Early pathogenesis of *Yersinia ruckeri* infections in rainbow trout

Els Tobback

*Promotors:*
Prof. Dr. K. Chiers
Prof. Dr. K. Hermans
Prof. Dr. A. Decostere

Ghent University
Faculty of Veterinary Medicine
Department of Pathology, Bacteriology and Avian Diseases
PhD Defense: September 2, 2009
• General introduction & Scientific aims
  - *Yersinia ruckeri*
  - Enteric redmouth disease (ERM)
• Experimental studies
  1) Virulence determination
  2) Route of entry and tissue distribution
  3) Interactions with gill and gut explants
  4) *In vitro* markers for virulence
• General discussion
• General introduction & Scientific aims
  - *Yersinia ruckeri*
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• General discussion
World aquaculture production of rainbow trout

Yersinia ruckeri

Bacterium
- rod shaped
- Gram negative
Enteric redmouth disease (ERM)
Enteric redmouth disease (ERM)

Septicemic disease
Mainly in salmonids (rainbow trout)
Most acute in small fish
Portal of entry?

Gills?

Skin?

Intestine???
Adhesion to host tissues

Introduction & aims

Exp. study 1
Exp. study 2
Exp. study 3
Exp. study 4

General discussion

Exp. study 4
Exp. study 3
Exp. study 2
Exp. study 1

General discussion

Adhesion to host tissues

3 capsule
flagellum
fimbriae
LPS
Adhesion to host tissues

mediated by:

- proteins
- sugars
- proteins & sugars (lectins)
Invasion of host tissues

Zipper

Trigger

Invasome
Invasion of host tissues

**Zipper**  **Trigger**  **Invasome**
Invasion of host tissues

Zipper  Trigger  Invasome
Invasion of host tissues

Zipper  Trigger  Invasome
Invasion of host tissues

Zipper

Trigger

Invasome

dansylcadaverine

cytochalasin D

colchicine
Scientific aims

- To reveal route of entry & tissue distribution

- To study adhesion to and invasion of gill and gut tissue

- To study different traits for virulence *in vitro*
• General introduction & Scientific aims
  - *Yersinia ruckeri*
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• **Experimental studies**
  1) Virulence determination
  2) Route of entry and tissue distribution
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• General discussion
Exp. study 1: Virulence determination

**AIMS:**

To determine the **virulence** of 7 *Y. ruckeri* strains at 2 different temperatures

→ **immersion infection** studies in rainbow trout
Immersion infection model

1 h

+ Yersinia ruckeri
Virulence at 2 temperatures

- 7 Y. ruckeri strains
- Immersion at 23°C
  16°C
- Recording clinical signs and mortality
- Sampling: dead and moribund fish

↓
Bacteriology
Histology
Immunohistochemistry
## Virulence at 2 temperatures

**Clinical signs, mortality and necropsy findings**

<table>
<thead>
<tr>
<th>Fish that died</th>
<th>Infection at 23°C</th>
<th>Infection at 16°C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Y. ruckeri 5</td>
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</tr>
<tr>
<td></td>
<td>Y. ruckeri 9</td>
<td>Y. ruckeri 9</td>
</tr>
<tr>
<td>Other strains</td>
<td></td>
<td>Other strains</td>
</tr>
<tr>
<td></td>
<td>6 / 20</td>
<td>4 / 20</td>
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### Virulence at 2 temperatures

#### Bacteriological examination

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<td>+</td>
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<td>Other strains</td>
<td>/</td>
<td>-</td>
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</table>
Virulence at 2 temperatures

Histopathological changes

Gills: moderate – severe oedema
Virulence at 2 temperatures

Histopathological changes

Spleen: necrosis
Virulence at 2 temperatures

Histopathological changes

Kidney: degeneration and/or necrosis of tubules
increased cellularity of glomerular tuft
increase in # (melano)macrophages
Virulence at 2 temperatures

Conclusions

- Two strains (5 & 9) caused disease and mortality
  → Differences in virulence exist between different *Y. ruckeri* strains

- Immersion at 2 temp: no different outcome
• General introduction & Scientific aims
  - *Yersinia ruckeri*
  - Enteric redmouth disease (ERM)

• **Experimental studies**
  1) Virulence determination
  2) **Route of entry and tissue distribution**
  3) Interactions with gill and gut explants
  4) *In vitro* markers for virulence

• General discussion
Exp. study 2: Route of entry and tissue distribution

**AIMS:**

I) Reveal the route of entry

II) Investigate the tissue distribution at different time intervals

performing immersion infection studies in rainbow trout
I) Route of entry

- 4 *Y. ruckeri* strains
- Infection dose: ~ $2 \times 10^8$ CFU ml$^{-1}$

**Sampling:** 2 fish at $t = 0, 1.5$ and $2.5$ h p.i.

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<th>Exp. study 1</th>
<th>Exp. study 2</th>
<th>Exp. study 3</th>
<th>Exp. study 4</th>
<th>General discussion</th>
</tr>
</thead>
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Bacteriology
Histology
I) Route of entry

Bacteriological examination

**Y. ruckeri 5**

**Y. ruckeri 17.00(2-1)**

**Y. ruckeri CCUG 14190**

**Y. ruckeri E842-95**
Histological analysis

Localisation of *Y. ruckeri* 5 in the gills after immersion in the mucus in a capillary
Histological analysis

Localisation of *Y. ruckeri* 5 in the gut after immersion:
in the crypts, attached to the villi, within the mucosa
II) Tissue distribution

- 4 *Y. ruckeri* strains
- Infection dose: \( \sim 2 \times 10^7 \) CFU ml\(^{-1}\)
- **Sampling:** 2 fish at \( t = 1, 2, 4, 6, 9, 12, 24, 48 \) and 72 h p.i.

![Diagram of a fish with labeled tissues]
II) Tissue distribution

Bacteriological examination

**Y. ruckeri 5**

**Y. ruckeri 17.00(2-1)**

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II) Tissue distribution

Bacteriological examination

*Y. ruckeri* 5

*Y. ruckeri* 17.00(2-1)

*Y. ruckeri* CCUG 14190

*Y. ruckeri* E842-95

![Graphs showing bacterial distribution in different tissues over time.](image-url)
Route of entry and tissue distribution

Conclusions

- **Gills** = important portal of entry
  1) highest numbers in the gills immediately after infection
  2) invasion

- **All strains caused sepsis**
  all sampled tissues were *Y. ruckeri* - positive
• General introduction & Scientific aims
  - *Yersinia ruckeri*
  - Enteric redmouth disease (ERM)

• **Experimental studies**
  1) Virulence determination
  2) Route of entry and tissue distribution
  3) *Interactions with gill and gut explants*
  4) *In vitro* markers for virulence

• General discussion
Exp. study 3: Interactions with gill and gut explants

AIMS:

To study *Y. ruckeri* adhesion to and invasion of
I) gill tissue and
II) gut tissue of rainbow trout
using standardized *perfusion models*
I) Gill perfusion model
I) Gill perfusion model
II) Gut perfusion model
II) Gut perfusion model

20°C
Interactions with gill and gut explants

- 5 *Y. ruckeri* strains:
  - 5 virulent
  - 9 virulent
  - E842-95 avirulent
  - 17.00(2-1) avirulent
  - CCUG 14190 avirulent

- *E. coli* strain:
  - DH5α = non-invasive → control
## Interactions with gill and gut explants

### Bacteriological examination

<table>
<thead>
<tr>
<th></th>
<th>Gill explants</th>
<th>Gut explants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adhesion</td>
<td>Invasion</td>
</tr>
<tr>
<td><strong>Virulent Yr strains</strong></td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Avirulent Yr strains</strong></td>
<td>+++</td>
<td>+ / +++</td>
</tr>
<tr>
<td><strong>E. coli DH5α</strong></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Interactions with gill explants
Histological and immunohistochemical analysis

Adhesion

Invasion
Interactions with gut explants
Histological and immunohistochemical analysis
Interactions with gill and gut tissue

Conclusions

- Virulent and avirulent *Y. ruckeri* isolates adhere to and invade gill and gut tissue

- Gills and gut = portal of entry

- Perfusion models suitable to study bacterial invasion
• General introduction & Scientific aims
  - *Yersinia ruckeri*
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• **Experimental studies**
  1) Virulence determination
  2) Route of entry and tissue distribution
  3) Interactions with gill and gut explants
  4) *In vitro* markers for virulence

• General discussion
Exp. study 4: *In vitro* markers for virulence

**AIMS:**

To study different traits that have been associated with bacterial virulence:

- **Adhesion** to mucus and cell lines
- **Invasion** and **intracellular survival** in cell lines
- **Serum resistance**
Adherence to mucus

- Gill mucus
- Intestinal mucus

+ $10^8$ CFU *Y. ruckeri* ml$^{-1}$, 1h, 20°C

+ WST-1 2h

**OD$_{450}$**

Adhering bacteria
Adherence to mucus

* A significantly higher adhesion to the gill mucus compared to the intestinal mucus (P < 0.05)
Adhesion inhibition to mucus

Pre-treatment of *Y. ruckeri* strains:
- proteolytic enzymes (pronase, trypsin)
- sodium metaperiodate
- carbohydrates

[Diagram of adherence assay to mucus]

OD$_{450}$
Adherence inhibition to gill and intestinal mucus

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Proteolytic Enzymes</th>
<th>Sodium Metaperiodate</th>
<th>D-galactose</th>
<th>D-fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yr 5</td>
<td>+ / ++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Yr E842-95</td>
<td>+ / ++</td>
<td>+++</td>
<td>±</td>
<td>+</td>
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<tr>
<td>Yr 17.00(2-1)</td>
<td>+ / ++</td>
<td>+++</td>
<td>±</td>
<td>+</td>
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Adherence to cell lines

3 fish cell lines: CHSE-214, FHM, R1

10^5 cells/well
10^6 CFU *Y. ruckeri*/well, 1h, 20°C

Scanning electron microscopy
Hemacolor staining
# Adherence to cell lines

<table>
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<tr>
<th></th>
<th>CHSE-214</th>
<th>FHM</th>
<th>R1</th>
</tr>
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<tbody>
<tr>
<td>$Yr \ 1$</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$Yr \ 5$</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$Yr \ 9$</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$Yr \ 2198(6)$</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$Yr \ E842-95$</td>
<td>+</td>
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<td>+ + +</td>
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<td>+</td>
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Adherence inhibition to CHSE-214

Pre-treatment of *Y. ruckeri* 17.00(2-1):
- proteolytic enzymes (pronase, trypsin)
- sodium metaperiodate
- carbohydrates

\[\downarrow\]

Adherence assay to CHSE-214

\[\downarrow\]

Hemacolor staining
# Adherence inhibition to CHSE-214

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<tr>
<td><strong>Proteolytic enzymes</strong></td>
<td>++</td>
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<tr>
<td><strong>Sodium metaperiodate</strong></td>
<td>+++</td>
</tr>
<tr>
<td><strong>D-glucose</strong></td>
<td>++</td>
</tr>
<tr>
<td><strong>D-galactose</strong></td>
<td>++</td>
</tr>
<tr>
<td><strong>D-maltose</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>D-sucrose</strong></td>
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Invasion and intracellular survival in cell lines

3 fish cell lines: CHSE-214, FHM, R1

10^5 cells/well
10^6 CFU *Y. ruckeri*/well, 1h, 20°C
Invasion and intracellular survival in cell lines

![Graph showing recovery of bacteria for different Y. ruckeri strains and cell lines.](image-url)
Invasion and intracellular survival in cell lines
Invasion inhibition in cell lines

Pre-treatment of cell lines CHSE-214, FHM, R1:

dansylcadaverine

cytochalasin D

colchicine

Invasion assay
Invasion inhibition in cell lines

- Dansylcadaverine: -
- Cytochalasin D: + (-)
- Colchicine: + (-)

depending on *Y. ruckeri* strain and cell line
Serum resistance

serum + *Y. ruckeri* →

0 h
3 h
Serum resistance

% viable bacteria after 3 h

Y. ruckeri strain
In *vitro* markers for virulence

**Conclusions**

- *Y. ruckeri* adheres to fish mucus and cell lines via lectins
- *Y. ruckeri* invades fish cell lines in actin filament- & microtubule-dependent way
- Serum resistant and serum sensitive strains exist

→ adherence to mucus and serum resistance seems to be correlated with *in vivo* virulence
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• General discussion
Adhesion to mucus (m)
Lectins → D-galactose
↓
D-fructose
Fimbriae? Flagella?
↓
Movement through mucus
↓
Adhesion to epithelium (e)
Lectins → D-galactose
↓
D-glucose
Fimbriae? Flagella? D-maltose
↓
Fimbriae? Flagella? D-sucrose
↓
Invasion of epithelial cells
= actin filament + microtubule dependent
Via invasin homologue? TTSS? Yrp1? YhlA?
Intracellular
No survival or for short period
Host cell lysis by YhlA?
↓
Invasion of capillary (c)
Serum resistance
↓
Dissemination to the internal organs
Adhesion to mucus (m)
Lectins → D-galactose
↓
D-fructose
↓
Fimbriae? Flagella?
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Dissemination to the internal organs
Prof. Chiers – Prof. Hermans – Prof. Decostere – Prof. Haesebrouck
Prof. Van den Broeck – Prof. Duchateau – Prof. Favoreel
Prof. Bossier – Prof. Turnbull – Dr. Lieffrig – Prof. Ducatelle
Prof. Pasmans – Prof. Martel – Prof. Van Immerseel

Thank you

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Evelyne – Pascale – Annemieke – Audrey – Roselien

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