

Proliferative kidney disease in brown trout *Salmo trutta*: further evidence of a myxosporean aetiology

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ABSTRACT: Two groups of brown trout *Salmo trutta* L. were studied, one after natural exposure to PKD-infected waters, the other after laboratory challenge by intraperitoneal inoculation with PKD-infected kidney tissue from rainbow trout *Salmo gairdneri* Richardson. Subclinical PKD developed in both groups; renal intraluminal myxosporean organisms were observed in both groups. The implications of these findings are discussed in relation to the aetiology of PKD.

INTRODUCTION

Prior to 1985 the aetiology of proliferative kidney disease (PKD) (reviewed by Clifton-Hadley et al. 1984a) had variously been described as nutritional (Besse 1956), viral (Schäperclaus 1954) and protozoan (Plehn 1924, Ghittino et al. 1977, Seagrave et al. 1980). The protozoa implicated included members of the phyla Sarcocystidophora (Plehn 1924, Ghittino et al. 1977) and Ascetosporea (Seagrave et al. 1980), while the possibility of a myxozoan causation was not excluded (Ferguson & Adair 1977). In 1985 intratubular myxosporean organisms (phylum Myxozoa) were described from the kidneys of 3 Pacific salmonids in association with clinical PKD (Kent & Hedrick 1985a). Transmission experiments and further light and electron microscopical studies indicated that these intratubular protozoa were probably later developmental stages of the interstitial PKX cell (Kent & Hedrick 1985b, 1986), with similarities to members of the genera *Sphaerospora* and *Parvicapsula*, both members of the phylum Myxozoa. However, a precise taxonomy was not possible as no mature spores, the basis of current classification, were seen.

In the UK, despite detailed histopathological studies of PKD under field and laboratory conditions (Clifton-Hadley et al. 1984a, 1987, Clifton-Hadley et al. 1984b,

Clifton-Hadley 1986), no intraluminal, myxosporean protozoa have been seen in PKD-infected rainbow trout *Salmo gairdneri* Richardson. However, intraluminal trophozoites similar to those described by Kent & Hedrick (1985a) have been noted in Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and grayling *Thymallus thymallus* L. exposed to PKD-infected waters (Bucke et al. in press). In the present paper results are reported of investigations into the possible association of the presporogonic, interstitial PKX cells in rainbow trout and these intraluminal, myxosporean trophozoites in brown trout.

MATERIALS AND METHODS

Two groups of fish were used: (1) fingerling rainbow trout (average weight 3 g, length 70 mm) from a spring-fed hatchery, and (2) fingerling brown trout (average weight 3 g, length 62 mm) from a borehole-fed hatchery, both sites having no previous history of PKD.

Natural challenge with PKD was arranged at a farm site on the River Avon in Wiltshire (S England) where PKD occurs annually. Fish were held in circular concrete tanks supplied directly with river water at ambient temperature. Laboratory challenge was by injection of PKD-infected kidney tissue. In this case fish were held in rectangular, glassfibre tanks supplied with a continuous flow of aerated dechlorinated tap-

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Table 1. *Salmo trutta* and *S. gairdneri*. Numbers of fish at each sampling with PKX infection and/or evidence of renal intraluminal protozoa in Expts 1 and 2

Expt	Fish species	Sample date (wk after challenge)	No. of fish in sample	Interstitial PKX cells	Intraluminal trophozoites	Number of fish with:	
						Developing spores	Both PKX cells and intraluminal trophozoites
1. Field challenge	Brown trout	3	5	0	0	0	0
		5	5	5	0	0	0
		7	5	1	0	0	0
		9	10	6	2	0	2
		11	5	0	0	0	0
		14	5	0	2	1	0
		18	5	1	3	1	0
2. Laboratory challenge	Rainbow trout	13	5	3	0	0	0
		20	4	1	0	0	0
	Brown trout	10	10	1	8	6	1
		13	3	1	2	2	1
		17	5	0	4	3	0

water, maintained throughout at a temperature of $16 \pm 1^\circ\text{C}$. At both sites fish were fed a proprietary pelleted diet at a maintenance level.

Development of PKD infection was followed by histopathological examination of tissue specimens processed to $5\ \mu\text{m}$ paraffin wax sections, and stained either with haematoxylin and eosin, or by Giemsa's method for parasites (Bancroft & Stevens 1977). Kidney samples were also examined fresh, for the presence of intratubular stages, using interference contrast microscopy.

Experimental procedures. Control fish. Prior to the start of experiments, 10 brown trout and 10 rainbow trout were examined for histological evidence of PKD and renal intratubular protozoa.

Expt 1: In the first week of July 1986, 60 brown trout were introduced to the farm site for exposure to PKD infection. These fish were sampled in batches of 5 or 10 fish 3, 5, 7, 9, 11, 14 and 18 wk after introduction, and examined for evidence of infection. Tissues studied for all fish included the anterior intestine plus caecum, gills, kidney, liver, pancreas and spleen.

Expt 2: In the second week of July 1986, 20 brown trout and 10 rainbow trout were anaesthetized in a 0.01% solution of MS222* (Sandoz, Basle, Switzerland) and injected intraperitoneally with 0.2 ml aliquots of a coarse suspension of PKD-infected rainbow trout

kidney tissue (2.2 g) in phosphate-buffered saline (7.5 ml) as previously described (Clifton-Hadley et al. 1984b, Clifton-Hadley 1986). The infected kidney tissue, derived from 3 rainbow trout with subclinical PKD, was screened for histological evidence of PKD by examining 5 sections from each mesonephros. Brown trout were sampled 10, 13 and 17 wk after injection with this material and rainbow trout 13 and 20 wk after injection.

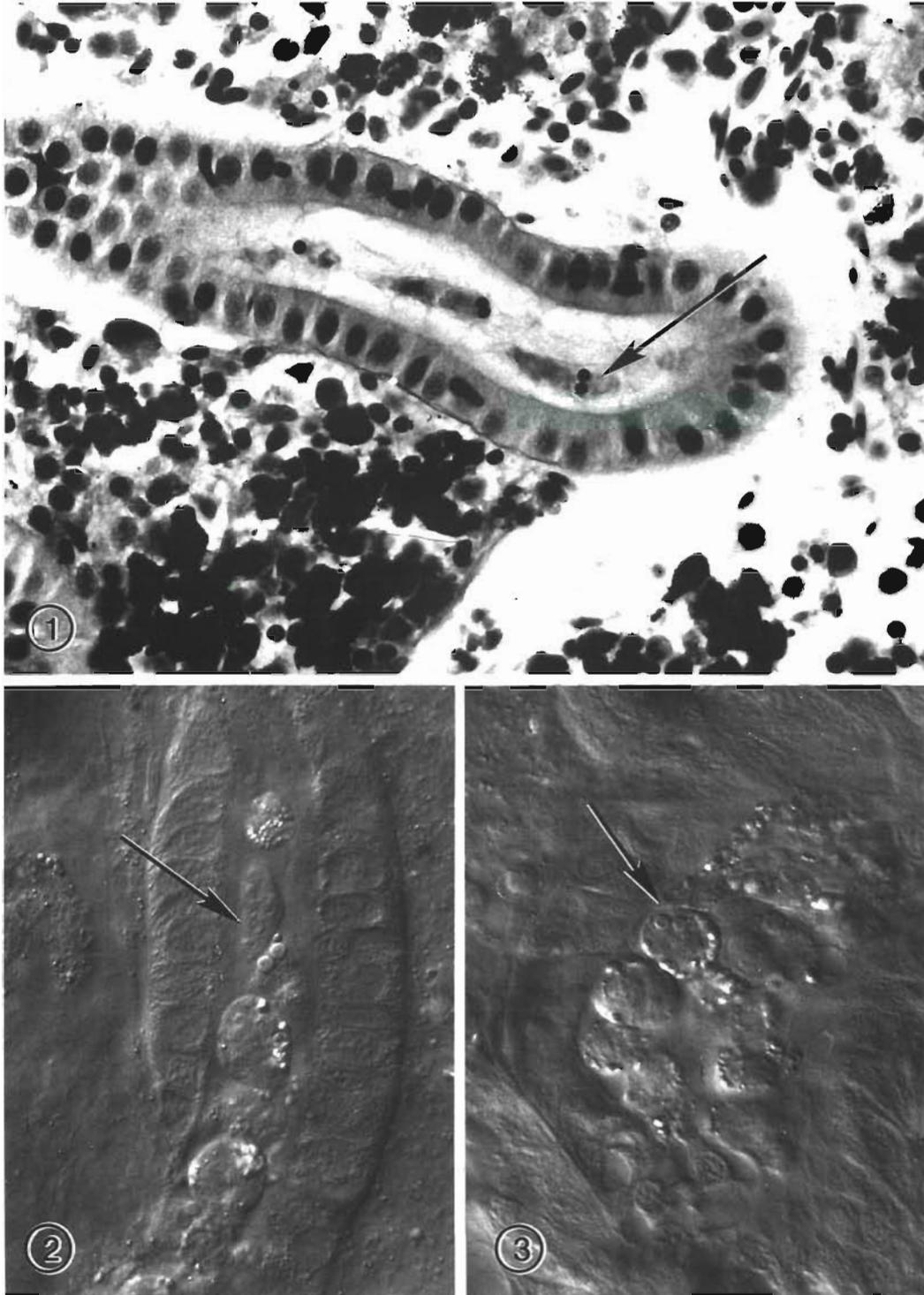
RESULTS

No evidence of systemic protozoan infection was detected in control fish sampled prior to challenge.

Expt 1

PKX cells were detected in brown trout kidney and spleen samples collected 5 to 18 wk after introduction to the farm site (Table 1). Histopathological changes associated with infection included haemopoietic hyperplasia, chronic diffuse inflammation with evidence in the kidney of limited nephron destruction, and occasional cell aggregation within blood vessels. These changes were sufficient in 5 fish (sampled at 7 to 9 wk) to cause macroscopic renal swelling. No other macroscopic pathology or clinical signs were seen. Intraluminal cells, similar to myxosporean trophozoites, were found in kidney specimens collected from 9 to 18 wk. Developing spores were detected in the 14 and 18 wk samples (Table 1); (Fig. 1).

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Figs. 1 to 3. *Salmo trutta* Fig. 1. Intratubular sporoblasts from brown trout kidney. Note elongate shape of parasite and small spherical polar capsules (arrow). Giemsa stained paraffin wax section ($\times 620$). Figs. 2 and 3. Infected brown trout renal tubules. Note the variability in sporoblast morphology, ranging from elongated forms (Fig. 2) to spherical forms (Fig. 3) (arrows). Polar capsules consistently appear in close proximity with each other. Fresh preparation, Normarski interference contrast ($\times 980$)

Expt 2

Histological examination of kidney tissue for inoculation showed PKX cells throughout the interstitial tissue but no evidence of intraluminal parasites. After inoculation with PKX-infected tissue, 4 of 9 rainbow trout and 2 of 18 brown trout were detectably infected with PKX cells at sampling. No intraluminal protozoa were seen in the rainbow trout. Intraluminal forms resembling myxosporean trophozoites and sporoblasts were found in the majority of brown trout (Figs. 2 and 3). Two brown trout kidneys contained both PKX cells and intraluminal developing spores. These results are summarized in Table 1.

DISCUSSION

In Expt 1, 13 of 40 brown trout became detectably infected with PKX cells. Clinical PKD did not occur, perhaps due to water temperatures lower than expected in the River Avon through the summer (mean maximum in July 17.2 °C, in August 14.8 °C and in September 11.8 °C) and the July introduction of the fish. Histopathological changes associated with the infection in brown trout were similar to, but not as extreme as, those previously described in rainbow trout (Clifton-Hadley 1986, Kent & Hedrick 1986) and in brown trout (Ellis et al. 1985). In particular, the vascular changes were less severe. The intraluminal trophozoites and developing spores were similar, under light microscopy, to those described by Kent & Hedrick (1985a, 1986), but there were few present.

In Expt 2, inoculation of rainbow trout tissue containing PKX cells but apparently no intraluminal organisms resulted in subclinical PKD in both recipient rainbow trout and brown trout. However, as with previous field and laboratory studies in the UK, no intraluminal protozoa were seen in rainbow trout in association with the infection. This contrasts with findings in salmonids, including rainbow trout, in the USA where intraluminal forms, considered to be further developmental stages of the PKX cell, were regularly found during PKD (Kent & Hedrick 1985a, 1986). The reason for this difference is unclear. However, in brown trout, which showed no evidence of intraluminal parasites prior to injection with rainbow trout kidney material, both intrarenal, interstitial PKX cells and intraluminal trophozoites and developing spores of a myxosporean nature were found. This would indicate that the PKX cell develops further in brown trout than in rainbow trout in the UK and is a vegetative stage in the life cycle of a myxosporean protozoan with similarities to some members of the *Parvicapsula* and *Sphaerospora* genera. These similarities include the site of infection, small spherical

polar capsules and indistinct valves. However, as can be seen from Figs. 1 to 3, the intratubular stages show pleomorphism, especially with regard to the overall shape of the maturing spore. This may represent natural variation or an artefactual change dependent on the method of examination employed. Stages of a *Sphaerospora* sp., including mature spores, have been described in brown trout from areas of West Germany where PKD is endemic (Fischer-Scherl et al. 1986), and perhaps this link deserves further investigation. However, in the present study, no mature spores were found in brown trout in either experiment, and this still precludes a precise taxonomic classification. It also suggests that brown trout, as with other salmonids so far studied, is not the definitive host for PKX. Recent studies into the occurrence of PKD in wild fish stocks have not revealed the true host of the PKX parasite, although the recorded host range has been widened (Bucke et al. in press). Further research into this aspect of PKD is in progress, with emphasis on the identification of the source of infection to cultured salmonids.

Acknowledgement. This study was carried out as part of a research project financed by the Chief Scientist's Special Research Fund, Ministry of Agriculture, Fisheries and Food, London, England.

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Responsible Subject Editor: Professor W. Körting; accepted for printing on January 30, 1989