Oral administration of PLGA-encapsulated CpG ODN and *Campylobacter jejuni* lysate reduces cecal colonization by *Campylobacter jejuni* in chickens

By

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Introduction

- *C. jejuni* is a Gram-negative, spiral-shaped, microaerophilic bacterium
- *Campylobacter* is one of the most common causes of bacterial food borne illness in humans
- Over 90% of all human cases of campylobacteriosis originate from farm animals, among which chickens are the main source of infection
- *Campylobacter* was detected in 59% of samples from chicken meat from retail stores
- A recent economic analysis in the US revealed that human *Campylobacter* illnesses cost up to $8 billion
General problem

Commensal → Poultry products → 500 bacteria

Diarrhea, abdominal pain and vomiting

**Severe cases:** Gastrointestinal perforation and Guillain-Barré syndrome

*Last from 5-10 days*
How to control *Campylobacter*

**Intervention strategies**
- Biosecurity measures
- Sanitation in slaughter plants
- Dietary manipulation
- Antimicrobial alternatives
- Genetic selection
- Vaccination

*Key part of controlling infection*

No commercial *Campylobacter* vaccines are currently licensed for use in chickens
Exploitation of TLR ligands to control *Campylobacter* colonization

**TLR ligands:** Microbial components and their synthetic analogs
* e.g. LPS, flagellin, lipoproteins, glycolipids and nucleic acids
Exploitation of TLR ligands to control *Campylobacter* colonization

- **Activation of cellular responses**
- **Secretion of effector molecules**
- **Cellular proliferation and differentiation**
- **Regulation of local and systemic inflammation**
Characterization of host responses induced by Toll-like receptor ligands in chicken cecal tonsil cells

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Exploitation of TLR ligands to control *Campylobacter* colonization

- **TLR ligands**: Microbial components and their synthetic analogs
e.g. LPS, flagellin, lipoproteins, glycolipids and nucleic acids

- Administration of CpG ODN (a synthetic analog of bacterial DNA) can protect chickens against bacterial infections (Gomis et al., 2003; Taghavi et al., 2008).
e.g. *Escherichia coli* and *Salmonella enterica serovar Typhimurium*

*CpG ODN can serve as a potent mucosal vaccine adjuvant*
Delivery of CpG ODN to mucosal effector sites in the intestine

Oral delivery

CpG

Induction of intestinal immune responses

Commensal relationship

➢ Oral delivery of CpG ODN remains a challenge

Short half-life
Potential degradation by gastric juice and digestive enzymes

Formulation in protective delivery systems such as PLGA NPs

CpG

NPs

Facilitate target delivery to antigen presenting cells

NPs

Enhance vaccine immunogenicity

NPs

Protect encapsulated ligand from enzymatic degradation

NPs

Extend the duration of immune system stimulation
Objectives

- Examine the effects of administration of TLR ligand CpG ODN 2007 or lyzed *C. jejuni* on protection against colonization with *C. jejuni*

- Develop and optimize approaches and formulations for delivery of vaccine and adjuvants to the chicken intestine

- Investigate the mechanisms of immunity conferred by CpG ODN and lyzed *C. jejuni*
In vivo trials

- Effects of administration of CpG ODN or lyzed C. jejuni on protection against colonization with C. jejuni
- **A) Comparing different routes and doses of CpG ODN**

14-day-old SPF layer chicks

- CpG ODN 5 or 50 µg
- Orally & IM

Oral challenge 10^7 C. jejuni

- 2, 5 or 8 days
- CFU/gram cecal content

Layers

- Low dose CpG ODN = 1.23 log_{10}
- High dose CpG ODN = 1.32 log_{10}

Day 8 post-infection

Oral route and sampling at day 8 to 22 post-infection were chosen for subsequent experiments
In vivo trials

B) Oral administration of encapsulated CpG ODN and C. jejuni lysate in layer and broiler chickens

14-day-old SPF layer chicks

5 µg encapsulated-CpG
5 µg CpG free form

lyzed C. jejuni

Orally

Oral challenge $10^7$ C. jejuni

24 h

8, 15 or 22 days

CFU/gram cecal content

Geometric mean CFU/gram (log10)

Days post-infection

Days-8 Days-15 Days-22

PBS CpG E-CpG C. jejuni lysate

E-CpG ODN = $1.89 \log_{10}$
C. jejuni lysate = $2.24 \log_{10}$
In vivo trials

Broilers

14-day-old broiler chicks

5 µg encapsulated-CpG
25 µg encapsulated-CpG
lyzed C. jejuni (low and high dose)

Orally

Oral challenge $10^7$
C. jejuni

24 h

Orally

8, 15 or 22 days

Blood samples
serum IgG Abs

CFU/gram cecal content

14-day-old broiler chicks

E-CpG ODN = 1.46 $\log_{10}$
C. Jejuni lysate = 2.24 $\log_{10}$
In vivo trials

*C) Combined effects of encapsulated CpG ODN and C. jejuni lysate*

**Broilers**

25 µg encapsulated-CpG, 10⁷ lyzed *C. jejuni* or a combination of encapsulated CpG ODN and *C. jejuni* lysate

14-day-old broiler chicks **Orally** 25 µg encapsulated-CpG, 10⁷ lyzed *C. jejuni* or a combination of encapsulated CpG ODN and *C. jejuni* lysate **Orally**

Oral challenge 10⁷ *C. jejuni* 8,15 or 22days

CFU/gram cecal content

Geometric mean CFU/gram (log_{10})

Day-8 Day-15 Day-22

Days post-infection

PBS  E-CpG  *C. jejuni* lysate  E-CpG + *C. jejuni* lysate

E-CpG ODN + *C. jejuni* lysate = 2.42 log_{10}
It has been reported that a reduction of 2 log$_{10}$ in the number of *Campylobacter* bacteria on contaminated chicken carcasses would lower the public health risk by 30-fold (Rosenquist et al., 2003).

- **CpG ODN**
- **PLGA-CpG ODN**
- **C. jejuni lysate**
- **PLGA-CpG ODN + C. jejuni lysate**

Fold reduction of public health risk:
- 18.5
- 28.5
- 32.1
- 36.3

Fold reduction of *Campylobacter* burden in chickens:
- 23
- 46
- 114
- 142
Mechanisms involved in protection against *C. jejuni*

- **Serum IgG responses**

Although higher serum IgG was detected in the groups treated with CpG ODN or low dose of *C. jejuni* lysate, there was a positive correlation between serum IgG Ab titers and cecal CFUs. This suggests that other mechanisms, such as mucosal immune responses, may be involved in protection against *Campylobacter*. 
Mechanisms involved in protection against *C. jejuni*

• Gene expression profiles

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<th>IL-1B</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-13</th>
<th>IL-10</th>
<th>TGF-β4/1</th>
<th>AvBD1</th>
<th>AvBD2</th>
<th>CATH-3</th>
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<td><strong>C. Jejuni lysate</strong></td>
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Keywords: Up-regulation, Down-regulation, No change

Oral-mucosal delivery of soluble CpG ODN upregulated the expression of IFN-γ, IL-1β, IL-8, TGF-β4/1, IL-10 and IL-13

PLGA-encapsulated CpG ODN upregulated the expression of IL-1β, IL-8, TGF-β4/1, IL-13, AvBD1, AvBD2 and CATHL-3

*C. jejuni* lysate upregulated the expression of IFN-γ, IL-1β, TGF-β4/1, IL-13, AvBD1, CATHL-3
Conclusion of gene expression results

- Enhanced expression of Th1-type and pro-inflammatory cytokines in response to CpG ODN (either soluble or encapsulated) and *C. jejuni* lysate may reflect their capacity to reduce intestinal colonization with *Campylobacter*.

- The induction of TGF-β4/1 and IL-13 in cecal tonsils (in response to soluble CpG ODN and *C. jejuni* lysate) and ileum (in response to encapsulated CpG ODN) may play a role in enhancing mucosal IgA antibody responses against *C. jejuni*.

- The elevated expression of antimicrobial peptides in the PLGA-encapsulated CpG ODN and *C. jejuni* lysate groups, but not in the soluble CpG ODN treated group, may explain, at least in part, the greater reduction in *C. jejuni* load in chicken ceca in these groups compared to the soluble CpG ODN group.
Conclusion

- Oral administration of **CpG ODN alone** significantly reduced intestinal colonization of chickens with *C. jejuni*

- **PLGA NPs formulations** enhance the ability of **CpG ODN** to reduce *C. jejuni* load in chicken ceca

- Oral administration of a combination of **PLGA-encapsulated CpG ODN** and *C. jejuni* lysate could potentially serve as a **candidate vaccine** for control of *C. jejnui* in broiler chickens
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References

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Thank you