

In Process Quality Control Factors Affecting Potency of Fowl Cholera Vaccine

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Abstract

Fowl cholera (FC) is one of the respiratory syndromes of commercial layer, breeder and broiler farms in Pakistan. The disease is mainly controlled by vaccination. In the present study, effect of “*in process quality control*” factors such as amount of immunogen, chemical nature of adjuvant, fractions of bacterial culture and its storage on potency of the vaccine was investigated. Immunogen amount (10^{11} CFU/ml) induced serum *anti-Pasteurella multocida* ELISA (anti-PM-ELISA) antibody titer in the vaccinated birds on 32 days post vaccination that was significantly higher ($P < 0.05$) than that of 10^9 and 10^{10} CFU/ml. Montanide based whole culture vaccine induced better antibody response as compare to aluminum hydroxide gel. Washed culture of *P. multocida* (bacterial sediment) induced significantly higher anti-PM-ELISA antibody titer as compared to the vaccine prepared from purified LPS or whole culture vaccine ($p < 0.05$). Storage of oil based FC vaccine at refrigerated temperature for six months did not affect its immunogenicity.

It is concluded that amount of immunogen, chemical nature of adjuvant, freeing of bacterial culture from LPS and media components and maintenance of cold chain are in process quality control factors affecting potency of the vaccine.

Keywords: Fowl Cholera, *Pasteurella Multocida*, Montanide, ELISA

1. Introduction

Fowl Cholera (FC) is one of the contagious bacterial diseases of domestic and wild avian species. It is characterized by facial edema, blackening of comb and wattles, off feed and water, gasping, dull depressed with high morbidity (up to 50%) and mortality up to 10% (Choudhury et al., 1992; Glisson et al., 2003). Disease severity is enhanced in hot humid environment, overcrowding, age, pH and sucrose level of clay (Bredy & Botzler, 1989; Hutyra et al., 1949; Petrides & Bryant, 1951; Samuel et al., 1999a; Titcher, 1979).

It is caused by *Pasteurella multocida* (*P. multocida*). The organism is serotyped on the basis of capsular (A) and somatic antigens. These serotypes are A:1, A:3, A:5 and A:9 serotype and rarely cause a problem in mammalian species (Benkirane & De Alwis, 2002; Mohamed et al., 2012; Rhoades & Rimler, 1989). Serotype A:1 of *P. multocida* is the common cause of the disease in Asian countries (Farooq et al., 2007; Shivachandra et al., 2006). Being acute nature of the bacterial disease, treatment with antibiotics is not successful unless carried out at an early stage. In poultry industry the disease is mainly controlled through mass vaccination program. The commonly used vaccine against FC is oil or gel based inactivated whole culture bacterial vaccine or live attenuated bacterial vaccine (Boyce et al., 2000). Killed vaccines have certain limitations. Alum precipitated vaccine although used extensively but, induces immunity for short period of time 4-6 months, whereas oil based vaccine provides protection up to one year but oil based vaccines in poultry induces facial edema, abscess formation and post vaccination shocks (Littledike, 1993). These problems might be due to bacterial capsule (lipopolysaccharide-LPS) that is produced extensively during bacterial growth in bio-fermentor (latest advanced technique of bacterial biomass production). The immunity against *P. multocida* is due to LPS as well as its outer

membrane protein (OMP). The present study is therefore, planned to investigate in process quality control factors such as amount of immunogen per dose, chemical nature of adjuvant, fractions of the bacterial culture and shelf life of the vaccine.

2. Materials and Methods

2.1 Cultivation of *P. Multocida*

Fresh culture of *P. multocida* (serotype A) was obtained from Ottoman Pharma 10-Km Raiwind road, Lahore for preparation of different types of vaccine. *P. multocida* was grown in biofermentor containing BHI broth at 37°C for 24 hours with constant stirring (Shah et al., 2008). The broth culture was inactivated with 0.5% (v/v) formalin (Merck). Sterility of the chemically inactivated culture was determined by its inoculation on BHI blood agar plates and subsequently their incubation at 37°C for 24 hours and safety was determined by its inoculation (1ml) through deep intramuscular route in two susceptible birds.

2.2 Preparation of Vaccines

Vaccines containing different amount of immunogens (10^{11} , 10^{10} , 10^9 CFU/dose), chemical nature of adjuvants (Montanide ISA70MVG, aluminium hydroxide gel, no adjuvant) and fractions of the culture (bacterial washed culture, whole culture and supernatant) were prepared (Sarwar et al., 2015).

2.3 Experimental Design

Eighty broilers (Six day old) were selected and randomly divided into eight groups each having ten birds. Each bird of group 1, 2 and 3 was vaccinated with montanide ISA 70MVG based FC vaccine containing 10^9 , 10^{10} and 10^{11} CFU/ml, respectively. Each bird of group 4 and 5 was vaccinated with FC vaccine containing, aluminium hydroxide gel and without adjuvant, respectively. Each bird of group 6 and 7 was primed with oil based vaccine containing supernatant and sediment culture, respectively. The birds of group 8 served as unvaccinated control.

2.4 Monitoring of Seroconversion of Vaccinated Birds

Blood sample (2ml) was collected from wing vein of each of birds of group one to eight on zero, 16, 32 and 48 days post priming in disposable syringes. Each of the blood samples were kept undisturbed at refrigeration temperature and subsequently at 37°C for 2 hours. The serum from each of the sample thus oozed out was transferred to properly labeled vials and stored at -20°C for monitoring of its anti FC ELISA antibody titer using (IDEXX PM) kit (Avakian et al., 1985).

2.5 Statistical Analysis

Mean anti-PA-ELISA antibody titer of each group of the vaccine was analyzed statistically using one way variance of analysis (ANOVA) and subsequently Duncan multiple range test (Day & Quinn, 1989).

3. Results and Discussion

Fowl cholera is common problem of commercial poultry farms particularly in countries having hot humid environment and is controlled by vaccination (Benkirane & Alwis, 2002; Hutyra et al., 1949). Causative agent of the disease (*Pasteurella multocida*) contains capsular LPS and somatic antigen/OMP. The vaccine is prepared using its capsulated form with the idea to develop antibodies specific to its capsular antigen (Chung et al., 2001; Tsuji & Matsumoto, 1988b). Moreover, efficacy of the vaccine depends upon density of its culture per dose of vaccine. The density of bacterial culture is controlled by enrichment media BHI both or CYS broth, incubation temperature and time and aeration of the culture during growth period (Shah et al., 2008). The bacterial count/ml of the culture was directly proportional to its biomass. Moreover, FC vaccine containing 7mg/ 10^9 CFU/ml of the dose induced 438 units of anti PM-ELISA antibody titer. Antibody response of the broilers was directly proportional to the amount of immunogen per dose of the vaccine (Figure-I). Higher the amount of immunogen per dose of the vaccine, higher will be the antibody response of the vaccinated host (Ali et al., 2000).

The vaccine without adjuvant showed poor antibody titer in the vaccinated birds that directly related with the retention time of vaccine at the injection site. Poor response of the birds is incriminated to rapid absorption of the immunogen from inoculation site. In case of live attenuated vaccine against the disease, the bacteria retained in the body on account of their multiplication in non-essential sites such as larynx/pharynx of the host. Adjuvants are chemicals that make the immunogen of the vaccine insoluble, hence, enhance its retention time at the inoculation site. These are therefore, used in the veterinary and medical inactivated vaccines. Commonly used adjuvants are mineral oils, aluminium salts (alum and gel), sodium alginate, saponin, lanolin and vegetable oils (Jankovic et al., 1997; Wu et al., 1994; Audibert & Lise 1993). Mineral oils encapsulate the bacterial antigen and make it water insoluble. Hence, protects rapid absorption of the vaccine from inoculation sites (Wu et al., 1994). Moreover, oil causes irritation at the inoculation site that result in development of local granuloma. As there are

no lymph nodes in birds and development of such granuloma at vaccine injection site play cardinal role in production of immunity (Igyártó et al., 2006). Aluminium hydroxide gel is not irritant so is commonly used in veterinary and medical vaccines. The gel adsorbs the vaccinal antigen, mitigates its direct removal from the inoculation site and hence removed from inoculation site through antigen processing cells. The vaccine material may be processed by local antigen processing and presenting cells (APC) such as macrophages, dendritic cells or B cells and the protein antigens are presented on its surface in association with class MHC-II antigen/immune associated antigen (Ia Ag) (Jankovic et al., 1997). Avian T helper (T_h) cells can only recognize the foreign specific antigen associated with Ia antigen on surface of the APC and undergo the process of blast formation, proliferation, differentiation into effector and memory cells. The effector cells those survive up to seven days secrete cytokines such as IL-2, IL-4, IL-5, INF- γ etc. Production of the cytokines is antigen specific and their action is antigen nonspecific so, these cytokines now specifically activates macrophages, Natural killer (NK) cells, Cytotoxic T cells (T_c), B cells etc. In this way cytokines potentiate the specific and nonspecific immune responses of the vaccinated birds (Tizzard, 1998). Similarly, avian bursal dependent lymphocytes (B cells) recognize specific free antigen from the inoculation site and undergo the process of blast formation, proliferation and differentiation into plasma cells without development of memory cells. These plasma cells survive for short time and secrete out immunoglobulins (IgM). Such antibody response is always primary response. Such birds can never mount boosting response as a result of boosting. The IgM is effectively detected by IHA test but not by ELISA test. This could be possible reason that indirect ELISA showed undetectable level of antibody response of the birds vaccinated with supernatant of *P. multocida* culture. However, cytokines production may modulate the T cell independent antigen (carbohydrates/lipids/nucleic acids) processing pathway and results in development of memory cells as well as switching over of IgM synthesis followed by IgG and IgA. The montanide based FC vaccine induced mean anti-PM-ELISA antibody response in the vaccinated broilers that gradually mounted and reached the peak level (570) on 48 days post priming while, the gel based FC vaccine induced anti-PM-ELISA antibody titer that reached at peak level (375) on 48 days post priming (Figure-II). The antibody response of the birds in both the cases started declining thereafter. In this experiment, oil based vaccine induced significantly higher antibody response as compared to gel based vaccines ($p < 0.05$). Gel based vaccines induce antibody response of short duration and less effective (Audibert et al., 1993; Muneer et al., 2005).

Washed culture of *P. multocida* (bacterial suspension free from LPS and medium component) induced significantly higher antibody titer as compared to the vaccine prepared from purified LPS or whole culture vaccine ($p < 0.05$; Figure-III). Outer membrane proteins (OMPs) of *P. multocida* have been studied as potential immunogens, which make them potential vaccine candidates (Lugtenberg et al., 1986; Rhoades & Rimler, 1989; Lu et al., 1983; Adler et al., 1996). Purified OMP of *P. multocida* or whole culture induces antibody response in rabbit and show resistance to challenge infection (Adler et al., 1996). The immunity against whole culture is more effective than that LPS alone. Several antigenic components have been investigated as an immunogen against *P. multocida* infection including purified lipopolysaccharide (LPS) (Rhoades & Rimler, 1991) and LPS-protein complex (Tsuji & Matsumoto, 1988b). It was observed that vaccine is stable at refrigerated temperature for six months (Figure-V). The vaccines are stored or transported at refrigerated temperature and are protected from sunlight to minimize the decay of the macromolecules. All chemically inactivated microbial cultures are admixed with preservatives such as thiomersal sodium @ 0.05% in the culture suspension before addition of adjuvants (Kulcsar et al., 2008).

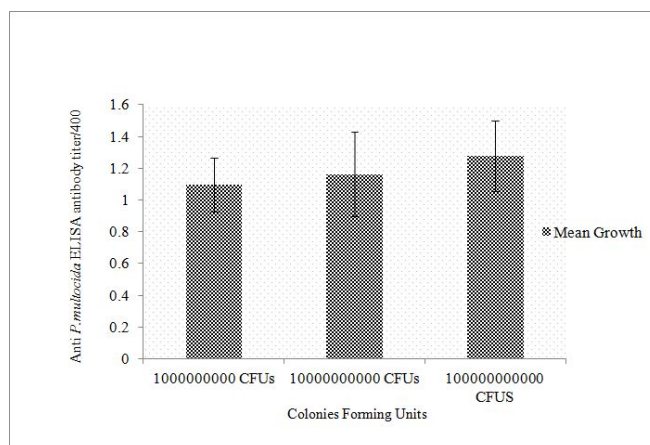


Figure 1. Effect of immunogen (bacterial count/dose of *P. multocida* vaccine) on antibody response of broiler

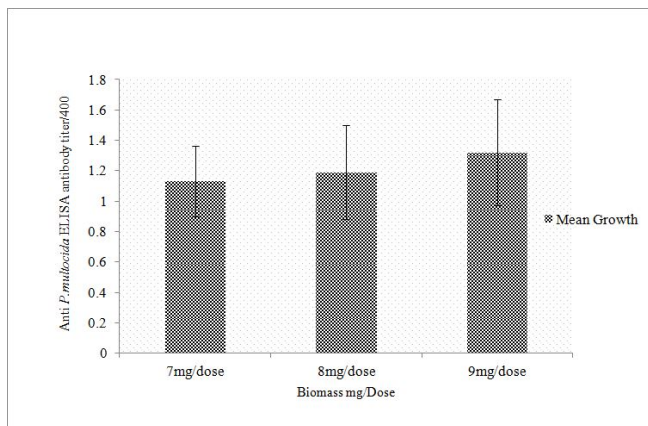


Figure 2. Effect of dry mass of immunogen on antibody response of broilers to oil based *Pasteurella multocida* vaccine

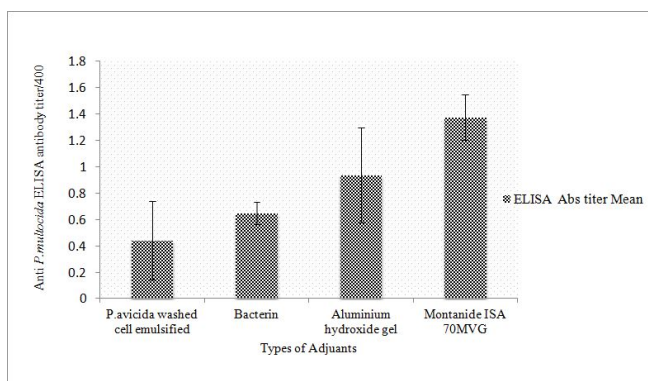


Figure 3. Effect of Adjuvant on Antibody response of Broilers to *Pasteurella multocida* vaccine

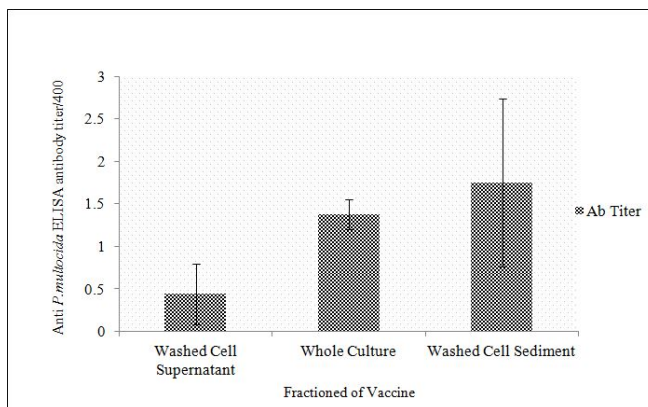


Figure 4. Antibody response of broiler to oil based vaccine containing sediment of *P. multocida*

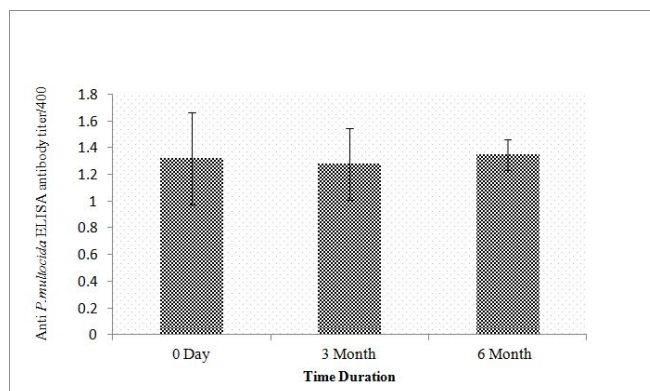


Figure 5. Effect of storage of vaccine on antibody response

It is concluded that amount of immunogen, chemical nature of adjuvant, freeing of bacterial culture from LPS and media components and maintenance of cold chain are in process quality control factors affecting potency of the vaccine.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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