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COMPARISON OF EFFICACY OF VACCINES BETWEEN MINERAL OIL AND ALUMINIUM HYDROXIDE ADJUVANT VACCINE AGAINST *MYCOPLASMA GALLISEPTICUM* INFECTION IN LAYER CHICKEN

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Abstract: To assess the efficacy of vaccines, ten layer chicks in each group were vaccinated with 0.5 ml per bird subcutaneously with aluminium hydroxide gel and mineral oil adjuvanted vaccines respectively at 6th week of age. Similarly ten chicks were kept as unvaccinated control. Post vaccinated sera samples were collected from all the groups at weekly intervals up to 15 weeks of age for estimation of antibodies. The antibody levels started to show gradual increase and attained highest level in fourth week post vaccination then gradually decreased both in commercial vaccine and mineral oil group where as in aluminium hydroxide adjuvanted groups, the antibody levels reached highest level in second week post vaccination then gradually decreased up to sixth week post vaccination. Prepared oil-emulsion vaccine produced high level of antibody titres when compared to aluminium hydroxide adjuvanted vaccine.

Keywords: *Mycoplasma gallisepticum*, Layer, aduvanted vaccine, immunity.

Introduction

Mycoplasma gallisepticum (MG) infections are characterized by respiratory rales, coughing, nasal discharge and conjunctivitis. Mycoplasma infection is wide spread in poultry farms in Tamil Nadu (Ramadass *et al*., 2006). To control Mycoplasmosis effective vaccination is renderer by using by very safe adjuvants, however they do occasionally produce sterile abscesses and persistent nodules, particularly if they are injected subcutaneously rather than intramuscularly. Effective adjuvanticity depends on complete adsorption of the antigen to the aluminium salts. Yagihashi *et al*. (1986) used aluminium hydroxide gel (5mg/ml of bacterin) to aliquots of the plain bacterin for adjuvant bacterin preparation. The aluminium hydroxide gel adjuvanted EDS-76 vaccine had advantages over the oil adjuvant vaccine that it was easy to inject, induced lesser inflammatory response at the site of injection and was economical to produce vaccine commercially (Garg and Garg, 1994). Oil-emulsion vaccines prepared with

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aqueous and oil-phase emulsifiers having low viscosity, were stable for more than 12 weeks at 37°C, and induced a marked primary antibody response in chickens (Stone *et al*., 1978). The first commercially available MG vaccines were oil-emulsion bacterins (Hildebrand *et al.,* 1983). Field trials at a commercial egg operation comparing production efficiency showed that chickens vaccinated with the oil-emulsified MG bacterin had higher egg production, a greater percentage of eggs graded large and over, a smaller percentage of under graded and better feed conversion than chickens vaccinated with a live-culture, low virulence Conn-F strain vaccine. The result of these studies indicated that the oil-emulsified MG bacterin was safe and highly efficacious. Hence, the present study was undertaken to Comparison of efficacy of vaccines between mineral oil and aluminium hydroxide adjuvant vaccine against MG.

Materials and Methods

Biological material: Mycoplasma gallisepticum vaccine, oil emulsion and aluminium hydroxide adjuvanted vaccine as MG isolate was cultivated in Frey's broth and the Mycoplasma cells were harvested and washed with Phosphate buffered saline (PBS) by centrifugation at 12,000 X G for 30 min. After three washings, a final suspension of antigen was prepared to contain one per cent packed cell volume in phosphate-buffered saline and this concentration was matched with McFarland standard tube no. 6 (1.8×10^9 CFU per ml). Then it was inactivated by treatment with 0.1 per cent formalin overnight at room temperature and incubated at 37°C for 24 hr in orbital shaker incubator. After 24 hr, a loopful of bacterin was inoculated in Frey's broth and also Frey's agar plates and incubated for seven days to ensure the inactivation. Sterility was assessed by inoculating the inactivated bacterin onto nutrient agar and Sabouraud's dextrose agar plates under aerobic and anaerobic incubation at 37°C for 48 hrs and stored at 4°C.

Development of vaccines: All MG inactivated vaccines developed with bacterin and adjuvants were with a final concentration of one per cent packed MG cells per dose / 0.5 ml and this concentration was matched with McFarland standard tube no. 6 (1.8×10^9 CFU per ml) and stored at 4°C until further use. Development of various adjuvanted inactivated vaccines against MG prepared and stored in the Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal was used .Thirty number of day old male layer chicks were procured from an organized commercial hatchery at Namakkal and maintained in experimental house till the end of trial. Thirty number of day old layer chicks were divided into three groups consisting 10 chicks in each group namely G_1, G3, and G_5. .

G_1 was kept as unvaccinated control group. At the age of sixth week, G_3 was vaccinated with 0.5 ml of oil emulsion adjuvanted vaccine. G_5 was vaccinated with 0.5 ml of aluminium hydroxide adjuvanted vaccine. All the inactivated vaccines were injected subcutaneously in the dorsal anterior neck region of layer chicks. Sera samples were collected from all the groups at weekly intervals up to 15 weeks for assessment of antibody level.

Assessment of antibody level by HI test (OIE, 2004): Mycoplasma gallisepticum antigen*,* HA antigen was prepared as per the method described by OIE (2004). A concentrated washed suspension of MG in PBS was used as haemagglutinating antigen. All the serum samples collected at weekly intervals from each group were subjected to HI test. Chicken blood was collected from serum antibody negative birds, maintained in the Department of Veterinary Microbiology, VCRI, Namakkal in Alsever's solution (Dextrose-2.5 g, Sodium citrate-0.8 g, Sodium chloride-0.4 g, Distilled water to make-100 ml) and washed thrice in PBS. Chicken erythrocytes 0.5 per cent suspension in PBS was used for carrying out HA and HI tests. The 96 well V bottom microtitre plates supplied by M/S. Tarson products private limited, Kolkata were used.

Haemagglutination test: 1:2 dilution of mycoplasma culture was made using phosphate buffered saline, pH 7.2 in the first well of V-bottom plate.Fifty microliter of PBS was added in other wells. Serial two-fold dilutions were made from the first well. Fifty microliter of a 0.5 per cent chicken RBC suspension was added to each well and mixed well.RBC control: 50 µl of 0.5 per cent RBC suspension was added to 50 µl of PBS and mixed well. Incubated for 1 h at room temperature.The endpoint was the highest dilution which showed complete haemagglutination.

Haemagglutination Inhibition test: Fifty microliter of PBS was added in first well of each row of V bottom plate.Fifty microliter of 8 HA units of antigen was added in the second well and 50µl of 4 HA units of antigen was added in other wells (third, fourth, etc.). Fifty microliter of 1/5 diluted serum was added in the first well. Mixed well and 50µl was transferred to the second well, and so on, and discarded 50µl from the last well. The first well was the serum control well.

Fifty microliter of a 0.5 per cent RBC suspension was added to all wells. The plate was lightly shaken to ensure thorough mixing of the well contents, and was read after allowing it for approximately 50 min at room temperature. The end-point was the highest serum dilution exhibiting complete inhibition of HA.

Results

Assessment of antibody level by HI test: The sera collected from experimental trial birds were subjected to HI test for the assessment of immunity. The result of HI test is presented in Table 21 and Fig.1. Statistically significant difference ($P<0.01$) was observed between vaccinated groups and unvaccinated control groups. Insignificant difference was observed between the vaccines adjuvanted with aluminium hydroxide, and mineral oil. During second week pv the titres of the aluminium hydroxide adjuvanted and commercial MG killed vaccine groups showed significant increase from that of mineral oil adjuvanted group. Aluminium hydroxide adjuvanted vaccine group showed peak HI titre of 102.4 whereas Mineral oil group showed HI titre of 48. Significant difference ($P<0.01$) was observed between vaccinated groups and unvaccinated control groups. During third, fourth and fifth week pv insignificant difference could be observed between control, and aluminium hydroxide adjuvanted vaccines whereas these groups differed significantly from mineral oil and commercial vaccine group.

During sixth week pv insignificant difference could be observed between control, and aluminium hydroxide adjuvanted vaccines whereas these groups differed significantly from mineral oil and commercial vaccine group.

During seventh week pv insignificant difference was observed between control and aluminium hydroxide adjuvanted vaccine group. The titres of mineral oil and aluminium hydroxide adjuvanted groups showed insignificant difference .Slight elevation of HI titre was noticed in aluminium hydroxide adjuvanted group.

During eighth week pv insignificant difference was observed between control and aluminium hydroxide adjuvanted vaccine group. The titres of the saponin and aluminium hydroxide adjuvanted groups showed insignificant difference where as there was insignificant difference could be observed between mineral oil adjuvanted group and commercial vaccine group.

During eighth week pv insignificant difference was observed between control and aluminium hydroxide adjuvanted vaccine group. The titres of the saponin and aluminium hydroxide adjuvanted groups showed insignificant difference where as there was insignificant difference could be observed between mineral oil adjuvanted group and commercial vaccine group. Significant difference ($P<0.01$) was observed between vaccinated groups except aluminium hydroxide adjuvanted and unvaccinated control groups.

During ninth week pv significant difference ($P<0.01$) was observed between vaccinated groups except aluminium hydroxide adjuvanted and unvaccinated control groups. Insignificant difference was observed between control and aluminium hydroxide adjuvanted

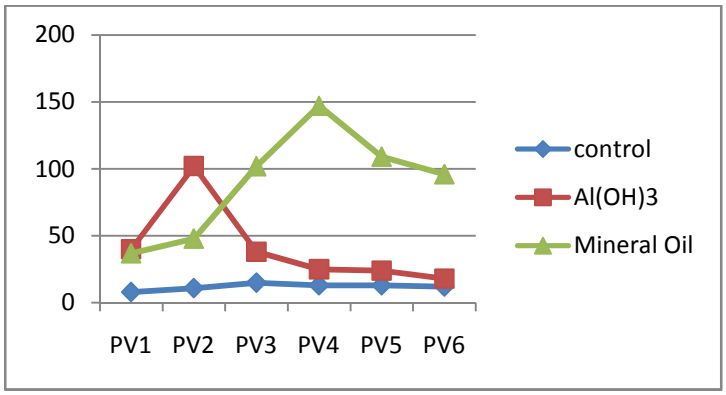
vaccine group. The titres of the saponin and aluminium hydroxide adjuvanted groups showed insignificant difference where as there was no significant difference could be observed between mineral oil adjuvanted group and commercial vaccine group. The titres of the saponin and mineral oil adjuvanted groups showed insignificant difference. In this study, the antibody levels started to increase gradually and attained highest level during fourth week pv then gradually decreased both in commercial vaccine and mineral oil groups. In aluminium hydroxide adjuvanted groups the antibody levels started to increase and reached highest level during second week pv then showed gradual decrease up to sixth week pv and slightly increased later during seventh week and then declined during eighth and ninth week pv.

Table-1: Antibody Response of Different Inactivated Vaccines (Mean Hi Titre Values)

GROUP	POST VACCINATION IN WEEKS								
	1	2	3	4	5	6	7	8	9
Control	$8^{a}\pm1.032$	$11.2^{a}\pm1.66$	$15.2^{a}\pm3.2$	$13.6^{a}\pm1.22$	$13.6^{a}\pm1.22$	$12^{a}\pm1.68$	$12.8^{a}\pm1.30$	$12^{a}\pm1.68$	$12^{a}\pm1.68$
Aluminium hydroxide adjuvanted vaccine	$40^{b}\pm5.46$	$102.4^{c}\pm10.45$	$38.4^{a}\pm5.93$	$25.6^{a}\pm4.88$	$24.8^{a}\pm5.12$	$18.4^{a}\pm3.16$	$40^{ab}\pm10.73$	$36.8^{ab}\pm6.33$	$27.2^{ab}\pm2.44$
Mineral oil adjuvanted vaccine	$36.8^{b}\pm6.33$	$48^{b}\pm5.33$	$102.4^{b}\pm10.45$	$147.2^{b}\pm25.33$	$108.8^{b}\pm9.77$	$96^{b}\pm10.66$	$70.4^{bc}\pm10.45$	$70.4^{c}\pm5.22$	$44.8^{cd}\pm5.22$

Mean bearing at least one common superscript do not differ significantly at 1 % level.

Fig-1: Antibody Response curve of Different Inactivated Vaccines



Discussion

Inactivated bacterins have proved to be efficacious in prevention of respiratory signs and lesions in chickens (Yoder, 1979) and have been demonstrated to be beneficial in reducing egg production losses and egg transmission (Glisson and Kleven, 1984). *Mycoplasma*

gallisepticum bacterins with oil emulsion adjuvant protected young chickens from intrasinus challenge with MG and commercial egg layers from MG induced drop in egg production. Khan *et al.* (1985) found that the vaccinated group responded a few weeks earlier than the unvaccinated controls indicated that the bacterin did indeed induce an active antibody response. In general, vaccinated pullets had higher agglutination and HI titres than unvaccinated pullets in our study also noticed high HI titre. Talkington and Kleven (1985) reported that the bacterins readily produced immune response in birds after only one dose, as shown by SPA and HI tests. In this study, the antibody levels started to increase gradually and attained highest level in fourth week pv and then gradually decreased both in commercial vaccine and mineral oil group after single dose of vaccination,

Stone *et al*. (1978) reported that parenterally inoculated antigens containing oil-emulsion adjuvants generally stimulated higher and more persistent antibody titres than equivalent of antigen inoculated without adjuvant or with aluminium hydroxide adjuvant. Similarly in this study also commercial and mineral oil adjuvanted group produced higher and persistent antibody titres than aluminium hydroxide adjuvanted vaccine. In comparison with commercial oil-emulsion adjuvanted vaccine, the prepared oil-emulsion vaccine shows low level of antibody titres. This might be due to the emulsion composition and method used to emulsify the aqueous and oil phases. This is in agreement with the findings of Becher (1975). Yoder *et al*. (1984) reported that inactivated MG oil-emulsion bacterin produced HI titres of 1:160 or better at the time of MG challenge four week post vaccination. Similarly in this study the commercial vaccine and mineral oil group showed peak HI titres of 204.8 and 147.0 respectively during fourth week.

Panigrahy *et al*. (1981) used two immunizations with oil emulsified bacterins six weeks apart and obtained higher HI titre than did a single immunization. In this study, single immunization was carried out. This might be the reason for falling of HI titre to low level during ninth week of pv. The study concluded that the bacterins with Oil-emulsion adjuvants stimulated higher and more persistent antibody titers than aluminium hydroxide gel adjuvanted vaccine.

REFERENCES

[1] Becher, Paul. Emulsions: Theory and Practice. Rheinhold Publishing Corporation, New York. 1957. Cited in Stone *et al*., 1978.

[2] Garg, S.P. and R.P. Garg. 1994. Studies on egg drop syndrome-76 virus immunizations with killed, adjuvanted vaccines. *Indian Vet. J*., **71**: 325-328.

[3] Hildebrand, D.G., D.E. Page and J.R. Berg. 1983. *Mycoplasma gallisepticum* (MG)-laboratory and field studies evaluating the safety and efficacy of an inactivated MG Bacterin. *Avian Dis*., **27:** 792-802.

[4] Khan, M.I., D.A. McMartin, R. Yamamoto and H.B.Ortmayer, 1985. Observation on commercial layers vaccinated with *Mycoplasma gallisepticum* bacterin on a multiple-age site endemically infected with *Mycoplasma gallisepticum*. *Avian Dis***. 30:** 309-320.

[5] Glisson, J.R. and S.H. Kleven. 1984. *Mycoplasma gallisepticum* vaccination: Effects on egg transmission and egg production. *Avian Dis.,* **28**:406-415.

[6] Lei, J.C. 1985. Aluminium hydroxide gel-guidelines for adsorption. *Vaccine.,* **3:** 154-155.

[7] Panigrahy, B., L.C. Grumbles and C.F. Hall. 1981. Immunogenic Potency of Oil-Emulsified *Mycoplasma gallisepticum* Bacterins. *Avian Dis*., **25:** 821-826.

[8] Ramadass, P., R. Ananthi, T.M.A. Senthilkumar, G. Venkatesh and V. Ramaswamy. 2006. Isolation and Characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from poultry. *Indian J. Anim. Sci.*, **76:** 796-798.

[9] Stone H.D., M. Brugh, S.R. Hopkins, H.W. Yoder and C.W. Beard.1978. Preparation of inactivated oil-emulsion vaccines with avian viral or mycoplasma antigens. *Avian Dis*., **22:** 666-674.

[10] Talkington, F.D. and S.H. Kleven. 1985. Evaluation of protection against colonization of the chicken trachea following administration of *Mycoplasma gallisepticum* bacterin. *Avian Dis*., **29**: 998-1003.

[11] Yagihashi, T., T. Nunoya and M. Tajima. 1986. Immunity induced with an aluminium hydroxide-adsorbed *Mycoplasma gallisepticum* bacterin in Chickens. *Avian Dis.,* **31**:149-155.

[13] Yoder, H.W., Jr. 1979. Serologic response of chickens vaccinated with inactivated preparations of *Mycoplasma gallisepticum. Avian Dis*., **23:** 493-506

[14] Office International des Epizooties. 2004. Avian Mycoplasmosis (*Mycoplasma gallisepticum*). In 'Manual of standards for diagnostic test and vaccines'. 4th edn, O.I.E., Paris. Chapter, 2.7.3.