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Short communication

Chitosan/alginate microparticles for the oral delivery of fowl typhoid vaccine: Innate and acquired immunity

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ABSTRACT

Oral fowl typhoid (FT) vaccine is necessary for improved flock vaccinations and economic growth. This study was undertaken to evaluate the immune responses of birds given oral fowl typhoid vaccine coated with chitosan/alginate microparticles and comparing it with the conventional subcutaneous route of administration. Preliminary studies were done to evaluate the particle size, encapsulation efficiency and agglutination. Sixty day-old chicks were divided into three groups of twenty birds each. This comprised a negative control group NEG 451 (non-vaccinated and non-challenged used as control for cytokine quantification), SC 634 (live 9R vaccine by the injection route) and OCV 567 (live 9R vaccine coated with chitosan/alginate microparticles). Vaccination was done at 10 weeks and 14 weeks of age followed by challenge at 16 weeks of age. IgG was measured using ELISA. mRNA fold expression of IFN-γ in spleen was calculated using qRT-PCR. Particle sizes ranged between 0.55 μm and 10 μm. Encapsulation efficiency was above 60%. ELISA showed E-values of 0.10 ± 0.14, 0.07 ± 0.01 and 0.02 ± 0.01 for OCV 567, SC 634 and NEG 451 respectively after primary vaccination. Also E-values were 0.25 ± 0.16, 0.19 ± 0.04 and 0.0008 ± 0.005 for SC 634, OCV 567 and NEG451 respectively after boost vaccination. The expression of IFN-γin spleen using $2^{-\Delta\Delta\ CT}$ calculation was upregulated with values of 1.97 and 0.75 for OCV 567 and SC 634 respectively. After challenge with the 85-kb virulence plasmid SG9, there was 100% protection of the birds in both OCV 567 and SC 634 groups with no mortality. In conclusion, there was no significant difference at $p < 0.05$ of the means ± SD in immune responses between the oral fowl typhoid vaccine coated with chitosan/alginate microparticles and the subcutaneous route of administration. However, it is noteworthy to mention that the protective efficacy of the oral route is due to the chitosan/alginate biopolymers which coated the vaccine preventing destruction in the gastrointestinal tract.

1. Introduction

Salmonella enterica serovar Gallinarum causes fowl typhoid, a systemic disease of susceptible birds. Clinical signs of fowl typhoid in poultry include anaemia, depression, laboured breathing, diarrhoea causing adherence of faeces to the vent, increased mortality and poor quality chicks hatched from infected eggs [1]. Vaccination against fowl typhoid is best given as a subcutaneous injection because the vaccine undergoes digestion in the gastrointestinal tract prior to induction of immune responses [2–4]. It is currently available as injections but the parenteral live 9R vaccine is cumbersome and uneconomical in the vaccination of large flock. However, oral delivery of fowl typhoid vaccine has not been fully exploited [5,6]. *Salmonella gallinarum* is a mucosal pathogen, therefore, the oral route of fowl typhoid vaccination could be exploited but adequate protection from the acid degradation in the stomach and first-pass metabolism would be a prerequisite [7]. Oral vaccination is economical, easier, safer and time saving. It also requires less stringent regulatory requirements [4]. The ability to administer the fowl typhoid vaccine orally would encourage many farmers to adopt vaccination against fowl typhoid. Fowl typhoid vaccination is of economic importance especially in developing countries where the biosecurity is low and vaccination regimens are not fully established [8,9]. Chickens are the natural host of *Salmonella gallinarum* and the severity depends upon the susceptibility of the infected breed of chicken [1,10]. Substantial knowledge of the immune responses to Salmonella is essential for effective

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vaccination of the birds [11]. The IgG immunoglobulin may not be sufficient to clear the pathogen from the mucosa and may require the presence of IgA to limit the replication within the mucosal lumen [12]. Salmonella may resist the mucosal IgA and travel through the lymphatics to the blood stream and systemic tissues [13]. Recent studies have confirmed the role of interferon gamma (IFN-γ) in the control of Salmonellosis, with gene knock-out of IFN- γ increasing susceptibility to Salmonella infection. IFN-γ has been shown to be crucial in eliminating initial bacterial infection [14]. Our research is directed towards an alternative route to fowl typhoid vaccination which is comparable to the parenteral route with respect to immune responses. Delivery of a vaccine via the oral route in a sufficient dose to induce a protective immune response depends on overcoming the loss of antigen integrity that occurs during intestinal passage. There are several strategies that have been employed with robustness and versatility which can act as antigen carriers with adjuvant effect to prevent the loss of antigenicity and improve the delivery of vaccinogen by the oral route, this include vaccine vectors, transgenic plants, particulate formulations such as microparticles, micro/nanoparticles, liposomes, etc [15]. Employing potent adjuvants or effective vaccine delivery systems to elicit sustainable immune responses that would be compatible with the vaccine is paramount [16,17] which can surmount problems such as degradation, instability, and incompatibility [18]. The oral nanoparticles are mainly absorbed by Peyer's patches in small intestine and enter the bloodstream through the lymphatic system [19]. In this work, we developed an oral vaccine carrier based on alginate-coated chitosan microparticles as an alternative strategy for the parenteral live 9R vaccine. Chitosan and sodium alginate biopolymers are non-toxic, biocompatible, biodegradable as well as mucoadhesive [20,21]. Chitosan has been explored as a nasal delivery system for H1N1, influenza vaccine, diphtheria toxoid [22,23]. Chitosan, the polymer of choice in this research is a linear heteropolymer of N-acetyl-D-glucosamine linked by β-(1-4) glycosidic bonds. Sodium alginate is also a hydrophilic polymer and comprises D-mannuronic (M) and L-guluronic acid (G) residues joined linearly by 1,4-glycosidic linkages. Chitosan-alginate microparticles were prepared by ionotropic gelation method. The microparticulate formulation was optimized using their particle sizes and entrapment efficiency for cellular uptake. We discovered in our preliminary research that live 9R vaccine given orally is ineffective probably due to its destruction by the harsh environment of the gastrointestinal tract hence the entrapment in a mucoadhesive alginate-coated chitosan microparticles. Alginate-coated chitosan microparticles protects acid-labile drugs better from degradation in acidic solution (pH 1.5) than the chitosan microparticles alone [24]. It has also been reported to enhance antigen uptake by mucosal lymphoid tissues especially at the Peyer's patches [15].

2. Materials and methods

2.1. Chemicals and reagents

The following materials were used as procured without further purification: sodium alginate, low molecular weight chitosan, calcium chloride, acetic acid, Tween® 80 and sodium tripolyphosphate (Sigma Aldrich Co., Germany), distilled water, deionized water (National Commission for Energy Research and Development, University of Nigeria, Nsukka), Fowl typhoid vaccine (National Veterinary Research Institute (NVRI), Vom, Nigeria, Batch 05/2017), Group B and D Antibody ELISA test kit from Biochek, Netherlands, pullorum antigen stained antigen polyvalent, Salmonella polyvalent Groups B and D SPA, negative SPA serum (Charles River, Wilmington, MA, USA). The chicks were raised from 1 day old until termination of the experiment. All bird handling and experiments were conducted following the guidelines stipulated by University of Nigeria Research Ethics Committee on animal handling and use.

2.2. Bacterial strain and challenge

The 85-kb plasmid *Salmonella gallinarum* 9 virulent strain was a donation from Professor Paul Barrow, University of Nottingham, UK.

2.3. Preliminary studies: microparticle preparation

A 5 ml volume of 0.25% sodium alginate solution was added to a beaker and stirred at 50 rpm using an automatic magnetic stirrer for 5 min. Then 1 ml of calcium chloride was added dropwise to above solution while stirring. The prepared Ca/Alg pregel was stirred for a further 30 min, then 10 ml of 0.25% chitosan cross-linked with 2 ml of TPP solution at pH 5.6 loaded with the fowl typhoid vaccine was added dropwise to the Cal/Alg pregel, while stirring for 30 min to form the complex particles. This was done in seventeen runs following Box-Behnken experimental design as seen in Table 1.

2.4. Particle size determination

The particle sizes of the microparticles were determined by photon correlation spectroscopy (PCS) using a Zetamaster analyser system, at 25 °C (Malvern Instruments, Malvern, UK). The diameter of the microparticles was determined after dispersion in ultrapure water (1/10) and measured at 25 °C with a dynamic light scattering angle of 90○. The zeta potential was determined as follows: 200 μL of the samples were diluted in 2 ml of a 0.1 mM KCl solution adjusted to pH 7.4. All measurements were performed in triplicate.

2.5. Percent drug encapsulation efficiency (%)

For quantitative determination of the fowl typhoid vaccine loading, samples were centrifuged in a cold centrifuge (Sorvall RT 6000 D) at 7500 rpm for 5 min and then the absorbance of the solutions in the tubes was measured at 267.5 nm using a UV/VIS spectrophotometer ((Spectrumlab 752S). The amount of Fowl typhoid vaccine associated with the microparticles was calculated indirectly by the difference between the initial amount of fowl typhoid vaccine added to the chitosan and the amount measured in the supernatant. The following equation was used to determine the EE.

$$Encapsulation\ efficiency(EE\%) = \frac{FT_{total} - FT_{supernatant}}{FT_{total}} \times 100 \qquad (1)$$

2.6. Slide agglutination test

Fifty day-old brown pullets were were used for the experiment. At 7 weeks of age, Group A was given 0.2 ml (5×10^7 viable 9R

Table 1
Variables used in the Box Behnken experimental design.

Independent variables	Symbol	Levels		
		−1	0	1
Chitosan (%)	A	0.25	0.5	0.75
Sodium alginate (%)	B	0.2	0.25	0.5
Calcium chloride (mM)	C	0.55	1.0	1.8

Constraints: Maximize Encapsulation efficiency (>90%).
Minimize particle size (<1 μm).