as an acute septicaemia and is often fatal. Protection against the causative bacterium, Pasteurella multocida, has been achieved safely and effectively through the inactivated vaccines (Rimler and Glisson, 1997). The commercially available vaccines are commonly adjuvanted with mineral oil or aluminum hydroxide. The major disadvantage of such adjuvants is their unacceptable local reactions at the site of injection. Additionally vaccines that are oil based has been administrated with difficulty as their marked viscosity may slow the continuous outflow of that vaccines via the injecting needles (Walker, 1997).

The double emulsion system is an alternative adjuvant system created by re-emulsifying a simple water in oil emulsion in an outer water phase. Several bacterial and viral vaccines containing such adjuvant system did not cause any adverse side effects and were highly protective (Mittal et al., 1979; Raid and Blackall, 1987 and Revyemamu et al., 1986).

The objective of the current study was to fully evaluate an experimental batch of P.multocida double emulsion vaccine in vitro studies as well as in vivo (chickens and rabbits).

MATERIAL and METHODS

Vaccinal Strains:

Four local reference P.multocida strains (5:A, 8:A, 9:A and 2:D) were kindly supplied by Aerobic Bacterial Vaccines Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

Vaccine Preparation:

Four experimental polyvalent pasteurellosis vaccines (containing approximately 3.4×107 CFU/dose) were prepared and evaluated in the present study. The traditional local vaccines for chickens and rabbits were of oil emulsified type and coded OE-1 and OE-2, respectively. The corresponding newly formulated vaccines based on a double emulsion adjuvant system were coded ME-1 and ME-2, respectively. Vaccines were prepared as described below.

Oil Emulsified (OE) Vaccine:

This vaccine was produced by the method described by Stone et al. (1978) with an aqueous: oil ratio of 1:2 using the mineral oil whiterex 307 (Mobil, Sydney, Australia).

Double Emulsion (ME) Vaccine:

For this purpose the previously prepared OE vaccine was re-emulsified in an outer water phase (tween 80 in formol saline) and blended as mentioned by Herbert (1968) and Mittal et al. (1979).

Stability And Emulsion Type Of The Vaccines:

The stability of the experimentally prepared vaccines was assessed by storage; a) at 4oC for 48 weeks, b) at 37oC for 48 weeks, or c) alternatively at 4oC and then 37oC for maximum periods of 72 hours. The emulsion type of these vaccines was determined as described by Herbert (1968).

Experimental Animals:

Forty five day-old chickens as well as twenty five Giza rabbits (1.4-1.7 kg), were obtained from commercial source and tested randomly to assure their freedom from P.multocida antibodies. Additionally, forty Swiss mice (weighing 20-25 gm) were similarly obtained for the purpose of safety testing of the experimental vaccines.

Vaccination And Challenge Exposure:

The procedures for vaccination, challenge, assessment of clinical signs, lesions, and protection percentage, were as described previously. Briefly, all experimental vaccines were subcutaneously given for either chickens or rabbits as two doses (0.5 ml each) at two weeks intervals. Two weeks following the primary and secondary vaccination, experimental chickens and rabbits were intramuscularly challenged by

infection with 0.2 ml/animal of 18 hours broth culture 106 dilution of virulent P.multocida. In both experiments, a group of unvaccinated control were similarly challenged. All experimental animals were examined regularly after each vaccination for local and systemic reactions to the vaccines.

Gross and Histopathology:

In both experiments the degree of local tissue reactions at the vaccine inoculation sites was examined grossly as well as histopathologically, following the procedure of Drury et al. (1980).

Serology:

All vaccinates (chickens and rabbits) were bled at a weekly intervals post primary and secondary vaccination. Sera were separated and kept frozen at -20oC until tested for P.multocida antibodies in ELISA method (Borkowska et al., 1997). The commercially available P.multocida coated plates (Idexx, Flock Check, USA) were used for that purpose. It contains capsular extract of X-73 and CU strain of P.multocida.

RESULTS and DISCUSSION

During the last 70 years many adjuvants have been developed but they were never accepted for routine vaccination because of their immediate toxicity and possibility of delayed side effects (Levine et al., 1997). The current attitude regarding risk-benefits of vaccination favor safety and efficacy concurrently for any given adjuvanted vaccine. The most frequent side effects associated with such vaccines is the formation of painful moderate to large nodules at the site of injection as seen with aluminum hydroxide gel and oil based vaccines, respectively (OIE, 2000 and Walker, 1997). In the way of such adverse reactions at the site of inoculation, the multiple emulsion (ME) vaccines may be an advantageous alternative adjuvanted vaccines (Weir, 1976 and Mittal et al., 1979).

Physical properties of emulsion vaccines: In the current study both of the OE and ME vaccines were stable at both 4oC and 37oC over a period of 48 weeks. From the point of view of ease of injection, ME vaccines are less viscous than OE ones (Weir, 1979). The nature of our OE vaccines was simple water-in-oil while that of ME vaccines was water-in-oil-in-water. The aforementioned properties meet with the requirements for satisfactory vaccine production as mentioned by Walker (1997).

Post vaccinal reactions in vivo: In the present study, no deleterious systemic effects on the general health of immunized chicken and rabbits injected with either ME or OE vaccines were observed. No pyrogenic properties induced by such vaccines as also reported by Opacka et al. (1996) and Levine et al. (1997). However, localized hot swellings were developed at more than half the vaccination sites. Such swellings (3-4 cm in diameter) were painful (upon palpation) especially in vaccinated rabbits but did not interfere with their growth. In this respect, Walker (1997) mentioned that different species show different susceptibilities to the adverse effects of OE vaccines. In contrast, the ME vaccines used in the present study caused only minor and transient swelling at the injection sites and completely disappeared within 1-3 days. Reid and Blackall (1987) concluded that the method of formulation of ME vaccines has some moderate effect upon the toxicity of oil-based vaccines. The severe local reactions induced by OE vaccines were histopathologically evident (Figs 1&2). Such adverse histopathological changes was previously reported by Amina et al. (1998). In ME vaccines, the antigen(s) has been enclosed within the most outer water phase, while the OE vaccines contained its antigen(s) entirely enclosed within the oil phase. Consequently, the ME vaccines exhibit a much lower viscosity than the OE vaccine. Such physical property of ME vaccine may be responsible for rapid dispersion of the vaccinal antigen from the site of injection. In contrast, the higher viscosity of OE vaccines resulted in formation of a depot at the injection site, from which the vaccinal antigen(s) has been slowly released (Talmage and Dixon, 1953; Weir, 1976 and Herbert, 1968).

Serological and post-challenge findings: An earlier and higher antibody responses were consistently detected in chickens and rabbits receiving ME pasteurellosis vaccines than in corresponding vaccinates receiving OE vaccines. Such immunological advantages of ME vaccines over OE ones were more detected at the 1st and 2nd week post primary vaccination and to a lesser extent at 1st and 2nd week post secondary vaccination (Figs 3, 4, 5 & 6). In this respect, Weir (1976) mentioned that the ME vaccines produce a much earlier but less persistent antibody response while OE vaccines stimulate a lowly rising but more persistent antibody responses. The superior immunological value of ME vaccines was previously reported for several bacterial and viral vaccines (Mittal, 1979; Reid and Blackall, 1987 and Lucio and Hitchner, 1979).

Protection against P.multocida infection has been shown to be directly correlated to the specific IgG ELISA antibody level

(Borkowska, 1997). However, the only acceptable method of assaying such protection was by actual challenge with live homologous organism. Half of the vaccinated chickens and rabbits were subjected to an early challenge exposure at the 2nd week post primary vaccination (2nd wppv), while the other half was similarly challenged at the 2nd week post secondary vaccination (2nd wpsv). The aim of such early challenge exposure was to detect an early protection value of ME vaccines, if any. In tables (1&2), upon 1st challenge exposure (at 1st wppv), the ME vaccines protected 53% and 50% of the challenged chickens and rabbits, respectively. Corresponding protection values induced by OE vaccines were 27% and 12.5%, respectively. Despite such an early partial protection produced by ME vaccines still of low magnitude (50 to 53%), however it may be of great epidemiological importance especially in areas with endemic pasteurellosis. Under such conditions, the aim is to establish any degree of early protection for combating such infection. The concern about our serological and protection findings has nercly supported by Avakian et al. (1986) who found that anti-pasteurella ELISA antibody titers ranged from 0-550 and 551-1100 which were associated with 85% and 65% protection level in challenged vaccinated birds, respectively.

The 2nd challenge exposure (at 2nd wpsv) resulted in 80% and 85% protection level in chicken vaccinated with ME and OE vaccine, respectively, while either vaccine induced 80% protection in challenged rabbits. Correlation between such protection findings and antibody titers nearly agree with Avakian et al. (1986) findings as he concluded that anti-pasteurella antibody titer above 1100 (in ELISA) resulted in 90% protection in challenged vaccinates.

Conclusively, our successful multiple emulsion pasteurellosis vaccine indicates that it can represent safe and satisfactory vaccine inducing an early partial protection in vaccinated chickens and rabbits. It can be recommended with OE vaccine for primary and secondary vaccination, respectively.

REFERENCES

Amina A. El-Bayomy; Ghoneim Wafaa; Hala A. Fadl and Thoria F. Salam (1998): "Immunological and local cytological changes associated with Pasteurella multocida vaccines in rabbits". Fourth Sci.Vet.Med.Conf., pp.125-139.

Avakian, A.P.; Dick, J.W.; Derieux, W.T. and Henry (1986): Comparison of various antigens and their ability to detect protective antibodies against Pasteurella multocida using enzyme linked immunosorbent assay. Avian Dis., 30(3): 527-535.

Borkowska, B.; Opaka, A.; Kednak, A. and Tuszezynski, M. (1997): Application of the ELISA for determination of anti Pasteurella multocida IgG in sera of rabbits vaccinated against pasteurellosis under field conditions. Bull.Vet.Inst.Pulawy, 41: 17.

Drury, A.A.B., Wallington, E.A.F. and Carletons, S. (1980): Histological Technique. 5th ed. Oxford, New York, Toronto.

Herbert, W.J (1968): Multiple emulsion, A new form of mineral oil antigen adjuvant. Lancet II, 771.

Herbert, W.J. (1968): The mode of action of mineral oil emulsion adjuvants on antibody production in mice. Immunology, 14: 301.

Levine, M.M.; Woodrow, G.C.; Kaper, J.B. and Cobon, G. (1997): Adjuvant for the future, in New Generation Vaccines. 2nd ed., Marcel Dekkery Inc., New York.

Lucio, B. and Hitchner, S.B. (1979): Infectious bursal disease emulsified vaccine : Effect upon neutralizing antibody levels in the dams". Avian Dis., 23(2).

Mittal, K.R; Jalswal, T.N and Gupta, B.K. (1979): Studies on haemorrhagic septicaemia oil adjuvant and multiple emulsion vaccines". Indian Vet.J., 3: 445-454.

OIE (2000): Manual of The Office International De Epizootic. France.

Opacka, B.B.; Truszezynoki, M.; Jurga, I.R. and Kadrak, A. (1996): Field trial of the active immunity in rabbits against pasteurellosis". Bull.Vet.Inst.Pulway, 40: 11-16.

Reid, G.G. and Blackal I.P.J. (1987): Comparison of adjuvants for an inactivated infectious coryza vaccine". Avian Dis., 31: 59-63.

Reveymamu, M.M.O.; Umehara, D.; De Lucaneto, M.C.; Baltazar, F.E.; Vicente, R.R and Medeiros-Neto. (1986): Efficacy of avridine as an adjuvant for Newcastle disease virus antigen in chickens. Am.J.Vet.Res., 47: 1243-1248.

Rimler, R.B. and Glisson, J.R. (1997): Fowl cholera in Diseases of Poultry. Pp.143-159. 10th ed. By Calnek, Iowa State University Press.

Stone, H.B.; Brugh, M.; Hopkins, S.R.; Yoder, H.W. and Beard, C.V. (1978): Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. Avian Dis., 22: 666-674.

Talmage, D.W. and Dixon, F.J. (1953): The influence of adjuvants on the elimination of soluble protein antigens and the associated antibody responses. J.Infect.Dis., 93: 176.

Walker, P.D. (1997): Bacterial vaccines, in Vaccine Manual, the production and quality control of veterinary vaccines for use in developing countries". FAO, Rome, Italy.

Weir, D.M. (1979): Mineral oil adjuvants and the immunization of laboratory animals. In Handbook of Experimental Immunology in three volumes. Vol.3, application of immunological meth hods, 3rd ed.

Table (1) Post challenge assessment of immunity induced in chickens which had been vaccinated with pasteurellosis inactivated vaccines containing different adjuvants

Group	Vaccinated with	Immunity assessment					
		2 weeks post primary vaccination			2 weeks post secondary vaccination		
		Titer at challenge	Dead/total	Protection %	Titer at challenge	Dead/total	Protection %
A	Oil emulsion vaccine (OE1)	727	11/15	27	2474	3/20	85
B	Multiple emulsion vaccine (ME1)	1778	8/15	53	3077	4/20	80
C	Non-vaccinated control	20	3/3	0	20	3/3	0

Table (2) Post challenge assessment of immunity induced in rabbits which had been vaccinated with pasteurellosis inactivated vaccines containing different adjuvants

Group	Vaccinated with	Immunity assessment					
		2 weeks post primary vaccination			2 weeks post secondary vaccination		
		Titer at challenge	Dead/total	Protection %	Titer at challenge	Dead/total	Protection %
I	Oil emulsion vaccine (OE2)	441	7/8	12.5	1549	2/10	80
II	Multiple emulsion vaccine (ME2)	1132	4/8	50	3197	2/10	80
III	Non-vaccinated control	20	3/3	0	20	3/3	0

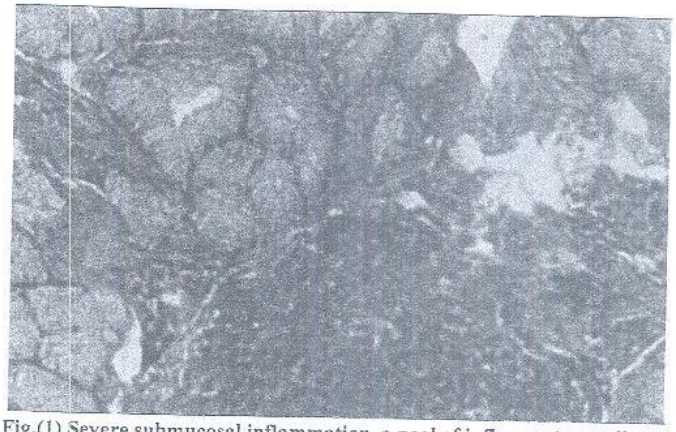


Fig.(1) Severe submucosal inflammation, a pool of inflammatory cells at the site of OE vaccine injection with desquamation of the affected tissues (H&E x40)

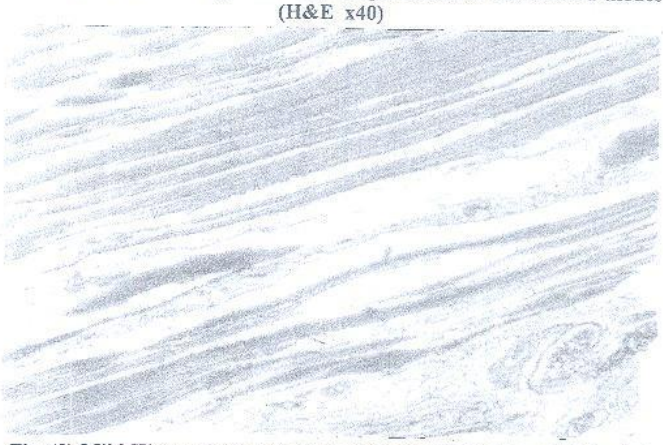


Fig. (2) Mild Histopathological changes in the skeletal muscle injected with ME vaccine as represented by vascular congestion, ecthyma and leucocytic infiltration (H&E x40)

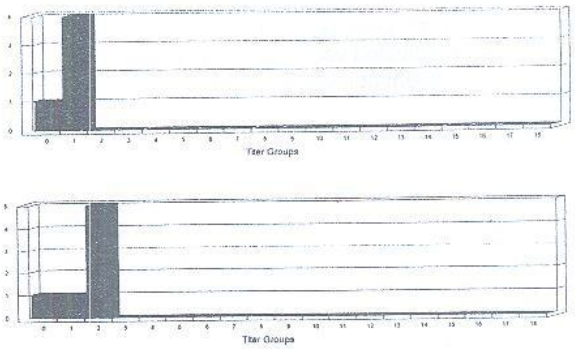


Fig. (3) representative ELISA histogram of *P.multocida* serum antibody titers in rabbits vaccinated with pasteurellosis inactivated vaccines containing different adjuvants. Titers were determined two-weeks post primary vaccination with oil-emulsion vaccine (above) or multiple-emulsion vaccine (below)

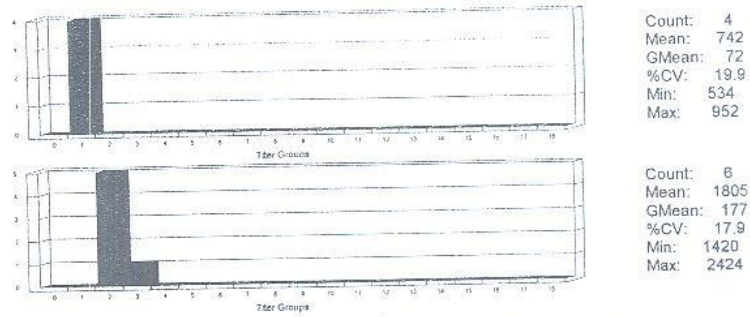
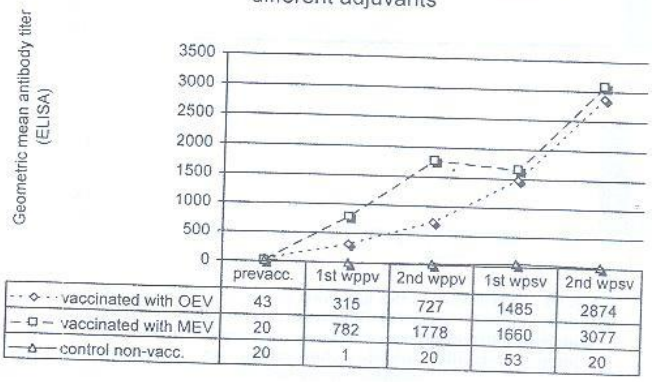


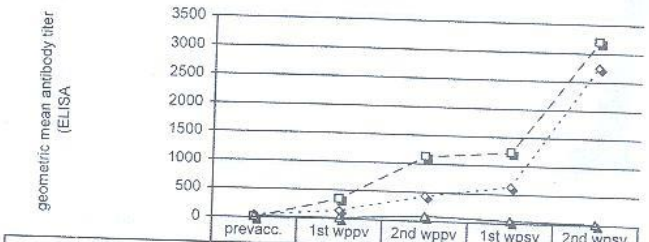
Fig. (4) representative ELISA histogram of *P.multocida* serum antibody titers in chickens vaccinated with pasteurellosis inactivated vaccines containing different adjuvants. Titers were determined two-weeks post primary vaccination with oil-emulsion vaccine (above) or multiple-emulsion vaccine (below)

Fig.(5) Serological response of chickens to pasteurellosis inactivated vaccines containing different adjuvants



	prevacc.	1st wppv	2nd wppv	1st wpsv	2nd wpsv
vaccinated with OEV	43	315	727	1485	2874
vaccinated with MEV	20	782	1778	1660	3077
control non-vacc.	20	1	20	53	20

Fig.(6) Serological response of rabbits to pasteurellosis inactivated vaccines containing different adjuvants



	prevacc.	1st wppv	2nd wppv	1st wpsv	2nd wpsv
vaccinted with OEV	43	148	441	634	2749
vaccinated with MEV	1	348	1132	1233	3197
control non-vacc.	20	20	93	45	20