



Novel mRNA isoforms identified in abomasal lymph node tissue of sheep affected by *Teladorsagia circumcincta* gastrointestinal nematode

Krishani Sinhalage, Stephanie Lam, Victoria Asselstine, Angela Cánovas

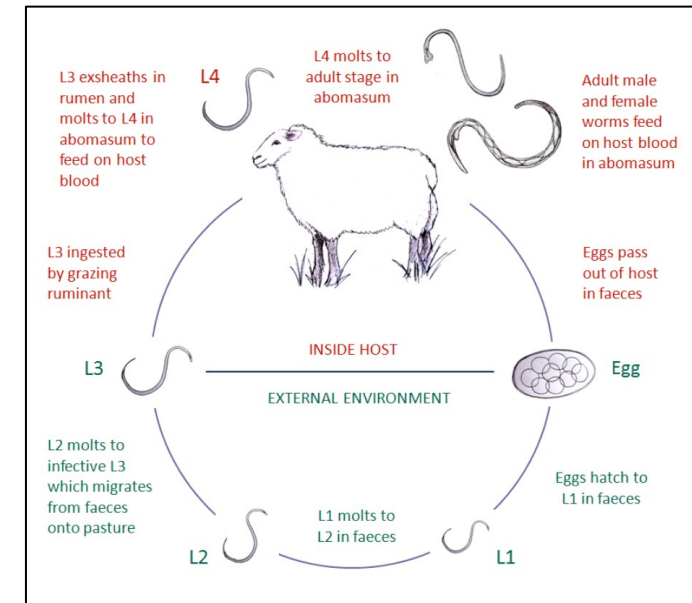
Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada



Introduction

Gastrointestinal Nematode (GIN) Infections

- Major problem affecting the sheep industry
- Infect when grazing contaminated pasture
- Mixture of nematode species that infect
 - abomasum and intestine
- ***Teladorsagia circumcincta* (TC)**
 - Pathogenic nematode
 - Infects **abomasum**
 - Causes **diarrhea, weight loss, poor productivity, and death** in infected animals in severe cases



Source: <http://www.farmanimalhealth.co.uk>

Introduction

Gastrointestinal Nematode (GIN) Infections

Animal health and welfare issues

Reduced sheep
productivity

Increased treatment
and management costs

High economic
losses

Quality of

- wool
 - skin
 - milk
 - meat
- production

Introduction

How to Control GIN Infection?

- Treatment with anthelmintics
- Pasture management



- Anthelmintic resistance (AR) in parasites
- Residues of anthelmintics
- AR is heritable in nematodes



Source: <https://www.vetsouth.co.nz>

Sustainable approach:

- **Genetic selection for sheep with resistance to GIN**
(resistance to GIN $h^2 \sim 0.3$)

Objectives

To identify **differentially expressed mRNA isoforms** via alternative splicing to understand **the genes and mechanisms involved in protective immunity/ resistant phenotype** and the **factors that regulate** these processes using large gap read mapping

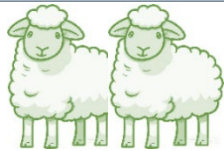
Materials and Methods

RNA Sequencing and Experimental Design

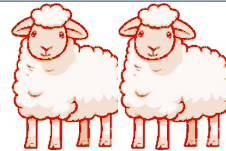
RNA-seq data from **NCBI GEO database** (GSE63547)

Experimental Design

Pre-selected **Scottish Blackface lambs** (n=20) based on fecal egg count (**FEC**)



Low FEC/ LFEC (n=10)
(Most resistant)



High FEC/ HFEC (n=10)
(Most susceptible)

Artificial challenge of *Teladorsagia c.* L3

Abomasal lymph nodes



RNA-Seq

At 7 dpi



(n=5) (n=5)

At 14 dpi



(n=5) (n=5)



(*dpi –days post infection)

Transcripts Discovery

RNA-seq data from NCBI GEO database (GSE63547)



Quality control (CLC Genomic Workbench)



Sequence reads aligned to **Oar_rambouillet_v.3**
ovine reference genome



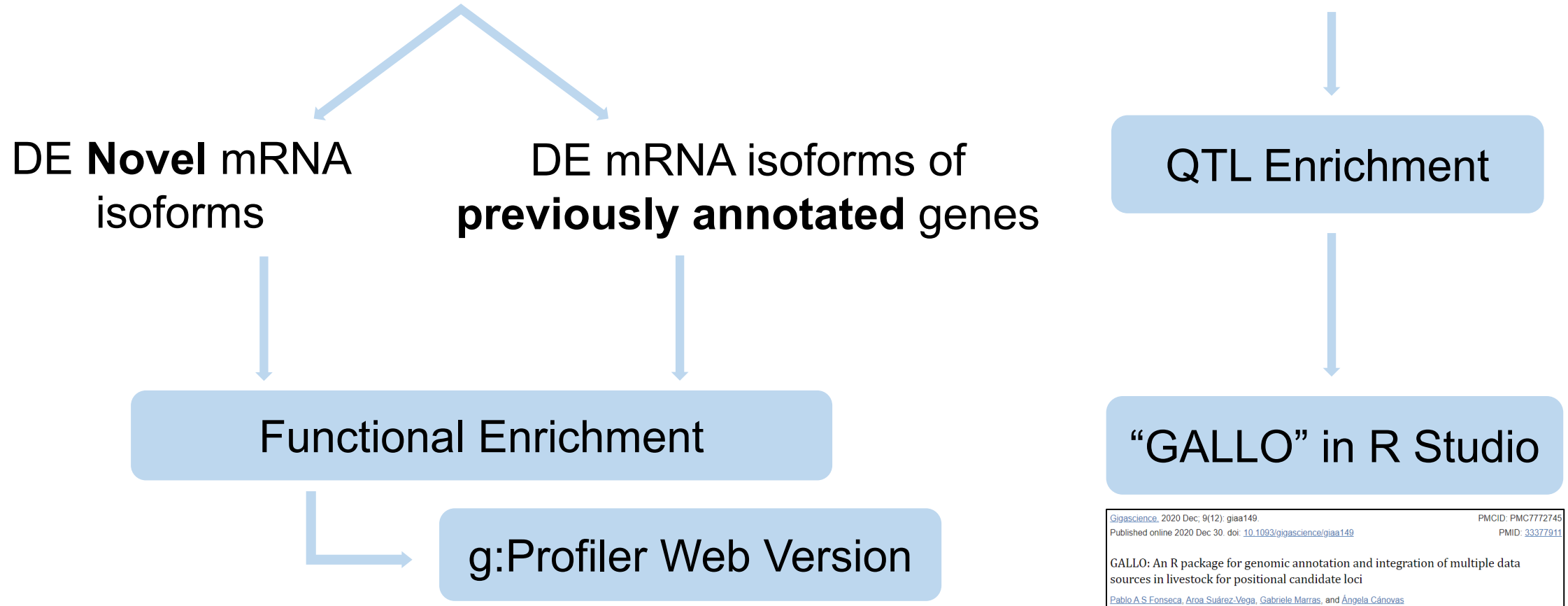
Large gap read mapping de novo assembly



Differential mRNA isoform expression analysis

Functional and QTL Enrichment Analyses

Differentially Expressed (DE) mRNA Isoforms **between LFEC and HFEC lambs at 7 dpi and 14 dpi** (P-value < 0.05; Fold change > |2|)



Results and Discussion

Differentially Expressed (DE) mRNA isoforms between LFEC (control) and HFEC Scottish Blackface lambs

7 days post-infection (7 dpi)

Upregulated	Total	Downregulated
52	103	51



- **82** → annotated in the ARS-UI_Ramb_v3.0 reference genome
 - **44** → novel lengths
- **21** → novel mRNA isoforms with novel lengths of non-annotated genes

14 days post-infection (14 dpi)

Upregulated	Total	Downregulated
63	95	32



- **76** → annotated in the ARS-UI_Ramb_v3.0 reference genome
 - **42** → novel lengths
- **19** → novel mRNA isoforms with novel lengths of non-annotated genes

DE mRNA isoforms of previously annotated genes between LFEC and HFEC Scottish Blackface lambs

7 DPI

82 DE mRNA isoforms

33 Upregulated Genes

34 Downregulated Genes

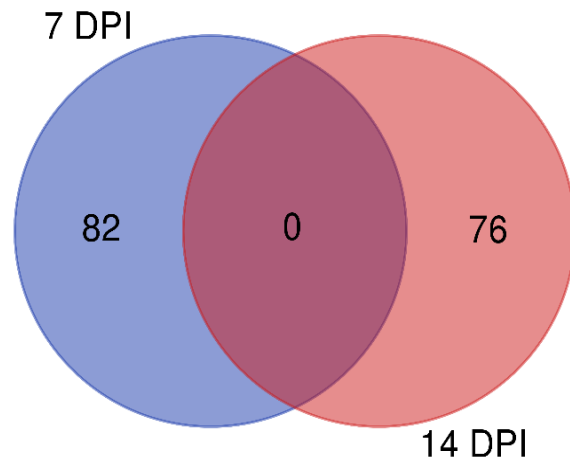
14 DPI

76 DE mRNA isoforms

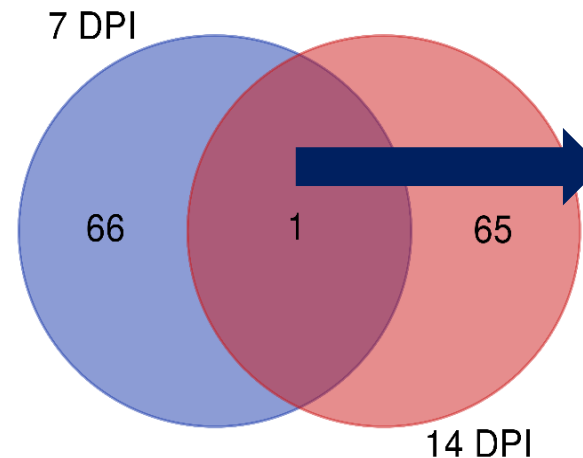
46 Upregulated Genes

21 Downregulated Genes

Overlapping DE mRNA isoforms



Overlapping DE Genes



U6 spliceosomal RNA:

Coordinates the magnesium ions required for splicing chemistry

Functional Enrichment: DE mRNA isoforms of previously annotated genes

7 dpi upregulated genes

Biological Processes	No. of Genes (NG)	FDR Value
Regulation of cellular metabolic process	11	2.221×10^{-5}
Glial cell differentiation	1	4.063×10^{-2}
Negative regulation of apoptotic process	3	4.3×10^{-2}

7 dpi downregulated genes

JAK3 (Tyrosine-protein kinase)
IL4R (Interleukin 4 receptor)
BCL2L1 (Bcl-2-like protein 1)
ZNF782 (Zinc Finger Protein 782)

Biological Processes	NG	FDR Value
Regulation of T-helper 2 cell differentiation	2	1.997×10^{-3}
T cell activation	2	3.624×10^{-3}
Interleukin-4-mediated signaling pathway	2	2.140×10^{-2}
Zinc-finger protein	2	6.5×10^{-2}

g:Profiler
LFEC vs HFEC

Functional Enrichment: DE mRNA isoforms of previously annotated genes

g:Profiler

LFEC vs HFEC

14 dpi upregulated genes

Biological Processes	NG	FDR value
Regulation of chemokine ligand 4 production	2	4.881×10^{-2}
Interferon binding	2	1.174×10^{-2}
Antigen processing	3	4.716×10^{-2}

14 dpi downregulated genes

Biological Processes	NG	FDR value
Adipocytokine signaling pathway	2	1.807×10^{-2}
Th17 cell differentiation	2	1.807×10^{-2}

DE Novel mRNA isoforms and their predicted genes

7 dpi LFEC vs HFEC

*Predicted gene using 'BLAST'

Feature ID	Biological Processes	FDR value
Gene_1744_2	Lysine degradation	3.429×10^{-2}
Gene_1970_1		
Gene_2399		
Gene_278_4	Regulation of cell morphogenesis	4.396×10^{-2}
Gene_406_3		
Gene_851_4	Biological Processes	FDR value
Gene_7883_7	Cardiac muscle hypertrophy in response to stress	1.486×10^{-5}
Gene_9913_3		
Gene_3247_10	Muscle hypertrophy in response to stress	1.486×10^{-5}
Gene_4943	Regulation of blood circulation	2.632×10^{-3}

DE Novel mRNA isoforms and their predicted genes

14 dpi LFEC vs HFEC

*Predicted gene using 'BLAST'

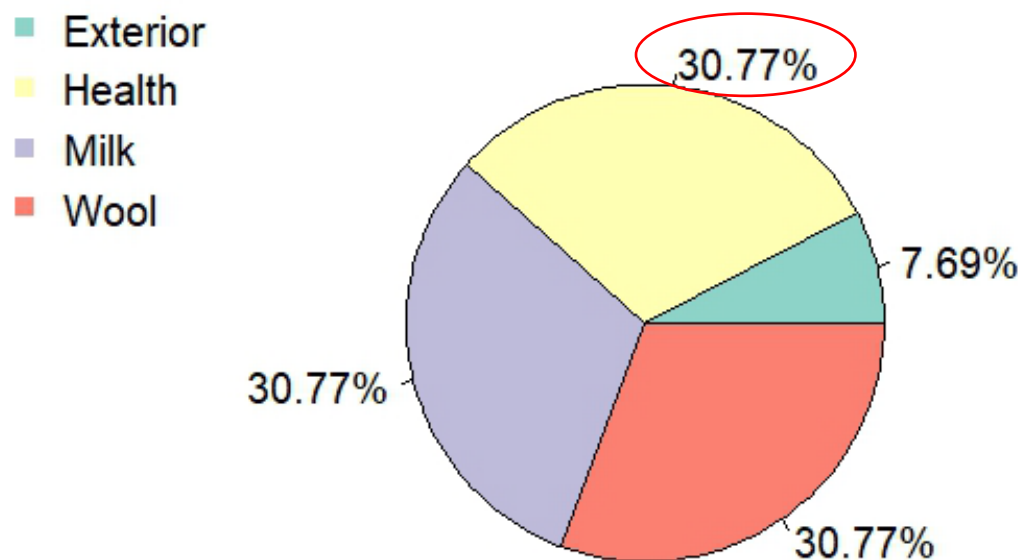
Feature ID	Biological Processes	FDR value
Gene_5121_3	Regulation of CD8-positive, alpha-beta T cell proliferation	2.603×10^{-2}
Gene_2024	Regulation of toll-like receptor 7 signaling pathway	2.603×10^{-2}
Gene_2263_1	Interferon-alpha production	3.982×10^{-2}
Gene_4230_7		
Gene_4732_1		
Gene_634_1	Regulation of intracellular signal transduction	1.521×10^{-2}
Gene_1177_9	Positive regulation of cell communication	1.521×10^{-2}
	Lipid metabolism	9.592×10^{-3}

QTL Enrichment

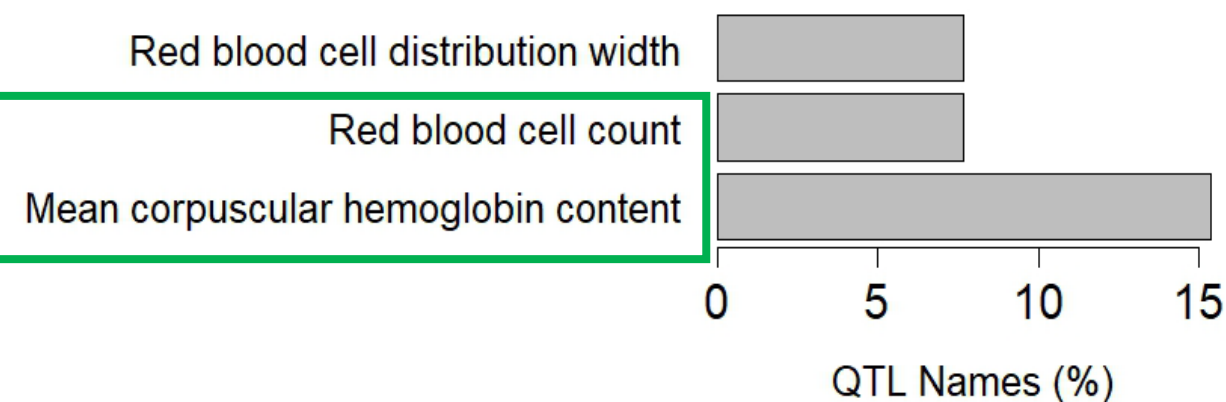
GALLO in R Studio

Gigascience, 2020 Dec; 9(12): giaa149. PMID: 33377911
Published online 2020 Dec 30. doi: 10.1093/gigascience/giaa149
GALLO: An R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci
Pablo A S Fonseca, Aroa Suárez-Vega, Gabriele Marras, and Ángela Cánovas

7-Days Post-infection LFEC vs HFEC



Percentage of DE mRNA isoforms co-localized with QTL types

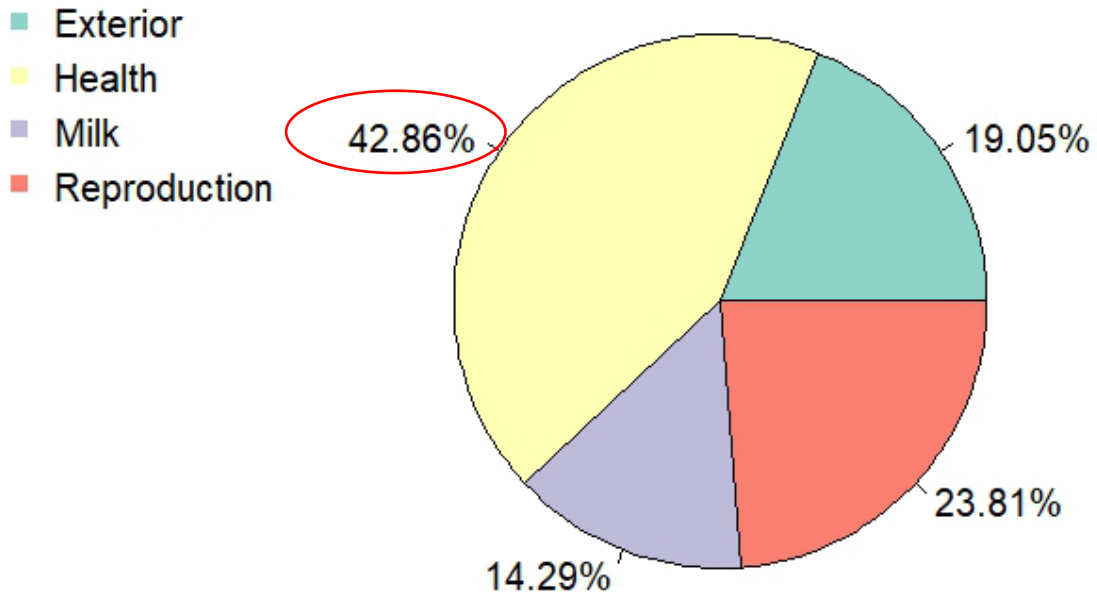


Health QTL types represented 3 health traits

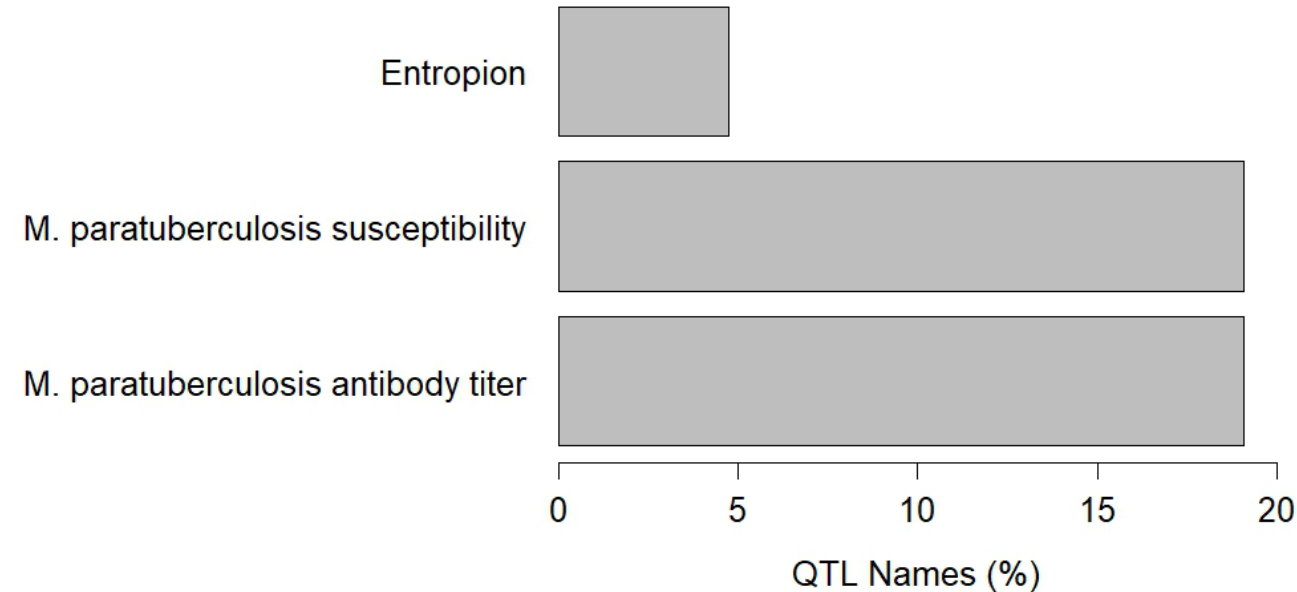
QTL Enrichment

GALLO in R Studio

14-days post-infection LFEC vs HFEC



Percentage of DE mRNA isoforms co-localized with QTL types



Health QTL types represented 3 health traits

Conclusions

- **DE novel mRNA isoforms** were identified between resistant (LFEC) and susceptible (HFEC) lambs at 7 dpi and 14 dpi.
- **Functional enrichment analysis** demonstrated,
 - Significantly enriched **upregulated biological processes** → primarily **responsible for cellular physiological events**, including **immune response-associated factors** in susceptible sheep.
 - **Susceptible sheep** showed **delayed immune responses over time**
- **QTL enrichment analysis** identified,
 - DE mRNA isoforms co-localized with **health QTL types**, and the health QTLs identified at 7 dpi represent **gastrointestinal nematode infection** associated health traits

Acknowledgement



**CANADA
FIRST**
RESEARCH
EXCELLENCE
FUND

**APOGÉE
CANADA**
FONDS
D'EXCELLENCE
EN RECHERCHE



**UNIVERSITY
of GUELPH**

**FOOD
FROM
THOUGHT**



Cánovas Lab



At the Red Rose farm; 747710 Township Road 4, Drumbo, Ontario, N0J 1G0



Krishani Sinhalage
ksinhala@uoguelph.ca