PREVALENCE OF *Renibacterium salmoninarum* IN FARMED AND WILD FISH IN ENGLAND AND WALES

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Aim:

To determine the background levels of *Renibacterium salmoninarum* (*R.s.*) in both farmed and wild fish in England and Wales.

Problem:

Renibacterium salmoninarum, the causative agent of bacterial kidney disease, is predominantly an intracellular bacterium (Daly J. G. 2001). Despite many improvements in culture media over the years it remains notoriously difficult to grow on solid agar. It is both fastidious in its growth requirements and very slow growing, taking in some instances up to 10 weeks to culture on initial isolation. During this time agar plates are prone to contamination from more quickly growing environmental bacteria and fungi (Figure 1, Figure 2).

Solution:

To develop a more sensitive PCR based detection method and use this method to survey both rivers and fish farms in England and Wales.

Method

- The PCR method used was a modification of the approach adopted by McIntosh *et al.*, 1996, based on the testing of crude fish macrophage preparations.
- A crude lymphocyte preparation was made from 50mg of fish head kidney and DNA was extracted using a modified DNAzol protocol.
- The PCR reaction mix contained 25mM of each dNTP, 1.5mM $MgCl_2$, 100pmol of each primer, 10µl reaction buffer IV, 10µl of template and 0.025U/µl of Redhot Taq (Abgene) in a total volume of 100µl.
- A manual hotstart was used followed by 33 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 2 min, followed by one cycle of 94°C for 1 min, 57°C for 1 min and 72°C for 10min.
- PCR products were visualised by gel electrophoresis after staining with ethidium bromide. Expected products were 358bp long and were confirmed by sequencing (Figure 3).

Figure1: Typical R.s. colonies on an SKDM plate

Sensitivity

This PCR technique detects between 10 and 100 *R.s.* cells per 50mg of head kidney tissue.

Wild Fish Survey

Rivers were selected to include

- A river catchment containing no fish farms and with no recent history of restocking
- River catchments containing fish farms currently negative for the presence of *R.s.* by conventional culture methods
- River catchments containing fish farms currently positive for the presence of *R.s.* by conventional culture methods (Figure 4).

Species sampled include

- Brown trout (*Salmo trutta*)
- Rainbow trout (*Oncorrhynchus mykiss*)
- Grayling (*Thymallus thymallus*)
- Salmon (*Salmo salar*)
- Pike (*Esox lucius*)
- Eel (*Anguilla anguilla*)

All fish were examined individually

Farmed Fish Survey

150 Rainbow trout (*Oncorrhynchus mykiss*) were sampled from each of 10 fish farms from England and Wales (Figure 4).

Experiments showed that fish could be pooled into groups of five and examined without significant loss of sensitivity.

Figure 2: Large fast growing contaminants found on an SKDM plate obscuring potential R.s. growth

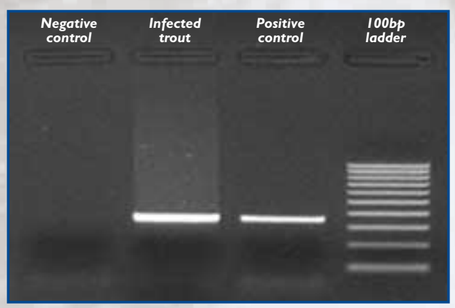

Figure 3: Gel electrophoresis showing 358 bp R.s. DNA fragment

Lane 1 Negative control
Lane 2 Infected trout
Lane 3 Positive control
Lane 4 100bp ladder

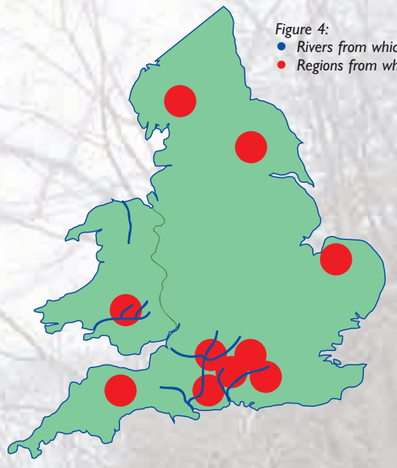

Figure 4:
- *Rivers from which wild fish were sampled*
- *Regions from which farmed fish were sampled*

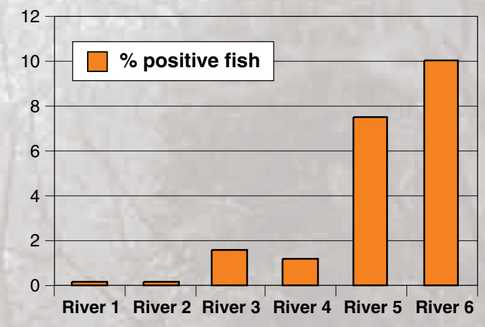

Figure 5: Results of Wild Fish Survey by river

Key
River 1 Has no fish farms and no recent history of restocking
Rivers 2 and 3 Had fish farms negative for R.s. by the culture method at the time of sampling
River 4, 5, and 6 Had fish farms positive for R.s. by the culture method at the time of sampling

Results of Wild Fish Survey by species and river

Shows numbers of each species sampled from each river followed by number and percentage positive

Species	River 1	River 2	River 3	River 4	River 5	River 6
Grayling		105 (0)	7 (0)		116 (12) 10.3%	172 (20) 11.6%
Brown trout	122 (0)	4 (0)	140 (2) 1.4%	88 (1) 1.1%	9 (0)	10 (0)
Salmon	28 (0)	6 (0)	1 (0)			49 (4) 8.2%
Rainbow trout		14 (0)	2 (0)	7 (0)	2 (0)	8 (0)
Pike		8 (0)			2 (0)	2 (0)
Eel						45 (1) 2.2%

Results of Farmed Fish Survey

A total of 1500 Rainbow trout were sampled (150 from each of 10 fish farms). No PCR positive bands were found from any of the fish sampled.

Discussion

From the results of the wild fish survey it appears that there is a higher prevalence of *R.s.* in grayling and salmon than in the other species tested. Only two wild fish (one salmon and one grayling) showed clinical signs of BKD. Both were from River 6. There appears to be a link between *R.s.* positive wild fish and rivers which have farms currently positive for the presence of the bacterium. However it is not clear whether that link is due to the greater prevalence of susceptible species (salmon and grayling) in these rivers or to the presence of the fish farms themselves. The farms could be acting as a reservoir of infection for the susceptible wild fish, or indeed, the wild fish could be re-infecting the fish farms and under farming conditions inducing the disease in rainbow trout - perceptively a less susceptible species.

This is the first reported finding of the presence of *R.s.* in eel. This fact that eel may carry *R.s* could have implications for the transmission of the disease between watercourses as eel are migratory over land.

It is not possible to generalise the results generated by the farmed fish survey as the farms were not truly randomly selected. One was chosen from each of the River catchments 2, 3 and 5 and two were chosen from River catchment 6. (Sampling was carried out two years after sampling for the wild fish survey. By this time all farms were negative for *R.s.* by the standard culture method). The other farms were from different areas of the country. The fact that none of the fish sampled yielded a positive result does not mean that *R.s.* is non-existent in these farms or indeed nationwide. Rather, if the farms had been truly randomly selected it would only have been possible to conclude, from a sample of 10 farms, that the nationwide farm level prevalence of *R.s* was less than 28% (Cameron and Baldock 1998). To ascertain the true farm level prevalence of *R.s.* a larger scale survey of farmed fish would need to be carried out.

Studies of *R.s.* in farmed and wild fish would need to be carried out simultaneously so that the interaction between the disease in both environments could be explored.

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