USE OF IN-SITU HYBRIDISATION TO ELUCIDATE ROUTE OF ENTRY OF *Tetracapsula bryosalmonae*, THE CAUSATIVE AGENT FOR PROLIFERATIVE KIDNEY DISEASE, IN EXPERIMENTALLY CHALLENGED RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Tetracapsula bryosalmonae, the causative agent of Proliferative Kidney Disease, is an important degenerate metazoan parasite of both wild and farmed salmonids. Until 1999, the lifecycle of this parasite outside of the fish host was unknown. It was expected that, like other myxozoans, the parasite would utilise an alternate host such as oligochaete worms in order to complete its lifecycle. The use of Polymerase Chain Reaction (PCR) and DNA sequencing techniques demonstrated that the DNA of a myxozoan parasite of freshwater bryozoans was identical to that of extrasporogonic stages of "PKX" in rainbow trout kidneys (Anderson *et al*., 1999; Canning *et al*., 1999, 2000; Longshaw *et al*., 1999). Experimental transmission of this parasite and subsequent induction of PKD in naïve rainbow trout demonstrated that the parasite of bryozoans is infective to fish (Feist *et al*., 2001). Whether stages released from the fish host are infective to bryozoans has yet to be demonstrated. The aim of the current study was to improve understanding of the route of entry and subsequent migration of the parasite to the kidney.

Figure 1: A single zooid of the colonial *bryozoan Fredericella sultana*, one of the hosts for *Tetracapsula bryosalmonae*

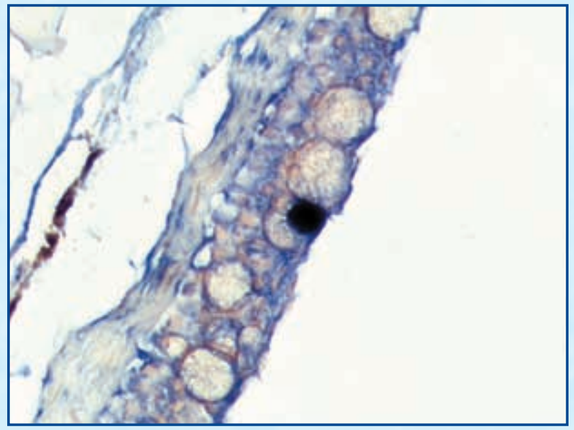

Figure 3: Tissue section through the skin of rainbow trout after exposure to bryozoans infected with *Tetracapsula bryosalmonae*. The infective stage is stained blue by the *in-situ* hybridisation technique

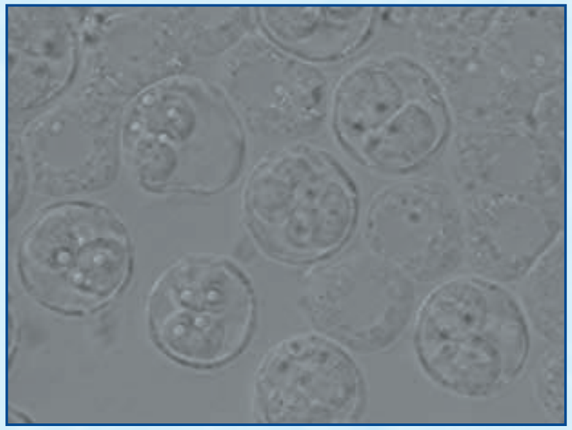

Figure 2: *Tetracapsula bryosalmonae* spores released from a sac within the bryozoan host. Note presence of four polar capsules on each spore

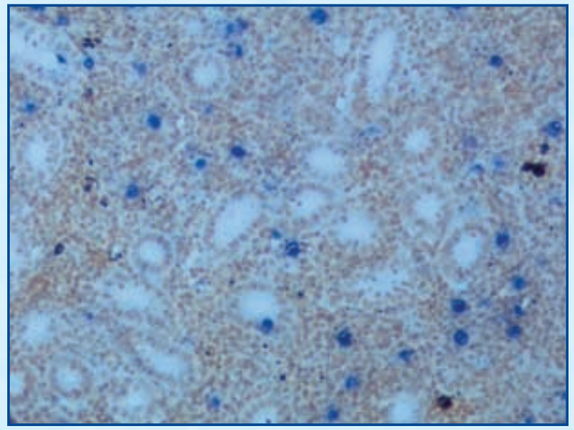

Figure 4: Extrasporogonic stages of *Tetracapsula bryosalmonae* stained by *in-situ* hybridisation within the kidney of rainbow trout eight weeks post exposure to infected bryozoans

Methods

Naïve rainbow trout were experimentally challenged with *Tetracapsula bryosalmonae* spores released from naturally infected zooids of *Fredericella sultana* collected from a river feeding a PKD-enzootic fish farm. Sub-samples of whole fish were taken for routine histology and *in-situ* hybridisation (ISH) following continuous exposure to *T. bryosalmonae* spores at 1, 2, 3, 5, 10, 30, and 90 minutes. Subsequently, samples for histology and ISH were taken 1, 2, 3, 4 days post-exposure and then every two weeks until clinical signs of PKD were observed.

In addition, rainbow trout were exposed for 10, 30 and 90 minutes to disrupted bryozoans and transferred to clean water. Subsamples were taken every two weeks until onset of the disease.

Results

Tetracapsula bryosalmonae was transmitted from the bryozoans to the fish and clinical PKD was induced. Results of the *in-situ* labelling of the parasite indicated that the portal of entry was through the epidermis of the skin. No organisms were detected entering via the gill. Within 24 hours, the parasite migrated into the dermis, but then could then not be detected by the use of ISH or routine histology until four weeks post exposure, when the presence of extrasporogonic stages of the parasite were detected in the kidney by ISH. By eight weeks post-exposure, clinical signs of the disease including splenomegaly and renal hypertrophy were observed in all groups.

Discussion

The present results confirm the studies of Feist *et al*., (2001) who demonstrated that bryozoans were involved in at least part of the lifecycle of *Tetracapsula bryosalmonae*. The entry into the fish via the skin contrasts with the results of Morris *et al*., (2000) who demonstrated that one of the portals of entry was via the gill and it is therefore likely that the parasite utilises a number of epithelial surfaces including gills and skin. The results of these authors were obtained from fish that had been exposed for three days in a river enzootic for PKD and not under experimental conditions. Twenty-four hours post-exposure, parasites were not visualised in the tissue sections examined. This may be the result of the relatively low numbers of parasites present during the early stages of infection. It is likely however that the parasite travels to the kidney and other organs via the blood system, undergoing limited divisions during its passage.

PKD was transmitted to fish exposed to infected bryozoans for 10 minutes suggesting that the infective stage is highly efficient at infecting fish. As previously shown by Feist *et al*., (2001), even a low dose of infective stages is capable of inducing clinical PKD. This suggests that the parasite is able to rapidly enter the host and is highly efficient in establishing a systemic infection. Although the longevity of the parasite outside the bryozoan host is currently unknown, future work in this area will target the development of the parasite following release from the bryozoan host and the early stages of host-parasite interaction.

References

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