USE OF LIPOSOME INCORPORATED COMPLETE LIPOPOLYSACCHARIDE CORE TYPES R1, R2, R3 AND R4 TO CONTROL INFECTIONS CAUSED BY *ESCHERICHIA COLI* IN CHICKENS

Dadigamuwage Ruchirangani Anuruddhika Dissanayake

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ABSTRACT

Infections caused by *Escherichia coli* (*E. coli*) make an economically significant impact in the poultry industry and non-serotype specific vaccine appears to be the most logical method of controlling it. The core oligosaccharide region of bacterial lipopolysaccharide is well conserved and highly immunogenic. This study focused on developing a broadly cross protective complete lipopolysaccharide core vaccine to control *E. coli* infections in chickens. Five distinct lipopolysaccharide (LPS) core types namely R1-R4 and K12 have been identified in *E. coli* and the distribution of those oligosaccharide core types among avian pathogenic *E. coli* is important to determine the LPS core types to be incorporated in the vaccine.

To identify putative avian pathogenic *E. coli*, serum resistance and the presence of three virulence genes namely temperature sensitive haemagglutination (*tsh*), increase serum survival (*iss*) and col V plasmid (*cva C*) were determined. Of the 143 clinical isolates examined 62% were R1, 22% were R3, 13% were R4 and 3% were R2. Fifty commensal isolates consisted of 58% with R1 core, 38% with R3 core, 4% with R4 core, and none with R2. None of the isolates were K12 core type. Distribution of core oligosaccharide types in clinical and commensal isolates were not statistically significant (P=0.51). Three genes, *tsh*, *iss* and *cva C* were found in *E. coli* of all four core types. The genes *tsh* (P>0.001) and *iss* (P=0.03412) were significantly associated with R4 core oligosaccharide type and the isolates containing LPS with R4 core type were mainly confined to phylogenetic group D. The widely-spread R1 core type showed less ability to process virulence genes and 83% were in the phylogenetic group A. The *E. coli* with R4 core type were less common among

commensals, possessed more virulence genes and were related to phylogenetic groups pathogenic for poultry. Results indicated the *E. coli* with R1, R2, R3 and R4 were important in causing infections in chickens.

Four rough *E. coli* strains possessing R1, R2, R3 and R4 core types were identified by polyacrylamide gel electrophoresis. A mixture of heat-killed bacterin prepared from those rough strains representing R1, R2, R3 and R4 showed significantly high levels of anti-LPS core antibody titres ($P<0.002$) that protected the birds from heterologous challenge.

Lipopolysaccharide extracted from the four selected strains by aqueous phenol method were incorporated into liposome consisted egg phosphatidylcholine and bovine brain phosphatidylserine and cholesterol to reduce toxicity. Endotoxicity of liposome incorporated LPS and free LPS were measured by Limulus amoebocyte lysate assay. Liposome incorporated LPS were at least 1000 times less toxic than free LPS. Induction of nitric oxide production and expression of inflammatory genes by free LPS and liposome incorporated LPS when tested on chicken macrophage cell line (HD11) showed that liposome incorporated LPS produced significantly less amount of nitric oxide (2-3.5 μM) than free LPS (18-22 μM). Expression of the gene interleukin-1beta (IL-1β) and inducible nitric oxide synthase (iNOS) were lower in cells treated with liposome incorporated LPS (IL-1β) 23.1ng/μl) than that of cells treated with free LPS (31.6ng/μl).

Chicks when immunized with 0.2μg, 1 μg and 5 μg of liposome encapsulated mixture of complete core types showed that the antigenic response increased with increasing dose and the birds received 5μg of liposome encapsulated LPS had significantly high ($p<0.001$) anti-LPS core antibody titres than the chicks in all other groups and also protected the birds against lethal challenge with *E. coli* O78.

The liposome encapsulated, mixture of complete LPS core vaccine was non toxic and showed greater potential to protect chickens against heterologous challenge.