Red Mark Syndrome of rainbow trout (*Oncorhynchus mykiss* Walbaum)

Michelle Pond, Stephen Feist, Edmund Peeler, Birgit Oidtmann, Georgina Rimmer, Myriam Algoet, Jason Mewett, Keith Jeffery, Keith Way and David Verner-Jeffreys*
Centre for Environment, Fisheries & Aquaculture Science, Weymouth
www.cefas.co.uk

Figure 1: Rainbow trout infected with Red Mark Syndrome (RMS)

Introduction

Red Mark Syndrome (RMS), otherwise known as Coldwater Strawberry Disease (CWSD), is a disease of rainbow trout, characterised by the appearance of multiple ulcerated skin swellings, of varying intensity, on the flanks of affected fish (Ferguson *et al.* 2006; Verner – Jeffreys *et al.* 2006).

The condition was first noted in Scotland. By 2005, it was found on 12 sites in Scotland, all of which were sites receiving live fish from the hatchery where the disease first emerged (the index farm). In early 2005, the condition was diagnosed for the first time in fish farmed in England. Farmers in both Scotland and England report that the disease is prevalent at low temperatures (less than 15 °C). Early signs can include scale loss, prior to the emergence of the characteristic external lesions and there are no systemic signs of infection (i.e. no affect on appetite, growth or mortality). The condition causes severe economic losses to farmers both in treatment costs and in that affected fish are down-graded at harvest.

The results of investigations of affected farms in England and Wales and subsequent multidisciplinary studies to determine an aetiological agent are reported. As a recent study has implicated the rainbow trout bacterial pathogen *Flavobacterium psychrophilum* as potentially being linked to the condition (Ferguson *et al.* 2006), particular effort was made to identify whether this, or a closely related organism, was associated with diseased fish.

Outbreak investigations

Epidemiological outbreak investigations of farms in England and Wales, suffering from RMS, were carried out. Two of the farms investigated, Farm A and Farm B, were also used as a source of fish for transmission experiments. At each visit, 10 fish with RMS were examined and samples taken for diagnostic testing (bacteriology, mycology, virology, histopathology, parasitology, biochemistry). Farm records and fish movements were also discussed with the farmers.

- The initial spread of RMS was associated with movement of live fish from Scotland to sites in England (including Farm A).
- The condition subsequently spread to other production sites linked to the initially infected farms.
- Within a farm, spread from affected stocks to previously unaffected naïve fish was demonstrated.
- There was another outbreak of RMS in a farm in Wales (Farm B) in 2005 with likely spread to other farms supplied by Farm B.
- Prevalence was variable (between 5-70% of fish in a stock affected).
- Treatment of affected fish with antibiotics reportedly controls the development of the disease, which may indicate a bacterial aetiology.

Acknowledgments

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* david.verner-jeffreys@cefas.co.uk

Transmission experiments

Attempts were made to transmit the condition from RMS affected fish to naïve fish in laboratory aquaria to help determine whether it has an infectious aetiology.

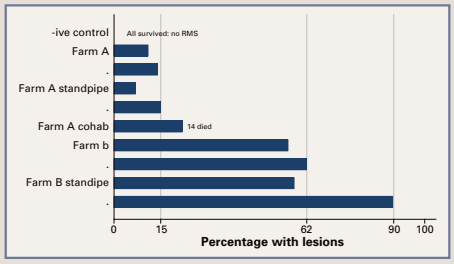

Figure 2: Results of cohabitation transmission trial. For most treatments, 15 RMS-affected fish were held on one side of a mesh screen with 40 naïve fish on the other. Treatments: Farm A or Farm B fish as the cohabitant source, with or without a surface-draining standpipe to allow accumulation of feed and faeces; Farm A cohab, a direct cohabitation challenge with naïve fish in direct contact with Farm A fish; negative control, 40 naïve stock fish only. Fish were held in 300 litre tanks at 10 °C in freshwater and examined for the presence of lesions after 110 days.

Laboratory investigations

Diagnostic bacteriology, virology and mycology of field samples and laboratory infected material

- No bacterial agents consistently isolated.
- No fungal agents isolated.
- Lesion samples from trial infected fish and cultured bacteria negative for *F. psychrophilum* by PCR (Figure 2).
- Lesion samples from 4/5 Farm A infected fish, used as infection source in trial, tested positive for *F. psychrophilum* (Figure 2).
- Lesion samples from 0/5 Farm B infected fish, used as infection source in trial, tested negative for *F. psychrophilum* (Figure 2).
- No virus cultured on any of the six cell lines used, under a range of incubation conditions.

Figure 3: RMS lesions on fish exposed to Farm B RMS affected fish at termination of infection trial.

Culture independent analysis of cohabitation trial lesion material for 16S rRNA genes of bacterial origin

- 198 partial 16S rRNA genes were cloned from lesion and skin (negative control) material and identified by a combination of Restriction Fragment Length Polymorphism (RFLP) and gene sequence analysis (as described by Pond *et al.* 2006).
- 83% of the resultant phylotypes were assigned to three main bacterial groups: *Acidovax*-like β-Proteobacteria (51%), *Methylobacter* and *Bradyrhizobium*-like α-Proteobacteria (15%) and *Porphyrobacter*-like α-Proteobacteria (17%).
- All the main phylotypes were also recovered from the negative control as well as lesion material.

Histopathology

- Epidermis generally unaffected, possibly some lymphocytic infiltration.
- Lifting and dissolution of scales with osteoclast involvement.
- Predominantly lymphocytic inflammation, focal to multifocal lesions, initially in the connective tissue of the dermis and extending into the underlying adipose and muscle tissue.

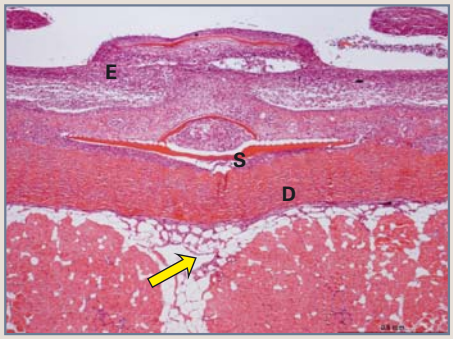

Figure 4: Typical transverse section through epidermis (E) and dermis (D) of a moderate RMS lesion. Note lymphocytic infiltration into the connective tissue of the dermis and extending into the underlying adipose and muscle tissue (arrow). S = scale.

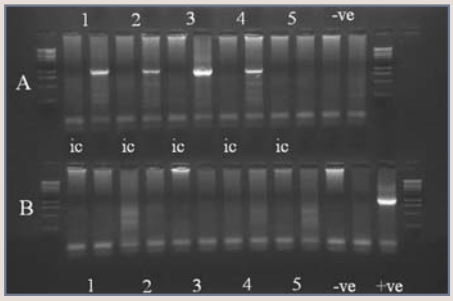

Figure 5: F. psychrophilum PCR of lesion material from 5 Farm A (row A) and 5 Farm B (row B) rainbow trout used in the cohabitation transmission trial. ic = internal control sample (unaffected area of skin and muscle); –ve = skin and muscle from a control population of unaffected rainbow trout; +ve = F. psychrophilum NCIMB 1947; 1 kbp ladder.

Conclusions

- RMS was apparently transmitted from affected to naïve fish in the field and in the laboratory, demonstrating it has an infectious aetiology.
- RMS has a long latency: greater then 50 days when fish are held at 10°C.
- Differences in the rate of transmission of condition between fish in contact with Farm A as opposed to Farm B were observed. It is not clear whether this was related to differences in virulence of the responsible agent or due to the interfering effects of observed concurrent infections in the Farm A-affected fish.
- Environmental factors (water quality) did not noticeably affect transmission rate in the laboratory.
- *F. psychrophilum* DNA was not consistently isolated from field or laboratory infected fish, lessening the likelihood that it, or a closely related organism is the sole responsible aetiological agent.

Further work required

- Risk to other farmed and wild fish needs to be established.
- Aetiological agent needs to be identified.
- Prevalence and spread of disease needs to be better understood.
- Suitable control methods need to be identified (as an alternative to continued application of antibiotics).
- Case definition needs to be established.