

The Immunity-AMR Project: A new Op+lait initiative

Infections pose a major threat to the vital industry of agriculture, which depends on their prevention and treatment to avoid economic losses. Dairy cows are frequently affected by mastitis and reproductive disorders, which sometimes require antibiotic therapy. However, antimicrobial resistance (AMR) is an emerging problem that compromises treatment efficacy. Most studies investigate the bacterial and pharmacological aspects of AMR, but neglect the immunological factors that influence treatment outcome and symptom recurrence. Hence, the identification of genetic variants that modulate the cow's immune response to diseases is essential.

AMR can develop in bacteria even before we initiate antibiotic therapy. Our immune system plays a key role in combating these resistant bacteria, but most research overlooks its contribution. This implies that treatment failure and relapse could be attributed to a weak immune system unable to completely clear the infection, rather than solely to antibiotic resistance. This notion has been highlighted during the COVID-19 pandemic, where new virus variants have emerged in immunocompromised individuals. Researchers have explored the genetics of the immune system in dairy cows, but we still need to elucidate how genetic factors affect the cow's susceptibility to infections and whether a poor immune response can facilitate the emergence of AMR.

The immune system helps animals fight infections and stay healthy. But some people and animals have rare diseases that make their immune system weak or unable to work properly. These diseases are called inborn errors of immunity (IEI) and they are caused by changes in their genes. These changes affect how the immune cells grow, work, communicate, and fight pathogens. People and animals with IEI can have problems with infections, allergies, etc. These 'genetic lesions' can be translated in a wide range of clinical manifestations, such as recurrent and severe infections, autoimmune and inflammatory disorders, malignancies, allergies, etc. The severity, onset, and pattern of clinical manifestations depend on the specific genetic defect and the affected components of the immune system.

The diagnosis of IEI usually involves a combination of clinical evaluation, laboratory testing, and genetic analysis. The clinical assessment includes a comprehensive medical history, physical examination, and analysis of immune function and serology tests. The different



diagnostic tool used for identifying IEI may include complete blood count, immunoglobulin levels, lymphocyte subsets, cytokine profiles, and functional assays. Genetic analysis can help identify the specific gene defects that underlie the IEI and inform the prognosis, inheritance pattern, and potential therapeutic options. However, discovery and reporting of these mutations is currently very limited, especially in livestock.

There is growing scientific literature pointing to a link between IEI and AMR. Individuals with IEI have shown increased susceptibility to different microbial infections, which leads to repeated and prolonged use of antibiotics and other antimicrobial agents. This could be a major factor contributing to the emergence and spread of AMR pathogens. Moreover, IEI can pose diagnostic and therapeutic challenges, as the infections caused by opportunistic or unusual pathogens may not respond to conventional antibiotics or may require specific treatments. This can result in delayed or inadequate treatment, which can exacerbate the infection and increase the risk of development of AMR. IEI can also affect the microbiota, which is the collection of microorganisms that inhabit various body sites. Different alterations of these commensal communities can lead to an increased risk of colonization and infection by pathogenic or AMR bacteria.

A new multidisciplinary research team composed of Christopher Fernandez-Prada, David Langlais, Jocelyn Dubuc, Éric Paquet, and Simon Dufour, based in the province of Québec, and involving multiple centers (UdeM, McGill, and U. Laval), has decided to explore how genetic mutations may contribute to cases of antibiotic treatment failure, AMR, and persistent or recurring infections in dairy cows. If these mutations



can be identified, the research team could develop new tests to predict how easily a cow will get sick. In addition, the research team will also shed new light on how a weak immune system can lead to an increased frequency of AMR infections in dairy cows.

The 'Immunity-AMR' project has enrolled 250 Holstein dairy cows from which the research team is collecting blood samples to assess their immune status over time. In addition, the herd is being continuously monitored to detect any animals with recurrent infections, potential AMR infections (mastitis, metritis, etc.), or unexpected immune susceptibilities. To this end, the research team is conducting clinical exams on each animal twice a week until they reach 30 days in milk. All those animals with bacterial infections that were treated with an antibiotic will be subjected to a strict follow-up. The AMR profile of resistant/relapsing infections is being characterized by a combination of MALDI-TOF MS analyses and classic bacteriological approaches. Using a big data approach, that includes both laboratory (cytokines, immunoglobulins, and cell types, among others) and clinical parameters,

the animals will be classified according to their risk to carry IEI. Selected individuals will be subjected to whole-genome sequencing to identify IEI in protein-coding genes. Potential damaging genetic variations will be evaluated for their frequency of in the general population, and investigated for their association with the different phenotypic variations.

This project could have a major impact on the dairy industry by increasing the availability of products derived from cows raised without antibiotics, and by improving animal health and welfare. By exploring the possible connection between IEI and AMR in dairy cows, this study can reveal crucial insights into the development and spread of AMR leading to better public health interventions and livestock management. Global growth of milk and beef production systems inevitably leads to an augmented risk of infectious disease emergence and spread – including AMR infections. Improved understanding of cattle IEI is needed to provide optimal tools to manage and prevent pathogens, while avoiding the emergence of AMR. *M²*



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