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# Molecular, enzymatic and cellular responses of rainbow trout exposed to herbicide and challenged with infectious hematopoietic necrosis virus (IHNV)

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**Keywords:** rainbow trout, herbicide, infectious hematopoietic necrosis virus (IHNV), qPCR

Streams and ground water all around the world are contaminated by pesticide and several studies have shown that contaminants can modulate fish immune system and reduce host resistance to pathogens.

Main objective of this study was to evaluate the susceptibility of rainbow trout, *Oncorhynchus mykiss* to an experimental challenge with infectious hematopoietic necrosis virus (IHNV) after a chronic exposure to pendimethalin, an herbicide frequently used in agriculture and measured at high level in river of several countries.

After 28 days exposure to chemical, fish have been challenged by immersion in water containing  $10^4$  TCID<sub>50</sub> mL<sup>-1</sup> of IHNV. Four conditions were tested: 1) control, 2) contaminated by pendimethalin, 3) challenged with IHNV and 4) exposed to pendimethalin and IHNV. Mortalities were recorded during the 44 days post-infection (dpi) and organs were collected from dead fish for virological examination. After the chemical contamination and 24h, 96h and 6 weeks after the bath immersion, survivor fish were sampled to analyze several specific and non-specific immune markers. Lysozyme concentration, complement activity and the detection and quantification of trout anti-IHNV antibodies were assessed in trout plasma. Moreover, expression of 8 genes implicated in immune system (complement C3-1 and C3-4, interferon  $\gamma$ , interleukin 1 $\beta$ , tumor necrosis factor  $\alpha$  1 and 2, toll-like receptor 3 and  $\beta$ -defensin 3) were followed in spleen.

Pendimethalin exposure seems to have no direct impact on fish immunity but the chemical contamination modulates the susceptibility and the immune response of rainbow trout in presence of IHNV. Few significant differences were observed at the cellular level whereas more fish were positive to virus with lower viral particles concentration in the group exposed to herbicide and virus. Moreover,  $\beta$ -defensin expression was down-regulated and TNF expression seems early and up-regulated too in this group than those of the group only challenged with virus. In conclusion, even if no direct effect of pollutant could be demonstrated, the fish immune response in presence of virus could be modulated by contamination.

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## In vitro polarization of bovine macrophages

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**Keywords:** bovine, M1 macrophage, M2 macrophage

Macrophages are major cells of the innate immunity. Macrophages derived from monocyte precursors undergo specific differentiation depending on the local tissue environment. Similar to the T helper type 1 and T helper type 2 polarization, two distinct states of polarized activation for macrophages have been defined in mouse and humans: the classically activated (M1) macrophage and the alternatively activated (M2) macrophage phenotypes. On the other hand, these different patterns of macrophage differentiation drive adaptive responses during the stages of infection, hence restraining inflammation and favoring tissue repair. In vitro generation and characterization of these subpopulations are essential to perform relevant studies understanding the host-pathogen interactions. Currently, several in vitro differentiation and polarization protocols are used to induce M1 or M2 mouse and human macrophages but none have been developed for the bovine species.

We developed a method for in vitro differentiation and polarization of bovine macrophages using GM-CSF and IFN $\gamma$  to induce M1, and IL-4 to induce M2 phenotype. We characterized M1/M2 macrophages by specific morphology, production of cytokines (IL-10, IL-12, TNF- $\alpha$ ), NO production, and phenotypic markers such as CD206 and TLR2.

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