



Short Communication

Immunomodulatory Effect of New Castle Disease Virus on Inactivated *Mycoplasma gallisepticum* Vaccine Response in Broilers

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ABSTRACT

In Pakistan the poultry sector is an important and vibrant segment of agriculture in Pakistan with a significant contribution to the national GDP (1.3%). *Mycoplasma gallisepticum* (MG) causes avian mycoplasmosis and is only controlled through mass vaccination. Antibody response of birds to inactivated MG vaccine is poor. On 7th day of age (broilers: n=40) each bird of group A, B, C and D (n=8) was vaccinated (subcutaneous: 0.3ml) with G-MG, O-MG, G-MG+NDV (New castle Disease Virus) and O-MG vaccines, respectively. Serum samples were collected on 21, 28 and 35 days post vaccination for anti-MG-ELISA antibody titer. Cumulative mean anti-MG-ELISA antibody titer of birds to G-MG, O-MG, G-MG+NDV, O-MG+NDV and control was 80.30, 137.49, 91.59, 169.76 and 0.15 units respectively on 35 days post-vaccination. It is concluded that NDV has insignificant immunomodulatory effect on antibody response of birds to the MG vaccine.

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Authors' Contribution

KM and MR designed and supervised the study. RR and SS conducted the experiment. MAR and ARK provided technical support to write the manuscript. MN and KM helped in publishing the manuscript.

Key words

Mycoplasma gallisepticum, CRD, Immunogen, Montanide ISA70, MG Bacterin

In Pakistan, poultry industry emerged as a most dynamic and well organized sector with high growth rate (11.8%) (GOP, 2014). Contrary to all, respiratory infections pose a great economical threat (Ali and Reynold, 2000). One of them is avian mycoplasmosis or chronic respiratory disease (CRD). Among 22 serotypes of mycoplasma, *Mycoplasma gallisepticum* (MG) is highly infectious pathogen (Kleven, 2008; Yilmaz *et al.*, 2011) that causes high mortality, disposal of carcass, low feed conversion ratio (Mukhtar *et al.*, 2012). It affects both meat and egg type poultry and is frequently encountered within poultry industries worldwide (Evans *et al.*, 2007). The disease is characterized by respiratory signs including conjunctivitis and closing of eyelids with frothy ocular exudates, sneezing, and coughing, nasal discharge, air sacculitis, breathing through partially opened beak. This organism also multiplies in reproductive organs such as ovules and oviducts leads to reduced egg production and poor hatchability (Ahmad *et al.*, 2008). The infected birds remain asymptomatic carriers and immunocompromised as MG evades from immunity and multiplies in macrophage (Gondal *et al.*, 2013). It transfers the through ovaries to next generation and aerosol

way to the birds in their vicinity (Feizi *et al.*, 2013). Eradication is only possible through control of the disease in poultry breeder flocks (Barbour and Newman, 1990). The disease is therefore controlled through live (Butcher, 2009) and killed MG vaccines (Olanrewaju *et al.*, 2011). The present study is therefore designed to evaluate effect of lentogenic Newcastle disease virus-NDV on antibody response of broilers to monovalent and bivalent inactivated gel and oil based MG vaccines. Glycoprotein of Newcastle disease virus (NDV) is T-cell dependent antigen that induces cytokine production, and is strong interferon gamma inducer, which is mainly responsible for development of effective humoral as well as cellular immunity in, vaccinates.

Materials and methods

One-day old Hubbard chicks (n=40) were purchased from Hi-Tech Hatchery, Multan road, Lahore and reared at the experimental shed, Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore. The chicks were raised under standard management conditions for a period of 42 days. On 7th day of age, the chicks were divided into A, B, C, D and E groups (n=8).

Local isolate of MG confirmed through polymerase chain reaction was procured in active form from University Diagnostic Laboratory (UDL), University of Veterinary and Animal Sciences (UVAS) Lahore. For revival of MG culture Frey's broth culture medium (250ml contains

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0.074g thallium acetate, 5.62 g PPLO broth base, 1.25g yeast extract, 0.0074 g phenol red, 0.074 g glucose (10% w/v), 0.025 g nicotinamide adenine dinucleotide (1% w/v), 0.025 g cysteine HCL (1% w/v), 0.156g penicillin G and 30ml horse serum) was used.

Active MG culture (10% v/v) was added in the Frey's broth culture medium in a flask of 250 ml capacity and incubated at 37°C in 10% CO₂ incubator (70% humidity) for 48 h. Broth was observed daily for growth. After 48 h of incubation, there was change in color of medium from red to yellow. At this stage, freshly prepared sterile broth (250ml) was added after every 48 h till the volume became 1 liter. The whole culture was centrifuged at 3000xg for 20 min. The pellet was suspended in 10ml PBS in Hopkins's tube (Kimax[®], USA) that was further centrifuged at 3000xg for 20 min to estimate packed cell volume (PCV) per ml of the medium and the result was recorded. The mass of the bacteria was admixed in PBS to make 1% suspension (Yoder *et al.*, 2009).

The bacterial suspension admixed with 0.125% of formaldehyde (37%) (Scharlau, Spain) was incubated at 37°C with 10% CO₂ for 12 h. The inactivated bacterial suspension was cultured on the broth and agar, incubated at 37°C with 10% CO₂ for 7 days to confirm inactivation (Koskiet *al.*, 1976).

Newcastle disease virus (lentogenic strain) used for vaccine preparation was taken from UDL, UVAS Lahore. The virus was grown in 9 days old chicken embryos inoculating through chorioallantoic sac route. The allantoic fluid (AAF) thus harvested from embryos was processed for determination of embryo infected dose 50 (EID50) (Villegas, 1998). AAF was processed for sterility on tryptose broth and tryptose agar and safety test in the non-vaccinated 30 days old broilers (Anonymous, 2008).

Gel based MG (G-MG) vaccine was prepared (0.3 ml/dose) by mixing 0.06 ml of one percent inactivated MG suspension, 0.06 ml PBS and 0.18 ml of 4% aluminum hydroxide gel. Oil based MG (O-MG) vaccine was prepared (0.3 ml/dose) by mixing 0.06 ml of one percent inactivated MG suspension, 0.06ml PBS and 0.18 ml montanide ISA-70 (SEPPIC, France). Oil based MG+NDV (O-MG+NDV) vaccine was prepared (0.3 ml/dose) by mixing 0.06 ml of one percent inactivated MG suspension, 0.06ml NDV having 10⁶ unit of EID50 and 0.18 ml montanide ISA-70. Gel based MG+NDV (G-MG+NDV) vaccine was prepared (0.3 ml/dose) by mixing 0.06 ml of one percent inactivated MG suspension, 0.06ml NDV having 10⁶ unit of EID50 and 0.18 ml of 4% aluminum hydroxide gel.

On 7th day of age, the chicks were randomly divided into A, B, C, D and E groups (n=8) and each chick of group A, B, C and D was primed (sub-cut injection at mid-dorsal side of the neck) with 0.3 ml of O-MG, O-MG+NDV, G-MG+NDV and G-MG vaccine, respectively. Each chick

of group E served as un-vaccinated control.

Blood sample was collected aseptically from wing vein of each chick of each of the groups on 21, 28 and 35 days post vaccination. The serum sample from each blood sample was separated, transferred to properly labeled serum vials and stored at -40°C till further processing. Each serum sample was processed for determination of anti-MG-ELISA using ProfFLOK MG ELISA kit (Synbiotics Corporation, USA) (Avakian *et al.*, 1988).

The data thus achieved processed through one-way analysis of variance (ANOVA) and subsequently Duncan Multiple Range Test (DMRT) using SPSS version 16.0 (Steel *et al.*, 1997) (Table I).

Results and discussion

MG is a persistent, highly transmissible chicken pathogen yield significant losses in performance and economics to all sectors of the poultry industry. Stringent biosecurity and bio surveillance practices are needed to control MG within the poultry industry (Evans *et al.*, 2005) as there is vertical transmission of MG results progeny flocks infected or carrier (Gharaibeh and Al Roussan, 2008). MG shows poor growth in the agar culture medium. MG grew well in Frey's broth and its growth was further augmented by its continuous growth method. It might be due to continuous supply of enrichment additives over 10 days of incubation. MG does not produce any toxin (exotoxin, endotoxin) as it is wall less bacteria but the components of the broth are antigenic so are removed by centrifugation of the culture suspension. It yielded 0.75ml/10ml of the medium. Its biological titer for production of vaccine may be one percent (1%) of MG in phosphate buffered saline (PBS) solution PH 7.2. Formaldehyde inactivated the MG suspension when admixed at rate of 0.15% at 37 C within 12 h. The suspension was therefore inactivated through mixing formaldehyde (37 per cent: Merck) @ 0.05 per cent (Karaca and Lam, 1987). Formaldehyde molecules inactivate its viability when it binds with amine group of amino acids in protein molecule of the organisms. Its higher concentration reduces the antigenicity of the organisms. Killed vaccines require the adjuvant as opposed to live attenuated vaccines (Petrovsky and Aguilar, 2004). Different adjuvants when admixed with vaccinal antigen enhance the immuneresponse (Coffman *et al.*, 2010). Mineral oil (Montanide ISA 70) encapsulates the immunogen and hence enhances its retention time at the inoculation site (Shakya and Nandakumar, 2013). However, aluminum hydroxide gel is also used for the purpose in veterinary and medical vaccines. Aluminum-containing vaccines are prepared by adsorption of antigens onto aluminum hydroxide or aluminum phosphate gels or by precipitation of antigens in a solution of alum (Hogenesch, 2002).

Table I. Comparative mean anti-MG-ELISA antibody response of broilers to adjuvated MG vaccines.

Type of vaccine	Anti-MG-ELISA antibody titer on days post-vaccination (DPV)			Cumulative mean antibody titer
	21	28	35	
G-MG	32.21±23.46	74.6±64.41	134.13±68.11	80.30b
O-MG	34.04±28.04	55.34±34.3	323.1±401.04	137.49c
G-MG+NDV	33.78±57.85	101.92±56.86	139.09±41.95	91.59b
O-MG+NDV	68.17±32	162.95±69.11	278.18±91.34	169.76c
Control	0.12±0.23	0.16±0.34	0.17±0.34	0.15a

Cumulative mean values of anti-MG-ELISA antibody titer in last column with different superscripts differ significantly ($P < 0.05$). MG, *Mycoplasma gallisepticum*; NDV, Newcastle disease virus; O, oil based; G, gel based.

Antibody response of the host is directly proportional to the retention time of the immunogen. This could be plausible reason of higher antibody response of birds to oil based vaccines than that of gel based vaccines. Vaccines induce specific immune responses in the vaccinated birds in the form of production of specific antibodies, and nonspecific factors / cytokines particularly interferon gamma that activate antigen stimulated B cells, macrophages, cytotoxic T cells, Natural Killer (NK) cells, etc. (Boyaka and Mcghee, 2001; Hilton *et al.*, 2002).

Birds vaccinated with G-MG vaccine showed 32.21±23.46, 74.6±64.41 and 134.13±68.11 anti-MG-ELISA mean antibody titer at 21, 28 and 35 days post vaccination. The broilers vaccinated with O-MG vaccine showed 34.04±28.04, 55.3±34.3 and 323.11±401.04 anti-MG-ELISA mean antibody titer at 21, 28 and 35 days post vaccination. These results are similar to the observation of Barbour *et al.* (2002). This study revealed significant immunoglobulin (Ig) response specific to MG in sera of chickens collected 3 weeks after the first and second vaccination with MG adjuvanted with oil-emulsion as compare to other adjuvants. That might be because oil has more antigen retention and slow degradation power as compare to gel so oil based vaccine has long term effect and titer decreases slowly.

Birds vaccinated with G-MG+NDV vaccine (live Newcastle disease virus) showed 33.78±57.85, 101.92±56.86 and 139.09±41.95 anti-MG-ELISA antibody titer at 21, 28 and 35 days post vaccination. Birds vaccinated O-MG+NDV showed 68.17±32, 162.95±69.11, 278.1±91.34 anti-MG-ELISA antibody titer at 21, 28 and 35 days post vaccination. The possible reason is the production of cytokines by T cell due to T cell dependent antigen. In the immune-compromised birds, interferon gamma is not produced effectively in response to the vaccine so macrophages are not properly activated that ultimately fail to kill phagocytosed mycoplasma (Muneta *et al.*, 2006). CD8⁺ cells activated in response to interferon gamma may inactivate the intracellular mycoplasma in macrophages or other cells (Gobel, 1996). A carrier

form of MG infected flock may be treated by inducing any antigen to such chicken as can produce interferon gamma that can subsequently activates macrophages for microbicidal activity. The major advantage of oil-emulsion bacterin is protection against economic losses can be obtained without the introduction of any live-vaccine strain. Whereas, disadvantages include: cost, requirement of handling individual chicken, and the relative lack of protection against colonization of field challenge strains of MG. Although vaccination for MG can be a useful tool, especially on multi-age commercial egg-production sites yet vaccination should be limited to situations where maintenance of infection free flocks is not feasible.

Antibody response of vaccinated broilers to combined MG-NDV vaccine was not significantly different from the birds vaccinated with either of the monovalent NDV or MG vaccine ($p > 0.05$). Moreover, it was observed that oil based monovalent (MG) or bivalent (MG+NDV) vaccine induced significantly higher antibody response in the vaccinated broilers than that of the birds vaccinated with gel based monovalent (MG) or bivalent (MG+NDV) vaccine ($p < 0.05$).

Conclusion

It is concluded that bivalent MG+NDV vaccine can be prepared to induce effective immunoprophylaxis against both the immunogens simultaneously. Moreover, oil-based vaccine is more effective in induction of immunity than that of the gel based vaccine.

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Statement of conflict of interest

There is no conflict of interests regarding the publication of this paper.

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