Mycobacterium salmoniphilum infection in a farmed Russian sturgeon, Acipenser gueldenstaedtii (Brandt & Ratzeburg)

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SHORT NOTE

Mycobacterium salmoniphilum infection in a farmed Russian sturgeon, *Acipenser gueldenstaedtii* (Brandt & Ratzeburg)

[1]M Righetti, [1]L Favaro, [2]E Antuofermo, [3]M Caffara, [2]S Nuvoli, [1]T Scanzio, [1]M Prearo

[1] Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy
[2] Dipartimento di Patologia e Clinica Veterinaria, Università degli Studi di Sassari, Italy
[3] Dipartimento di Scienze Mediche Veterinarie, *Alma Mater Studiorum*, Università di Bologna, Italy

Correspondence: marino.prearo@izsto.it

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The Russian sturgeon, *Acipenser gueldenstaedtii* (Brandt & Ratzeburg) is a threatened fish, which is indigenous in Eastern Europe and Western Asia (Hochleithner & Gessner 2012). This species is listed in CITES Appendix II and is considered ''critically endangered'' by the IUCN (Gesner, Freyhof & Kottelat 2010). Nevertheless, it is of high commercial value for caviar production (Vlasenko, Pavlov, Sokolov & Vasil'ev 1989). For these reasons, Russian sturgeon's farming has created an on growing interest in Europe and Asia in the last 20 years for both commercial and reintroduction purposes.

Fish mycobacteriosis is a chronic disease caused by *Mycobacterium* spp. (Inglis, Roberts & Bromage 1993; Gauthier & Rhodes 2009), characterized by numerous variably sized granulomas in fish tissues. Target organs include spleen, kidney and liver. Affected fish usually show clinical signs including weight loss (anorexia), melanosis and, occasionally, vertebral deformities as well as exophthalmia (Decostere, Hermans & Haesebrouck 2004).

Piscine mycobacteriosis is known to occur worldwide in a variety of wild (Jacobs, Stine, Baya & Kent 2009), farmed (Rodgers & Furones 1998; Bozzetta, Varello, Giorgi, Fioravanti, Pezzolato, Zanoni & Prearo 2010), and ornamental fish (Prearo, Latini, Proietti, Mazzone, Campo dall'Orto, Penati & Ghittino 2002; Zanoni, Florio, Fioravanti, Rossi & Prearo 2008; Evely, Donahue, Sells & Loynachan 2011). Among the Acipenseridae family, atypical mycobacteriosis was reported by Ucko, Colorni, Kvitt, Diamant, Zlotkin & Knibb (2002) in Siberian sturgeon *Acipenser baeri* (Brandt), while, to our knowledge, no previous records of this infection have been reported in the Russian sturgeon.

In July 2011, a dead *Acipenser gueldenstaedtii* was sent to the Fish Diseases Laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin from a commercial fish farm in NW Italy. The fish was 3 years old, 25 cm length and 250 g weight with evident cachectic syndrome.

The necropsy showed the presence of several nodular lesions in the liver and kidney (Fig. 1). During necropsy, swabs from the kidney and liver were aseptically collected, streaked onto a Columbia blood agar (Microbiol®) plate with 5% sterile sheep blood and incubated at 22±2°C for 72 h for bacterial isolation. Bacteria other than mycobacteria were not isolated from the liver or kidney samples.

Portions of the liver and kidney were homogenized and decontaminated using 1.5% cetylpyridinium chloride monohydrate (AppliChem, Germany) solution for 30 min and 10 µl were inoculated on two Löwenstein-Jensen slant-tubes (VWR®) and one Stonebrink's slant-tube (Microbiol®). The Löwenstein-Jensen tubes were incubated at 28±2°C and 37±2°C while the Stonebrink's tube was incubate at 28±2 °C. All tubes were examined weekly for 60 days. All suspected mycobacterial colonies were microscopically checked after Ziehl–Neelsen staining and, the acid-fast positive colonies, were subjected to biochemical identification (Kent & Kubica, 1985). *Mycobacterium abscessus* (*M. chelonae*-complex) was identified from all the cultures by these tests. We did not observe co-infections by other *Mycobacterium* species.

A fragment of ~439 bp of the 65-kDa heat shock protein gene (*hsp*65) was amplified with the primers TB11 and TB12 and then subjected to PCR-RFLP by *Bst*EII and *Hae*III enzymes (MBI Fermentas) (Telenti, Marchesi, Balz, Bally, Bottger & Bodmer 1993). The isolate showed a restriction pattern identical to *Mycobacterium salmoniphilum* (*M. chelonae*-complex), with a band of 308-132 bp with *Bst*EII and 195-114 bp with *Hae*III. The PCR-RFLP profile were in contrast to the biochemical identification, for this reason the *hsp*65 gene of the isolate was sequenced with an ABI 3730 DNA analyser at StarSEQ GmbH (Mainz, Germany). The DNA trace files were assembled with Vector NTI Advance 11 software (Invitrogen Carlsbad, CA). A multiple sequences alignments, with related sequences retrieved from GenBank, were constructed using BioEdit 7.1.11 and pairwise distance with Kimura 2-parameter model (K2P) were calculated by MEGA 5.05. The BLAST search gave 98% identity with *M. salmoniphilum* (DQ866778), with a K2P distance, among the *M. salmoniphilum* sequences, ranging from 1.0 to 2.5%. The sequence obtained was deposited in GenBank under accession number KC839822.

Moreover, samples of all organs were formalin fixed, paraffin embedded, and cut into 4 µm thick sections for histopathology. Slides, stained with Hematoxylin and Eosin and Ziehl-Neelsen, were subjected to microscopic observation.

The liver and kidney exhibited multifocal to coalescing nodules (Fig. 2A-2B) characterized by a severe granulomatous inflammation mainly composed by high number of macrophages, ephitelioid cells and few lymphocytes (Fig. 2C). Throughout the kidney the presence of scattered foci of mineralized material was also evident. Phagocytized red, rod-shaped acid-fast bacteria were present in high number in the liver and kidney macrophages (Fig. 2D). No lesions due to *Mycobacterium* infection were observed in the other organs examined.

The increasing commercial importance of sturgeon farming throughout the world requires detailed investigation on diseases causing mortality among this fish group. In this study, we have described for the first time a severe *M. salmoniphilum* infection in Acipenseridae, in general, and in the Russian sturgeon in particular. To the best of our knowledge, *M. salmoniphilum* was only isolated from salmonid fish (Whipps, Buttler, Pourahmad, Watral & Kent 2007; Zerihun, Nilsen, Hodneland & Colquhoun 2011), burbot (Zerihun, Berg, Lyche, Colquhoun & Poppe 2011) and Atlantic cod (Zerihun, Colquhoun & Poppe 2012). The present case report, also underlines the importance of comparing biochemical identification with molecular techniques to obtain an accurate identification of the etiological agent. In particular, biochemical methods are time-consuming and often do not clearly identify the microbial pathogen. On the contrary, PCR-based techniques have been extensively used in recent years and represent a modern, reliable, and rapid alternative to traditional biochemical methods.

Mycobacteriosis has the potential to affect the fish industry causing high economic losses (Kusuda & Kawai 1998). The ingestion of mycobacteria with food - including cannibalism - is suspected to be the major source of fish infection (Jacobs *et al*. 2009) even if a direct transmission from contaminated waters - e.g. through injured skin – should be taken into consideration as well (Inglis *et al*. 1993). Several Authors (Chinabut 1999; Zanoni *et al*. 2008) suggested that abnormal environmental stress due to poor tank management - e.g. high concentration of nutrients, scarce water supply, and sudden temperature variation - might increase the probability of infection. To date, there are no reliable treatment for this disease, and depopulation followed by complete fish tank disinfection is often the only effective solution (Jacobs *et al*. 2009). For these reasons, we underline the importance of surveillance and monitoring measures, such as randomly testing dead fish for *Mycobacterium* infections, to prevent the manifestation and diffusion of this disease in sturgeon farming.

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Figure legends

Figure 1

Visceral organs of the Russian sturgeon infected by *Mycobacterium salmoniphilum*. The arrows point to the variably sized (2-3 mm) off-white nodules dispersed throughout the liver (A) and kidney (B).

Figure 2

(A) Liver. Multifocal to coalescing, irregular to round, granulomatous foci surrounded by degenerate hepatocytes. (H&E, bar = 50 μm).

(B) Kidney. Renal tubuli surrounded by severe granulomatous inflammation. Glomeruli and hematopoietic tissue are also present. (H&E, bar = 50 μm).

(C) Renal interstitium. Mononuclear cells infiltration characterized by macrophages, and lymphocytes. (H&E, bar = 10 μm).

(D) Kidney. Numerous acid-fast bacteria phagocytized by macrophages. (Ziehl-Neelsen acid fast stain, bar = 10 μm).