



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

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Yersinia ruckeri

Gram negative rod

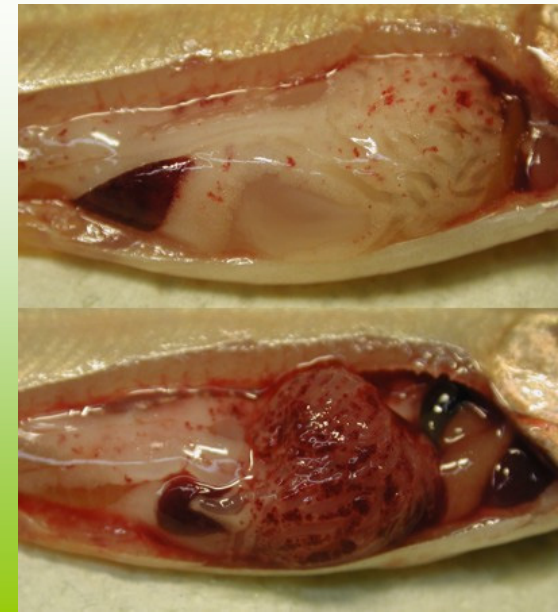


Enteric Redmouth Disease

mainly in salmonids (rainbow trout)

septicaemia

haemorrhages, exophthalmia



Main goals

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

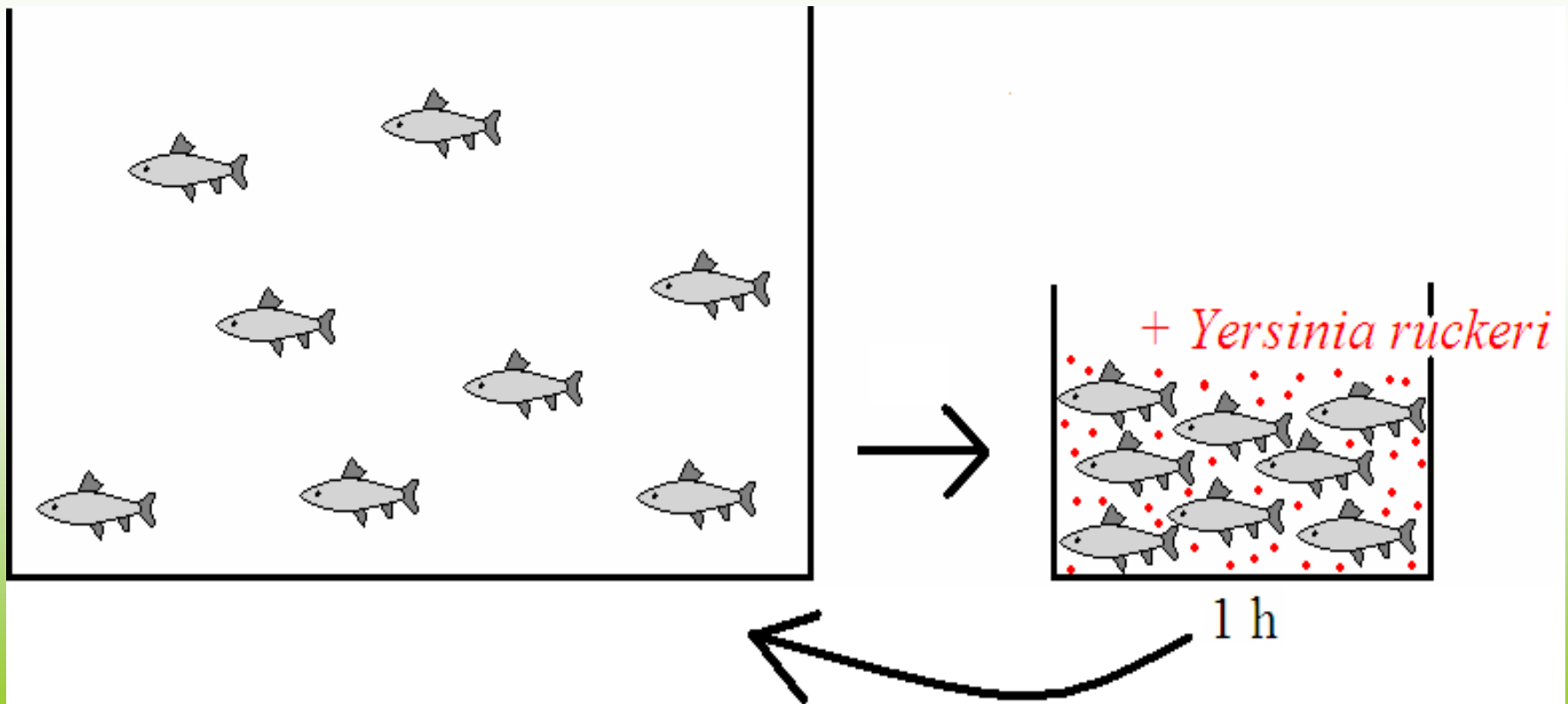
Materials and Methods

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

	inoculum (CFU ml ⁻¹)	sampling
(1) virulence	2×10^7	- dead/moribund fish - euthanasia: 14-34 days p.i.
(2) route of entry	2×10^8	0, 1.5, 2.5 hours p.i.
(3) tissue distribution	2×10^7	1, 2, 4, 6, 9, 12, 24, 48, 72 hours p.i.

Results

In vivo: Experimental infections

(1) virulence

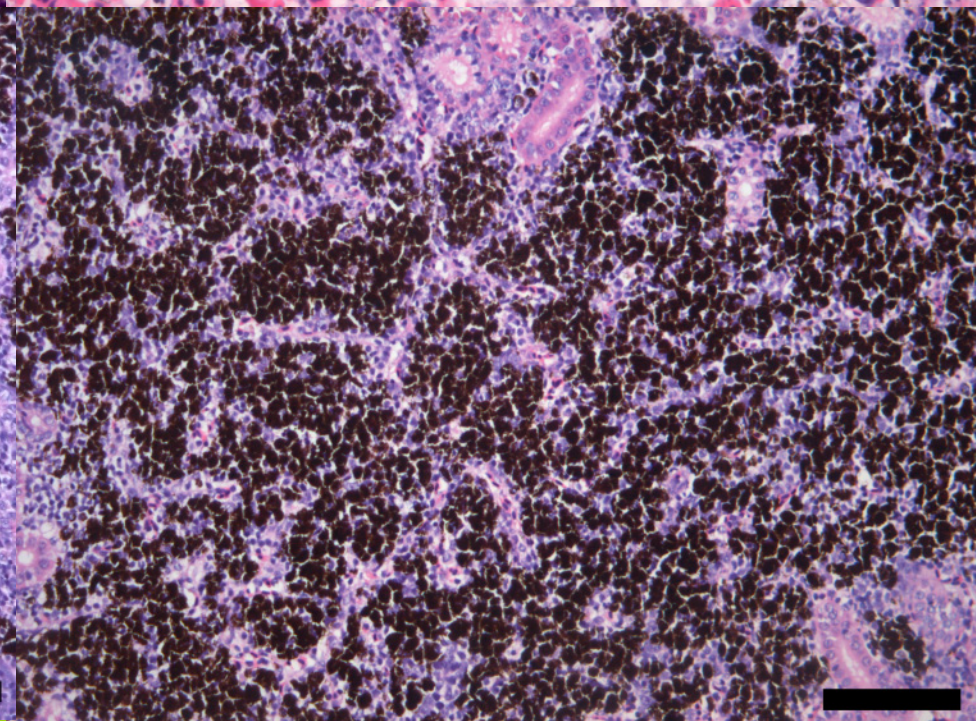
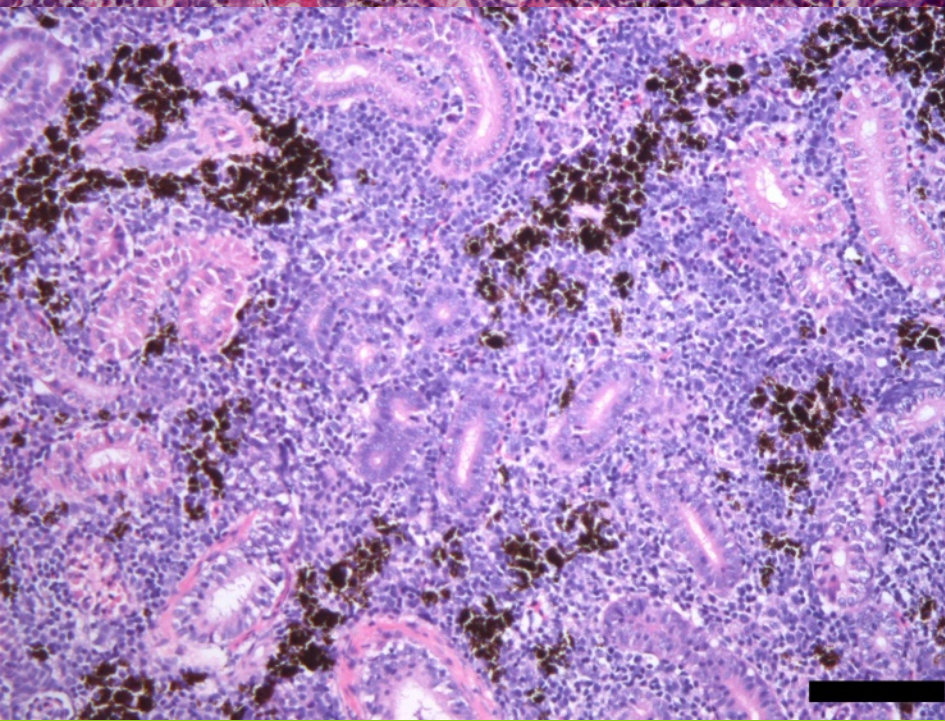
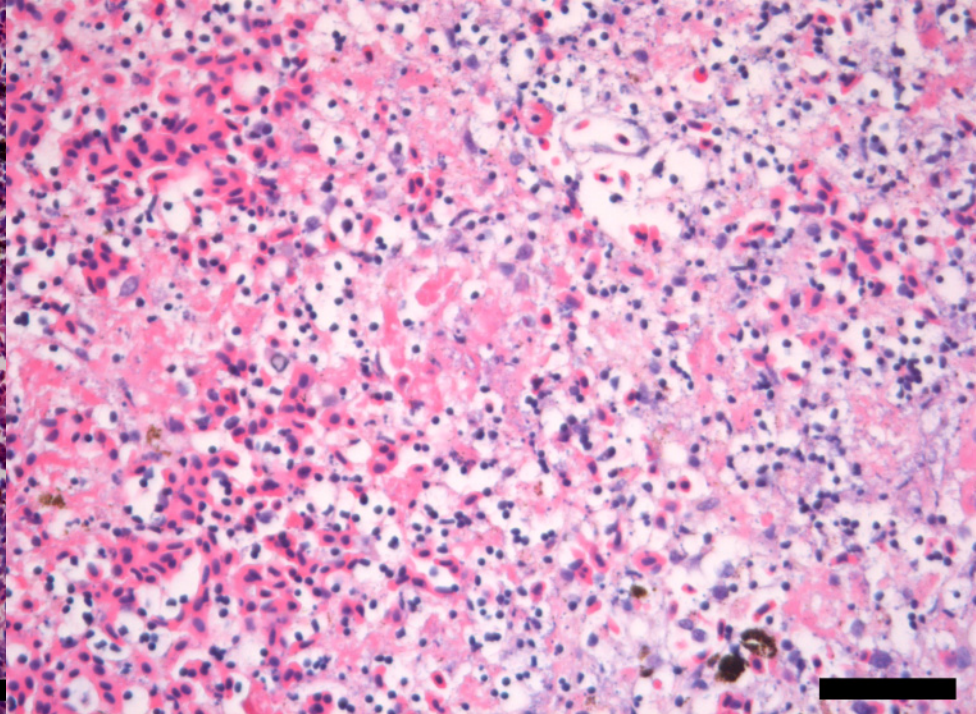
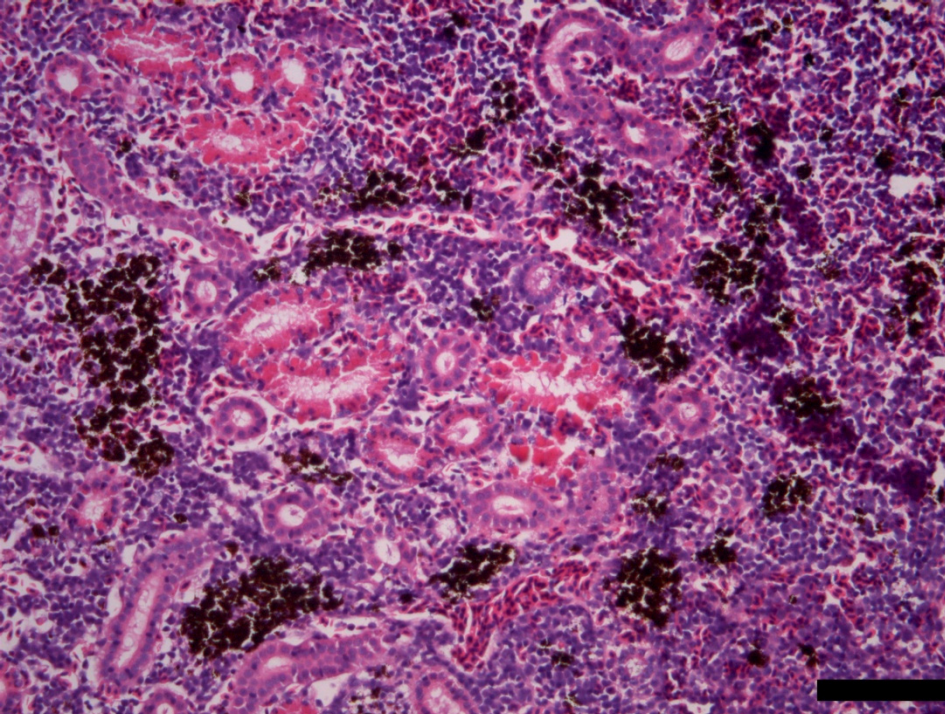
<i>Y. ruckeri</i> strain	# fish with clinical signs	# fish that died	Mean time of death (days p.i.)	Bacteriological examination (gills,gut,liver,kidney,spleen; CFU g ⁻¹)	
				Fish that died	Fish that survived
5	4 %	23 %	7.5	10 ⁶ -10 ⁹	10 ² -10 ³
17.00(2-1) CCUG14190 E842-95	0 %	0 %	/	/	0

Results

In vivo: Experimental infections

(1) virulence

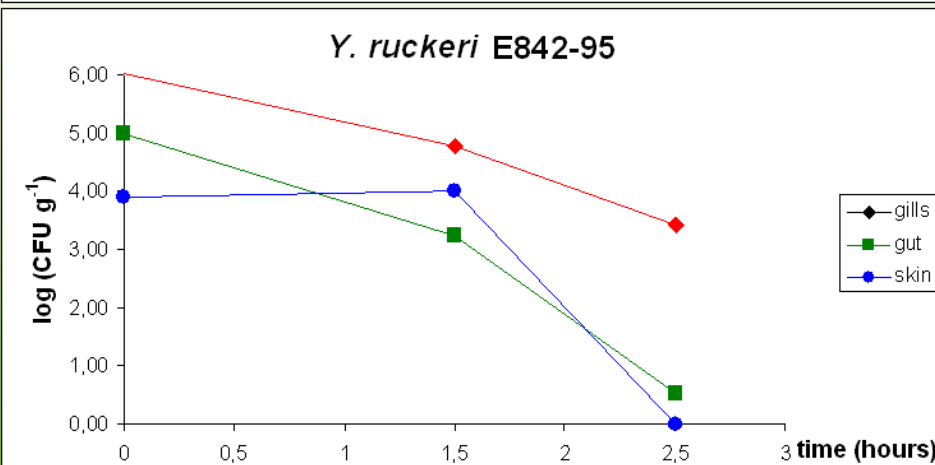
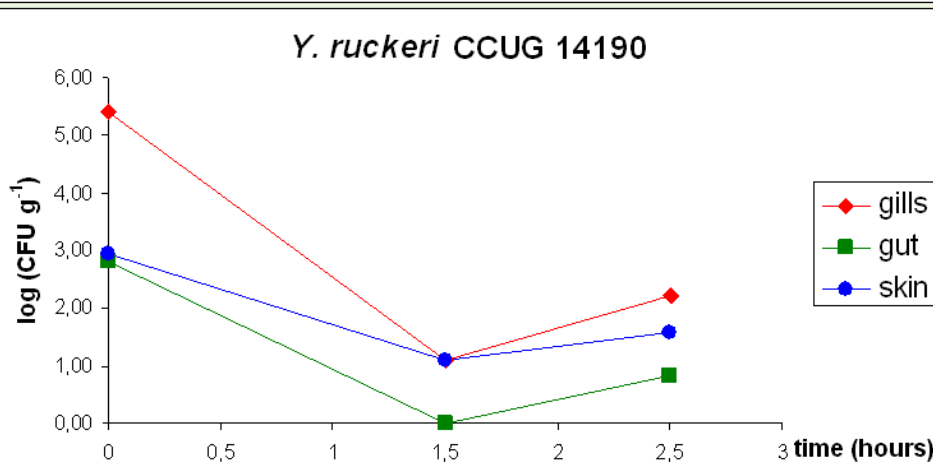
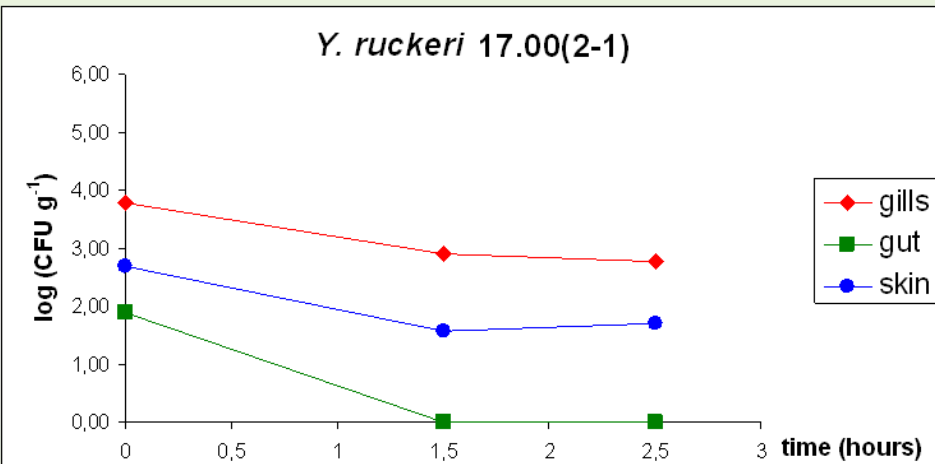
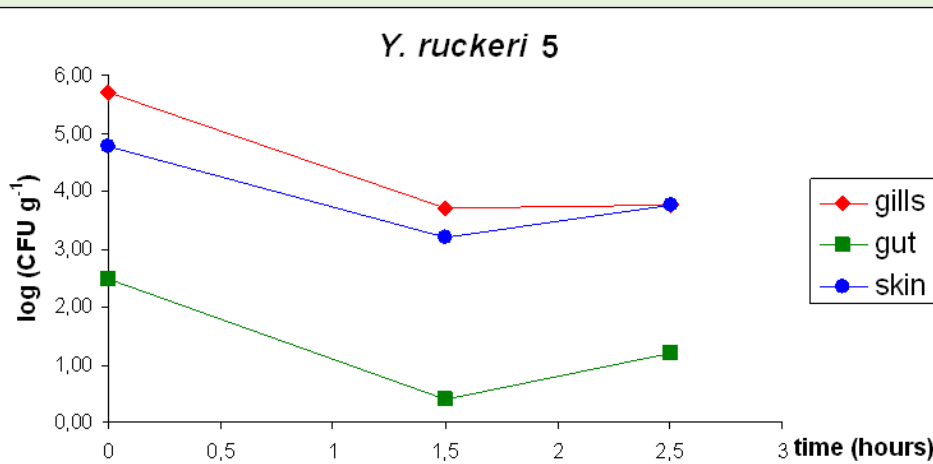
<i>Y. ruckeri</i> strain	Histopathological changes		
	gills	spleen	kidney
5	-moderate/severe oedema	-necrosis	-degeneration and/or necrosis of tubules -increased cellularity of glomerular tuft -increase in # (melano)macrophages
17.00(2-1) CCUG14190 E842-95	/	/	/



Results

In vivo: Experimental infections

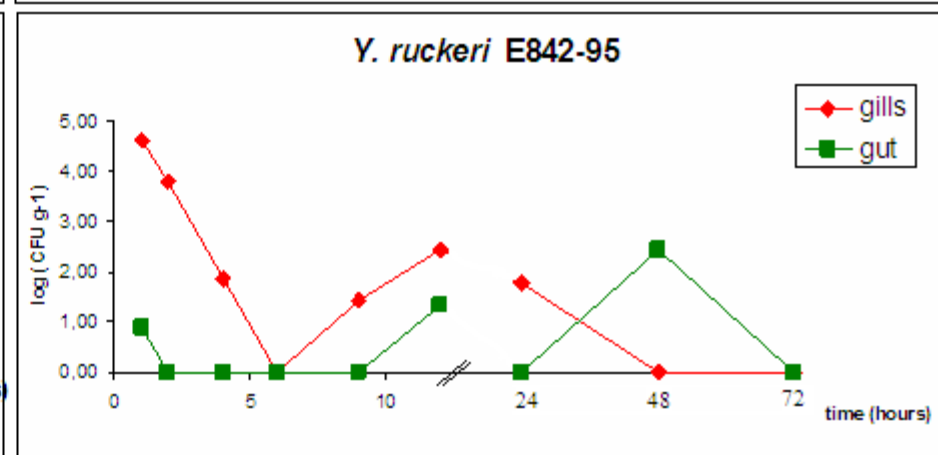
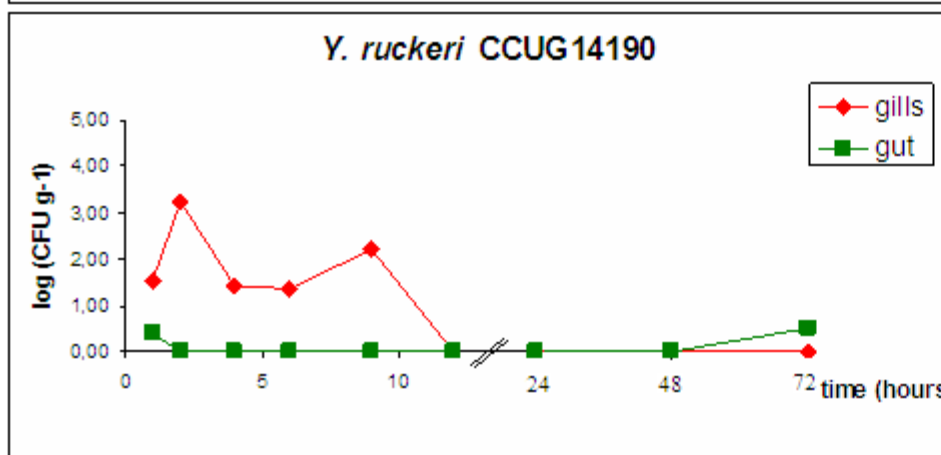
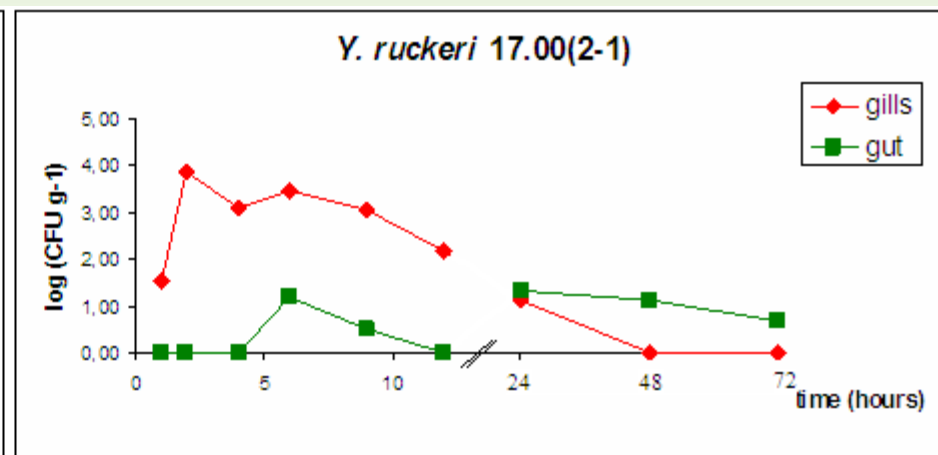
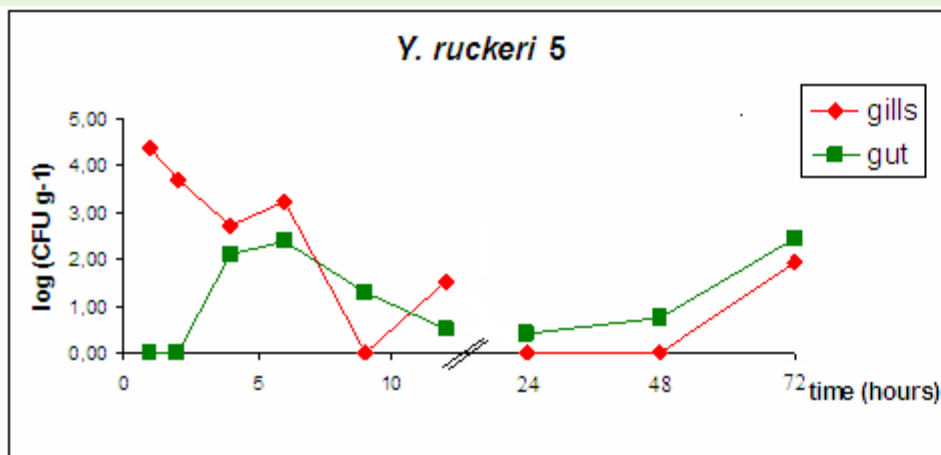
(2) route of entry: gills



Results

In vivo: Experimental infections

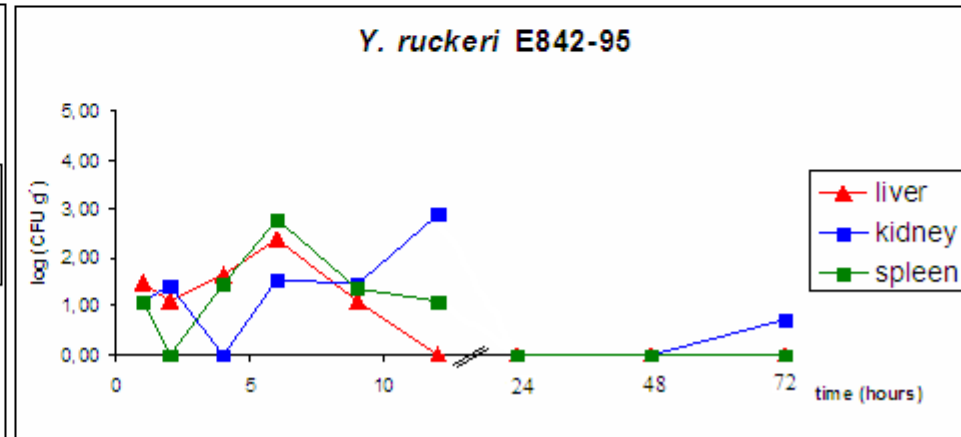
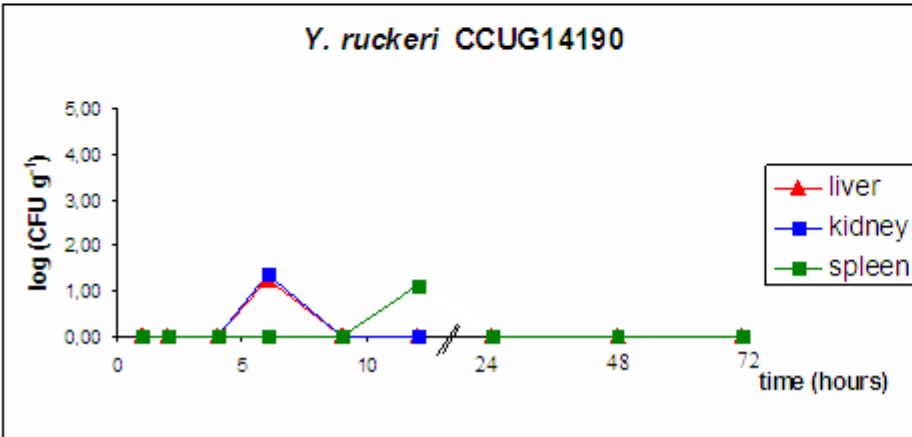
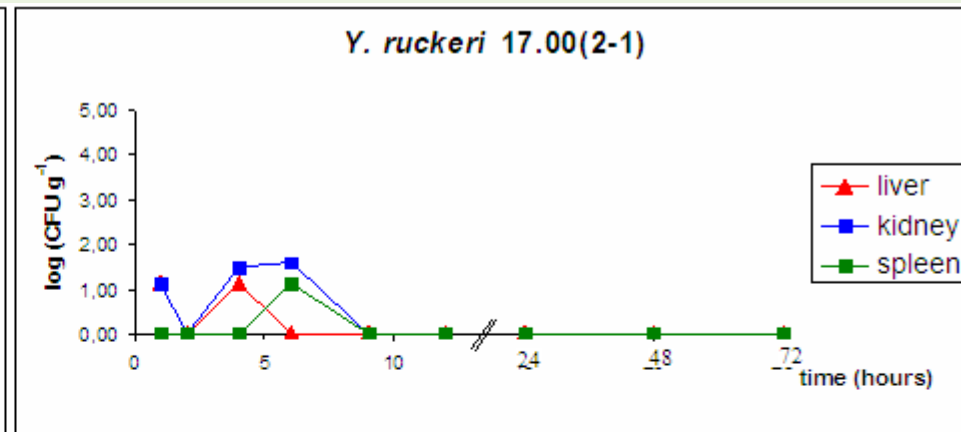
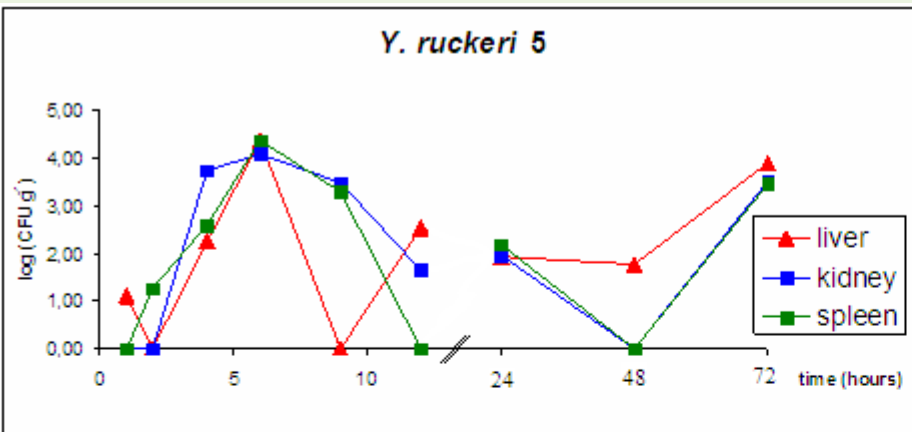
(3) tissue distribution



Results

In vivo: Experimental infections

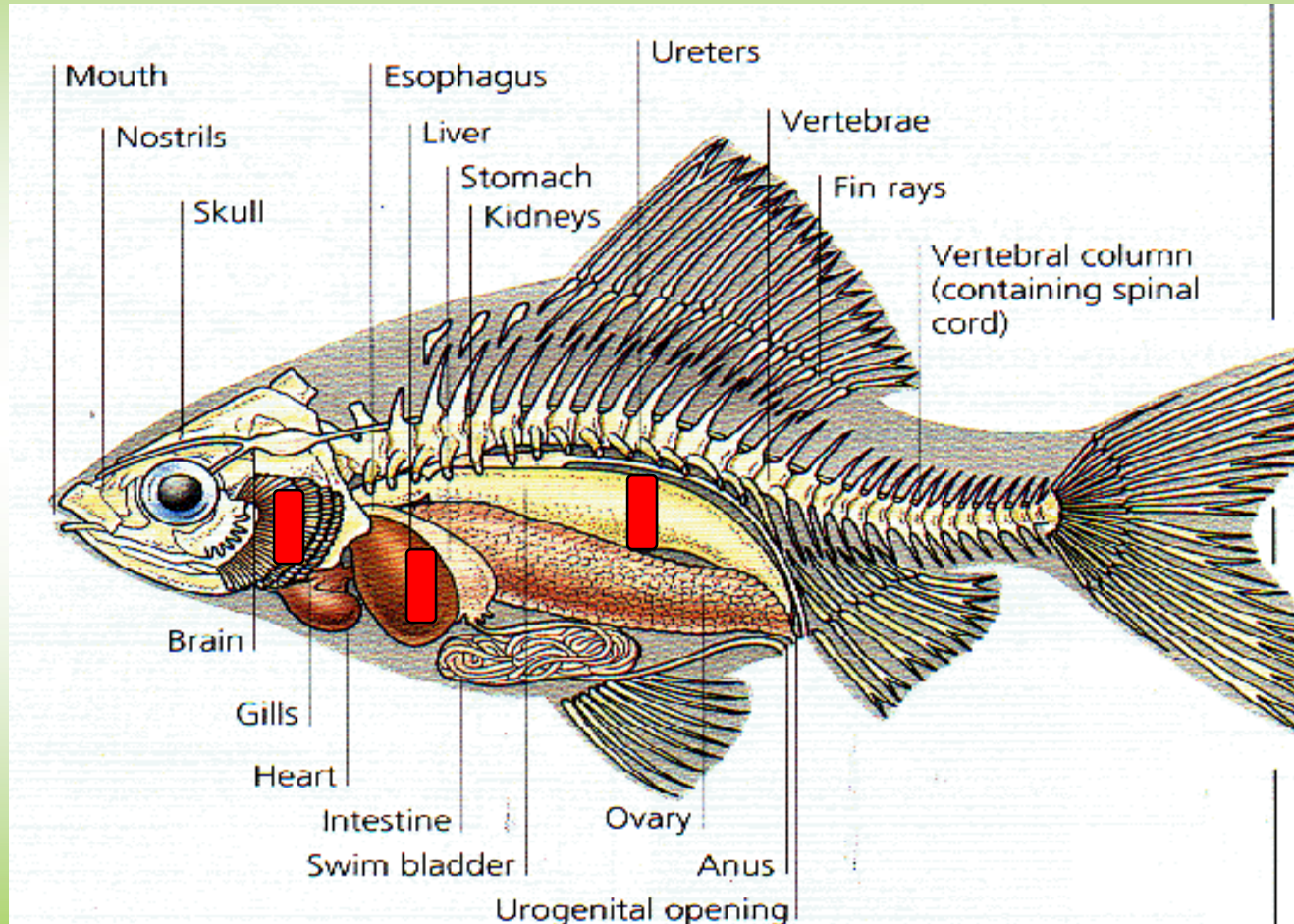
(3) tissue distribution



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?



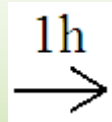
Materials and Methods

In vitro: Adhesion assays

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

10^5 cells/well

10^6 bacteria/well

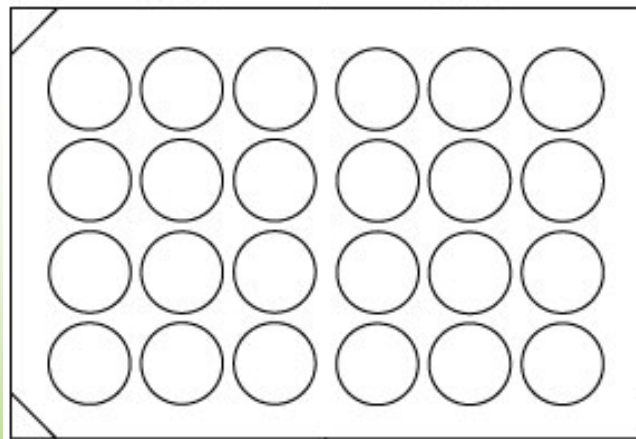


Scanning Electron
Microscopy (SEM)

Materials and Methods

In vitro: Invasion/intracellular survival assays

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



10^5 cells/well
 10^6 bacteria/well



- gentamicin
- triton-X100

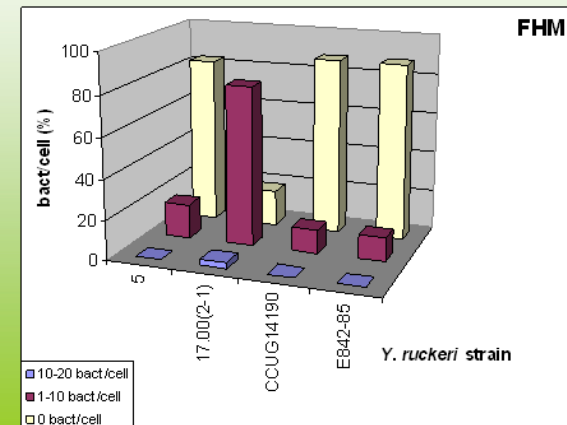
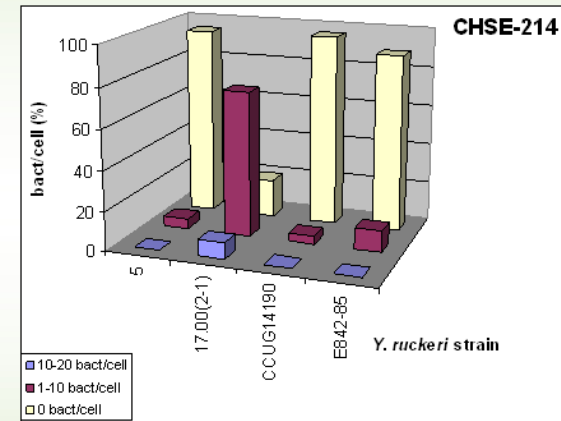
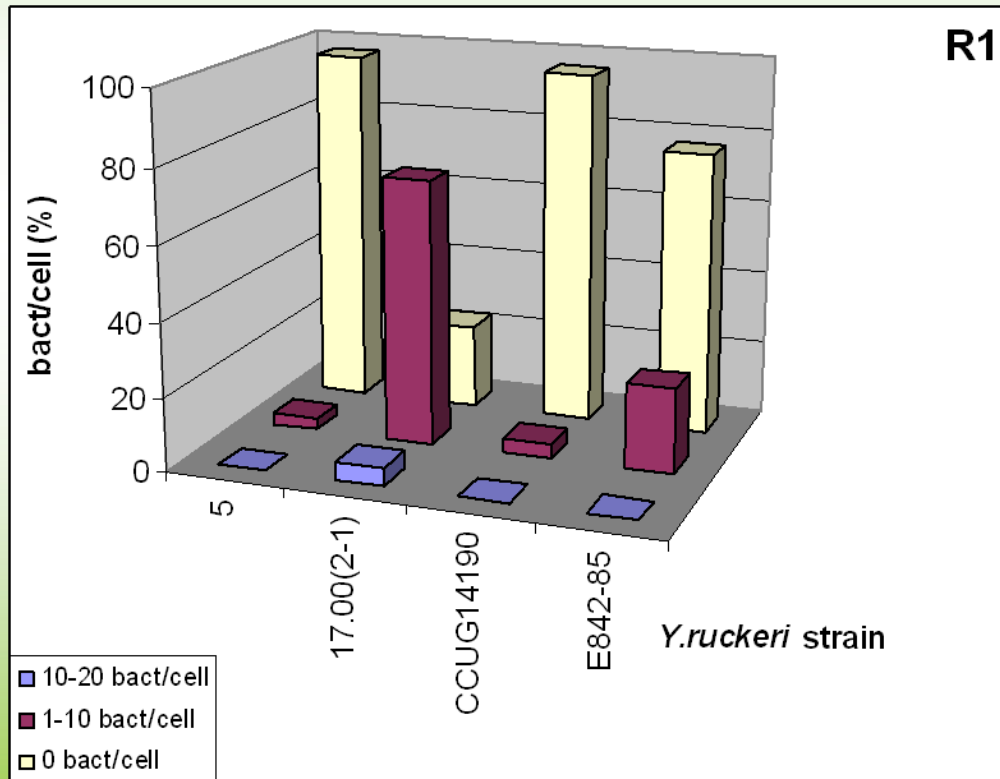
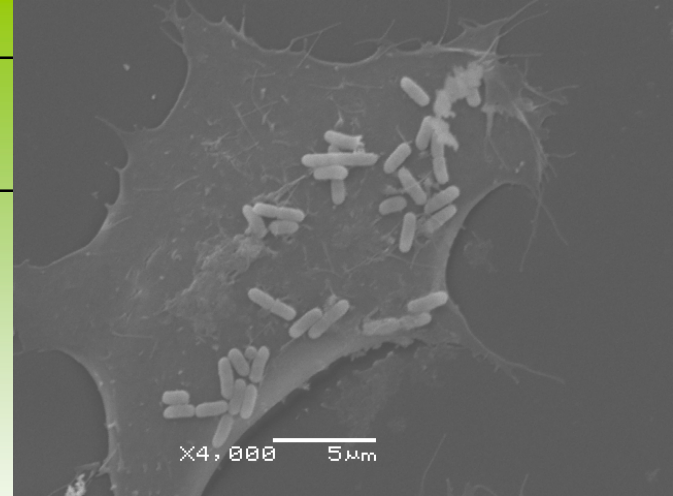


1h

6h

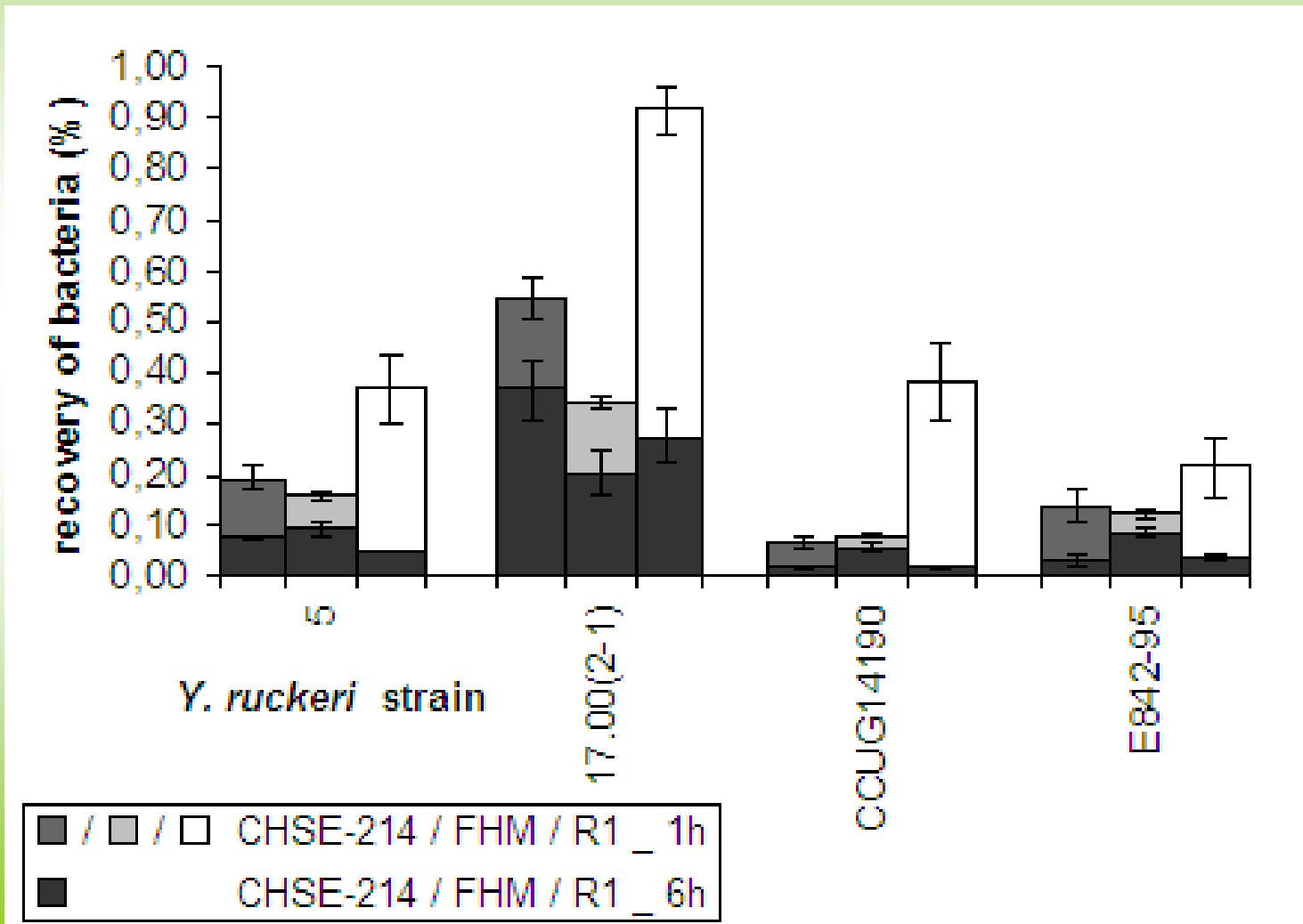
Results

In vitro: Adhesion assays



Results

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!

