Immunization of broiler chickens against necrotic enteritis: Progress and possibilities

John Prescott, Ravi Kulkarni
Necrotic enteritis

• Small intestinal infection of broiler chickens
• Caused by *Clostridium perfringens*
• Serious infection, through mortality and morbidity
• $6$ billion dollar disease
• High cost of subclinical disease and of prevention by antibiotics
• Need to stop preventive use of medically-important antibiotics
Immunization of broiler chickens against necrotic enteritis

• The challenges
• Can we immunize?
• What is the basis of immunity?
• What are the important antigens?
• What is an ideal vaccine?
• How can we deliver vaccine antigens?
• How should we test vaccines?
• Future possibilities
The challenges

• Complex disease
• Understanding the basis of protective immunity
• Immunizing in the face of maternally-derived immunity
• Identifying key antigen(s)
• Defining the best systems to test vaccines
• Safety, efficacy, robustness, cost
• Field versus lab testing
Keyburn et al. identified a novel toxin, NetB, that plays a key role in development of NE.
Major factors predisposing to NE:

- High Cp – NetB+ve strains
- Protein leakage
- Coccidiosis
- Gut stasis
- Wet litter
- Whole wheat
- Fish meal
- Whole wheat starch
- Tpel enhances virulence

NetB → NE
NE strains are unique

- NE genetic loci highly conserved, strongly correlated with disease
- *netB* critical, but more complex than this
- Several chromosomal loci associated with *netB*-positive isolates
Identification of NE-specific loci

**NELoc-1**
(42 kb)

**NELoc-2**
(11.2 kb)

**NELoc-3**
(5.6 kb)

Lepp et al., 2010
NE locus 1: Mucin colonization, degradation

Zinc metalloprotease

Putative role:
- Toxin
- Adhesion
- Regulation
- Mobilization
- Enzyme
- Membrane protein
- Other
- Plasmid
- Hypothetical protein

NELoc-1
(42 kb)

NELoc-2
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(5.6 kb)

Lepp et al., 2010
NE locus 1: Tissue adhesion

NELoc-1
(42 kb)

NELoc-2
(11.2 kb)

NELoc-3
(5.6 kb)

Lepp et al., 2010
NetB toxin damage

NELoc-1
(42 kb)

NELoc-2
(11.2 kb)

NELoc-3
(5.6 kb)

Putative role:
- **Toxin**
- **Adhesion**
- **Regulation**
- **Mobilization**
- **Enzyme**
- **Other**
- **Plasmid**
- **Hypothetical protein**

Lepp et al., 2010
Can we immunize?
Passive immunity: NetVax vaccine for layers provides maternal antibody for broilers

“Toxoid A”: supernatant antigens

Mortality in 22-28-day-old broilers
Passive immunization rNetB versus “toxoid” (Keyburn et al., 2013)

Day 21 chicks

Day 14 chicks
Key findings from passive immunization

• Yes, you can immunize
• Immunization with “PlcC [alpha-toxoid]” (netB-negative) gives good but incomplete protection
• rNetB plus “toxoid” > rNetB alone older chicks
• Useful strategy but not in later broiler production
• Need active immunization
Can we actively immunize?
Active immunization against *C. perfringens* in NE: Key lab findings

- Secreted proteins crucial
- Supernatants vary in protective ability
- Several different antigens provide reasonable protection
- Intestinal mucosal IgY >> IgA important
- Protection depends on challenge severity; system?
- Mixed antigens or chimeric proteins often better protection than individual antigens
What is the basis of immunity?
What is the basis of immunity?

• Not understood in detail, Th2 and Th17 cytokine mediated
• Antibodies to secreted virulence factors (PlcC, NetB, zinc metalloprotease) important
• Antibodies to secreted housekeeping (“moonlighting?”) proteins important
• Many different antigens provide some protection experimentally
• Effect may be by impairing bacterial growth rather than neutralizing toxin (IgY)
Defined antigens with value in immunization against NE

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Role</th>
<th>Value*</th>
<th>Reproducibility**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha toxin (PlcC)</td>
<td>Virulence? Phospholipase</td>
<td>++ .... ++++</td>
<td>++++</td>
</tr>
<tr>
<td>NetB toxoid</td>
<td>Virulence</td>
<td>++ .... ++++</td>
<td>++++</td>
</tr>
<tr>
<td>Zinc metalloprotease</td>
<td>Virulence? Mucin degradation</td>
<td>++ .... ++++</td>
<td>+++</td>
</tr>
<tr>
<td>Fructose biphosphate aldolase, FBA</td>
<td>Housekeeping-Moonlighting? Adhesion?</td>
<td>+ .... ++++</td>
<td>++++</td>
</tr>
<tr>
<td>Pilus</td>
<td>Virulence (collagen adhesion)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvate ferrodoxin oxidoreductase, PFOR</td>
<td>Housekeeping</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate oxidoreductase, GPD</td>
<td>Housekeeping</td>
<td>+ .... ++</td>
<td>+++</td>
</tr>
<tr>
<td>Phosphoglyceromutase, PGM</td>
<td>Housekeeping</td>
<td>++ .... ++++</td>
<td>+</td>
</tr>
<tr>
<td>Elongation factor-Tu</td>
<td>Housekeeping</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-N-acetylgluosaminidase</td>
<td>Housekeeping</td>
<td>0 .... ++++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Overall protection, + 25-49%, ++, 50-74%, +++ , ≥ 75%; ** , ++++, ≥ 4 studies; +++ , 3 studies, etc
NE lesion scores in chickens immunized IM with CP proteins and different challenge severity

Kulkarni et al., 2007
Vaccination with recombinant NetB toxoid (Keyburn et al., 2013)
Protection of broiler chickens against NE after SC immunization with PlcC-NetB chimeric toxoid

Hunter et al., 2019a
What is an ideal vaccine?

• Safe, effective, cost-effective, profitable
• Easily administered: in ovo or in drinking water
• Robust under field conditions
• Local intestinal immunity important, so best if orally administered
• 100% protection
How can we deliver immunization?
Live attenuated oral *Salmonella* vectored vaccines
Salmonella vaccine vectors

“Regulated expression” of foreign antigens; “delayed attenuation”, so virulent at time of infection; programmed lysis so disappear

Improved plasmid vectors expressing CP antigens
Intestinal lesion scores of birds immunized three times, aged 1, 7 and 14 days, with Salmonella vaccine vectors (χ3987, χ9241, χ9853, χ9945, χ11442, χ11445, vector only control group (χ3987), expressing the C. perfringens PlcC, and challenged at day 28 with C. perfringens CP4.

Prescott et al., unpublished
Impact of immunization with *Salmonella*-vectored PlcC and/or NetB toxoids on percentage of broiler chickens with severe lesions

Jiang et al., 2015
Mean intestinal lesion scores in broilers immunized with single *Salmonella* vaccine expressing different antigens

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> vector</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Non-vaccinated controls</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>plcC-netB</em></td>
<td>1.5*</td>
<td>1.9*</td>
</tr>
<tr>
<td><em>plcC-fba-netB</em></td>
<td>0.8**</td>
<td>0.9**</td>
</tr>
<tr>
<td><em>fba</em></td>
<td>-</td>
<td>0.7**¥</td>
</tr>
</tbody>
</table>

Hunter et al., 2019b
Live attenuated *C. perfringens* mutants for oral vaccination? No.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Mean ± SD (% control)</th>
<th>Number studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphodiesterase</td>
<td>190 ± 60</td>
<td>3</td>
</tr>
<tr>
<td><em>netB</em></td>
<td>95 ± 31</td>
<td>3</td>
</tr>
<tr>
<td>Diguanylate cyclase</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>CP1-3475 ABC transporter</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>Zinc metalloprotease</td>
<td>108</td>
<td>1</td>
</tr>
</tbody>
</table>

Prescott et al., unpublished
Future possibilities
Potential avirulent live oral vaccine vectors for necrotic enteritis

<table>
<thead>
<tr>
<th>Vector</th>
<th>Antigen</th>
<th>Mouse protection</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>PLC (alpha toxoid)</td>
<td>+++</td>
<td>Gao, 2019</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>PLC</td>
<td>+++</td>
<td>Alimolaei, 2017</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>PLC</td>
<td>+</td>
<td>Song, 2018</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>PLC</td>
<td>+++</td>
<td>Hoang, 2008</td>
</tr>
</tbody>
</table>

*Plainspores*
in ovo immunization?

- One study of efficacy *Eimeria* profilin and rNetB
- Partial protection against experimental NE
- Adjuvant important
- Far more work needed
How should we test vaccines experimentally?
Reproducing necrotic enteritis experimentally

**Induction of NE**

- **NetB** (critical)
- **TpeL** (for enhanced disease)
- Age of CP broth culture
- CP inoculation conditions: numbers, method of delivery
- Change to high animal protein diet at challenge
- Wheat - barley - based diet
- Coccidial infection: virulent, vaccine
- Immunosuppression: IBD vaccine

Shojadoost et al., 2011
Assessment of vaccine efficacy: Important issues

- No standard model to reproduce NE: Does *Eimeria* bias results?
- No challenge strain(s) criteria
- No standard assessment system
- No standard challenge severity
- No “gold standard” vaccine comparison

6 point NE scoring system:
Keyburn et al., 2008
The future

• Immunization has promise in reducing NE
• Very useful adjunct in control
• Oral attenuated *Salmonella* with “mixed” antigens promising
• Need for field testing attenuated *Salmonella* vaccines
• Explore other avirulent vaccine vectors
• *in ovo* immunization plus oral vaccine boost?
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