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Fish pathology

INTERESTING CASE OF PATHOLOGICAL CHANGES IN RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM) PROBABLY CAUSED BY MYXOSPOREA

INTERESUJĄCY PRZYPADEK ZMIAN PATOLOGICZNYCH U PSTRĄGA TĘCZOWEGO *ONCORHYNCHUS MYKISS* (WALBAUM) WYWOŁANYCH PRAWDOPODOBNIE PRZEZ MYXOSPOREA

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The causative agents of health problems of rainbow trout from a fish farm were investigated by parasitological and anatomo-pathological study of affected fishes. The anatomo-pathological changes in gills, swim bladder, kidney, and especially the deformations of backbone are described. The numerous trophozoites of unidentified species of Myxosporea, occurring in all damaged tissues are suspected to be a causative agent of the disease and mortality in the fish farm under study.

INTRODUCTION

In January 1995 we obtained information about health problems in a trout farm near Ełk (north-eastern part of Poland). Between December and January 1993/94 rainbow trout died with symptoms of weakness and erratic swimming. A portion of them exhibited body deformity, as well as darkening of skin, and for that reason the fish were rejected by consumers. This situation reoccurred between December and January 1994/95 with mortality reaching 15–20%, although the farm was supplied with new stock material (fingerlings) from another commercial hatchery. Due to difficulties with maintaining optimal water temperature the farm was compelled to order stock material and to stop trout breeding at the end of spring.

The rainbow trout transported to our laboratory were suspected of whirling disease, caused by the myxozoan *Myxobolus cerebralis* Hofer 1903, because of de-

formity of caudal segment of the body, spine deformation and disturbances of equilibrium that are clinical symptoms of this disease (Prost 1994). However, abnormal swimming, changes in body shape, scoliosis and lordosis, and dark coloration of the body can also occur as signs of nutritional deficiency or toxicity (Akiyama et al. 1986; Tacon 1992; Frischknecht et al. 1994). Vertebral curvature and whirling appeared during viral infection (Maemo et al. 1995), while kypholordosis was connected with bacterial disease of fish (Amlacher 1986). Abnormal body shape and heavy dystrophic changes occurred in eels with "beko disease" caused by strong microsporidian infection of *Pleistophora anguillarum* Hoshina, 1951 in muscles (T'sui and Wang 1988). Spinal abnormality and myofibril's dystrophy were observed in young fishes infected with metacercaria of *Posthodiplostomum cuticola* Nordman, 1932 (Prost 1994).

The pathological features observed in trout from Fish Farm Ełk could therefore have had many reasons. In the present paper we tried to detect those responsible for the disease in the fish farm under study, and we described the symptoms occurring in the affected fish.

MATERIALS AND METHODS

The examined fish, rainbow trout *Oncorhynchus mykiss* (Walbaum) obtained from the Fish Farm Ełk (FFE), situated in north-eastern Poland. The farm is supplied with fingerlings (0+) from commercial hatcheries. The trout are cultured to market size in earth ponds and fed with commercial pellet trout food. The ponds are supplied with water from eutrophic lake Sunowo. In January and May 1995, we examined respectively, 30 and 16 specimens trout 1+ (mean weight 110.6 ± 37.74 and 97.8 ± 27.08 g and total length 21.3 ± 2.57 and 19.4 ± 1.83 cm respectively).

The condition factor was calculated according to the Fulton formula

$$C = \frac{W \times 100}{L^3}$$

C – condition factor,
W – fish weight (g),
L – total length (cm).

Anatomo-pathological and parasitological examinations were made of all organs and tissues. Blood smears and imprints of kidney were stained with buffered Giemsa. Tissue samples (skin, muscles, gills, gut, liver, kidney, swim bladder, and brain) were fixed with Bouin's fluid, embedded in wax blocks and cut at 5 μm. The sections were stained with haematoxylin and eosin (H and E). Fresh scrapings and tissue squashes from all organs and stained materials were examined by light microscopy. Microphotographs were taken using a Nikon – Optiphot 2 microscope.

X-ray pictures were taken at the Department of Surgery and Roentgenology, Veterinary Medicine Faculty, University of Agriculture and Technology in Olsztyn, Poland.

Behaviour of the affected fish was observed in FFE ponds.

RESULTS

Infected rainbow trout exhibited disordered swimming. Many fish were weak and had forced respiration. Their skin was darker then normal. In some specimens scoliosis and lordosis occurred (Figs. 1, 2). The trunk-caudal peduncle region was shorter and deformed (Fig. 1). The change in proportion of the body, especially shortening of body length, resulted in some cases in very high value of the condition factor (1.62–1.88).

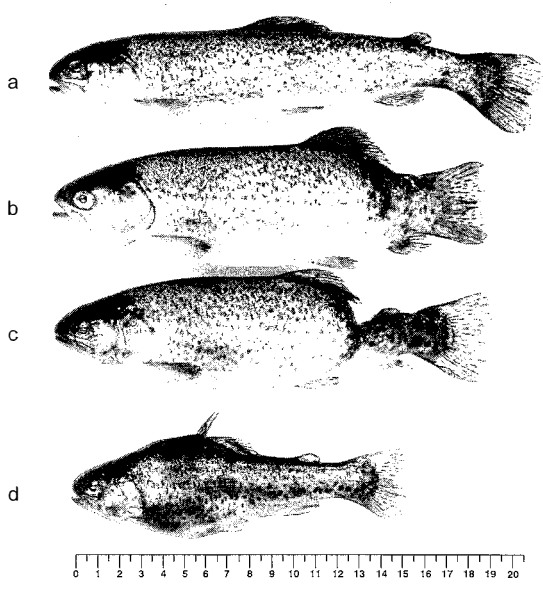

Fig. 1. Rainbow trout with the body deformation (a—normal, b—shortening of peduncle region of the spine, c—severe muscular atrophy, d—shortening of truncal region of the spine)

Photo by R. Bardega

The gills were pale, swollen and covered with a moderate layer of mucus. Some swim bladders were oedematous and filled with a light transparent fluid. The walls of this organ grew thicker and showed slight haemorrhage. The kidney was dark and enlarged in the middle and posterior part.

The observations of fresh material revealed the presence of myxosporean trophozoites in 87% of fish examined in January and in 90% of those examined in May 1995. The level of infection was high in gills and kidney, while low in skin, swim bladder and intestine. There was no "cyst"; only small plasmodia were observed. In the gills, most of the trophozoites occupied central part of lamellae near the big blood vessels and cartilage. Sometimes they exhibited slow movement. Big proliferation of the epithelium occurred. In wet material from the gills of one trout sampled in May a few spores of *Sphaerospora* sp. were detected. In the kidney, plasmodia with retractable granules were observed inside the tubular lumen and they were found less frequently in the interstitium. Intracellular stages were sporadic.

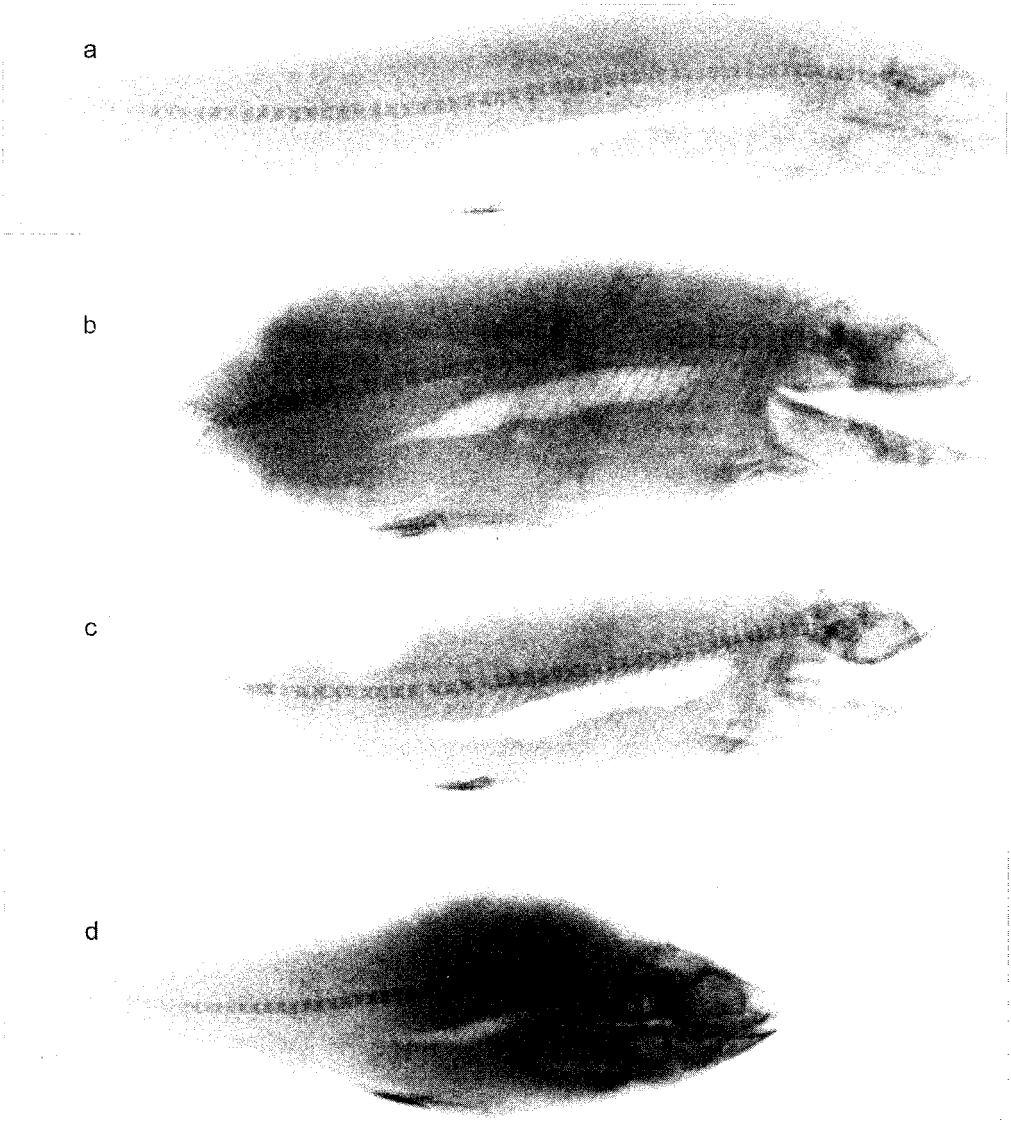

Fig. 2. X-ray pictures of rainbow trout. (a—normal, b—shortening of peduncle region of spine, c—atrophy of muscle tissue, d—shortening of truncal region of the spine)

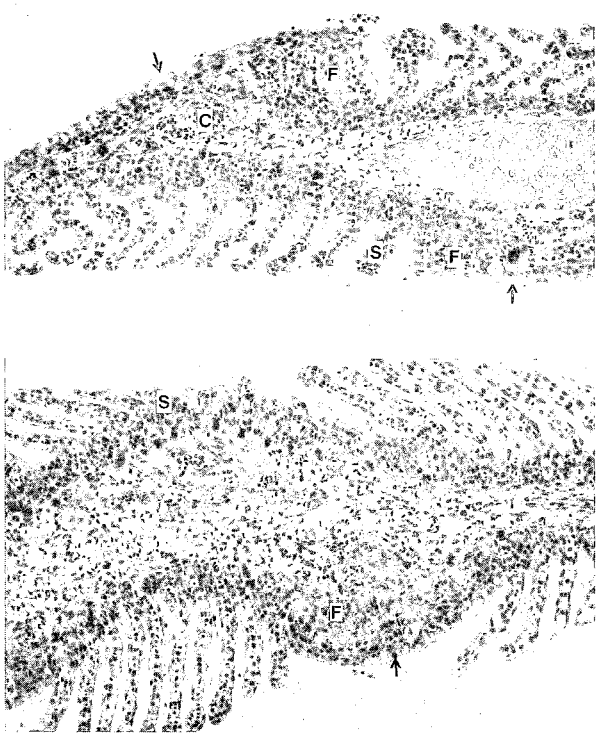

Fig. 3.The proliferation of respiratory epithelium in gills of affected trout. The fusion of secondary gill lamellae (F), shortening and thickening of secondary lamellae (S), numerous mucus cells (arrow), focal congestion (C) H&E, ×152

Other parasites rarely occurred in examined rainbow trout. Only low numbers of *Hexamita salmonis* Moore 1992 were encountered in January in gall bladder and intestine of 13% of examined fish. The skin of four trout was infected with *Gyrodactylus* sp. The metacercariae of *Diplostomum* sp. were present in 100% of trout but intensity of infection was low. The parasitic ciliates *Trichodina* sp. and *Capriniana piscium* (Bütschli 1889) Jankowski 1973, occurred in gills of 10–16% of fishes. Both prevalence and abundance of *Trichodina* sp. were low. Five specimens of 3rd stage larvae of *Anguillicola* sp. were present in the swim bladder wall of one fish in May.

The radiographs of deformed trout revealed enlarged swim bladders (Figs. 2 b, c), and damage of the structure and shape of vertebrae (Figs. 2 b, c, d). In some segments of the spine the vertebrae were very short and were grown together which limited spine mobility (Figs. 2 b, d). In such region proliferation and infiltration of connective tissue throughout myofibrils caused muscular atrophy (Fig. 2 c). Sometimes connective tissue formed big, hard nodules fixed to the backbone. Numerous myxosporean trophozoites infiltrating proliferated connective tissue were observed by light microscopy.

The strong proliferation of respiratory epithelium was observed in histological preparations of the gills. The secondary lamellae in some places completely disappeared due to fusion or became shorter and thicker than normally (Fig. 3). There was an increase in the number of mucus cells. Some capillaries were congested. In other fragments of lamellae extravasation of erythrocytes and focal necrosis of epithelium occurred. Trophozoites were not so frequently visible in stained sections as in wet preparations. In one fish (May sample) sporadic sporoblasts of *Myxobolus* (?) were

detected in the central part of the lamellae. There was no pigmentation in the gills. In the liver, muscles, and especially in the kidney melanomacrophage centres (MMC) were numerous. They occupied places near necrotic tissue. In kidney MMC were situated close to tubular lumen infected by myxosporean plasmodia (Fig. 4). Infiltration of lymphocytes was rather rare and dystrophic changes of kidney interstitium and tubular cells occurred. Some glomeruli were hypertrophied which resulted in the clogging of the space between the glomeruli and Bowman's capsule.

In some histological preparations of muscles from affected fish focal degeneration was observed.

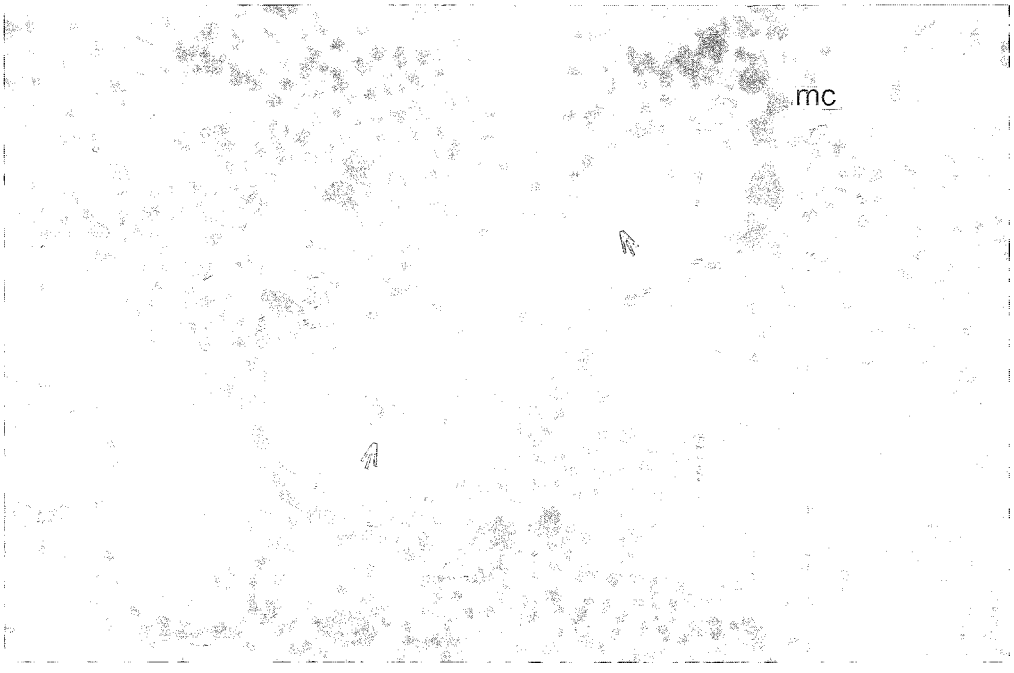

Fig. 4. The kidney of affected trout; myxosporean plasmodia (arrow) visible in the lumen of tubules. The presence of melanomacrophage centres (mc) close to the infected place

(H&E, ×304)

DISCUSSION

Health problems caused by parasites in trout farms that utilise water pumped up from underground water sources are not so frequent as in cage rearing of trout in lakes (Waluga et al. 1978) or in farms supplied with water from eutrophic lakes. The latter creates the danger of potential diseases caused by protozoans, ne-

matodes, monogeneans, and digeneans (Dechtiar and Christie 1988). That was the case with the fish from FFE in which several species of parasites were encountered. The highest abundance and prevalence were noticed for the myxosporeans, which infected the gills, kidneys, swim bladders, skin, and muscles. However, only trophozoites were frequent and numerous in many tissues and organs. The presence of myxosporean sporoblasts or spores in gills (*Sphaerospora* sp. and *Myxobolus* sp.) was sporadic. Several reasons support the conviction that myxosporean trophozoites are the major etiological agent of the pathological changes in affected trout from FFE.

Proliferative changes in gill epithelium of the trout from FFE were similar to the picture in yearling rainbow trout from salmonid farms in Germany with proliferative gill disease (PGD) probably caused by parasites (Hoffmann et al. 1992). Similar signs of PGD occurred in channel catfish *Ictalurus punctatus* in the United States, especially in cool months. It is very difficult to identify the PGD etiological agent as in many cases of outbreaks of the disease only vegetative forms of myxozoans are found on the gills of catfish; developing and mature spores usually do not occur (Styer et al. 1993). "PGD-Myxozoan" in catfish, suggested being a stage of *Aurantiactinomyxon* sp. from oligochaete, were diagnosed as a reason of PGD but other myxosporeans, *Henneguya exilis* Kudo 1929 and *Sphaerospora ictaluri* Hedrick, McDowell and Groff 1990 also occurred commonly in both healthy and PGD-infected channel catfish (Styer et al. 1993).

In the case of PGD in catfish the affected filaments revealed inflammation, haemorrhage, necrosis and distortion of cartilage. During infestation of *Myxobolus cerebralis* in trout the areas of lysis and inflammation of cartilage are observed in the gills, skull and vertebrae (Prost 1994). Deficiency of vitamin C is the cause of primary gill lamellae curvature, irregular distension and cartilage cell's degeneration. Atrophy of the bone-supported structures in gills was observed in a cichlid with vitamin C deficiency (Chávez de Martinez and Richards 1991).

We can refute nutritional deficiencies as a causative agent of the observed health problems as the fish were fed with good quality artificial pellet food and supplementary natural food supplied by water from the lake. Moreover, we did not observe degenerative changes or the presence of parasites within the cartilage but many myxosporean trophozoites were located very close to the cartilage of gill lamellae and vertebrae. In our study the shape of vertebrae was hardly altered. Very often several vertebrae were compacted into immobile blocks that resulted in deformation and shortening of the spine. Similar deformations of the backbone were observed in trout, common carp, *Cyprinus carpio*, and white crappies, *Pomoxis annularis* (Havelka et al. 1971; Bauman and Hamilton 1984). Havelka et al. (1971) divided spine deformation in carp and trout into two groups; spine defor-

mation (1) without and (2) with an alteration of the shape and structure of vertebrae. According to these authors the first group of deformations is related mostly to nutritional deficiency, toxicity, viral or bacterial diseases. The second group is connected with the presence of biological agents in the affected cartilage. Bauman and Hamilton (1984) differentiated two groups of spine deformations according to the mechanisms causing backbone damage: deformations caused by (1) alterations of structurally critical biological processes or (2) acute muscular contraction. According to these authors hereditary factors, defective embryonic development, radiation, dietary deficiency or toxic substances were responsible for the first category of damages, while parasitic infections, electric current, and toxic substances are the factors that could damage vertebrae by causing acute muscular contraction. It seems to us that myxosporeans can cause skeleton deformations by both destroying bone and cartilage tissue and by acute muscular contraction following degenerative changes in nervous tissue. It is probable too that the chronic inflammation existing near the backbone caused by the presence of myxosporean parasites results in poor nourishment and degeneration of the affected spine segment.

The condition factor (CF) reflects length-weight relationship. In poorly fed trout and in wild salmonids CF is below or about 1.0. For rainbow trout reared in ponds it reaches 1.2 (Backiel 1964). High value of CF is desirable by aquaculturists. During whirling disease or vitamin C and E deficiency rainbow trout exhibited reduced growth (Prost 1994; Frischknecht et al. 1994) i.e. reduced both weight and length so CF could appear "normal". In our study the presence of parasites did not influence the weight of fish. This fact together with pathological shortening of their body length made the value of CF abnormally high. Nevertheless in this case, as well as in the case of "normal" CF in whirling disease does not reflect good condition of fish.

In our study we failed to detect the presence of the spores of *Myxobolus cerebralis*. Such features as shortening and deformation of the caudal region of the body and impairment of balance could show that the fish under study suffered from whirling disease but lack of altered pigmentation does not agree with the symptoms of this disease.

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STRESZCZENIE

Przy zastosowaniu metod parazytologicznych i anatomopatologicznych podjęto próbę identyfikacji czynnika chorobotwórczego odpowiedzialnego za zaburzenia zdrowotne pstrąga tęczowego w jednym z gospodarstw rybackich północno-wschodniej Polski. Opisano zmiany anatomopatologiczne w skrzelach, pęcherzu pławnym, nerkach, a w szczególności deformacje kręgosłupa. Za najbardziej prawdopodobny czynnik odpowiedzialny za zmiany chorobowe i śmiertelność w objętym badaniami gospodarstwie rybackim uznano liczne trofozoity niezidentyfikowanego gatunku Myxosporea, występujące we wszystkich zniszczonych tkankach.

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