RENIBACTERIUM SALMONINARUM - THE CAUSATIVE AGENT OF BACTERIAL KIDNEY DISEASE - ITS PRESENCE IN WILD FISH FROM SELECTED RIVER SYSTEMS IN ENGLAND AND WALES

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Introduction

- Bacterial Kidney Disease caused by the Gram positive bacterium *Renibacterium salmoninarum* (*R.s.*) is considered one of the most serious and widespread diseases of salmonids world-wide.
- At present there are no effective vaccines or treatments.
- As such it is classed as a List III notifiable disease in the UK under current EU legislation (Council Directive 91/67/EEC). MAFF operate a control policy prohibiting the movement of live fish or eggs from farms or hatcheries found to harbour the bacterium.
- This policy has a serious impact on hatcheries.
- Some members of the Fish Farming community believe that the control measures are excessive as the bacterium doesn't appear to be as virulent in Rainbow trout - the predominantly farmed salmonid in England and Wales - as it is in other salmonid species farmed world-wide.
- MAFF is receptive to reviewing the current policy but change needs to be adopted on the basis of sound science.
- One area where further knowledge is required concerns the prevalence of the organism in wild fish populations.
- A survey of wild fish in England and Wales has been conducted using a novel validated molecular biology based technique to give a more accurate estimation of the presence of the bacterium in wild fish from selected river systems.

Wild Fish Survey

- Rivers were selected in England and Wales according to 3 criteria
  1. A pristine river system- a river with no fish farms and no recent history of restocking
  2. River systems containing fish farms which are at present culture negative for *R.s*
  3. River systems containing fish farms which are at present culture positive for *R.s.*
- Rivers were electrofished in conjunction with the Environment Agencies electrofishing programme or with riparian owners.
- Species sampled include
  - Brown trout (*Salmo trutta*)
  - Rainbow trout (*Salmo gairdneri*)
  - Grayling (*Thymallus thymallus*)
  - Salmon (*Salmo salar*)
  - Pike (*Esox lucius*)
- Attempts were made to sample a total of 150 fish, mixed species, from each river catchment.

Figure 1. Gross external symptoms of BKD include; darkening, petechial haemorrhaging, swollen abdomen.
Note - most fish are infected asymptomatically.

Figure 2. Gross internal symptoms of BKD include; swollen kidney and spleen, petechial haemorrhaging in muscle, characteristic lesions on kidney, spleen and liver.

Sampling

- Fish were sacrificed on-site and the head kidney swabbed onto a Selective kidney Disease Medium agar plate. These were brought back to the laboratory and incubated for up to 10 weeks at 15°C.
- The remainder of the fish were individually bagged, returned to the laboratory on ice and frozen at -20°C until processed.
- The head kidney was removed.
- DNA was extracted.
- A validated molecular biology based technique (single round PCR using two primers unique to *R.s.*) was applied. This technique was designed to detect a particular nucleic acid sequence of 358bp which is unique to *R.s.*
- Positive results appear as bands on a gel, measuring 358bp which correspond with the positive control.
- To confirm the presence of *R.s.* DNA all bands of this size are subjected to a nested PCR, using another 4 unique sets of primers, the product DNA cloned and sequenced and the sequence compared to that published for the p57 MSA gene of *R.s.*

Figure 3. Electrofishing with the Environment Agency.

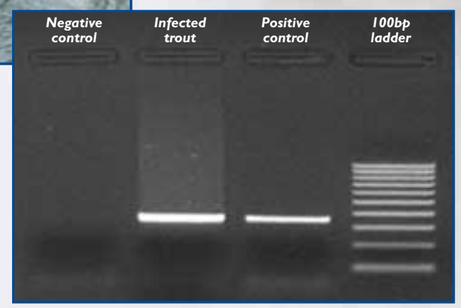

Figure 4. Gel electrophoresis showing 358bp R.s. DNA fragment.

Results

- There was no evidence for the presence of *R.s.* in the pristine river
- A low level of *R.s.* was found in the rivers which contain fish farms at present culture negative for *R.s.*
- There was a higher prevalence in the rivers containing fish farms currently culture positive for *R.s.*

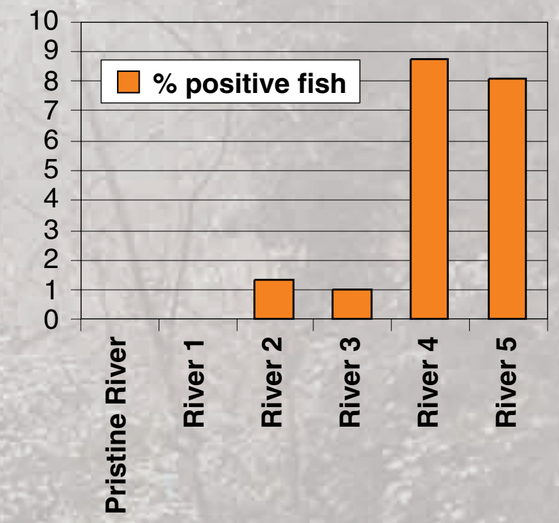

Figure 5. Chart depicting the prevalence of Renibacterium salmoninarum *in wild fish in selected river systems in England and Wales.*

Rivers 1&2 River catchments have fishfarms currently culture negative for *R.s.*

River 3 An opportunistic sample from the river around a fishfarm, culture positive for *R.s.*, undergoing a disinfection. Most fish were sampled downstream of the fishfarm. Weather conditions deteriorated prohibiting much upstream sampling.

Rivers 4&5 River catchments have fishfarms currently culture positive for the presence of *R.s.*

Conclusion

Renibacterium salmoninarum does not appear to be widespread in wild fish populations throughout England and Wales.

There appears to be a link between the presence of fish farms, their *R.s.* status, and the presence of the bacterium in the wild.

Future work

In conjunction with the British Trout Association a survey of fish farms - which are at present negative for the presence of *R.s.* by current approved culture methods - will be carried out using the new molecular biology technique. This will give a more accurate picture of the prevalence of the bacterium in fish farms.

Tank based experiments will be conducted to ascertain the effect of smolting on salmon asymptomatically infected by *R.s.*

Laboratory based transmission studies will be carried out using molecular biology based techniques to ascertain the possibility of spreading the bacterium between fish farms via infected nets, wellington boots, vehicle tyres etc.

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