

Granulomatous myositis in rainbow trout *Oncorhynchus mykiss* affected by proliferative kidney disease (PKD)

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ABSTRACT: A hatchery for brown (*Salmo trutta*) and rainbow (*Oncorhynchus mykiss*) trout has been repeatedly affected by proliferative kidney disease in summer. Rainbow trout was the only species affected, showing lesions principally in the kidney and in the red muscle of both lateral lines. Grossly, fish had protuberances in the kidney and on the skin surface corresponding to granulomatous inflammation of the kidney and of the red muscle, respectively. PKX cells were observed in granulomatous tissues of both muscle and kidney and were composed of macrophages, lymphocytes and plasma cells. Using lectin GS-I, extrasporogonic stages of myxosporean PKX cells were recognized in the kidney and muscle.

KEY WORDS: PKD · Rainbow trout · *Oncorhynchus mykiss* · Granulomatous myositis

INTRODUCTION

Proliferative kidney disease (PKD) is a disease of cultivated salmonids in Europe (Roberts & Shepherd 1974) and North America (Smith et al. 1984) which has an important economic impact. This disease is caused by an unclassified myxosporean (Kent & Hedrick 1986) that affects principally subyearling salmonids during the summer (Ferguson & Needham 1978) when the water temperature increases (Ferguson 1981, Clifton-Hadley et al. 1986, Foott & Hedrick 1987).

Extrasporogonic stages (PKX cells) of this myxosporean are initially found in the blood vessels and kidney interstitium. The increasing number of PKX provokes the appearance in the kidney of nodules of chronic inflammatory reaction. As the disease progresses, PKX cells are transported by the circulatory system and may reach other organs and tissues including spleen, liver, islets of Langerhans, gills, striated muscle, intestinal submucosa, heart, brain, spinal canal

and peritoneum. In heavily infected fish, parasites can be present in many of the mentioned tissues. The presence of PKX cells in these secondary tissues provokes a similar granulomatous response but to a lesser extent than it does in the kidney (Ferguson & Adair 1977, Ferguson & Needham 1978, Smith et al. 1984, Clifton-Hadley et al. 1985, Kent & Hedrick 1986, Clifton-Hadley et al. 1987, MacConnell et al. 1988).

Occasionally the reactive tissue, chiefly in the kidney and spleen, develops macroscopic white nodules of chronic inflammation measuring up to 10 mm in diameter and numbering up to 20 in one fish (Clifton-Hadley et al. 1984, Clifton-Hadley et al. 1985). These macroscopic nodules have been described (Ghittino et al. 1977) as white or reddish nodules in the kidney and also in other tissues such as liver, peritoneum and muscle.

In the late autumn of 1992, following an acute summer stage of PKD in a salmonid farm in the NE of Spain, the appearance of macroscopic nodules on the lateral lines of the fish was the main sign of the disease found in recovering fish. This report describes our macroscopic and microscopic observations of those

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inflammatory nodules caused by the long-standing presence of PKX cells in the striated muscle of fish affected by PKD.

MATERIALS AND METHODS

The affected hatchery produces 1 batch of brown trout *Salmo trutta* and 2 batches of rainbow trout *Oncorhynchus mykiss* per year; the first hatched around the first week of December and the second hatched around the second half of March. Brown trout is hatched around the first half of February.

Fifteen fish per batch were randomly netted in late May, mid-July, early October and late January. Sampled fish were examined for the presence of macroscopical lesions, mainly nodules on the skin surface and in the kidney. Samples of the kidney and striated muscle at the level of the dorsal fin, gill, spleen and liver were taken. Tissue samples were fixed at 10% buffered formalin and then embedded in paraffin. Routine sections of 4 μm were obtained and stained with haematoxylin and eosin (H&E) or periodic acid-Schiff aniline-orange (PAS-AO) for histopathological examination. The biotinylated lectin GS-I (*Griffonia simplicifolia* agglutinin-I) was applied using the avidin-biotin peroxidase (AB-P) technique (Castagnaro et al. 1991, Marín de Mateo et al. 1993). Histological sections of muscle and kidneys were examined for the presence of PKX cells, scanning the whole preparation.

RESULTS

Abdominal distension and anaemia of rainbow trout were the most obvious clinical signs during July and August, and the appearance of a roughened skin surface along the lateral lines was characteristic after an outbreak. Subyearling brown trout showed infected kidneys but neither macroscopical nor microscopical lesions were seen in the striated muscle and the parasite was never found in that tissue. However, gross and microscopical lesions, principally in the kidney and striated muscle, were observed in subyearlings of both batches of rainbow trout. Macroscopical nodules were observed in the red muscle of nearly 80% of the surviving subyearling fish, although they were hardly found at the time of the outbreak.

In May, parasites were first detected in the kidney of both species of trout. Prevalence and intensity of parasites increased through the summer, reaching maximum values in mid-July. PKX cells were no longer found in January. In the striated muscle, first appearance of parasites and maximum values of prevalence and intensity (Table 1) were detected 2 mo later than in kidney.

Small numbers of PKX cells in the late summer cause disorganised tissue foci in the red muscle. In early autumn, the number of parasites increased, and organised nodules of inflammatory reaction were observed. These nodules occasionally reached a large size (Fig. 1), and were easily detected in 80% of the affected fish



Fig. 1. *Oncorhynchus mykiss*. Large nodules in a rainbow trout affected by PKD

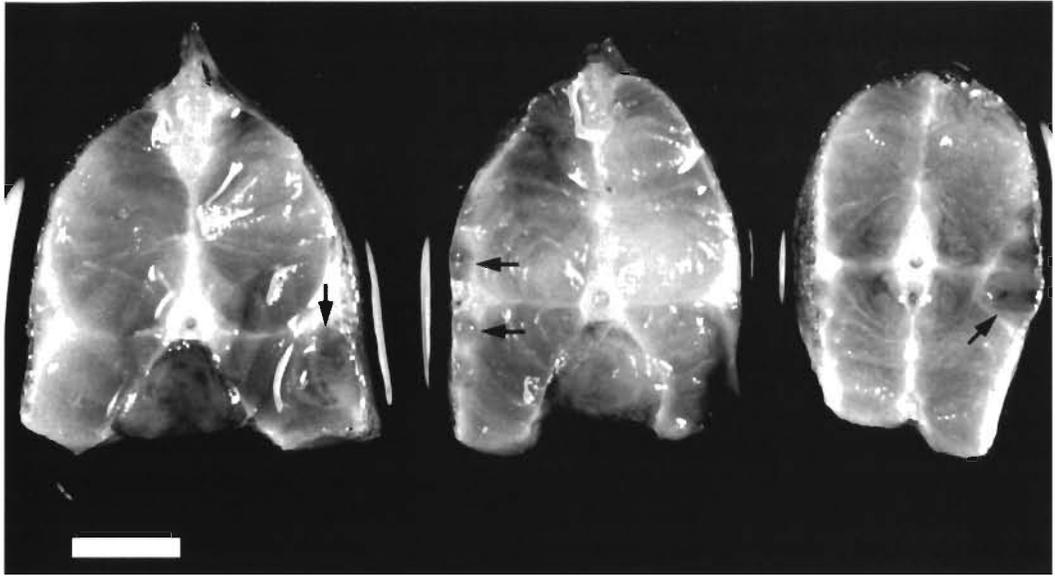


Fig. 2. *Oncorhynchus mykiss*. Nodules in the lateral line of rainbow trout with PKD lesions. Scale bar = 0.5 cm

when sectioned (Fig. 2). In mid-autumn, the inflammatory nodules could reach 7 to 8 mm in diameter, causing protuberances on the skin surface. These protuberances were responsible for the outward appearance of roughened skin surface along the whole length of both lateral lines, observed in approximately 20% of sub-yearling rainbow trout.

The nodules observed in the striated muscle were red or white, corresponding to a granulomatous in-

flammation. These nodules persisted until January. The striated muscle affected was the red muscle of one or both lateral lines (Figs. 3 & 4). The dorsal line was also infiltrated in a few cases. The white muscle was not affected, but in some cases, areas close to the red muscle were infiltrated by expansion. The inflammatory cells involved in this lesion were mainly macrophages, lymphocytes and plasma cells. A high density of PKX cells surrounded by macrophages were seen in

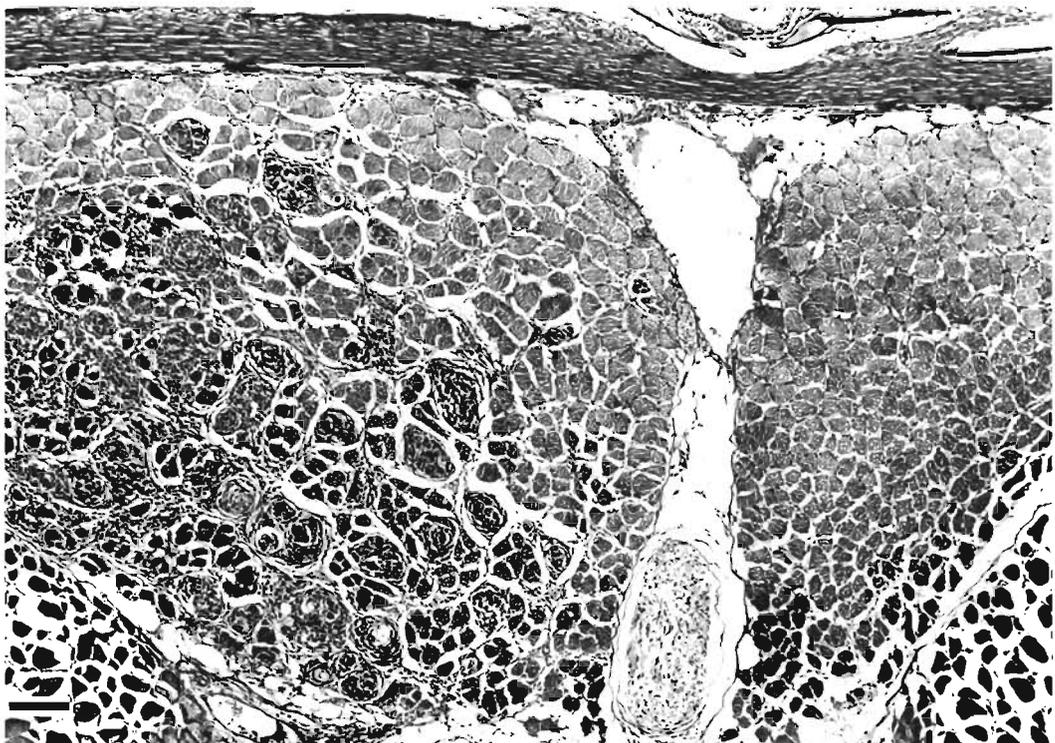


Fig. 3. *Oncorhynchus mykiss*. Granulomatous inflammation of red muscle in lateral line of a rainbow trout with PKD. H&E. Scale bar = 100 μ m

Table 1. *Oncorhynchus mykiss*. Prevalence and intensity of myxosporean parasites in the red muscle of rainbow trout

	Trout hatched in:		March			December			
	Survey in:	May	Jul	Oct	Jan	May	Jul	Oct	Jan
% of PKX-positive trout (in muscle) ^a	0	26.66	73.33	0	0	0	20	80	0
Mean no. of PKX cells per positive trout	0	1.25	710	0	0	0	1.33	741	0
Min. and max. no. of PKX cells	0	(1–2)	(76–2659)	0	0	0	(1–2)	(196–2247)	0
% of positive trout bilaterally infected	0	25	73	0	0	0	33	83	0

^an = 15

the inflammatory tissue (Fig. 5). Muscle fibers of the red and white muscle, nerves, vessels and skin suffered atrophy through compression by the inflammatory tissue. The kidney also showed the presence of PKX cells and an inflammatory infiltration mainly by macrophages, lymphocytes and plasma cells. Extra-sporogonic stages of PKX cells in the kidney and muscle were recognized by lectin GS-I (Fig. 5). PKX cells were also seen in the gills and spleen but not in the liver.

DISCUSSION

The diagnosed outbreak of PKD in rainbow trout is in accordance with other cases previously described from other countries (Ferguson & Adair 1977, Ghittino et al. 1977, Smith et al. 1984). Generally the season of appearance of the disease, the percentage of affected

fish, and the lesions found in the kidney and other secondary organs are similar. However, the difference to other outbreaks is the presence of large nodules in the red muscle of the lateral lines when the fish begin to recover. Muscle is the most affected secondary tissue, showing macroscopic inflammatory nodules. These kind of nodules are occasionally found in kidney, spleen, liver, muscles and peritoneum, but in this study these nodules were observed in the red muscles of nearly 80% of the surviving fish.

PKX cells are spread via the circulatory system and besides the kidneys they have been found frequently in other organs and tissues in heavily infected fish (Ferguson & Adair 1977, Ghittino et al. 1977, Ferguson & Needham 1978, Clifton-Hadley et al. 1984, Smith et al. 1984, Clifton-Hadley et al. 1985, Kent & Hedrick 1985, 1986, Clifton-Hadley et al. 1987). Although any irrigated tissue may be parasitized, major pathological changes are related to the kidneys and to a lesser extent to liver

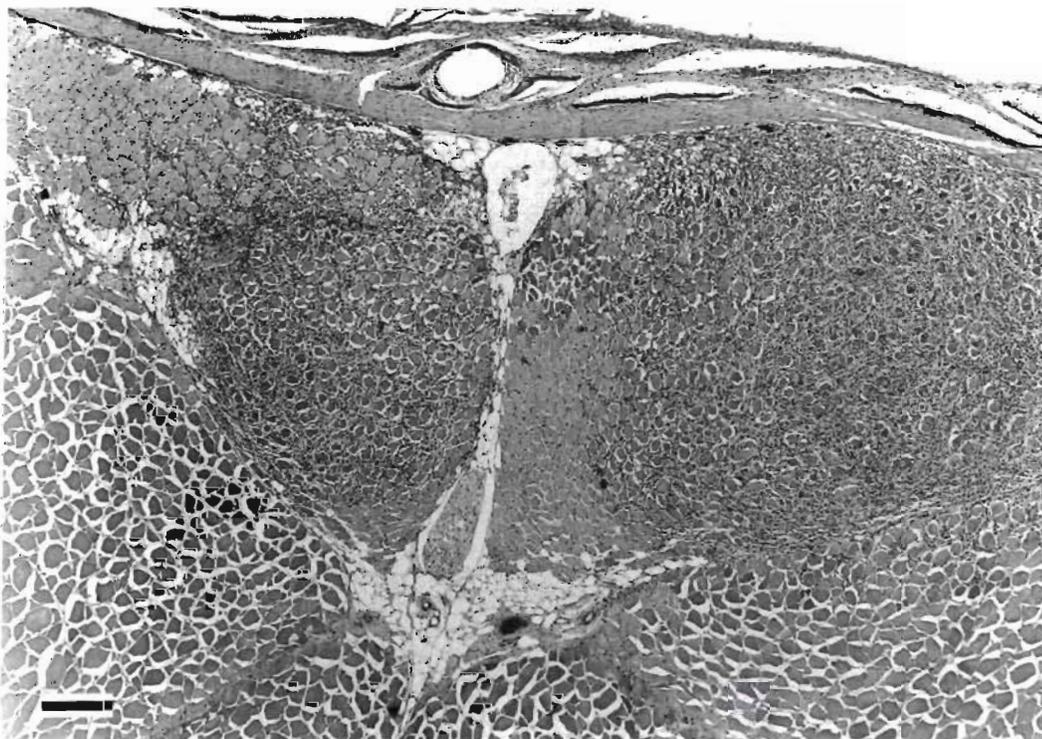


Fig. 4. *Oncorhynchus mykiss*. Heavy granulomatous inflammation of red muscle in lateral line of a rainbow trout with PKD. H&E. Scale bar = 200 μ m

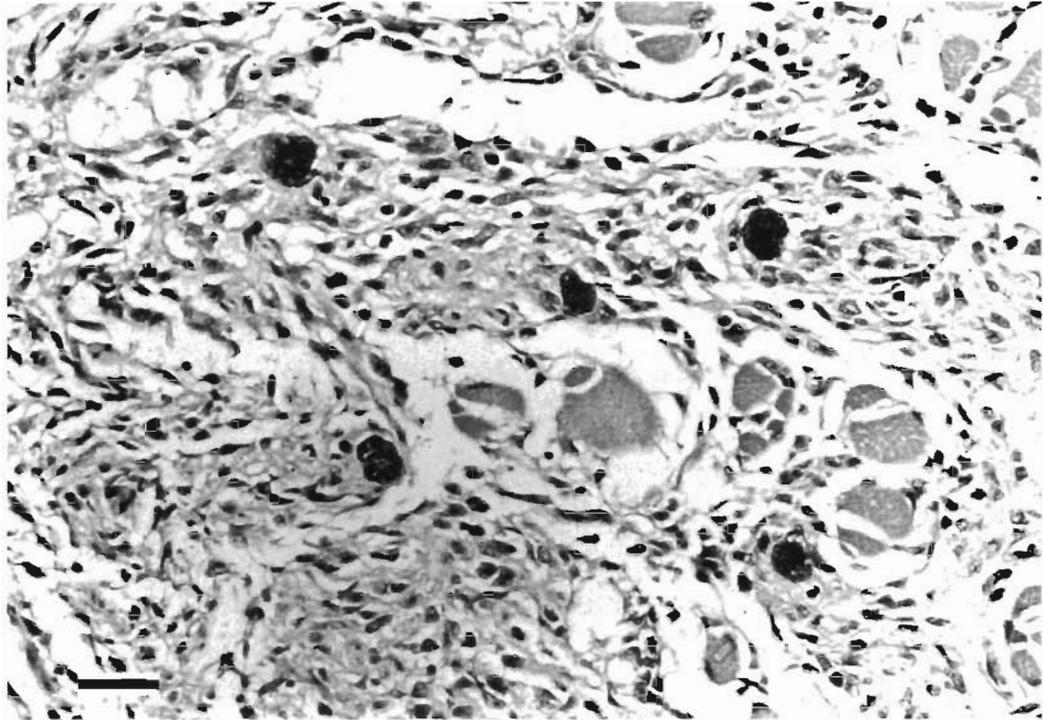


Fig. 5. *Oncorhynchus mykiss*. Granulomatous inflammation and PKX cells stained with lectin GS-I in red muscle of the lateral line in a rainbow trout with PKD. AB-P. Scale bar = 25 μ m

and spleen. Our findings demonstrate the presence of PKX cells in tissues other than kidney. However, the most affected tissues with microscopic and macroscopic changes and with a high number of parasites are the kidney and red muscles of the lateral line.

Presence of PKX cells in the red muscle of the lateral line was a consistent finding that we observed in PKD-affected rainbow trout during the late summer and autumn. The presence of extrasporogonic stages and, consequently, appearance of the inflammatory response in muscle follows a pattern that is similar to that of the well-known histopathological changes of the kidneys (Ferguson & Needham 1978, Clifton-Hadley et al. 1985, MacConnell et al. 1988, Hedrick et al. 1993) but is asynchronous between the 2 organs. PKX cells are first detected in muscle when they have reached a maximum number in the kidney. The number of parasites in muscle increases at the time that the kidney is recovering from the inflammatory response.

Macroscopical nodules of several millimetres in diameter have occasionally been reported in kidneys, spleen, liver, muscles and peritoneum. Gross lesions in the skeletal muscle were described in an outbreak where nodules were present in the liver, muscle and peritoneum (Ghittino et al. 1977). Histopathologically, a granulomatous myositis with the presence of PKX cells has been described in the muscle dorsal to the kidneys and in subdermal muscles (Ferguson & Adair 1977, Ferguson & Needham 1978). Also, inflammation of the skeletal muscle has been observed in different

cases of PKD but without the identification of muscles affected (Ghittino et al. 1977, Smith et al. 1984, Clifton-Hadley et al. 1985, 1987). In the outbreak studied here, it is evident that the inflammation of skeletal muscles was not due to the proximity to the kidneys. The presence of PKX cells and subsequent granulomatous myositis in the red muscles of the lateral lines could be explained by the high irrigation of this tissue. Probably, the myxosporean reaches this muscle through the circulatory system.

Previously, the presence of PKX cells eliciting an inflammatory response has mainly been related to the muscles dorsal to the kidneys. In our observations, the pathological changes were restricted to red muscle on the lateral lines, although solitary cells were found in the white fibres close to the red muscle.

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