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Strategies to reduce Campylobacter colonisation in chickens

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Abstract

Campylobacter jejuni is a gram negative bacterium which is one of the leading causes of bacterial related acute entritis in the developing world. *C. jejuni* is also linked to auto- immune diseases such as Miller- Fisher syndrome and Guillain- Barre Syndrome. *C. jejuni* is highly effective in colonizing chicken intestinal mucosa without causing any clinical symptoms and the consumption of poultry meat is the major source of transmission of bacteria to humans. One of the approaches to reduce *Campylobacter* related illnesses is to reduce the burden of *Campylobacters* in chickens. This can be achieved by vaccinating chickens against *Campylobacter*; however, various approaches to develop a vaccine against *Campylobacter* have yet to yield a commercial vaccine. One approach to develop a new class of vaccines against *Campylobacter* or other pathogens is to use an attenuated *Salmonella* autotrophic mutant as a vector to deliver antigens of *Campylobacter* origin to chickens. Our results indicate that *Salmonella* mutants can be effectively used as vector to deliver antigens of *Campylobacter* origin for vaccine purposes. However, before this method can be commercialized several parameters including the choice of suitable antigen or antigens needs to be evaluated.

Keywords: Salmonella mutants; Campylobacter; antigens; Vaccine.

I. Introduction

Campylobacter jejuni is a gram negative bacterium which colonises chicken without causing any clinical manifestations, and it is also one of the major causes of gastroenteritis in developed countries. Antibodies generated against *Campylobacter* can bind to some self- antigens causing autoimmune diseases such as Gullian-Barre syndrome and Miller Fisher syndrome in humans [1]. The most intriguing aspect of *Campylobacter* pathogenesis is their capability to evade chicken immune responses, although they heavily colonise (up to 10^9 bacteria/gm) chickens intestinal mucosa [2]. Post harvesting measures such as chilling and chlorine washing of poultry meat have been somewhat effective in reducing the transmission of *C. jejuni* to humans but these measures are not sufficient to eliminate Campylobacter related gastroenteritis. Also such treatments are costly and degrade the quality of meat [3]. Vaccinating chickens against *Campylobacter* spp. is one effective way to reduce the bacterial burden on chickens; this will not only reduce chicken to human transmission but also eliminate costly post harvest treatments [4]. Although several methods have been tested to design effective vaccination against selected *Campylobacter* spp., so far these methods have not materialised in developing a viable vaccine against *Campylobacter* [3]. Recently several studies have successfully tested the use of *Salmonella enterica enterica*, serovar Typhimurium (*S.* Typhimurium) mutants as delivery vector to deliver antigens from other pathogens for vaccine purpose [5, 6]. These antigens can be delivered either from a plasmid location or from the chromosomal location, expressed under the influence of various inducible promoters [7]. Similarly in our lab we have successfully tested the use of

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Salmonella Typhimurium mutant-1 (STM-1) to deliver various heterologous antigens for vaccine purposes [7, 8]. STM-1 is an aroA mutant i.e. the *aroA* gene has been deleted; this leads to a non-reverting aromatic biosynthesis defects. This renders the STM-1 non-virulent in respect to invasive infection, because aromatic metabolites such as paraminobenzoate (for synthesising folate), dihydroxybenzoate (for synthesising enterochelin) and the aromatic amino acids tyrosine are not available in host tissues [9] and so are not available to the STM-1 to support active replication. In this study we have demonstrated that STM-1 can successfully be used as vector to deliver *Campylobacter* related antigens for vaccines in chickens.

2. Choice of antigens and design of constructs

One of the major obstacles in developing vaccination against *Campylobacter* spp. is to identify suitable antigens. There are several proteins which play critical roles in *Campylobacter* pathogenesis [10]; however, the exact role of these proteins *in vivo* is not well studied, particularly with respect to facilitating chicken colonisation. As a strategy to test STM-1 for antigen delivery four antigens were selected [11, 12] and were cloned in the PMW2 plasmid (a medium copy number plasmid) and similar constructs were inserted into the STM-1 chromosome at the *aroA* site. Protein expression was analysed *in vitro* by western blotting. After validating the design of the constructs and protein expression, animal experiments were undertaken to analyse the efficacy of *Salmonella* Typhimurium mutant STM-1 to deliver these antigens *in vivo*.

3. Animal Trials

In this experiment groups of 4 Chickens (1 week old) were vaccinated with 10^7 cfu (colony forming units) of STM-1 expressing *Campylobacter* antigens either from the plasmid or chromosomal location. The vaccine was administered orally twice at an interval of two weeks. Table 1 shows the experimental design. One week after the second vaccination blood samples were collected to analyse humoral immune responses against the delivered antigens. On week four (two weeks after the second vaccination) all the groups were challenged orally with 10^9 cfu *C. jejuni* strain 81116.

Table1. Experimental design of the vaccination trial.

Group	Vaccine	Number
1	PBS control	4
2	STM-1	4
3	STM-1/PMW2	4
4	cjaA/Chromosome	4
5	cjaA/PMW2	4
6	cadF/chromosome	4
7	cadF/PMW2	4
8	ciaB/chromosome	4
9	ciaB/PMW2	4
10	cj1496/chromosome	4
11	cj1496/PMW2	4
12	Combined/chromosome	4
13	Combined/PMW2	4
	Total	52

All chickens were sacrificed two weeks post challenge and caecal contents from each chicken collected in sterile tubes. Each gram of caecal content was liquefied with 1mL Muller Hinton (MH) and serially diluted prior to plating in triplicate on blood- free charcoal based selective medium and incubating under microaerophilic conditions at 42 deg C. Colony counting was completed following incubation and the number of cfu from test groups was compared with control groups.

4. Result and Discussion

The result from this study (Fig.1) indicates a reduction in the colonisation of *C. jejuni* in chickens vaccinated with various *Campylobacter* antigens. Although the maximum reduction in colonisation was 2-3 logs, this is not yet sufficient for commercial vaccine purposes (5 log reduction is desired). However, these results indicate the efficiency of STM-1 mutant to deliver *Campylobacter* related antigens in chickens, and when an optimal antigen combination is found may lead to greater reduction in colonisation.

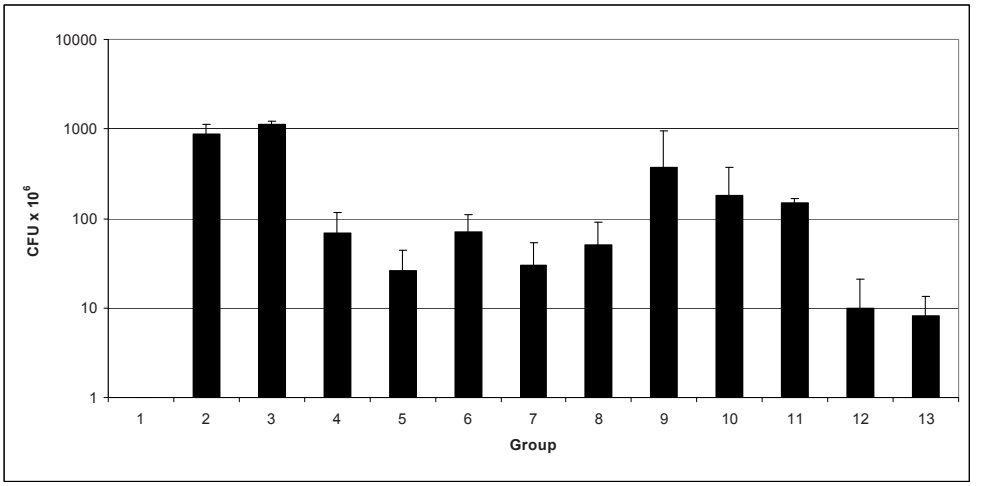

Fig. 1. Analysis of *Campylobacter* colony forming units after vaccination and subsequent challenge. Colonisation is CFU/ g caecal content.

Other modifications can include the choice of suitable promoters [13] which can facilitate higher expression of foreign protein, specifically from the chromosomal location [7], as this will not only generate higher immune responses against the delivered antigens but also will assure the stability of the foreign construct. Although the location of expression of foreign protein i.e. plasmid or chromosomal location, did not have a major effect on reduction in colonisation, the chromosomal location is preferable as this not only allows for the stability of the construct and protein expression, but for commercial use it will ensure no contamination of other gut flora with plasmid.

However, selection of optimal *Campylobacter* antigen or antigens is most important as the choice of antigenic protein is crucial to develop effective vaccination against the pathogens [4, 14-16]. This is one of the most difficult hurdles to overcome as we are still filling the gap in our understanding of

Campylobacter pathogenesis and metabolism. Genome mining approaches for candidate proteins are currently being undertaken in the search for such candidates.

5. References

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