Molecular characterization of Bovine Leukemia Virus strains in Israel and the specific biological markers of the disease in cattle for the development of diagnosis methods and treatments against BLV strains associated to different levels of the disease.

Abstract

Bovine Leukemia Virus (BLV) is a delta-retrovirus that causes Enzootic Bovine Leukosis. While most infected cattle remain asymptomatic carriers, approximately 30% of cases progress to persistent lymphocytosis, and about 5% develop malignant lymphoma leading to death. These outcomes result in severe economic losses for dairy and beef farmers. Consequently, there is a critical need for early assessment methods to predict disease progression, even in the absence of early clinical signs.

Current Diagnostic Gaps

Genotyping is typically performed using a segment of the envelope protein gene (env). Additionally, the pX gene, which encodes the virulent factor Tax protein, serves as a marker; specific mutations in Tax have been associated with viral virulence, proviral load (PVL), and disease severity. In Israel, previous surveys estimate that BLV is present in 40-50% of farms, with an average of 5% of tested cows being carriers.

Currently, Israeli diagnostics rely primarily on serological and molecular methods that:

- Do not differentiate between viral strains or sub-strains.
- Do not quantify the proviral load.
- Lack data regarding the distribution of Tax protein genetic polymorphisms and their link to farm-wide morbidity.

Research Methodology and Findings

This study conducted a detailed sequence analysis of the *env* gene segment and *tax* gene from 169 samples collected between 2015 and 2024 (with a focused collection in 2023-2024).

- Genotyping: Based on *env* sequences, Genotype 4 is dominant in Israel, showing phylogenetic proximity to isolates from Russia and Poland. Genotype 6 was identified in third of the samples during 2020-2021. But, in 2024, approximately 13% of sequences were identified as Genotype 6 (typically originating from the Far East).
- Recombination: Interestingly, all *tax* sequences clustered exclusively with Genotype 4, even in samples where the *env* sequence was identified as Genotype 6. This suggests a potential recombination event resulting in a unique local sub-strain.
- Virulence Markers: Four major variants of the Tax protein were identified. Specifically, the Y257C mutation was associated with samples from cows exhibiting clinical signs and disease progression.

The four identified variants were cloned into expression plasmids (with a 6xHis-tag) and successfully expressed in bovine MDBK cells. Given the link between the Y257C mutation and high proviral load, a real-time PCR-SNP (single nucleotide polymorphism) diagnostic method was developed to target this specific codon to identify high-virulence variants.

Conclusion: This work lays the foundation for future research into Tax-host protein interactions and the development of non-invasive tools to identify virulence-associated variants and host biomarkers. Standardizing these tests will provide veterinarians and breeders with vital tools to assess the probability of disease progression and implement informed management strategies.