Route of entry and tissue distribution of *Yersinia ruckeri* in experimentally infected trout

*E. Tobback*

*Supervisors: Prof. dr. K. Chiers  
Prof. dr. K. Hermans*

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
Yersinia ruckeri
Gram negative rod

Enteric Redmouth Disease
mainly in salmonids (rainbow trout)
septicaemia
haemorrhages, exophthalmia
In vivo:
To reveal the route of entry and investigate the tissue distribution of *Y. ruckeri* in rainbow trout

In vitro:
To characterize the adhesive, invasive and intracellular survival properties of *Y. ruckeri* to cell lines
Materials and Methods

In vivo: Experimental infections

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

- Experimental infections to determine:
  (1) virulence
  (2) route of entry
  (3) tissue distribution
Materials and Methods

*In vivo*: Experimental infections: contact challenge
**Materials and Methods**

*In vivo*: Experimental infections

<table>
<thead>
<tr>
<th></th>
<th>inoculum (CFU ml⁻¹)</th>
<th>sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) virulence</td>
<td>2 x 10⁷</td>
<td>- dead/moribund fish</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- euthanasia: 14-34 days p.i.</td>
</tr>
<tr>
<td>(2) route of entry</td>
<td>2 x 10⁸</td>
<td>0, 1.5, 2.5 hours p.i.</td>
</tr>
<tr>
<td>(3) tissue distribution</td>
<td>2 x 10⁷</td>
<td>1, 2, 4, 6, 9, 12, 24, 48, 72 hours p.i.</td>
</tr>
</tbody>
</table>
## Results

**In vivo: Experimental infections**

(1) Virulence

<table>
<thead>
<tr>
<th>Y. ruckeri strain</th>
<th># fish with clinical signs</th>
<th># fish that died</th>
<th>Mean time of death (days p.i.)</th>
<th>Bacteriological examination (gills, gut, liver, kidney, spleen; CFU g⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fish that died</td>
</tr>
<tr>
<td>5</td>
<td>4 %</td>
<td>23 %</td>
<td>7.5</td>
<td>10⁶-10⁹</td>
</tr>
<tr>
<td></td>
<td>Fish that survived</td>
<td></td>
<td></td>
<td>10²-10³</td>
</tr>
<tr>
<td>17.00(2-1)</td>
<td>0 %</td>
<td>0 %</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>CCUG14190</td>
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<td>/</td>
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<tr>
<td>E842-95</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
### Results

**In vivo**: Experimental infections

(1) virulence

<table>
<thead>
<tr>
<th><em>Y. ruckeri</em> strain</th>
<th>Histopathological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gills</td>
</tr>
<tr>
<td>5</td>
<td>-moderate/severe</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>17.00(2-1) CCUG14190 E842-95</td>
<td>/</td>
</tr>
</tbody>
</table>
Results

*In vivo*: Experimental infections

(2) route of entry: gills
Results

In vivo: Experimental infections

(3) tissue distribution
Results

*In vivo*: Experimental infections

(3) tissue distribution
Conclusions

- Highest bacterial numbers were found in gills soon after infection = portal of entry?
  → to be confirmed by histology/immunohistochemistry (future)

- *Y. ruckeri* was reisolated from liver, kidney and spleen of fish infected with low virulent strains only between 0 – 12 h p.i.

- The numbers of *Y. ruckeri* found in liver, kidney and spleen of fish infected with virulent strain sharply increased after 48 h p.i.
Hypothesis on pathogenesis?
In vitro: Adhesion assays

- 4 *Y. ruckeri* strains
- 3 fish cell lines: CHSE-214, FHM, R1

10^5 cells/well \rightarrow 1h \rightarrow 10^6 bacteria/well \rightarrow Scanning Electron Microscopy (SEM)
Materials and Methods

*In vitro*: Invasion/intracellular survival assays

- 4 *Y. ruckeri* strains
- 3 fish cell lines: CHSE-214, FHM, R1

![Diagram showing invasion process](image-url)
Results

In vitro: Adhesion assays

![Bar charts showing adhesion assays for Y. ruckeri strain in vitro.](image)
Results

\textit{In vitro}: Invasion/survival assays
Conclusions

- **Strain 5:**
  - caused mortality and disease signs *in vivo*
  - showed moderate invasiveness and adhesion *in vitro*

- **Strain 17.00(2-1):**
  - showed high invasiveness and adhesion *in vitro*
  - didn’t cause disease *in vivo*

→ Differences in virulence exist between different *Y. ruckeri* strains
→ A higher *in vivo* virulence was not reflected in a higher capacity to invade cell lines *in vitro*
Thank you for your attention!