INFECTIOUS BURSAL DISEASE VACCINE DELIVERY VIA TOPICAL APPLICATION IN 18-DAY-OLD SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS

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ABSTRACT

The purpose of this study was to investigate the effect of vaccination against infectious bursal disease (IBD) via topical application of IBD vaccine (MyVAC UPM93) either with or without liposomes as vaccine carrier in 18-day-old specific pathogen free (SPF) embryonated chicken eggs. The study demonstrated that the IBD vaccine group alone could induce high and protective level of IBD antibody titre (2545±1884 ELISA unit) in the chicks at 14 days of age. In contrast, IBD antibody titre was not detected in the liposomes, combination of liposomes with IBD vaccine and control groups of chickens. It appears that further study is needed to improve the liposome as a vaccine carrier to accelerate vaccine delivery to the target organ and enhance antibody titre. It was concluded that topical application of IBD vaccine (MyVAC UPM93) in 18-day-old SPF embryonated chicken eggs can be an effective vaccination route against IBD.
Keywords: Infectious bursal disease, vaccine delivery, topical application route, liposome

INTRODUCTION

Developments of nanoparticles as carrier-based vaccines receive a lot of attention in order to provide effective immunization. Nanoparticles are solid particles that have size around 10 to 1000 nm (Kreauter, 1996). Liposomes, being composed from phospholipids and can function adroitly complements the natural lining of nearly every cell. This therefore creates a natural bond and/or affinity for the liposomes to deliver their drugs or vaccines to the cells in the live body (Rosenblum and Chen, 1995). Infectious bursal disease (IBD) is one of the major viral diseases for the poultry industry worldwide. IBD causes death to susceptible chickens as well as immunosuppression which lead to variety of secondary infections and high mortality. IBD can be controlled and prevented by proper vaccination and biosecurity. Vaccination as early as 18-day-old embryonated eggs known as *in ovo* vaccination has been practiced, although puncturing the eggshell and membrane dramatically increased embryonic mortality, regardless of whether any material is injected into the egg (William, 2005). Alternatively, topical application onto eggshell could provide better solution and could reduce mortality. A few studies have been done related to the topical application of drugs onto the crocodile eggs (Muller *et al*., 2007) that have similar characteristic with chicken eggs (Astheimer *et al*., 1989). It was the objectives of this study to determine the effect of vaccination against IBD via topical application of IBD vaccine (MyVAC UPM93) either with or without liposomes as vaccine carrier in 18-day-old specific pathogen free (SPF) embryonated chicken eggs.

MATERIALS AND METHODS

Specific-pathogen free (SPF) embryonated chicken eggs and IBD vaccine (MyVAC UPM93) were obtained from Malaysian Vaccines and Pharmaceuticals (MVP) Sdn. Bhd. Positive liposome kit (Product No: L4395) was obtained from Sigma Malaysia. Sterile double distilled water (1.0 mL) was added into the vial that contains dry

powder of liposomes at room temperature. Then, by using vortex, the hydrated liposomes were shaking for 60 seconds. A sample was taken for determination of size and zeta potential. After that, by using sterile pipette, IBDV were combined with hydrated liposomes. The vial was stored at 4-6 ˚C. Twenty four, 18-day-old SPF embryonated chicken eggs were marked with pencil and the surfaces of eggshells were cleaned by using 70 % ethanol. Then, the eggs were divided into 4 groups namely the control, liposome, combination of IBDV and liposome and IBDV. Samples (0.1 mL) were sprayed onto eggshells accordingly. After half an hour, eggs were incubated at 37 °C until all the eggs hatched. Serum was collected from all groups of chickens at 14 days of age for detection of IBD antibody titre using ELISA technique.

RESULTS AND DISCUSSION

The mean size of pure liposome, IBDV and combination of liposome and IBDV were 1441±313 nm, 1827±182 nm and 2842±168 nm, respectively. These averages of sizes are quite far from the expected values and contradict with early hypothesis. This could be due to homogenization procedure which was not performed before sprayed onto eggshells in the study. The average of zeta potential of empty liposome, IBDV and combination of liposome and IBDV were +192 mV, -18.0 mV and -12.1 mV, respectively. In order to maintain stability and pH of liposomes, phosphate buffered saline (PBS) is a better choice to dilute the liposomes rather than using distilled water. Although the size IBDV, liposomes and combination of both are more than 1000 nm, but there are still under the range diameter size of pores in the eggshells of the chickens which ranged from 110 to 4140 nm (Tan *et. al*., 1992). Hence, there are possibilities of the vaccine and the carriers can pass through the eggshells of the chickens. Neither clinical signs nor gross and histological lesions of the bursa of Fabricius were observed in all groups of chickens throughout the study. It is interesting to note that the IBD vaccine group alone could induce high and protective level of IBD antibody titre (2545 ± 1884 ELISA unit) in the chicks at 14 days of age. In contrast, IBD antibody titre was not detected in the liposome, combination of liposome with IBD vaccine and control groups of chickens. It appears that further study is needed to improve the liposome as a vaccine carrier to accelerate vaccine delivered to the target organ and enhanced antibody titre. It was concluded that topical application of IBD vaccine (MyVAC UPM93) on 18-day-old SPF embryonated chicken eggs can be an effective vaccination route against IBD.

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