GENETIC ANALYSIS OF CATTLE LICE AND THE DEVELOPMENT OF NOVEL MOLECULAR DIAGNOSTIC TOOLS TO MONITOR BURDENS AND TREATMENT EFFECTIVENESS

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1 INTRODUCTION

- Parasitic lice are a significant problem in beef and dairy cattle, especially in colder climates[1].
- Lice can cause irritation, anemia, impact hide quality, and decrease production[2], with economic losses estimated at $120 million annually in North America[3].
- Many recent anecdotal reports have mentioned increased hair loss, skin lesions and lice infestations in Western Canada and Northern USA. Current control relies heavily on endectocides such as macrocyclic lactones and insecticides such as pyrethroids[4], but their widespread use leads to concerns about drug resistance and environmental impacts[5].
- Urgent action is needed to assess changing lice populations, develop field-deployable diagnostic tools, support evidence-based control and reduce insecticide use.

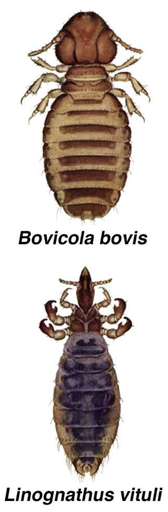

Figure 1: *Four species of cattle lice found in North America*

2 OBJECTIVES

The project aims to detect lice DNA from cattle skin swabs, with the goal of advancing diagnostic methods for lice infestations in cattle.

1: To develop 18S rDNA and COI mtDNA Illumina and Oxford Nanopore Technologies (ONT) to identify cattle lice species and characterize their genetic diversity.

2: To develop species-specific and "pan-louse" PCR and Loop-Mediated Isothermal Amplification (LAMP) assay to detect louse DNA on cattle skin swabs as a diagnostic and disease monitoring tool.

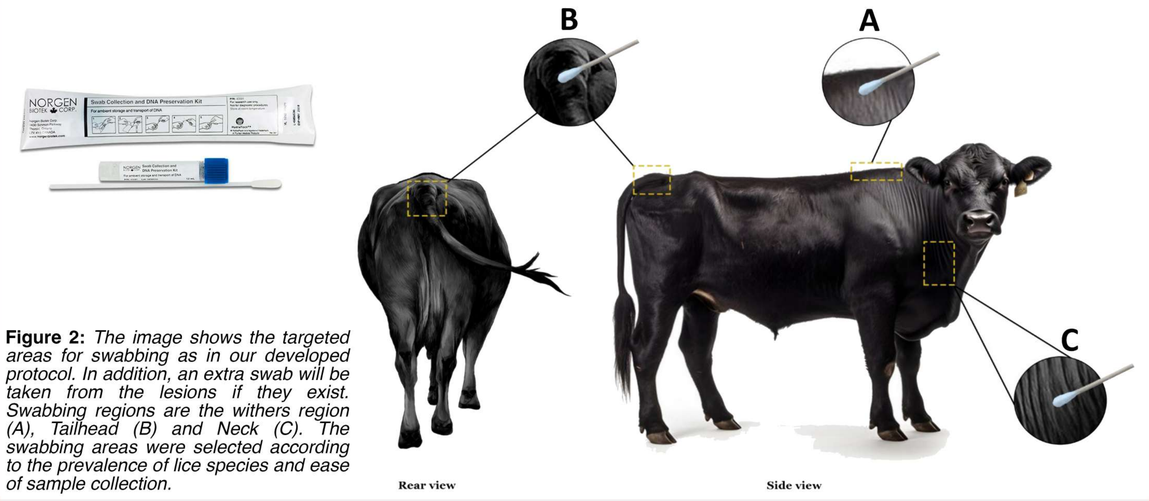

Figure 2: *The image shows the targeted areas for swabbing as in our developed protocol. In addition, an extra swab will be taken from the lesions if they exist. Swabbing regions are the withers region (A), Tailhead (B) and Neck (C). The swabbing areas were selected according to the prevalence of lice species and ease of sample collection.*

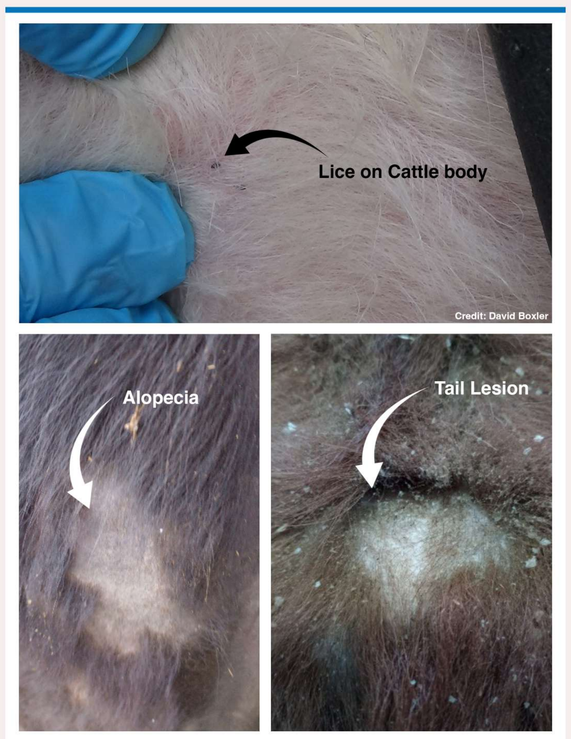

Figure 3: *Animal pictures showing lice on animal body, hairloss and lesions*

3 METHODOLOGY

- Genomic DNA extraction is performed from the collected lice specimens, followed by PCR to develop the 18S rDNA and COX-1 mtDNA Illumina and Oxford Nanopore Technologies (ONT) metabarcoding assays. Amplified Sequence Variants (ASVs) will be mapped against insect 18s rDNA and COX-1 reference sequence databases.
- We are developing Loop-Mediated Isothermal Amplification (LAMP) diagnostic tool employing the WarmStart® LAMP Kit (NEB) with FAM dye and quantifying fluorescent signal on a Thermo Fisher QuantStudio 5. LAMP Primer sets are designed using Primer Explorer V5 and the NEB primer design tool. The LAMP protocols are first developed on adult lice DNA and then tested for their ability to detect louse DNA from skin swabs taken from infected cattle.

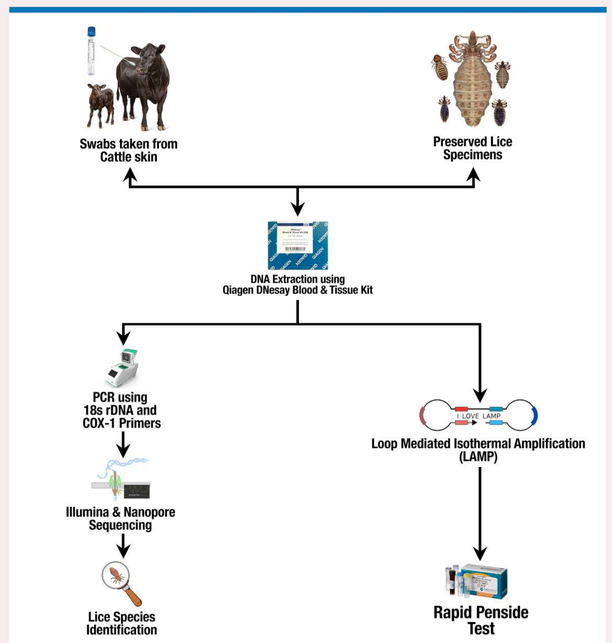

Figure 4: *Diagram illustrating methodology*

4 RESULTS

- The results show that we can detect the Lice DNA from the cattle skin swabs. We detected the presence of two major lice species: *Linognathus vituli* and *Bovicola bovis* from the swabs taken from 9 different herds.
- LAMP assay has been developed and optimized. Species-specific primer sets (PE1) for *L. vituli* and (PE3) for both *L. vituli* and *B. bovis* DNA have been developed.

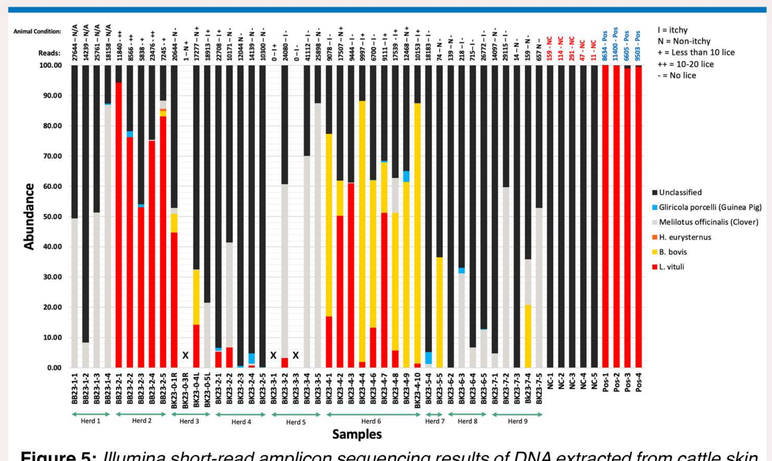

Figure 5: *Illumina short-read amplicon sequencing results of DNA extracted from cattle skin swabs taken from 9 different herds. In Herd 2 and Herd 3, a high prevalence of L. vituli was found, while in Herd 6, a high prevalence of B. bovis was present. All the other herds were negative.*

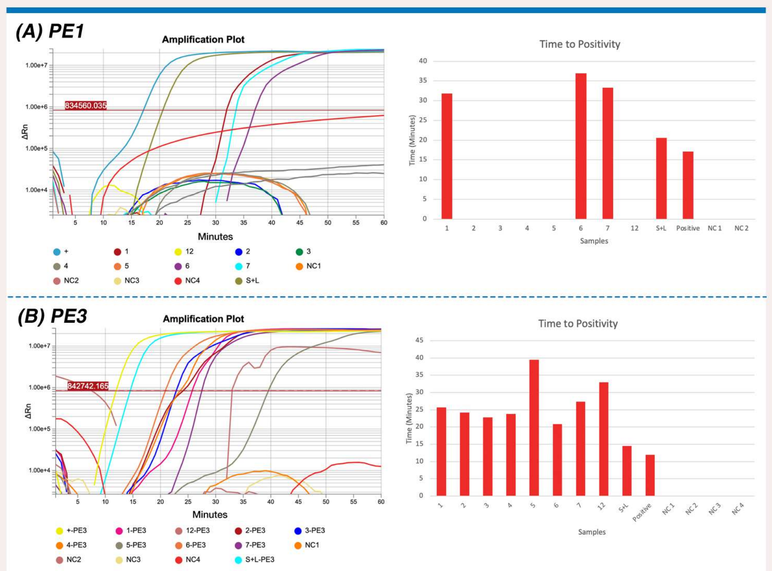

Figure 6: *Real-time LAMP (Rt-LAMP) results of cattle swab samples* ***(A)*** *PE1 Lamp primer set was used.* ***(B)*** *PE3 Lamp primer set was used. First the DNA was extracted, and then we used the NEB Warmstart LAMP kit with FAM dye and quantified using QuantStudio 5.*

5 CONCLUSION

This study provides evidence on the detection of lice DNA from cattle skin swabs through both PCR and LAMP. Lice DNA extracted from skin swabs allowed rapid detection via real-time LAMP within a 30-minute incubation period. This information and novel diagnostic tools should allow more targeted insecticide use and improve animal health and welfare while reducing the environmental impacts.

REFERENCES

[1]. Munro JA, 1943.
[2]. Bilkis M, 2011
[3]. Byford RL, 1992
[4]. Wells B, 2012
[5]. Briggs LL, 2006