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Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Schmidt, J. G., Donati, V. L., & Lorenzen, N. (2023). *Experimentally induced immunity against red mark syndrome in rainbow trout, Oncorhynchus mykiss*. Abstract from The 4th Congress of the International Society of Fish & Shellfish Immunology, Bodø, Norway.

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Experimentally induced immunity against red mark syndrome in rainbow trout, *Oncorhynchus mykiss*

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Abstract

Red mark syndrome (RMS) is a disease affecting rainbow trout under farming conditions, and which is characterized by large red skin lesions – often in market size fish. The causative agent is not formally established, but a bacterium related to *Midichloria mitochondrii* (thus termed *Midichloria*-like organism, MLO) is considered the most likely candidate. MLO belongs in the order Rickettsiales – a group of obligate intracellular bacteria. Previous studies have shown that while CD8+ lymphocytes infiltrate the lesion site at a relatively late stage in lesion development, B lymphocyte infiltration is more prominent throughout lesion development. The disease is not lethal but is costly for the fish farmer. Although antibiotics can reduce clinical disease, affected fish are usually close to slaughter and treatment with antibiotics is not allowed due to retention restrictions. Due to the apparently benign nature of the disease, we speculated whether early exposure to RMS as fingerlings could result in protective immunity throughout the remaining production cycle, thus preventing skin lesions in larger fish and thereby reducing the farmers' RMS-related problems. However, some trout farmers have reported occasional re-emergence of RMS in some batches of fish having recovered from the disease several months earlier. We here wanted to test this experimentally.

MLO has not been isolated nor propagated *in vitro*, and infection studies rely on cohabitation with RMS-affected fish. Thus, RMS-affected rainbow trout were cohabited with naïve SPF rainbow trout fingerlings at 12°C. After approximately 7 weeks the cohabitants started showing symptoms of RMS. After 3 months, RMS had been observed in 79% of the cohabitants and importantly there was no RMS-related mortality. The cohabitants were now maintained for 13 months during which no further pathology was observed. The cohabitants were then once again subjected to cohabitation with RMS-affected fish and naïve SPF cohabitants were included as controls. After another three months of cohabitation, RMS lesions had developed in all the naïve control fish but not in any of the previously exposed fish. Our results thus support the idea that immunization by early exposure might be useful to prevent appearance of clinical RMS at later life stages.

Keywords: Red mark syndrome, intracellular bacteria, immunity, skin disease.

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