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# Dissecting complex disease case in RAS by a validated microfluidic chip targeting salmonid pathogens

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**Abstract**

The production of rainbow trout (*Oncorhynchus mykiss*) in RAS system is intense. It normally involves high biomass in contained farm environments, high reutilization of water, and frequent introduction of new fish batches. This diverge from the paradigm of "all-in all-out" which has been a cornerstone of biosecurity and brings a number of health challenges for farmed stocks.

Along with known production bacterial and viral pathogens, new ones colonize this ecological niche. It is the case of *Piscine orthoreovirus* genotype 3 (PRV-3) was first reported in Denmark in 2017 in association with disease outbreaks with high mortality in rainbow trout. A surveillance conducted in 2017-2019, showed that although this was widespread in farmed rainbow trout, disease outbreaks with mortality were only observed in recirculating aquaculture systems, and primarily during the winter.

Importantly, preliminary field investigations have highlighted the presence of other pathogens during PRV-3 associated disease outbreaks. In order to deepen the understanding of PRV-3 associated disease, we developed and validated a high-throughput qPCR method (Fluidigm) for simultaneous detection of multiple pathogens. From March to September 2022, monthly sampling was performed from 10 fish from the same cohort. Along with tissue samples of heart and spleen, gill tissues and water were sampled. Fish were examined with traditional diagnostic methods, and results compared to the output of the microfluidic chip. Additionally, production data was recorded during the seven months, including weight, feeding, disease outbreaks, treatments, and water quality parameters. A severe disease outbreak affected the cohort we followed, the event lasted 5 weeks with an overall mortality yielding more than 2000 kg. Besides the constant presence of PRV-3, and the sporadic detection of *Yersinia ruckerii* and *Flavobacterium psychrophilum*, assays targeting Piscichlamydia and Brachiomonas yielded positive results in gill tissues, in connection with the outbreak.

A comprehensive diagnostic overview combined with the production data will be presented. The study show how disease outbreaks in RAS are complex and sustained by co-infections and possibly stressors induced by farm practices. Interestingly, mortality increased significantly after fish transfer and was not mitigated by water treatment with salt. The tool we have developed and validated can provide important information on solving complex disease cases and suggest adequate preventative measures and mitigating strategies.

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