LIFE CYCLE AND TRANSMISSION REQUIREMENTS OF THE PKD ORGANISM AND OTHER MYXOSPOREAN PATHOGENS OF FRESHWATER FISH (MAFF PROJECT F1138)

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Introduction

Myxozoans are well known parasites of fish and although most are relatively innocuous, several species are serious pathogens of both wild and farmed fish. Until the pioneering work of Wolf and Markiw (1), the lifecycle was believed to be direct from fish to fish. Subsequent studies showed that oligochaetes were implicated in the lifecycle, cycling between actinospore stages in the oligochaete and myxospore stages in the fish. In a previous MAFF funded project "Investigations into the lifecycles of myxosporean parasites, including the agent for proliferative kidney disease" studies were undertaken into pathogenic parasites that impact on wild cyprinid fry in freshwater systems and on proliferative kidney disease of salmonids. The aim was to elucidate those factors which affected transmission of the parasite and to attempt identification of the alternate host for the agent for PKD.

Previous studies on approximately twenty thousand oligochaetes showed that relatively few were hosts for actinospores. The parasites were classified on the basis of morphological features and samples kept for electron microscopy and molecular biology. Polymerase chain reaction (PCR) was carried out on all released actinospores using either *Tetracapsula bryosalmonae* (PKX)-specific or myxozoan-general primers. All released actinospores encountered were negative for *T. bryosalmonae* by PCR. Use of myxozoan-general primers and subsequent sequencing of the resultant product confirmed that they were not related to the PKD organism. Collaborative work with the Universities of Reading and London (Imperial College) lead to the description of the PKD organism from bryozoans as *Tetracapsula bryosalmonae* (2-5). Subsequent experimental trials at CEFAS Weymouth confirmed the sequence information obtained from parasites in bryozoans. Transmission of the parasite from bryozoans to naïve rainbow trout resulted in the induction of PKD (6).

The new project aims to exploit this new breakthrough in the understanding of the source of infection of PKD to fish and examine further the detail of infection as well as continuing research into myxosporean parasites of wild fish.

Collaborative links

For any project to succeed, collaboration between workers is essential. Work to date has succeeded as a result of collaborative links with, amongst others the Universities of Reading, London (Imperial College) and Stirling, with the fish farming industry and with colleagues abroad. It is a key objective that this should continue and where possible be strengthened.

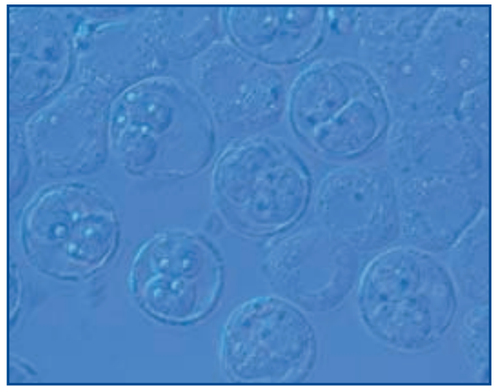

Figure 1: Several spores of Tetracapsula bryosalmonae, *the causative agent of proliferative kidney disease after release from* Fredericella sultana, *one of the bryozoans implicated in the lifecycle of PKD*

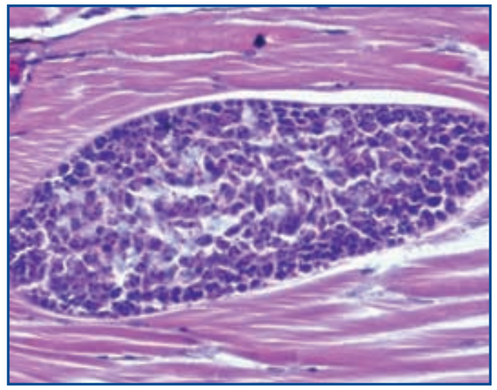

Figure 2: Histological section through a Myxobolus cyprini *cyst in the skeletal musculature of a juvenile chub (H&E)*

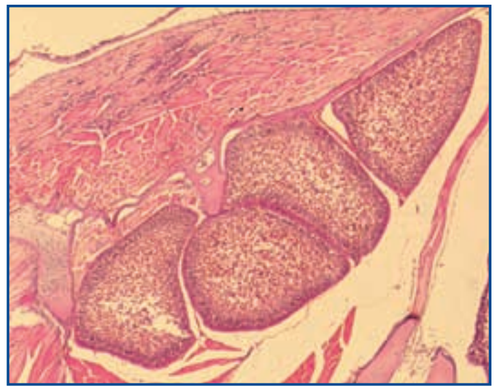

Figure 3: Low power view of several myxozoan cysts in the jaw muscle of a juvenile roach (H&E)

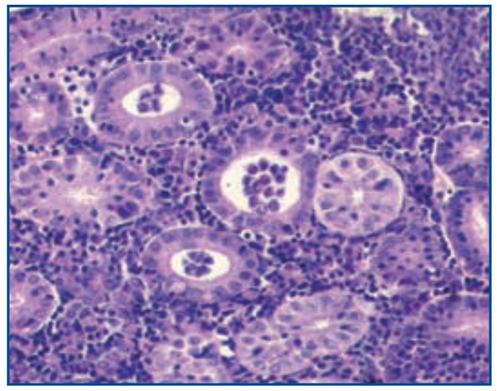

Figure 4: Sphaerospora *sp. in the kidney of dace. Note the dilation of the renal tubules due to the presence of the parasite (H&E)*

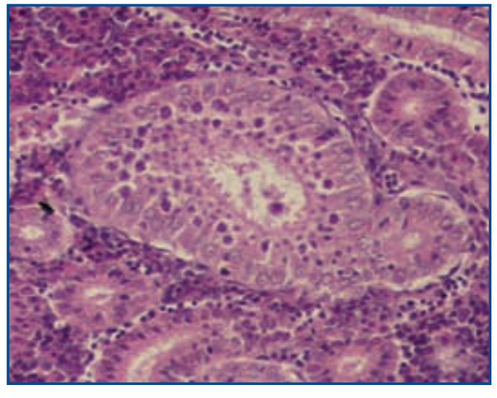

Figure 5: Early stages of a Hoferellus *sp. in the kidney of a juvenile dace (H&E)*

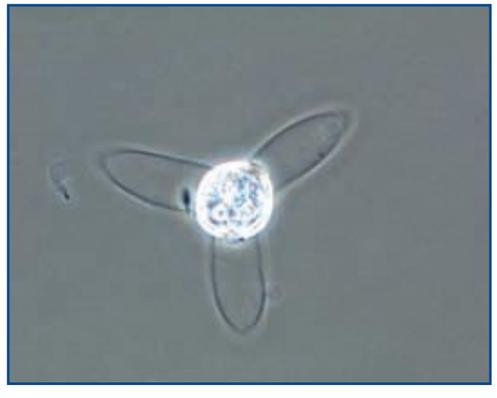

Figure 6: Aurantiactinomyxon*-type actinospore released from a tubificid worm. These stages are infective to fish*

Proposed work programme

Over the lifetime of the project the following areas of research are expected to be addressed. These match some of the key research priorities of the MAFF funded workshop held on November 15-17, 1999 at CEFAS Weymouth on "Proliferative Kidney Disease (PKD) in wild and farmed salmonids".

- Determine the types and geographical distribution of Myxozoan infections in freshwater bryozoans and to identify those species harbouring *Tetracapsula*
- Characterise *Tetracapsula* species and other myxozoan stages from bryozoans, oligochaetes and fish utilising molecular biology, light microscopy and electron microscopy
- Understand the behaviour of *Tetracapsula* spores in the water column prior to infection of fish or bryozoans and those factors which influence their infectivity
- To see if *Tetracapsula bryosalmonae* from different geographical areas are capable of inducing PKD and to assess the effect of bryozoan and fish host on severity of disease
- To survey oligochaetes for actinosporean infections in order to obtain long-term data on prevalence, intensity and seasonality of release
- To obtain DNA sequences of actinospores released from oligochaetes and myxospores from cyprinid fish and to compare the two in order to match stages
- Transmit selected actinospores from oligochaetes to naïve fish and vica versa
- Determine the early pathogenesis of Myxozoans in fish focusing on cellular interactions and triggers for defence in the host epithelium

References

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