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Full Length Research Paper

Preliminary evaluation of the immunoenhancement potential of fowl typhoid vaccine formulated as an oral cationic liposome

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Salmonella gallinarum **is a non-motile host-specific bacterium in domestic poultry. It causes fowl typhoid, especially in domestic poultry. The study was performed with a view to compare the antibody titre of commercial subcutaneous Fowl typhoid® vaccine with its oral liposomal formulation. The chicken, divided into three groups (n = 20 per group), were inoculated via oral or subcutaneous route. Group A was given commercial subcutaneous Fowl typhoid® vaccine, Group B served as the unvaccinated control and Group C was given the Fowl typhoid® vaccine encapsulated in a cationic liposome. Vaccination of the groups was carried out once at 42 days of age. Blood samples were collected from the jugular vein at 56 days of age to obtain the sera for slide and microplate agglutination test. At 63 days of age, the birds were challenged with a field strain of the *S. gallinarum*. From the microplate agglutination test (mean ± standard deviation (SD), at $p < 0.05$), it was found that there was no significant difference in the mean antibody titre of the birds by either the subcutaneous or oral vaccination of Fowl typhoid® vaccine.**

Key words: Fowl typhoid vaccine, *Salmonella gallinarum*, liposome.

INTRODUCTION

Salmonella gallinarum (SG) is the etiologic agent of fowl typhoid (FT), a severe systemic disease of chickens and other galliform birds (Shivaprasad, 2000). The disease is dose-dependent and differences in pathogenicity may be found depending upon the susceptibility of the infected genetic line of chickens (Oliveira et al., 2005). Mortality and morbidity rates due to fowl typhoid may reach up to 80% (Wigley et al., 2002, 2004). Fowl typhoid is still of considerable economic importance in many countries of Africa, Asia, and Central and South America (Pomeroy and Nagajara, 1991; Lee et al., 2003). Vaccination against SG is commonly used as preventive measure which is available as injections (Lee et al., 2007). However, oral delivery of Fowl typhoid® vaccine has not yet been fully exploited. Delivering Fowl typhoid® vaccine orally would not only be beneficial to farmers but would reduce the injuries and hazards caused by needle-stick vaccination. Overall, it would encourage rural farmers to adopt vaccination and this would ultimately improve livestock production and ensure food security. Liposomes have therefore, been employed in this research as carriers and adjuvants of Fowl typhoid® vaccine.

Twenty-five years after the discovery of the immuno-logical adjuvant properties of liposomes, they appear now as a major candidate adjuvant, with a liposome based vaccine (against hepatitis A) being licensed for use in humans (Gregoriadis, 1995). Liposomes are small, spherical, self-closed vesicles of colloidal dimension which consist of amphiphilic lipids, enclosing an aqueous core. (Valiante et al., 2003). Cationic liposomes remain

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at the forefront of vaccine design owing to their well documented ability to boost both humoral and cell-mediated immune response. In this study, DOTAP-based liposome was used to entrap a standard fowl typhoid vaccine for possible oral vaccination. The results suggest that fowl typhoid vaccine entrapped in a DOTAP-based liposome might be a suitable alternative to parenteral delivery.

MATERIALS AND METHODS

Cholesterol (Sigma Grade, minimum purity 99%, Sigma Aldrich Chemie, St. Louis, USA), 1,2-dioleoyl-3-trimethylammonium propane (DOTAP (Sigma-Aldrich, USA) phospholipon 90H (GmbH Nattermannallee, Köln, Germany), methanol (extra pure, Scharlau Chemie S.A.), chloroform (Sigma-Aldrich, GmbH Germany). All are of analytical grade.

Chickens

Sixty day old chicks (*Gallus gallus* domesticus) from CHI farms, Ogun State were used. The chicks were raised from day old until termination of the experiment. The birds in the different groups were housed in separate rooms and all cleaning and feeding was planned to minimise the risk of cross-contamination. All animal handling and experiments were conducted following the guidelines stipulated by University of Nigeria Research Ethics Committee on animal handling and use. Results of the study was presented as mean with standard deviations (SD) of each of the parameters.

Vaccine and challenge strain

The 9R Fowl Typhoid® vaccine strain [100 units] (National Veterinary Research Institute (NVRI), Vom, Nigeria) was used in this trial. The challenge strain used was the virulent *S. gallinarum* (1.0 Mc Farland suspension was prepared) strain Gr. D1, 1.9.12 (Instituto zooprofilatico sperimentale delle venezie Legnaro, Italy. It was stored at -20°C.

Experimentals

Preparation of dry lipid films

196 mg of phosphatidylcholine, 96.7 mg of cholesterol and 50 µg of DOTAP were weighed and dissolved in 3 ml of chloroform/methanol system (2:1) in a round bottom flask. The solvent mixture was evaporated at room temperature and the flask rotated until a smooth, thin, dry film on the wall of the flask was obtained (Azmin et al., 1985).

Hydration of dry lipid films

Fowl typhoid® (100 units) vaccine was reconstituted with 20 ml of normal saline. 5 ml volume of the reconstituted vaccine was used to hydrate the dry films of the liposome and agitated gently until multilamellar vesicles were formed.

Vaccination of the birds

Sixty birds were divided into three groups of twenty birds each. At 42 days of age, Group A was given 0.2 ml/bird of the commercial subcutaneous fowl typhoid vaccine, Group B served as the unvaccinated control and Group C was given 0.2 ml/bird of the fowl typhoid vaccine encapsulated in a cationic liposome. The vaccination was carried out once.

Blood collection

At 56 days of age, about 5 ml of blood was collected from the jugular vein of each bird using sterile syringe and emptied into marked individual Ethylenediaminetetraacetic acid (EDTA) tubes. Sera were prepared from blood samples to determine the circulating antibody titres by slide and microplate aglutination test.

Preparation of challenge strain

1.0 McFarland standard was prepared by adding 990.0 ml of 1% sulfuric acid to 10.0 ml of 1% barium chloride. The density of the *S. gallinarum* inoculum was adjusted to match the density of the standard solution. A 0.5 McFarland standard is comparable to a bacterial suspension of 10^8 cfu/ml.

Slide agglutination test

Clean white square tiles were used. Serum samples from all the birds were tested. One drop (0.05 ml) of crystal violet stained *S. gallinarum* antigen was placed at nine different points equidistant from each other. A drop of the serum samples of the immunized birds from the different groups, one group at a time, was placed next to the drop of antigen. The drops of antigen and serum were mixed using a glass rod which was wiped clean between samples. Then, using a gentle rocking motion the tiles were rotated for 2 min and signs of agglutination observed. The samples that showed agglutination were used for the plate agglutination test.

Plate agglutination test

The O somatic antigen was incubated overnight at 40°C. A 25 ul of 0.5% phenol saline was added to all the wells in a column in a microtitre plate. A 25 ul volume of the chicken serum was added to the first well and two-fold dilutions made. The last well in the row contained only saline as negative control. A 25 ul volume of O somatic diluted antigen was then added to all the wells and left for 4 h to incubate. The wells were examined for signs of agglutination and compared with the negative and positive controls. Statistical analysis was performed at $p < 0.05$, applying a one-way analysis of variance (ANOVA) test with least squared difference (LSD) multiple comparisons.

Challenge and post-challenge observation of chicken

At 63 days of age, ten chickens from each group were peritoneally inoculated with 0.5 ml of 1.0 Mc Farland suspension of the virulent *S. gallinarum.* Necropsy samples of the spleen, liver, bursa and thymus of infected were randomly collected from dead as well as live infected birds from each group for gross examination.

Safety test

The safety test had already been carried out in an earlier experiment using La Sota® vaccine (Onuigbo et al., 2012).

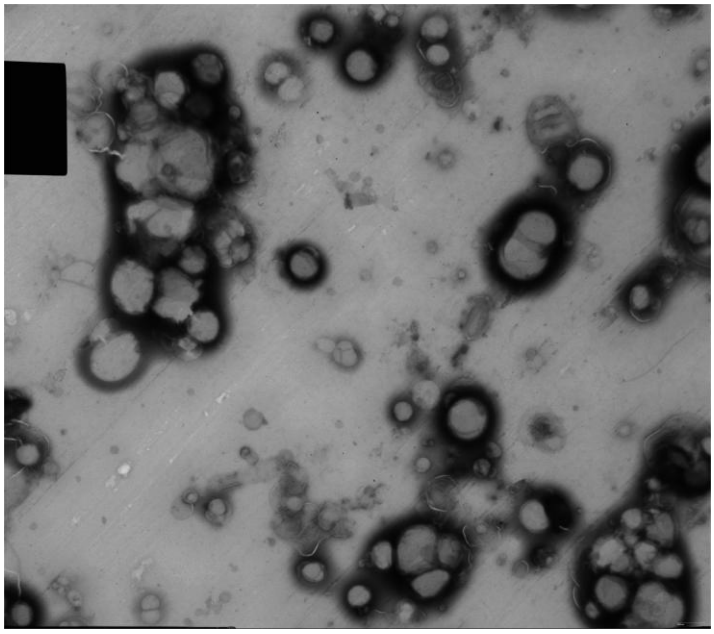

Figure 1. Transmission electron microscopy images of the cationic liposome incorporating the fowl typhoid vaccine (×10500).

RESULTS AND DISCUSSION

The technique used for the preparation of the liposome was lipid film hydration technique (hand shaking method) which formed a film on the wall of the flask and on hydration with phosphate buffer solution (pH 7·4), produced thick, gel-like, milky colloidal dispersion as shown in Figure 1. These vesicles incorporated the fowl typhoid vaccine within the core of the vesicles and protected it from the harsh environment of the gastrointestinal tract and peptidases which destroy antigen. It also prevents vaccine from being released immediately to the lymphoid tissues, thereby prolonging the contact of the vaccine with the vast mucosal associated lymphoid tissues. The net surface charge of the liposomes was modified by the incorporation of positively charged DOTAP lipid. This positive liposome can fuse with negatively charged cell membranes and deliver the vaccine endosomally.

Using a nanosizer, the particle sizes of the cationic liposome were determined. The mean size distribution was found to peak at 101.1 nm as shown in Figure 2. Particle size is a very important factor in vaccine delivery. Particle size is one of the determining factors for macrophage clearance. Large liposomes are rapidly removed from circulation. If the size of the liposome is below 200 nm, as obtained in the experiment, it would escape phagocytosis and the circulation time will be prolonged (Maurer et al., 2001). If the circulation time is prolonged, there will be more contact time of the liposomes with the immune cells, resulting in higher immunity.

In the slide agglutination test, the sera of the unvaccinated did not agglutinate which indicated negative result. The sera of the positive group and the liposome group showed strong agglutination. The post-vaccination plate agglutination titres of sera samples (mean ± SD) of the unvaccinated was 1.00 ± 0.0; the liposomal group was 7.75 ± 0.25 and positive control group given Fowl typhoid vaccine subcutaneously was 8.00 ± 0.0. From the microplate agglutination test (mean standard deviation (SD_0, at $p < 0.05$), it was found that there was no significant difference in the mean antibody titre of the birds by either the subcutaneous or oral vaccination route of fowl typhoid vaccine. The birds in the unvaccinated group were seen in a recumbent position after challenge. They had loss of appetite, looked depressed and had white mucoid stool. On the fifth day post-vaccination, a total of five birds from the unvaccinated group had died. The birds in the positive group and liposomal group looked healthy and none died.

Conclusion

FT vaccine encapsulated in a cationic liposome administered orally to chicken protected them from the field strain of *S. gallinarum* after vaccination. The antibody titre of the commercial FT vaccine administered subcutaneously was not significantly higher than the orally administered FT vaccine encapsulated liposome. More experiments would be carried to ascertain stability of the liposomal FT vaccine at room temperature. Positive results from this would be beneficial to farmers in rural areas and improve

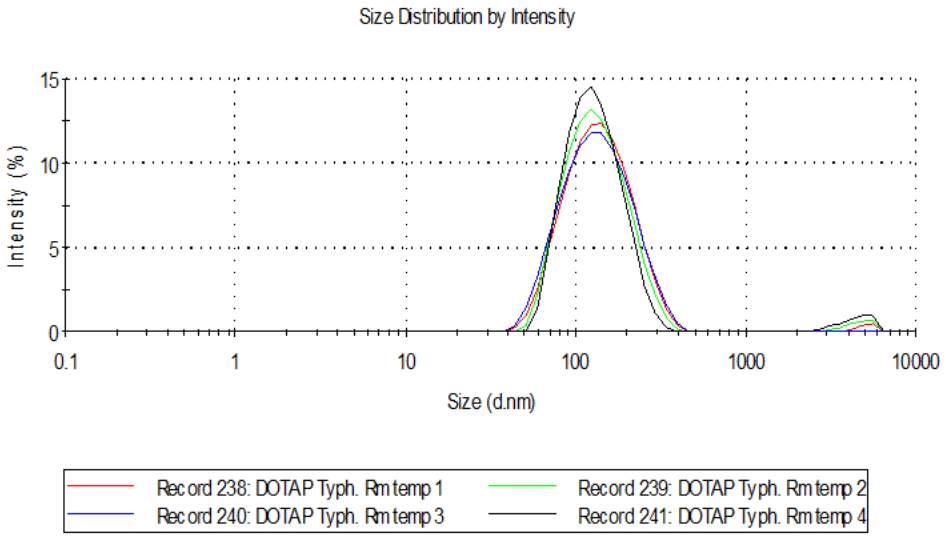

Figure 2. Particle size of the liposome using photon correlation spectroscopy.

vaccination compliance.

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