First report on proliferative kidney disease (PKD) in marble trout (Salmo trutta marmoratus, Cuvier 1817)

First report on proliferative kidney disease (PKD) in marble trout (*Salmo trutta marmoratus*, Cuvier 1817)

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Abstract

Proliferative kidney disease is a hyperplastic response of the principal lymphoid tissue of salmonid fish infected by *Tetracapsuloides bryosalmonae*, a myxozoan parasite. This parasite affects many rainbow trout farms in Europe and North America. The disease has also been reported in other salmonids as well as in pike (*Esox lucius*) and grayling (*Thymallus thymallus*). In autumn 2000, an outbreak of PKD induced mortality in a group of marble trout (*Salmo trutta marmoratus*) juveniles reared in a farm in north-east Italy. The fish were intended to restock public waters. Diseased fish showed a lethargic behaviour, skin darkening, abdominal dilatation, gill anaemia and, after necroscopy, increase in volume and a pale colour of the kidney. All fishes subjected to histological examination showed a marked granulomatous interstitial nephritis, as well as foci of pancreatic and hepatic necrosis. The immunohistochemistry and PAS stain allowed visualisation of the extrasporogonic phase of the parasite *Tetracapsuloides bryosalmonae* in several tissues of the host. This is the first reported outbreak of PKD in marble trout, and should receive full attention since this species is potentially under risk of extinction.

Introduction

Marble trout (*Salmo trutta marmoratus*) is a salmonid which is endemic in the rivers of the Po Valley, of Alpine tributaries of the River Po, and of the Friuli-Venezia Giulia region. Outside Italy, it can be found in streams of Slovenia, Croatia & Albania (Stoch et al., 1992; Pov• et al., 1996; Salviati et al., 1997).

Many authors believe that marble trout populations are constantly decreasing, both in number and in habitats of choice, although exact quantitative data are lacking.

Water pollution, overfishing, reduction of flow due to massive water drawings, river rectification, riverbed cementation, and the resulting decrease in spawning areas, have caused a decrease in the populations of this species (Alessio, 1986; Buda Danchevic et al., 1982; Amirante et al., 1987). In addition, the building of barrages in streams has hindered the migration of this salmonid, thus causing a lower genetic exchange and promoting crossbreeding with brown trout (Salmo trutta fario), which is genetically similar to marble trout and has been plentifully reintroduced into public waters in the last few years. Cross-breeding has led to a reduction of the genetic specificity of marble trout and to a risk of its complete extinction.

For this reason, various public and private initiatives to restore this species have recently

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been launched (Merati & Gallandra, 1994). Typically, these projects aim either to obtain fertile eggs and juveniles to be released into protected waters (Arlati, 1986; Forneris & Alessio, 1986; Bresolin & Loro, 1987), or to carry out a complete rearing cycle by capturing and domesticating the breeders, rearing the juveniles with commercial feed, and releasing them into the natural environment at the most suitable age.

In this context, and considering the effects of the application of EC directive 91/67 and of the subsequent Italian ministerial decree of 2 September 1996 concerning the introduction of fish species into public waters, it is also important to investigate thoroughly the diseases of marble trout, both in a natural environment and in captivity.

At present, knowledge of the diseases affecting this species is poor and limited to occasional reports of ecto-endoparasitic infections, caused by *Piscicola* sp., *Pomporhyncus* sp. (Forneris et al., 1990) and *Capillaria hepatica* (Masoero & Forneris, 1986) in wild fish. Under fish-farm conditions, a few mortality episodes have been ascribed to ichthyobodosis (*Icthiobodo necator*) and gill disease (Bresolin & Loro, 1987). A serious episode of infectious pancreatic necrosis (IPN) has recently been reported in marble trout fry reared to restock public waters (Bovo et al., 2001). Reports of bacterial diseases such as furunculosis by *Aeromonas salmonicida* and bacterial kidney disease by *Renibacterium salmoninarum* in juveniles and adults have come mainly from professional experiences in the field (Giavenni, personal communications).

This study should contribute to the knowledge of diseases affecting this salmonid species, by describing an episode of proliferative kidney disease (PKD).

Materials and methods

During October 2000, in a fish farm located in north-east Italy, a mortality episode occurred that involved fingerling trout (approximately one year old), intended to restock public waters. The fish were reared in outdoor glass-fibre reinforced plastic tanks which were fed by a surface stream and were subject to considerable temperature changes. During the mortality episode, the water temperature was 11-12°C.

Among the fish showing clinical symptoms, 10 trout were sampled in order to carry out a post-mortem examination, a parasitological test on fresh specimens, and a bacteriological test by isolation from the kidney on blood agar plates; in addition, smears of kidney-tissue squashes were prepared for Gram staining.

Ten fish showing clinical symptoms, after their abdominal cavities had been cut open, were fixed *in toto* in 4% buffered neutral formalin for histological processing. Five μm sections obtained with a microtome were stained with Haematoxylin-Eosin, PAS and Twort's Gram, and were observed under a light microscope (Leica DMRB). The histopathological pattern of the disease was documented by digital camera (Olympus). In order to rule out any concomitance of viral diseases and to confirm the PKD diagnosis, histological sections of the visceral organs were analysed with anti-IPN and anti-IHN (Microtek International Inc) and anti-

Tetracapsuloides bryosalmonis (Aquatic Diagnostic Ltd) polyclonal antibodies by indirect immunoperoxidase technique. The reaction, which involves the use of an avidin-biotin peroxidase complex (DAKO) as detection method, was developed in a solution of diaminobenzidine (DAKO) and hydrogen peroxide.

Results

The fish involved in the mortality episode showed clinical symptoms including lethargy, anorexia, skin darkening, a mild exophthalmos on both eyes and a slight abdominal dilatation. Infected trout remained isolated from the group, weakly responding to external physical stimuli.

In the post-mortem examination, these fish showed a marked hyperplasia of the kidney, both in the lympho-haematopoietic and excretory portions. The organ appeared paler than normal in colour and had assumed a marble-like appearance. A few individuals showed a mild anaemia in the visceral organs.

The sampled trout were negative in the bacteriological test and the observation of preparations obtained from kidney-tissue squashes did not reveal any presence of bacteria.

The parasitological test on fresh specimens of gills and skin scraping showed a mild monogenean infection, though this could not be correlated with the observed mortality.

The histopathological pattern was characterised by severe injuries located in the renal system. Under low magnification, the kidney appeared hyperplastic, in the excretory portion the glomeruli and renal tubules appeared atrophic due to the pressure exerted by interstitial tissue. Under higher magnification, the histopathological pattern could be ascribed to a serious granulomatous interstitial nephritis, characterised by the proliferation of epithelioid cells surrounding amoeboid cells. These are typical extrasporogonic stages of *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD) in salmonids. The trophozoite appeared amoeboid, 8-30µm in diameter frequently with three vegetative nuclei in the primary cells; the secondary and tertiary cells were also observed (Figure 1). The extrasporogonic stages had a foamy eosinophilic cytoplasm, moreover the presence and distribution in the tissues of extrasporogonic stages was revealed by PAS staining and immunohistochemistry examination. Apart from the renal system, the parasite was also detected in the liver, where it induced a serum hepatitis, accompanied by degeneration and foci of hepatic necrosis.

The spleen and pancreas were affected by chronic pancreatitis and splenitis, charac-

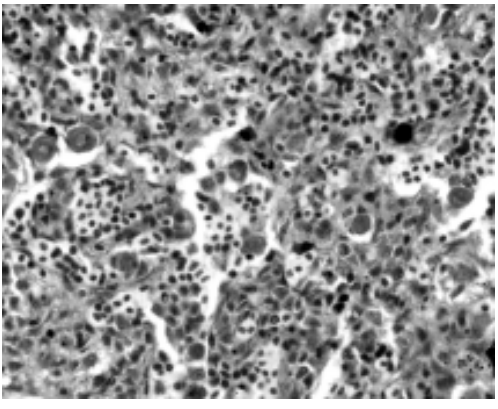

Figure 1. Extrasporogonic forms of *Tetracapsuloides bryosalmonae* in kidney interstitium. PAS, 40x.

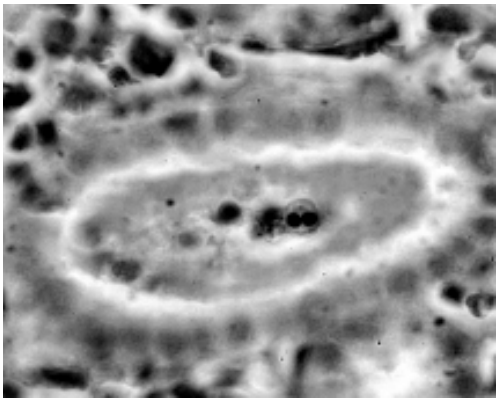

Figure 2. Mature spore with two polar capsules inside a renal tubule. Twort's Gram, 100x.

terised by infiltration of mononucleated leukocytes and epithelioid cells, which were responsible for upsetting the architecture of these organs. The inflammatory processes, which in some cases involved visceral fat and induced a pattern of steatonecrosis, were always associated with the presence of the parasite. Occasionally, a few trophozoites were detected inside the blood vessels associated with the gastrointestinal tract and inside the capillaries of secondary gill lamellae.

The PAS stain enabled detection of presporogonic forms inside renal tubules (detected in all examined fish), whereas Gram's stain showed fully formed spores (about 10-12µm wide and 6-8µm long) characterised by two spherical polar capsules (2µm in diameter) (Figure 2).

The immuno-histochemical investigations with anti-IPN and anti-IHN antibodies were negative.

Discussion

Proliferative kidney disease (PKD) is caused by a myxozoan parasite belonging to the class Malocosporea (family Saccosporidae), which is considered one of the most serious parasitic diseases of salmonids, causing high mortalities in fish farms and also wild fish (Okamura et al., 2001). The spread of the causative agent of PKD, *Tetracapsuloides bryosalmonae*, is closely linked to the presence of bryozoans, its invertebrate host. This parasite is particularly known in intensive-rearing rainbow-trout farms. It has been found in the USA (Smith et al., 1984; Hedrick et al., 1985) and in Europe (Hoffmann & Dangshat 1981; Ellis et al., 1982; Chilmonczyk et al., 1989). In Italy, the disease causes serious losses in farms where the water exchange comes from streams that flow on the surface and that during the summer reach temperatures up to 15°C (Prearo 1996). Under natural conditions, PKD has been described in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta fario*) (Ellis et al., 1982), Arctic char (*Salvelinus alpinus*) (Bucke et al., 1991), grayling (*Thymallus thymallus*) (Feist & Bucke 1993; Wahli et al., 2002), silver salmon (*Oncorhynchus kisutch*) and king salmon (*Oncorhynchus tshawytsha*) (Hedrick *et al*., 1985) as well as in cutthroat trout (*Salmo clarki*) (McConnel & Peterson, 1992).

This first episode of PKD described in marble trout allows, on the one hand, to include this species in those susceptible to the disease and, on the other hand, to make some etiological remarks about the disease.

The marble trout species, which is reared to restock public waters, is generally held in farms located in mountain territory, where they can take advantage of spring waters that are clean, well oxygenated and with low water temperatures, in any case lower than 15°C (Giavenni, personal communications). However, the choice habitat for this species is represented by streams in plains, therefore lying lower than the zone which is typical of brown trout. Usually, these streams have a low water quality, with slower flow and higher temperatures.

The epizootic outbreak described above seems mainly due to poor water quality and to the high temperatures reached during summer. The water supply to the outdoor tanks on the farm, where the fingerling trout were reared, is provided by a stream, which from its spring to the farm flows on the surface for about 1.5km and passes through a village and some cultivated fields. As for temperature, no precise data are available, but it is suspected that, during the summer and in view of the particular climate in that year's summer season, water temperature at the farm inlet exceeded 15°C. From an epidemiological view, it is very important that the infectious stages of *T. bryosalmonae* seem to be present in the water throughout the entire year and that the temperature, influencing the kinetics of the myxozoan multiplication in bryozoan and in fish, play a primari role in the development of PKD (Gay et al., 2001).

In addition, histological results suggest that marble trout, under certain environmental conditions, show poor resistance to PKD. In clinical-phase fish, a wide distribution of the parasite was observed in various tissues and organs: kidney, liver, spleen, pancreas and blood vessels. The low water quality could have induced stress and immunosuppression, thereby allowing systemic parasitic disease to develop. Research carried out in Germany and France corroborates this hypothesis, showing that the prevalence and severity of PKD increased with decreasing water quality (Schmidt et al., 1999; Geiser 2002).

We can therefore conclude that PKD represents a biological risk for the marble trout, which is already threatened with extinction for other reasons such as: water pollution, overfishing, reduction of flow due to massive water drawings, river rectification, riverbed cementation, and the resulting decrease in spawning areas (Alessio, 1986). In addition, the building of barrages in streams has hindered the migration of this salmonid, thus causing a lower genetic exchange and promoting crossbreeding with brown trout (*Salmo trutta fario*), which is genetically similar to marble trout and has been plentifully reintroduced into public waters in the last few years. Crossbreeding has led to a reduction of the genetic specificity of marble trout and to a risk of its complete extinction. Human activities could favour the PKD diffusion in different ways and the restocking of this trout species or other species could rapidly propagate the disease in natural environments.

Finally, it is appropriate to emphasise the occasional detection of fully formed spores in the lumen of renal tubules. The spores are subspherical with two spherical polar capsules. The appearance and size of the spores confirm what was observed by Kent et al. (2000). These authors suggested that the

sporogenesis of *Tetracapsula renicola* (currently *Tetracapsuloides bryosalmonae*) takes place inside the renal tubules of infected fish. More recently, Hedrick et al. (2004) detected the spores with similar features in rainbow trout urine. In spite of the recent discoveries that confirm the presence of a bryozoan in the life cycle of this parasite (Feist et al., 2001, Okamura et al., 2001, Canning et al., 1999 and 2000; Hedrick et al., 2004), the complete life cycle of *T. bryosalmonae* still remains unknown.

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