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SHORT COMMUNICATION

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A case of mycobacteriosis in farmed pikeperch (*Sander lucioperca*) cultured in a recirculating aquaculture system

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1 | INTRODUCTION

Mycobacteriosis caused by *Mycobacterium marinum* was first reported by Aronson (1926) in marine fish kept in the Philadelphia Aquarium. Subsequently, mycobacteriosis has been reported in many marine and freshwater fish species worldwide (Austin & Austin, 2016). All fish species should be considered susceptible (Bruno et al., 1998). Disease outbreaks have been described in a number of cultured teleostean and chondrostean species, such as striped bass (*Morone saxatilis*) (Hedrick, McDowell, & Groff, 1987; Plumb, 1999), turbot (*Scophthalmus maximus*) (dos Santos, do Vale, Sousa, & Silva, 2002), African catfish (*Clarias gariepinus*) (Antychowicz, Lipiec, & Matusiewicz, 2003), cobia (*Rachycentron canadum*) (Lowry & Smith, 2006), yellowtail (*Seriola quinqueradiata*) (Weerakhun et al., 2007), meagre (*Argyrosomus regius*) (Avsever et al., 2014), half-smooth tongue sole (*Cynoglossus semilaevis*) (Luo et al., 2018), Amur sturgeon (*Acipenser schrenckii*), Chinese sturgeon (*Acipenser sinensis*) (Zhang et al., 2015) and in hybrid sturgeons, that is *Acipenser baerii* x *Acipenser gueldenstaedtii* (Zhang et al., 2015) and *Acipenser baerii* x *Acipenser schrenckii* (Chang, Chen, Hsu, Chen, & Chen, 2014). Most of these outbreaks have been associated with high mortality and significant economic losses. For example, the cumulative mortality during *M. marinum* outbreak in hybrid sturgeon (*Acipenser baerii* x *Acipenser schrenckii*) could exceed 70% (Chang et al., 2014), in striped bass it is around 50% (Hedrick et al., 1987), in half-smooth tongue sole it was estimated to be 30%–50% (Luo et al., 2018), while in African catfish cumulative mortality reached 5% (Antychowicz et al., 2003). *M. marinum* outbreaks in cultured fish appear to be related to high stocking density (Avsever et al., 2014; Hedrick et al., 1987), poor diet (Jacobs et al., 2009) and water quality (Lewis & Chinabut, 2011). In fact, such stressors could reduce immune function (Lewis & Chinabut, 2011) and affect the progression (Slany, Makovcova, Jezek, Bodnarova, & Pavlik, 2014) and severity of the disease (Jacobs et al., 2009).

Pikeperch (*Sander lucioperca*) is an emerging aquaculture species in Europe. As an emerging species for commercial culture, pikeperch shows high sensitivity to stressors, which implies a reduced immune system and increased susceptibility to diseases (Nguinkal et al., 2019). The present report describes the occurrence of mycobacteriosis in farmed pikeperch cultured in a commercial recirculation system.

Five moribund pikeperch, 29–31 cm in length, were collected from a single rearing tank in a commercial fish farm located in Croatia. Stocking density in the tank was 48 kg/m^3. The source farm had a standing stock of approximately 6000 fish, and farming was based on juvenile pikeperch imported from Hungary. The average

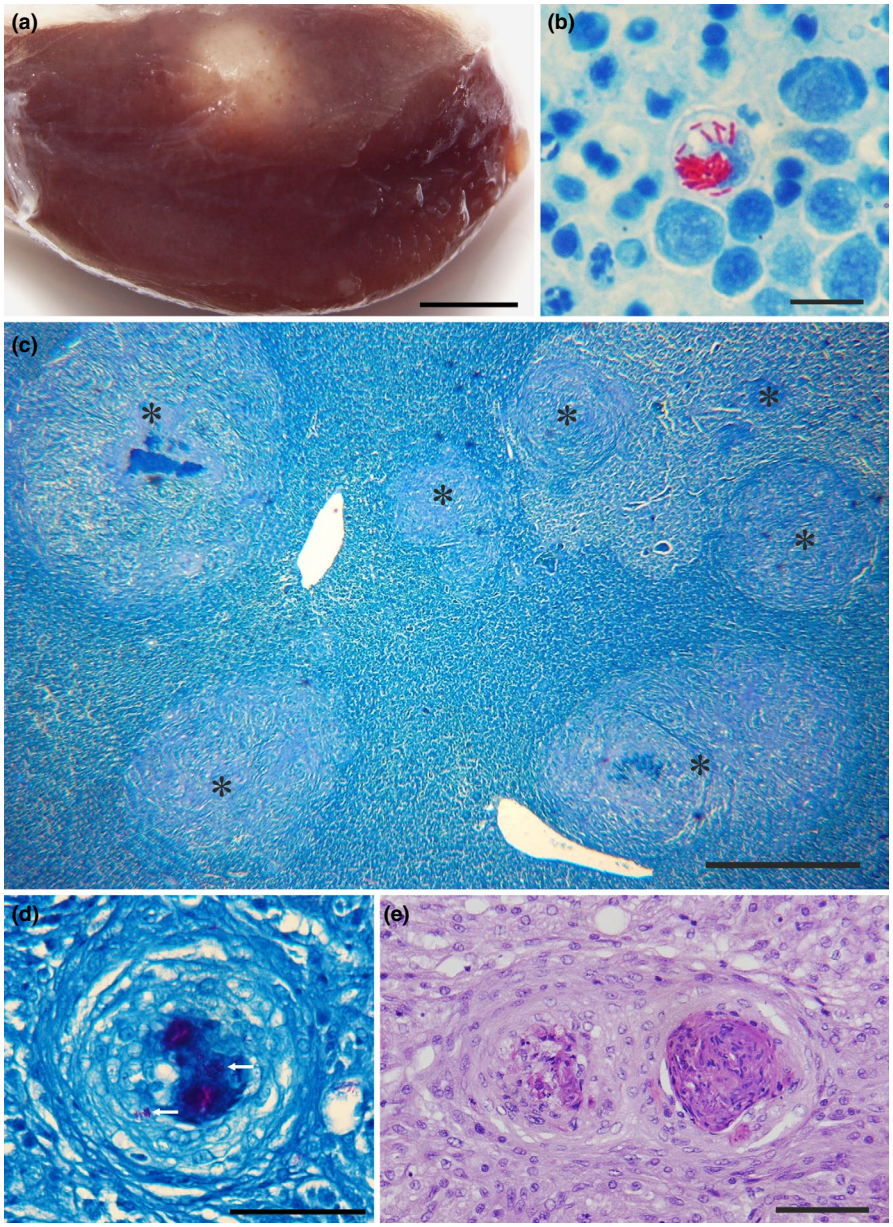

FIGURE 1 Mycobacteriosis caused by *Mycobacterium marinum* in pikeperch. (a) Superficial nodular lesion in the spleen. Scale bar = 2 mm. (b) Kidney imprint with acid-fast mycobacteria within the macrophage (ZN). Scale bar = 10 μm. (c) Histological section of a pikeperch kidney showing granulomatous response (ZN). Multiple poorly developed granulomas occupying a large portion of the anterior kidney (*). Note the central dark blue necrotic material within two larger granulomas. Scale bar = 500 μm. (d) High magnification of early granuloma with a small number of acid-fast mycobacteria (arrows) (ZN). Scale bar = 50 μm. (e) Histological section of a pikeperch spleen (H&E). Note a thick wall of epithelioid macrophages and necrotic debris in centre of granulomas. Scale bar = 50 μm

initial body weight of imported fish was 18.3 g. Approximately 14 months after stocking, the mortality of pikeperch suddenly increased from 1.5% to 6.0% per month. The farm used a recirculation system supplied by borehole fresh water. Water temperature in the system was between 20.2 and 22.5°C, pH was between 7.4 and 7.8, and dissolved oxygen was between 7.0 and 8.7 mg/L (additional water quality parameters were not available). Fish in the farm were fed a commercial pelleted diet produced for sturgeons (Supreme-10, Coppens International). In addition to the pikeperch, the farm also housed beluga sturgeon (*Huso huso*) with no clinical signs of mycobacteriosis.

The study was performed in accordance with the guidelines of the European Union on the protection of animals used for scientific purposes (Directive 2010/63/EU) and approved by the Institutional Ethics Committee (Faculty of Veterinary Medicine, University of Zagreb). Moribund pikeperch were transferred to the Laboratory for Fish Diseases at the Faculty of Veterinary Medicine and then euthanized in a separate tank by an overdose of MS-222 (Sigma-Aldrich, St. Louis, MO, USA) buffered with sodium bicarbonate. Following necropsy, spleen and kidney imprints were prepared and stained with Ziehl–Neelsen method (ZN). For histological examination, small samples of both organs were fixed in 10% neutral buffered formalin. Fixed material was embedded in paraffin, and 5 μm serial sections were prepared. Sections were stained with haematoxylin and eosin (H&E), periodic acid-Schiff reaction (PAS) and ZN method. Several sections were also stained with Masson's trichrome staining for collagen and by the von Kossa/van Gieson method to demonstrate mineralized tissue. Imprints and sections were analysed by light microscopy using an Olympus BX41.

For the isolation of non-acid-fast bacteria, samples from kidney were inoculated on tryptone-soya agar and 5% sheep blood agar, and were incubated at 25°C for up to 7 days. Isolation of acid-fast

bacteria was performed at the National/Supranational Reference Laboratory for tuberculosis (Croatia). Samples were homogenized and processed according to standard procedures as previously described (Pfyffer, 2007). Culture was performed using BACTEC MGIT 960 system (BD) and additionally Löwenstein–Jensen medium for 6 weeks at 25°C. Species identification was done by molecular methods, using GenoType Mycobacterium CM and GenoType Mycobacterium AS reverse hybridization assays (GenoType© CM/AS; Hain Lifescience).

A high mortality among farmed pikeperch (0.2%/d) cultured in a recirculating aquaculture system was recorded. Affected pikeperch were emaciated, lethargic, with clearly visible skin lesions and discolouration. At necropsy, multiple greyish-white nodules were found in the spleen and kidney. These nodular lesions were larger in the spleen (up to 2 mm in diameter) than in the kidney (0.06–0.95 mm in diameter). In contrast, severity of lesions was greater in the kidney than in the spleen. In the spleen, nodules were usually located superficially (Figure 1a). Gross pathological changes in moribund fish also included enlargement of the spleen and ascites. ZN-stained imprints and sections of both organs demonstrated acid-fast bacteria within tissues and phagocytes (Figure 1b,d). Histologically, granulomatous inflammation was evident (Figure 1c). Granulomas were composed mainly of epithelioid cells with or without central area of necrosis. Connective tissue capsule was absent. Neither multinucleated giant cells nor dystrophic calcification in the granulomas was detected. Granuloma morphology was similar in both organs. Occasionally, adjacent granulomas appeared to fuse, resulting in large multinodular lesions (Figure 1e). Acid-fast bacteria were mostly limited to the inside of the granulomas (Figure 1d).

A definitive diagnosis was made based on isolation and identification of acid-fast bacteria. The culture of kidney samples for mycobacteria was positive, and growth was observed on liquid media after 2 weeks of incubation at 37°C. The isolates were identified by molecular methods as *M. marinum*.

To our knowledge, this is the first report of mycobacteriosis caused by *M. marinum* in farmed pikeperch. In this outbreak, the mortality rate was 6.0% per month. In cultured fish, mycobacteriosis is usually associated with a high mortality rate (Antychowicz et al., 2003; Chang et al., 2014; Hedrick et al., 1987; Luo et al., 2018; Zhang et al., 2015).

The host response to mycobacterial infection was granulomatous inflammation. Granulomatous lesions were found only in the spleen and kidney. Similar finding in a half-smooth tongue sole is colloquially known as 'splenic and renal granulomas disease' (Luo et al., 2018). Granulomas were poorly formed and composed of epithelioid cells, with central area of necrosis in more advanced lesions. Observed structure of granulomas mainly corresponds to earlier descriptions by Bruno et al. (1998), Jacobs et al. (2009) and Novotny et al. (2010). However, these authors reported the presence of overlying fibrous capsule. The structure of granulomas depends on their maturity (Bruno et al., 1998; Novotny et al., 2010), causative agent, host species and some other factors (Chang et al., 2006; Gauthier, Vogelbein, & Ottinger, 2004). In this case, the absence of connective tissue capsule represents a relatively early stage of granuloma development. This supposition is in accordance with data published previously (Gauthier, Rhodes, Vogelbein, Kator, & Ottinger, 2003; Novotny et al., 2010). Furthermore, an experimental study involving striped bass placed on a reducing diet and infected with *M. marinum* did not demonstrate fibrous capsule at 4 weeks post infection (Jacobs et al., 2009).

The source of the pathogen is unknown. However, one cannot exclude the possibility that *M. marinum* was introduced into the affected farm by infected juvenile pikeperch particularly because the whole system was empty for a few years prior to stocking. As mentioned previously, there are a few possible predisposing factors for the onset of mycobacteriosis in cultured fish. In the current case, mycobacteriosis may have been triggered by poor diet and/or water quality. It is worth mentioning that beluga sturgeon could also have been a possible source of infection since sturgeons are recognized as a potential mycobacterial reservoir (Zhang et al., 2015). In this scenario, the absence of clinical disease in beluga sturgeon could be explained by a higher resistance to aquaculture stressors in some species of sturgeons (chondrostean species) compared to teleosts (Falahatkar, Akhavan, Efatpanah, & Meknatkhah, 2012). The use of advanced genetic tools and better understanding the virulence characteristics of this pathogen may aid in the detection of carrier fish and determination of the origin of the strains (Ostland et al., 2008).

2 | CONCLUSION

In conclusion, prevention strategy for mycobacteriosis of farmed pikeperch should include good management practice and avoidance of introduction of *M. marinum* to a recirculating system. Therefore, screening of fish at the supplier hatchery/farm is essential. Finally, mycobacteriosis is of particular interest because of its zoonotic potential (Lowry & Smith, 2006; Mason et al., 2016) and should be given special attention (Zhang et al., 2015). There is no effective treatment of fish mycobacteriosis (Hughes, Smith, & Duncan, 2002; Lowry & Smith, 2006), and implementation of strategies that Mason et al. (2016) used to manage and reduce the impact of the pathogenic mycobacteria in a recirculating system was not possible in this case. For this reason, depopulation and farm disinfection was recommended.

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DATA AVAILABILITY STATEMENT

All data analysed during this study are included in published article.

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