Characterisation of emerging strains of *Yersinia ruckeri*

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Introduction

Yersinia ruckeri is the Gram-negative pathogen responsible for outbreaks of enteric redmouth (ERM) disease amongst farmed rainbow trout (*Oncorhynchus mykiss* Walbaum; Figure 1) and Atlantic salmon (*Salmo salar* L.). Non-motile strains are typically associated with rainbow trout ERM in the UK, whilst motile strains are associated with ERM in mainland Europe and the US.

Figure 1: Rainbow trout mortalities due to ERM

ERM has been successfully controlled by vaccination, however recent outbreaks have been observed on rainbow trout farms in England and Wales despite vaccination. This has been attributed to an emerging biogroup of *Y. ruckeri* (Austin *et al.*, 2003). Similar cases have been reported in mainland Europe and the US. A non-motile strain has been implicated in recent Spanish outbreaks (Fouz *et al.*, 2006). Likewise, there are increasing reports of outbreaks among vaccinated UK Atlantic salmon.

Figure 2: A typical UK rainbow trout farm

Davies (1991) developed a detailed *Y. ruckeri* typing scheme based on O-serotype, biotype and outer membrane protein (OMP) profile. However, this scheme does not provide information on the genetic relatedness of strains.

Pulsed-field gel electrophoresis (PFGE)

A PFGE scheme was developed to supplement Davies (1991) typing scheme. PFGE is described as the 'gold standard' for differentiating between closely related strains, allowing short term evolution and spread of highly similar bacterial clones to be observed (Tenover *et al*, 1995). Endonuclease restriction patterns (pulsotypes; pt) are generated from total genomic DNA.

Multilocus Sequence Typing (MLST)

The genetic relatedness of globally distributed *Y. ruckeri* populations was investigated by MLST, a nucleotide sequencing-based approach that characterises slowly accumulating variation at a number of selectively neutral (housekeeping) genes. Shared alleles at each of these loci indicate the extent to which isolates within a species are related (Enright & Spratt, 1999).

Characterisation of strains

Pulsotypes were generated for 158 isolates collected between 1978 and 2007. These included: **Rainbow trout** 14 isolates from Davies (1991) typing scheme, 52 UK isolates including EX5 as an example of the emerging UK biogroup, 53 Danish isolates, 3 Spanish isolates, including a non-motile outbreak strain (Fouz *et al.*, 2006), 6 French isolates, 2 American isolates; **Atlantic salmon** 28 Scottish Atlantic salmon isolates. Isolates were restricted in *Not*I, and electrophoresed at 14°C for 18 hrs, 6.0 V/cm, with switch times between 1.0 s and 15.0 s.

The MLST scheme was developed using 14 fully characterised strains provided by R.L. Davies (Davies, 1991). A further 17 *Y. ruckeri* strains representing isolates from various host species, geographic sources and serotypes. 450-500 bp loci were sequenced for housekeeping genes *adk, aroA, glnA, gyrB, recA, thrA* and Y-HSP60. Sequences were assembled and aligned using Seqman software (DNAStar, US) and EMBL-EBI Kalign alignment software (http://www.ebi.ac.uk/kalign/). Unique alleles were numbered in order of discovery, generating a series of 7-digit allelic profiles. Sequence types were assigned based on the allelic profile of each isolate.

Results

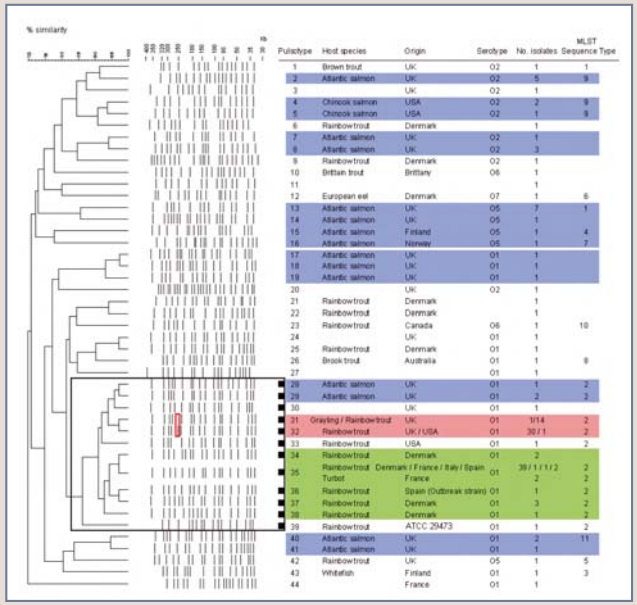

Figure 3: *Y. ruckeri Not*I PFGE scheme. UPMGA generated dendrogram showing pulsotypes clustered as percentage similarities was produced using BioNumerics software (Applied Maths, Belgium). Pulsotype, host species, origin, serotype, number of isolates and MLST sequence type is shown. Puslotypes 28-39 represent the major O1 pathogenic group (circled in black). Colours indicate significant clusters as follows: Atlantic salmon strains (blue); original UK O1: 2 and EX5 (pink); European rainbow trout strains and Spanish UK-like outbreak strain (green). The 250 Kb band difference between original UK O1: 2 and EX5 strains is circled in red.

Discussion

Typing of UK rainbow trout strains

- Davies (1991) scheme placed EX5 within the same clonal group as other UK rainbow trout strains (data not shown), but was shown to be a different, though closely related strain by PFGE.
- Historical analysis of farmed rainbow trout isolates in England and Wales identified just two pulsotypes between 1981 and 2006. Pulsotype 31 was predominant in the UK between 1981-1999, but since this time the EX5 pulsotype 32 has predominated amongst UK farmed rainbow trout.
- Pulsotypes 31 and 32 were identical with the exception that a single additional band of 250 Kb was observed in pulsotype 32 (EX5). Typically, an additional large band would be accompanied by the absence of two smaller fragments due to loss of a *Not*I restriction site. As this was not observed, it is possible that the fragment represents a horizontal gene acquisition.

Typing of European and US strains

- The non-motile Spanish outbreak strain (pt36) was more closely related to European *Y. ruckeri* strains (pt34-38) than UK strains (pt31-32). Loss of motility appears to be due to convergent evolution.
- Serotype O1 rainbow trout pathogenic strains from across the UK, mainland Europe and the USA were identical by sequence type, indicating a very recent common ancestor. The addition of more variable gene loci to the scheme may improve the resolution sufficiently to map the global spread of highly clonal O1 strains.
- The presence of an EX5-like strain in the US suggests that EX5 may have been introduced recently from the UK, or that exchange of strains between the US and UK is more common than previously thought.

Typing of Atlantic salmon strains

- PFGE and MLST data indicated that Atlantic salmon were infected by O1 UK rainbow trout strains in the early 1980s (pt28-29). However modern field isolates are more diverse and may not be related to the early O1 strains.
- Strains of serotypes O1, O2 and O5 were isolated from diseased fish, suggesting that serotypes other than O1 are pathogenic to UK Atlantic salmon.

Further work

- PFGE of representative isolates with a second restriction enzyme to improve robustness of scheme. Investigate significance of 250 Kb pt32 (EX5) band. Further characterization of European and US rainbow trout strains and Atlantic salmon strains.
- Improve MLST scheme resolution by addition of more rapidly evolving gene loci (Urwin & Maiden, 2003). Simplification of method with a nested PCR strategy.
- Vaccine trials to ascertain the efficacy of existing vaccines compared with vaccines based on modern field strains. Improve coverage of Atlantic salmon vaccination by identifying virulent O1, O2 and O5 strains associated with disease outbreaks.

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References

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