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Circulating Bovine Leukemia Virus Cell-Free DNA as a Promising Biomarker for Enzootic Bovine Leukosis

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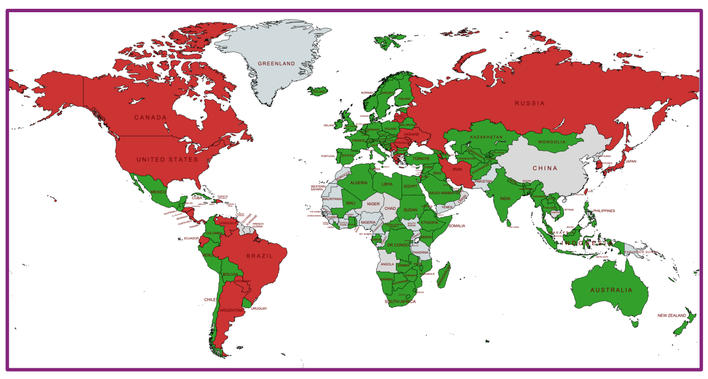
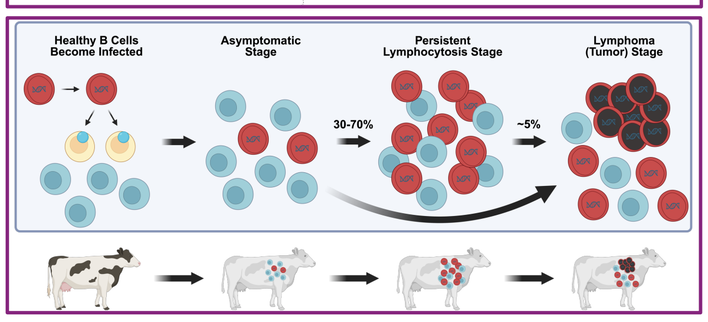
INTRODUCTION & AIM

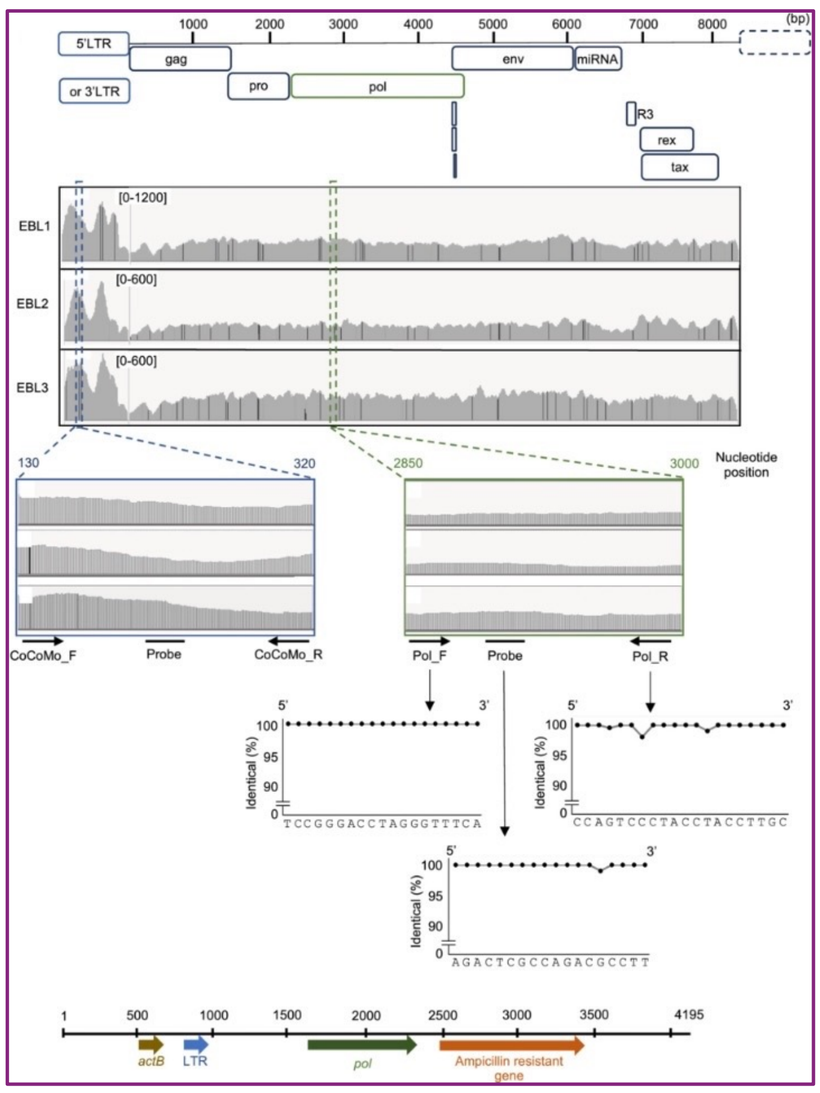


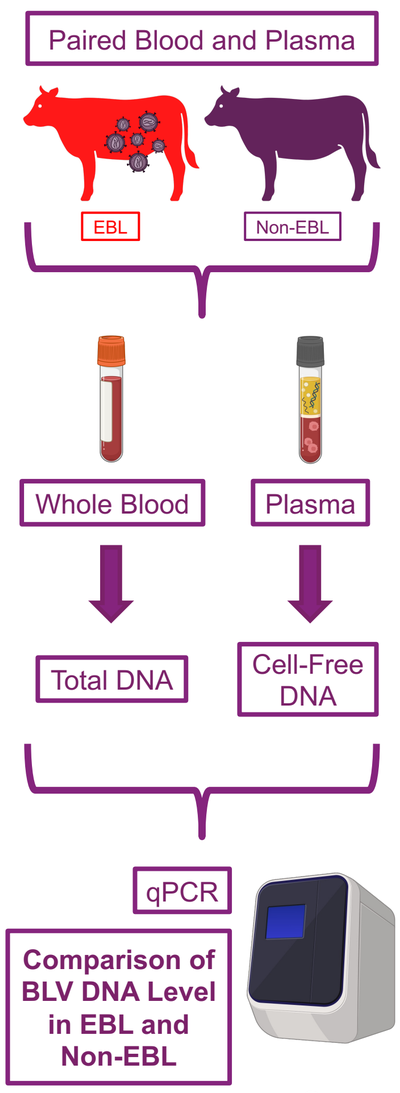


- 50 million cattle infected worldwide.
- No approved vaccines or antiviral drugs.
- Control Strategies:
  Biosecurity (quarantine, testing),
  Herd management, and
  Selective breeding.
- Current EBL diagnostics are invasive and time-consuming.
- Urgent need for non-invasive, rapid, and affordable diagnostic method to enable early detection and large-scale monitoring of EBL.
- Tumor cells release more cell-free DNA (cfDNA) due to higher turnover.
- cfDNA proviral load may distinguish EBL and non-EBL cattle.

- **Aim:**
  Development of an efficient cfDNA-based approach for improved diagnosis of EBL.

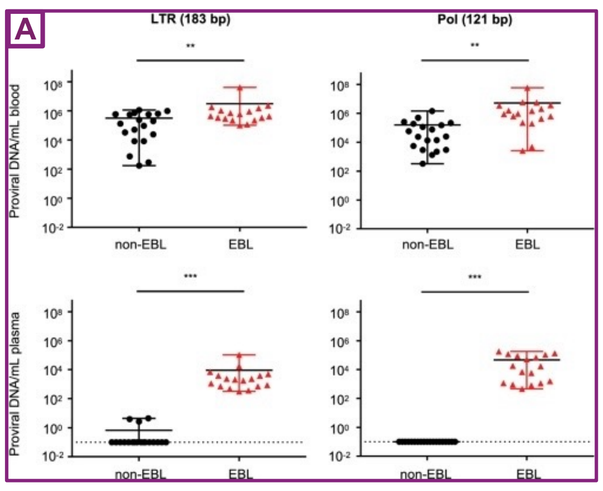
METHOD



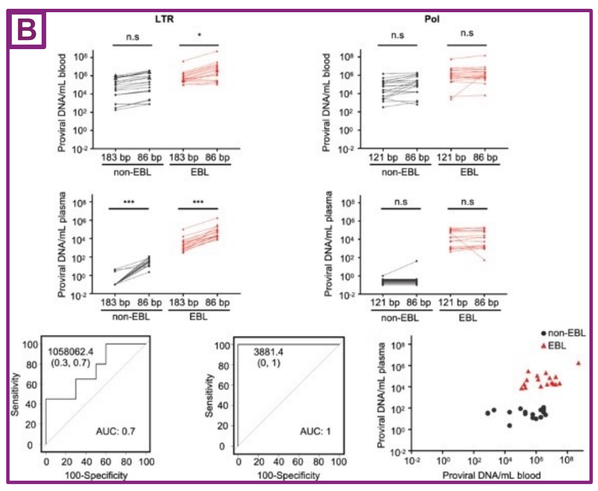


- DNA-capture sequencing of cfDNA from EBL cattle identified high-coverage regions for primer/probe design.
- *LTR* and *pol* regions selected for qPCR due to strong read density and sequence conservation.
- *Pol* target (121 bp): *Pol*_F (2878-2896), *Pol*_R (2978-2997), and Probe (2911-2928).
- Control plasmid constructed containing BLV *LTR*, *pol*, and bovine *actB* fragments as qPCR standards.

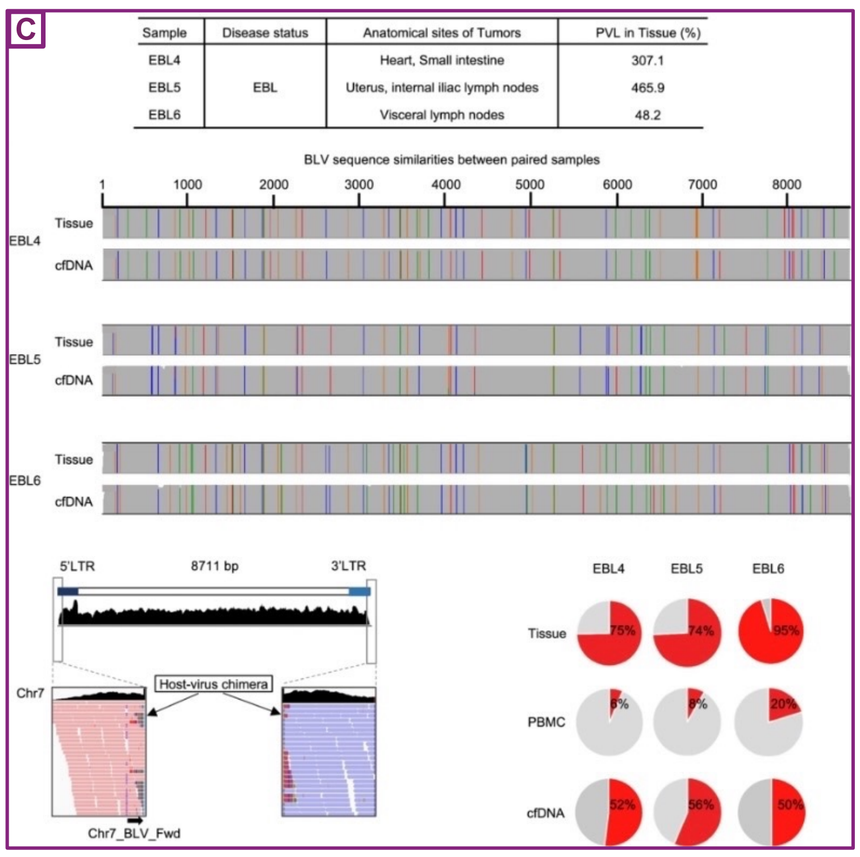
RESULTS & DISCUSSION



Comparison of Proviral Load in Whole Blood and Plasma



Comparison of Proviral DNA Detection Efficiency by Target Length



Comparative BLV Sequence Analysis in Tumor and cfDNA Samples

CONCLUSION

- cfDNA in plasma effectively distinguishes EBL cattle from non-EBL cattle, outperforming whole blood PVL measurements.
- Shorter PCR targets (86 bp *LTR*) improve detection sensitivity in cfDNA.
- cfDNA predominantly originates from tumour clones rather than PBMCs, reflecting malignant cell turnover.
- Plasma cfDNA shows 100% sensitivity and specificity for EBL detection, whereas whole blood shows lower accuracy.
- cfDNA-based testing could enable earlier, rapid, and cost-effective EBL diagnosis, reducing economic losses in cattle farms.
- Larger studies are needed to confirm the cfDNA origin and validate its use as a routine biomarker.

REFERENCES

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