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

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1 **SHORT NOTE**

2

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

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15 Keywords: Piscine mycobacteriosis, *Mycobacterium chelonae*-complex, PCR-RFLP,
16 aquaculture

17

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

25 Fish mycobacteriosis is a chronic disease caused by *Mycobacterium* spp. (Inglis, Roberts
26 & Bromage 1993; Gauthier & Rhodes 2009), characterized by numerous variably sized
27 granulomas in fish tissues. Target organs include spleen, kidney and liver. Affected fish
28 usually show clinical signs including weight loss (anorexia), melanosis and, occasionally,
29 vertebral deformities as well as exophthalmia (Decostere, Hermans & Haesebrouck 2004).
30 Piscine mycobacteriosis is known to occur worldwide in a variety of wild (Jacobs, Stine,
31 Baya & Kent 2009), farmed (Rodgers & Furones 1998; Bozzetta, Varello, Giorgi, Fioravanti,
32 Pezzolato, Zanoni & Prearo 2010), and ornamental fish (Prearo, Latini, Proietti, Mazzone,
33 Campo dall'Orto, Penati & Ghittino 2002; Zanoni, Florio, Fioravanti, Rossi & Prearo 2008;
34 Evely, Donahue, Sells & Loynachan 2011). Among the Acipenseridae family, atypical

35 mycobacteriosis was reported by Ucko, Colorni, Kvitt, Diamant, Zlotkin & Knibb (2002) in
36 Siberian sturgeon *Acipenser baeri* (Brandt), while, to our knowledge, no previous records
37 of this infection have been reported in the Russian sturgeon.

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

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

47 Portions of the liver and kidney were homogenized and decontaminated using 1.5%
48 cetylpyridinium chloride monohydrate (AppliChem, Germany) solution for 30 min and 10 µl
49 were inoculated on two Löwenstein-Jensen slant-tubes (VWR®) and one Stonebrink's
50 slant-tube (Microbiol®). The Löwenstein-Jensen tubes were incubated at 28±2°C and
51 37±2°C while the Stonebrink's tube was incubate at 28±2 °C. All tubes were examined
52 weekly for 60 days. All suspected mycobacterial colonies were microscopically checked
53 after Ziehl–Neelsen staining and, the acid-fast positive colonies, were subjected to
54 biochemical identification (Kent & Kubica, 1985). *Mycobacterium abscessus* (*M. chelonae*-
55 complex) was identified from all the cultures by these tests. We did not observe co-
56 infections by other *Mycobacterium* species.

57 A fragment of ~439 bp of the 65-kDa heat shock protein gene (*hsp65*) was amplified with
58 the primers TB11 and TB12 and then subjected to PCR-RFLP by *Bst*EII and *Hae*III
59 enzymes (MBI Fermentas) (Telenti, Marchesi, Balz, Bally, Bottger & Bodmer 1993). The
60 isolate showed a restriction pattern identical to *Mycobacterium salmoniphilum* (*M.*
61 *chelonae*-complex), with a band of 308-132 bp with *Bst*EII and 195-114 bp with *Hae*III.
62 The PCR-RFLP profile were in contrast to the biochemical identification, for this reason the
63 *hsp65* gene of the isolate was sequenced with an ABI 3730 DNA analyser at StarSEQ
64 GmbH (Mainz, Germany). The DNA trace files were assembled with Vector NTI Advance
65 11 software (Invitrogen Carlsbad, CA). A multiple sequences alignments, with related
66 sequences retrieved from GenBank, were constructed using BioEdit 7.1.11 and pairwise
67 distance with Kimura 2-parameter model (K2P) were calculated by MEGA 5.05. The
68 BLAST search gave 98% identity with *M. salmoniphilum* (DQ866778), with a K2P distance,

69 among the *M. salmoniphilum* sequences, ranging from 1.0 to 2.5%. The sequence
70 obtained was deposited in GenBank under accession number KC839822.

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

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

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

94 Mycobacteriosis has the potential to affect the fish industry causing high economic losses
95 (Kusuda & Kawai 1998). The ingestion of mycobacteria with food - including cannibalism -
96 is suspected to be the major source of fish infection (Jacobs *et al.* 2009) even if a direct
97 transmission from contaminated waters - e.g. through injured skin - should be taken into
98 consideration as well (Inglis *et al.* 1993). Several Authors (Chinabut 1999; Zanoni *et al.*
99 2008) suggested that abnormal environmental stress due to poor tank management - e.g.
100 high concentration of nutrients, scarce water supply, and sudden temperature variation -
101 might increase the probability of infection. To date, there are no reliable treatment for this
102 disease, and depopulation followed by complete fish tank disinfection is often the only

103 effective solution (Jacobs *et al.* 2009). For these reasons, we underline the importance of
104 surveillance and monitoring measures, such as randomly testing dead fish for
105 *Mycobacterium* infections, to prevent the manifestation and diffusion of this disease in
106 sturgeon farming.

107

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109

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114 **References**

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116 Bozzetta E., Varello K., Giorgi I., Fioravanti M.L., Pezzolato M., Zanoni R.G. & Prearo M.
117 (2010) *Mycobacterium marinum* infection in a striped bass farm in Italy. *Journal of Fish*
118 *Diseases* **33**, 781-785.

119 Chinabut S. (1999) *Mycobacteriosis and nocardiosis*. In: *Fish Diseases and Disorders –*
120 *Viral, Bacterial and Fungal Infections* (ed. by P.T.K. Woo & D.W. Bruno), pp. 319–340.
121 CAB International Publishing, Wallingford, UK.

122 Decostere A., Hermans K. & Haesebrouck F. (2004) Piscine mycobacteriosis: a literature
123 review covering the agent and the disease it causes in fish and humans. *Veterinary*
124 *Microbiology* **99**, 159–166.

125 Evely M.M., Donahue J.M., Sells S.F. & Loynachan A.T. (2011) Ocular mycobacteriosis in
126 a red-bellied piranha, *Pygocentrus nattereri* Kner. *Journal of Fish Diseases* **34**, 323-326.

127 Gessner J., Freyhof J. & Kottelat M. (2010) *Acipenser gueldenstaedtii*. In: *IUCN 2012.*
128 *IUCN Red List of Threatened Species. Version 2012.2.* <www.iucnredlist.org>.
129 Downloaded on 21 November 2012.

130 Gauthier D.T. & Rhodes M.W. (2009) Mycobacteriosis in fishes: a review. *Veterinary*
131 *Journal* **180**, 33–47.

132 Hochleithner M. & Gessner J. (2012) *The sturgeon and paddlefishes of the world - Biology*
133 *and Aquaculture*. AquaTech publications, Kitzbühel, A, 248pp.

134 Inglis V., Roberts R.J. & Bromage N.R. (1993) *Bacterial Diseases of Fish*. Blackwell
135 Science Publications, Oxford, UK, 312pp.

136 Jacobs J.M., Stine C.B., Baya A.M. & Kent M.L. (2009) A review of mycobacteriosis in
137 marine fish. *Journal of Fish Diseases* **32**, 119–130.

138 Kent P.T. & Kubica G.P. (1985) *Public Health Mycobacteriology. A Guide for the Level III*
139 *Laboratory*. US Department of Health and Human Services, Centres for Disease Control,
140 Atlanta, GA, 207pp.

141 Kusuda R. & Kawai K. (1998) Bacterial diseases of cultured marine fish in Japan. *Fish*
142 *Pathology* **33**, 221–227.

143 Prearo M., Latini M., Proietti M., Mazzone P., Campo dall'Orto B., Penati V. & Ghittino C.
144 (2002) Casi di micobatteriosi in pesci rossi d'acquario. *Bollettino Società Italiana Patologia*
145 *Ittica* **33**, 30–43.

146 Rodgers C.J. & Furones M.D. (1998) Disease problems in cultured marine fish in the
147 Mediterranean. *Fish Pathology* **33**, 157–164.

148 Telenti A., Marchesi F., Balz M., Bally F., Bottger E.C. & Bodmer T. (1993) Rapid
149 identification of mycobacteria to the species level by polymerase chain reaction and
150 restriction enzyme analysis. *Journal of Clinical Microbiology* **31(2)**, 175-178.

151 Ucko M., Colorni A., Kvitt H., Diamant A., Zlotkin A. & Knibb W.R. (2002) Strain Variation
152 in *Mycobacterium marinum* Fish Isolates. *Applied and Environmental Microbiology* **68(11)**,
153 5281-5287.

154 Vlasenko A.D., Pavlov A.V., Sokolov L.I. & Vasil'ev V.P. (1989) General introduction to
155 fishes - Acipenseriformes. In: *The freshwater fishes of Europe*. (ed. by J. Holcík), vol. 1/II,
156 pp. 294-345. Aula-Verlag, Wiesbaden, Germany.

157 Whipps C.M., Butler W.R., Pourahmad F., Watral V.G. & Kent M.L. (2007) Molecular
158 systematics support the revival of *Mycobacterium salmoniphilum* (ex Ross 1960) sp. nov.,
159 nom. rev., a species closely related to *Mycobacterium chelonae*. *International Journal of*
160 *Systematic and Evolutionary Microbiology* **57**, 2525-2531.

161 Zanoni R.G., Florio D., Fioravanti M.L., Rossi M. & Prearo M. (2008) Occurrence of
162 *Mycobacterium* spp. in ornamental fish in Italy. *Journal of Fish Diseases* **31**, 433–441.

163 Zerihun M.A., Berg V., Lyche J.L., Colquhoun D.J. & Poppe T.T. (2011) *Mycobacterium*
164 *salmoniphilum* infection in burbot *Lota lota*. *Diseases of Aquatic Organisms* **95**, 56-64.

165 Zerihun M.A., Nilsen H., Hodneland S. & Colquhoun D.J. (2011) *Mycobacterium*
166 *salmoniphilum* infection in farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*
167 **34**, 769–781.

168 Zerihun M.A., Colquhoun D.J. & Poppe T.T. (2012) Experimental mycobacteriosis in
169 Atlantic cod, *Gadus morhua* L. *Journal of Fish Diseases* **35**, 365–377.

170

171 **Figure legends**

172

173 Figure 1

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

177

178 Figure 2

179

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

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

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

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