PKX ORGANISM, A NEW SPECIES OF TETRACAPSULA DISCOVERED IN BRYOZOA

by B. Okamura[1], C.L. Anderson[2], M. Longshaw[3], A. Curry[4], S.W. Feist[3] & E.U. Canning[2]

Introduction

Proliferative kidney disease of salmonid fish is responsible for high mortality in fish farms and is prevalent in Europe and North America. The causative organism PKX (Seagrave *et al.*, 1980) was recognised as a myxozoan (Kent & Hedrick, 1985) but could not be named for lack of mature spores in the fish. Wolf & Markiw (1984) contributed significantly to a knowledge of myxozoan life cycles when they reported that actinosporean parasites in tubificid worms are stages in the life cycle of *Myxobolus cerebralis*. The involvement of actinosporeans and oligochaetes has since been demonstrated for several other myxozoans parasitic in fish but a search for PKX stages in invertebrates has until now been fruitless. We report on the presence of PKX in several species of Bryozoa.

Methods

In 1998 bryozoans were collected in Ohio and Michigan states of the USA from lakes where salmonid fish were absent or rare. Myxozoan sacs similar to those of *Tetracapsula bryozoides* (Canning *et al.*, 1996) were found in specimens of *Pectinatella magnifica*, *Plumatella rugosa* and *Cristatella mucedo*. DNA from the infected bryozoans and from PKX derived from rainbow trout was extracted, and fragments of the 18S rDNA were amplified by PCR, using general ribosomal DNA primers and/or primers specific for PKX (Saulnier & de Kinkelin, 1997). The amplified products were sequenced and aligned with a published sequence for PKX (Anderson *et al.*, 1999a, b). Similar sacs were collected in 1999 from *Plumatella emarginata* and *Fredericella sultana* upstream of rainbow trout farms in the United Kingdom. 18S rDNA from these sacs was also sequenced and some sacs were fixed in 2.5% glutaraldehyde in 0.1% cacodylate buffer and were processed for electron microspcopy.

Conclusions

1. The presence of PKX (*Tetracapsula* sp.), revealed by 18S rDNA sequences and ultrastructure in bryozoans upstream of fish farms with PKD strongly suggests that bryozoans are the source of infection for fish.
2. The presence of PKX (*Tetracapsula* sp.) in bryozoans in North American lakes where there were no salmonid fish suggests that fish are accidental hosts and that the parasite may normally cycle between bryozoans.
3. At this stage we cannot eliminate the possibility that other invertebrates or non-salmonid fish can harbour PKX (*Tetracapsula* sp.) but these have been investigated extensively and a single host system seems more likely.
4. The susceptibility, to PKX (*Tetracapsula* sp.), of a broad range of bryozoans that inhabit ecologically diverse sites would account for the common occurrence of PKD.

Results

18S rDNA sequences

Sequences from myxozoan 18S rDNA were aligned and compared with a published sequence of PKX (Accession No. U70623; Saulnier & de Kinkelin, 1997). Sequences from North American material showed some variation (Figure 1) but three of them (PECT NA1, NA2 and CRIST NA2) fell within the range of variation obtained for PKX derived from rainbow trout and are identified as PKX. Others, outside this range, may be PKX or a closely related species.

Four isolates of myxozoans from the British material showed 98.5% homology with PKX (U70623) and 100% homology with each other (Longshaw *et al.*, 1999) and are also identified as PKX.

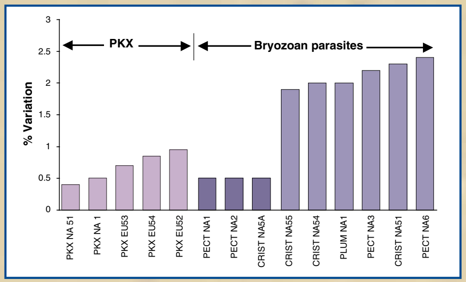

Figure 1 The percentage difference in the sequences of various myxozoan isolates (on x-axis) in comparison to PKX U70623, plotted as % Variation. Identities of sequence codes (accession numbers) (number of bp) are: North American PKX isolates = PKX NA1, 51 (AJ133408, AJ133409) (581, 570); European PKX isolates = PKX EU52-54 (AJ133560, AJ133561, AJ133562) (593, 526, 531); undescribed myxozoan from Pectinatella magnifica *= PECT NA1-3, 6 (AJ133416, AJ133416, AJ133414, AJ133415) (412, 412, 411, 580); undescribed myxozoan from* Cristatella mucedo *= CRIST NA5A, 51,54,55 (AJ133416, AJ133410, AJ133411, AJ133412) (412, 578, 580, 567); undescribed myxozoan from* Plumatella rugosa *= PLUM NA1 (AJ133418) (577).*

Ultrastructure of PKX from *Plumatella* and *Fredericella*

PKX sacs extruded from *P. emarginata* and *F. sultana* were spherical, measured about 300 µm diameter and contained developmental stages or mature spores (Figure 2). Mature spores showed 4 polar capsules and 2 sporoplasms (Figure 3) and were identified as a new species of *Tetracapsula*.

Electron microscopy showed that the walls of the sacs were composed of a single layer of cells (Figure 4) within which were packed irregular, uninucleate cells (Figure 4) or spores with 4 valve cells, 4 capsulogenic cells (Figure 5) and two sporoplasms, with secondary cells enclosed (Figure 6), confirming that PKX is a species of *Tetracapsula*.

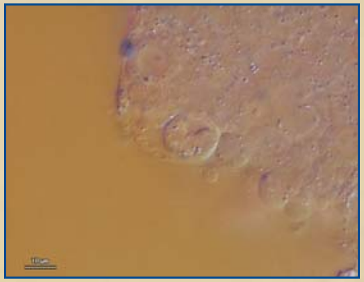

*Figure 2 Slightly disrupted sac of PKX (*Tetracapsula sp.*) recovered from* Fredericella sultana *zooids: the spherical stages within the sac are developing spores. Nomarski interference. Scale bar = 10 µm.*

*Figure 3 Two mature spores of PKX (*Tetracapsula sp.*) expressed from a sac recovered from* Plumatella emarginata*. One shows two sporoplasms within the wall of valve cells, the other shows the four polar capsules in surface view. Scale bar = 10 µm.*

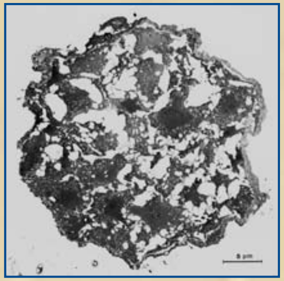

*Figure 4 A sac of PKX (*Tetracapsula sp.*) dissected from* P. emarginata *showing a mass of irregular developmental stages within a wall of flattened cells. Scale bar = 5 µm.*

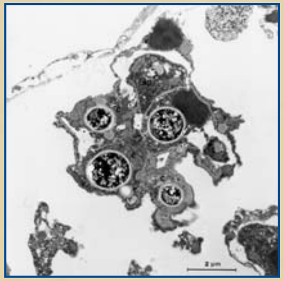

*Figure 5 Surface view of a spore of PKX (*Tetracapsula sp.*) within a sac dissected from* P. emarginata*. Four polar capsules are visible in capsulogenic cells (c) within the valve cells (v). Part of a sporoplasm (sp) is also visible. Scale bar = 2 µm.*

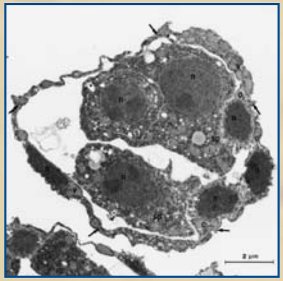

*Figure 6 Spore of PKX (*Tetracapsula sp.*) sectioned through the sporoplasms (sp) one of which shows a secondary cell (s). Valvogenic cells and their junctions (arrows) surround capsulogenic cells (c). n = nuclei. Scale bar = 2 µm.*

References

Anderson, C.L., Canning, E.U. & Okamura, B. (1999a). 18S rDNA sequences indicate that PKX organism parasitizes Bryozoa. *Bull. Eur. Ass. Fish Pathol.* **19**: 94-97.

Anderson, C.L., Canning, E.U. & Okamura, B. (1999b). Molecular data implicate bryozoans as hosts for PKX (Phylum, Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology* (in press)

Canning, E.U., Okamura, B. & Curry, A. (1996). Development of a myxozoan parasite *Tetracapsula bryozoides* gen.n. et sp.n. in *Cristatella mucedo* (Bryozoa: Phylactolaemata). *Folia Parasitol.* **43**: 249-261.

Kent, M.L. & Hedrick, R.P. (1985). PKX, the causative agent of proliferative kidney disease (PKD) in Pacific salmonid fishes and its affinities with the Myxozoa. *J. Protozool.* **32**: 254-260.

Longshaw, M., Feist, S.W., Canning, E.U. & Okamura, B. (1999). First identification of PKX in bryozoans from the United Kingdom - molecular evidence. *Bull. Eur. Ass. Fish Pathol.* **19**: 146-148.

Saulnier, D. & de Kinkelin, P. (1997). Polymerase chain reaction primers for investigations on the causative agent of proliferative kidney disease of salmonids. *J. Fish Dis.* **20**: 467-470.

Seagrave, C.P., Bucke, D. & Alderman, D.J. (1980). Ultrastructure of a haplosporean-like organism: the causative agent of proliferative kidney disease in rainbow trout. *J. Fish Biol.* **16**: 453-459.

Wolf, K. & Markiw, M. (1984). Biological contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. *Science* **225**: 1449-1452.

[1] Department of Animal and Microbial Sciences, University of Reading, Reading RG6 6AJ, UK
[2] Department of Biology, Imperial College of Science, Technology & Medicine, London SW7 2AZ, UK
[3] The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK http://www.cefas.co.uk
[4] Public Health Laboratory, Withington Hospital, Manchester M20 2LR, UK