The role of heat shock proteïns as immunostimulants against the ‘enteric redmouth syndrome’, caused by *Yersinia ruckeri*

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Goals

- Evaluation of the influence of HSPs on the course of a bacterial infection in fish

  In vivo

  - Testing in a model with platyfish
  - Validation of results in rainbow trout

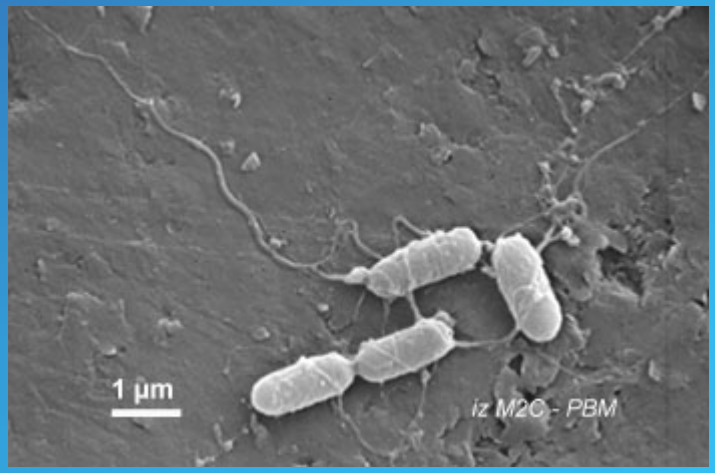
  In vitro

  - Role of HSPs in the immune response

Introduction: HSPs

- What?
  - Highly conserved cellular proteins
  - Present in all organisms
  - Molecular chaperones
  - Induced by exposure to stress
  - Role in the immune system
    - Danger signal
    - Modulator of PAMP signaling
    - Role in antigen presentation

Introduction: *Yersinia ruckeri*

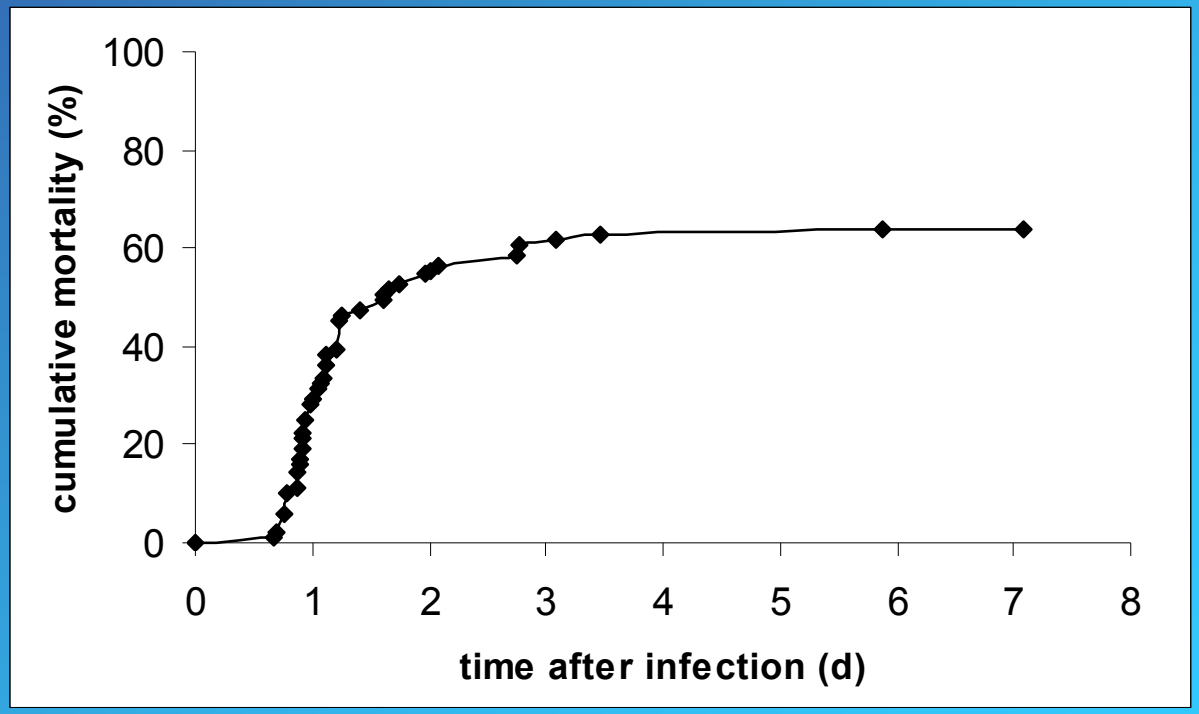


- Gram negative rod
- Causative agent of
  enteric redmouth disease
  - Septicemic disease
  - Host: salmonids
  - Most acute in small fish
  - Symptoms are: change in behaviour, haemorrhages, exopthalmia

Challengemodel: infection of platyfish with *Y. ruckeri*

- Parameters of the infection model:
  - Pathogen: *Y. ruckeri* 5 (serotype I)
  - Infection: i.c. injection, 30 µl PBS/fish, 10^6 CFU/fish

    → Expected mortality: 60-70% after 4 - 5 days

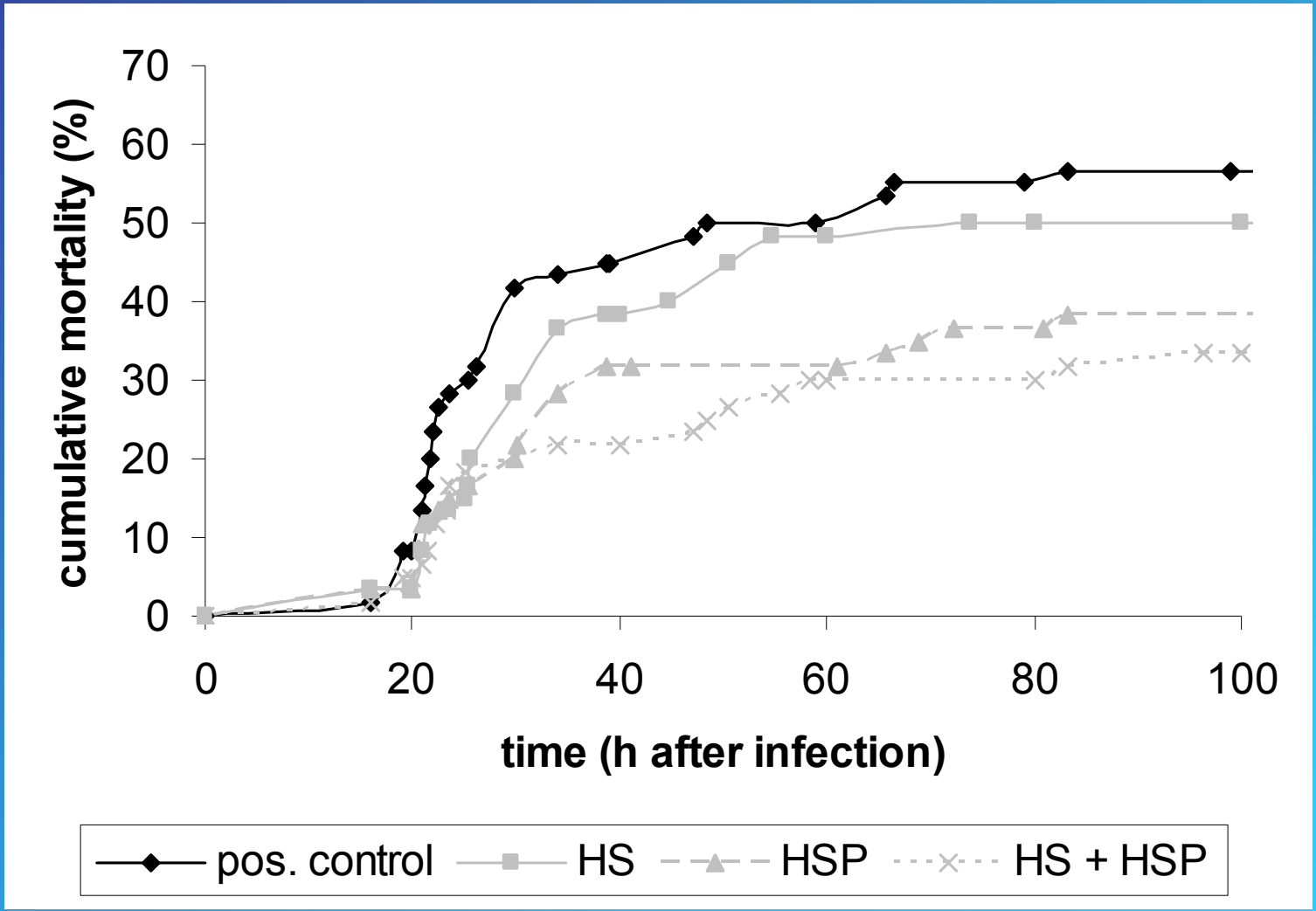


Effect of HSPs on a *Y. ruckeri* infection in platyfish

- Testing of 2 parameters: HS and bacterial HSPs
- 3 different treatments:
  - Heat shock
  - Injection with bacterial HSPs (1 µg)
  - HS + injection with bacterial HSPs (1 µg)
- 6h later: infection with *Y. ruckeri*
  - Intracoelomal injection
  - 10^6 CFU/fish, in 30 µl PBS

- Effect on survival?

Effect of HSPs on a *Y. ruckeri* infection in platyfish: results



Effect of HSPs on a *Y. ruckeri* infection in platyfish: results

- HS-HSP differs significantly from control with a hazard ratio equal to 0.503
- The comparison between HSP and control just fails to be significant (p=0.025) with a hazard ratio of HSP versus control equal to 0.547
- HS does not differ significantly from control (p=0.29)

Role of HSPs in the immune response

- Innate immune system: macrophages
  - Effect on intracellular survival
  - Effect on respiratory burst
  - Effect on NO production
- Acquired immune system
  - Effect on antibody titers

Effect on intracellular survival

- Isolation of macrophages from head kidney of rainbow trout
  - → yield: ca 1 . 10^7 macrophages/fish
  - → separation on a percoll gradient (34% / 51%)
  - → seeded in 96-well plates at 10^6 cells / well (in 100 µl)
  - → incubate overnight at 17°C + 5% CO_2
- Gentamicin protection test
  - → Incubation of the macrophages with *Y. ruckeri* for 1 h at MOI = 10
  - → Killing of extracellular *Y. ruckeri* with gentamicin for 1h at a concentration of 100 µg/ml
  - → Sampling at t = 0h, 6h, 16h and 24h

Effect on intracellular survival

- Effect of HS on intracellular survival
  - → Selection of optimal heat shock conditions: ΔT, duration and recovery
  - → Gentamicin protection test

Effect on respiratory burst

- Respiratory burst:
  - Rapid release of reactive oxygen species from immune cells as they come into contact with pathogens
  - Occurs in phagocytes to degrade internalized particles and bacteria
- Measurement of the production of oxygen radicals with chemoluminescence assay
  - → Unstable oxygen radicals go back to a lower energy level and emit photons = chemoluminescence
  - → Luminol will be added to amplify the signal
  - → Measurement with luminometer

Future?

- Purification of recombinant bacterial HSP DnaK
- Effect on NO production (innate immunity) and on antibody titers (acquired immunity)
- Validation of the results of the *in vivo* experiments in rainbow trout

Thank you for listening!

Any questions?