

Identification of functional INDELs responsible for splice site effect in acute stress response grazing sheep exposed to gastrointestinal nematode infection

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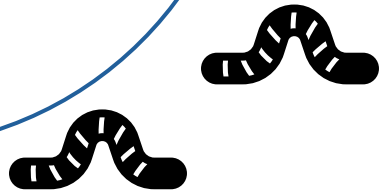
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Gastrointestinal nematode (GIN)



- **Gastrointestinal nematodes (GINs)** can infect grazing sheep and cause **morbidity** and **mortality**.
- GIN can also bring massive **economic losses** due to **production losses** and **treatments**.

McLeod, 1995; Saddiqi et al. 2012; Galyon et al. 2020.

Overreliance on anthelmintics



- Overreliance on **anthelmintics** to treat infected animals has led to **GIN resistance**.
- Increased concerns over the **residue** of drugs in the **food** and **environment**.

Khusro et al. 2004; Saddiqi et al. 2012; Falzon et al. 2013 Sallé et al. 2021

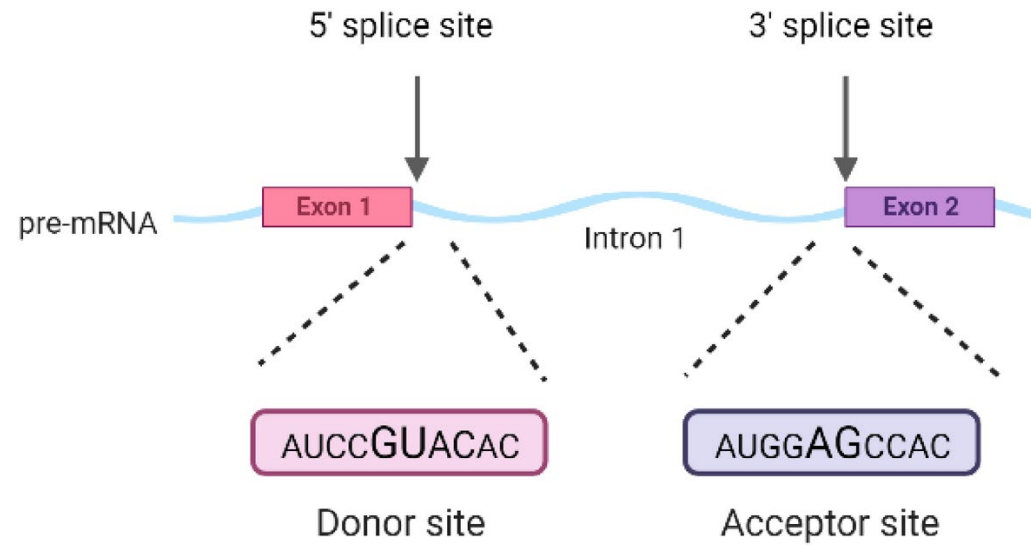


Immune response

- **Several studies** have been performed using **different** types of **data** and **methodologies**.
- The **immune response** to GIN infection in sheep is a **complex** and **polygenic** trait.
- Identify **variants** responsible for **functional consequences** in sheep with **different responses** to **acute inflammatory stress**.

You et al. 2008; Benavides et al. 2016; Lam et al. 2020

Splice Site



Adapted from Zabardast et al. 2023

- Regions responsible for **signaling** specific **places for cleavage of introns**, located at the **boundary of exons and introns**.
- **Creation** or **deactivation** of splice sites.
- **Exclusion** of **exon** and **inclusion** of **introns**.
- Change the **expression ratio** between mRNAs isoforms.

De Conti et al. 2013

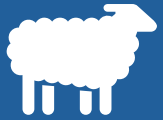
Objective



Identification of short insertion and deletions (**INDELs**) responsible for possible **splice site effect** in Rideau x Dorset crossbred sheep with **different immune profiles** under **natural exposure** to **gastrointestinal nematodes** using **RNA-Sequencing**.



Material and Methods



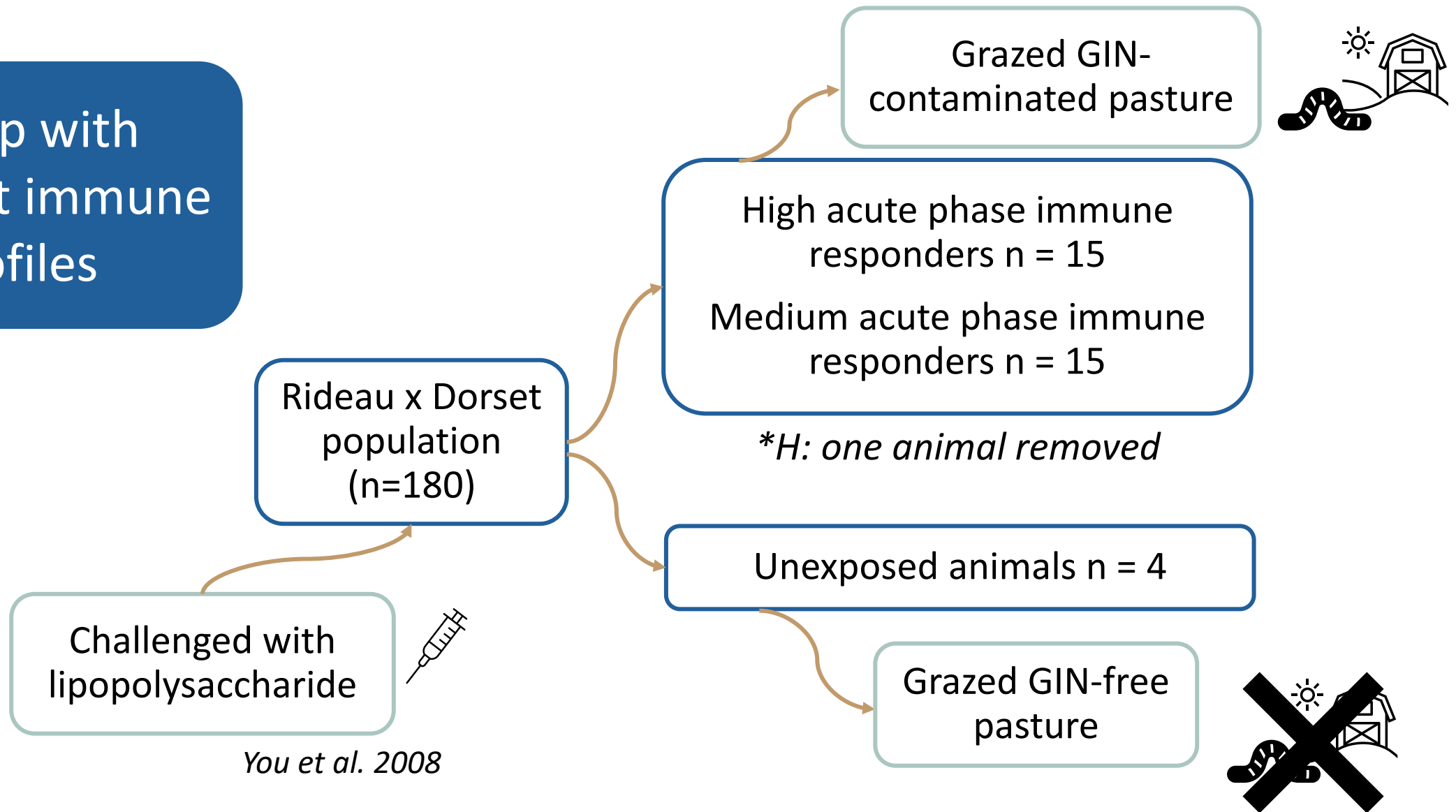
Sheep with different immune profiles

Tissue sampling

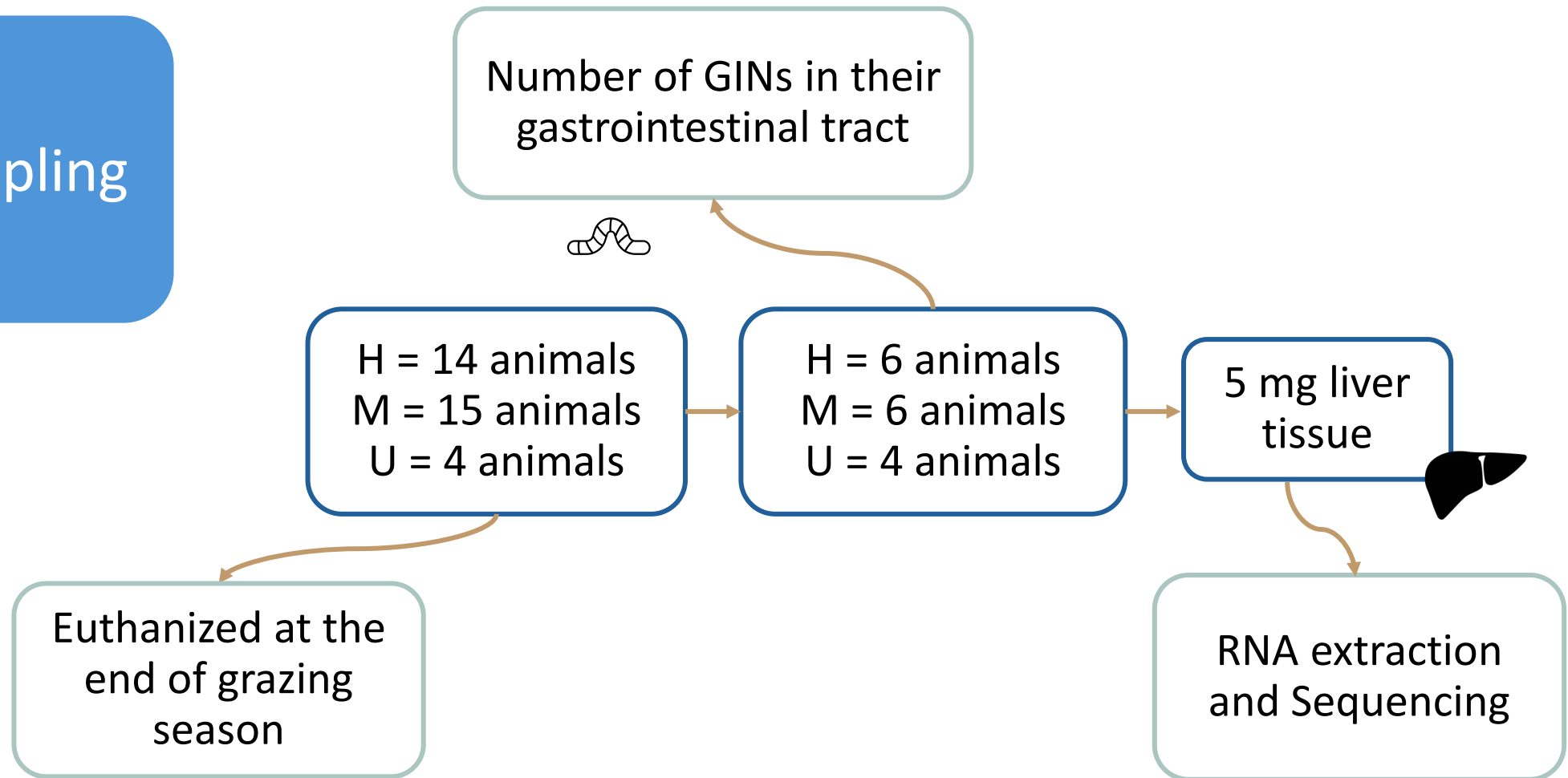
Variant Identification

Functional Annotation

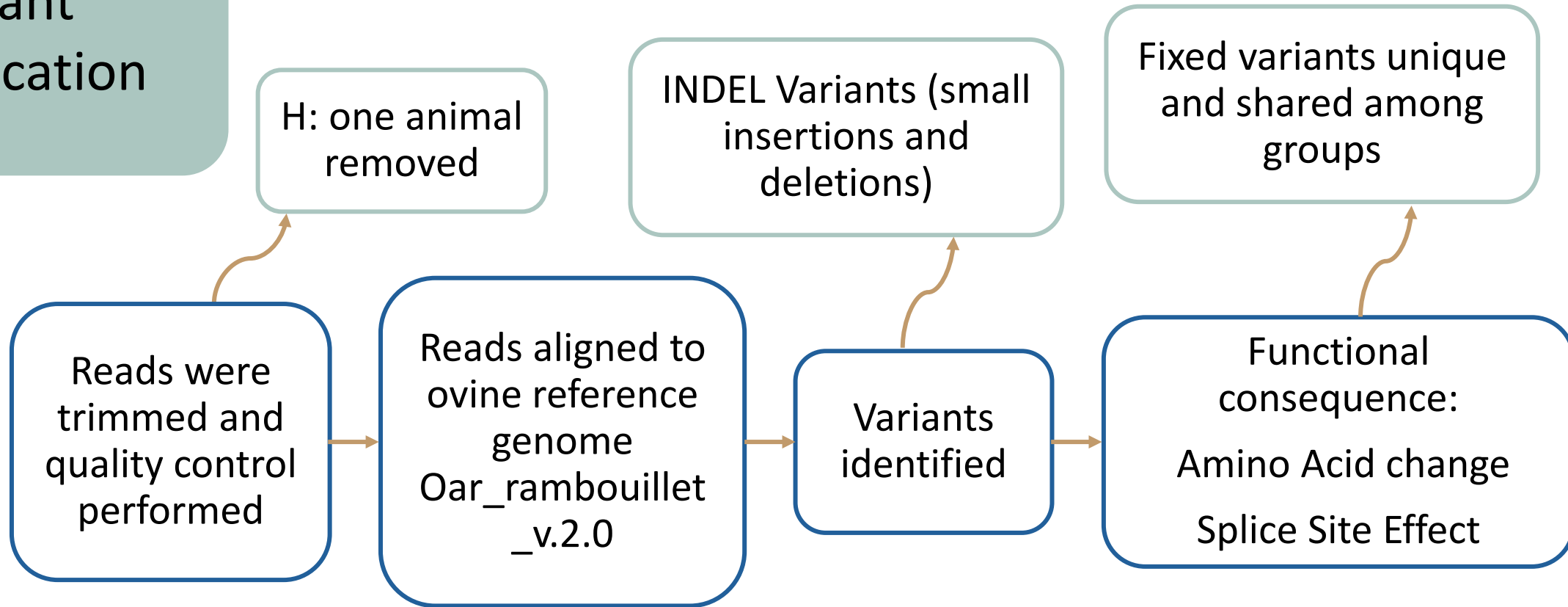
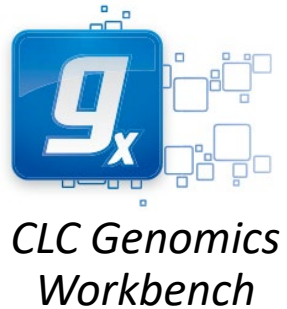
Sheep with different immune profiles



Animal Sampling



Variant Identification



Functional Annotation

INDEL Variants

Annotation of genes associated with each INDEL

e!Ensembl

Functional analysis:
Enriched metabolic pathways

 reactome

Results and Discussion



Variants

Genes

Metabolic Pathways

Variants

Table 1. Number total of INDEL variants, INDELs that led to amino acid change (AAC) and to splice site effect (SSE) unique and shared among groups

Group	Variants	AAC	SSE
H	34,168	1,938	1,614
M	39,380	2,026	2,980
U	19,610	1,414	1,396
H/M/U	27,032	1,587	1,777
H/M	12,531	571	719
H/U	5,381	307	376
M/U	8,301	405	558
Total	146,403	8,248	9,420

H: High-stress responders; M: Medium-stress responders; U: control group not exposed to gastrointestinal nematodes.

Variants

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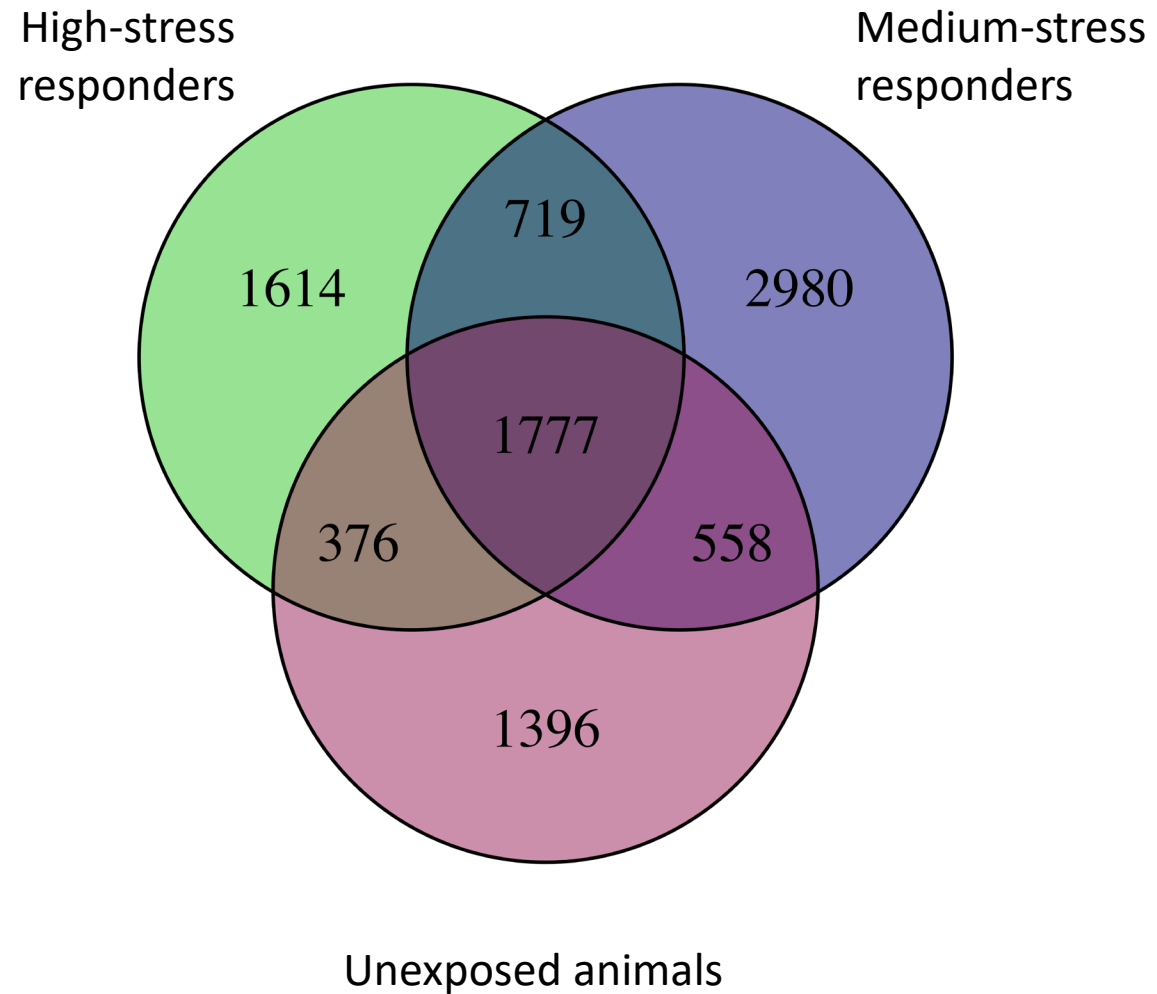


Figure 1. Number of INDELs identified

Variants

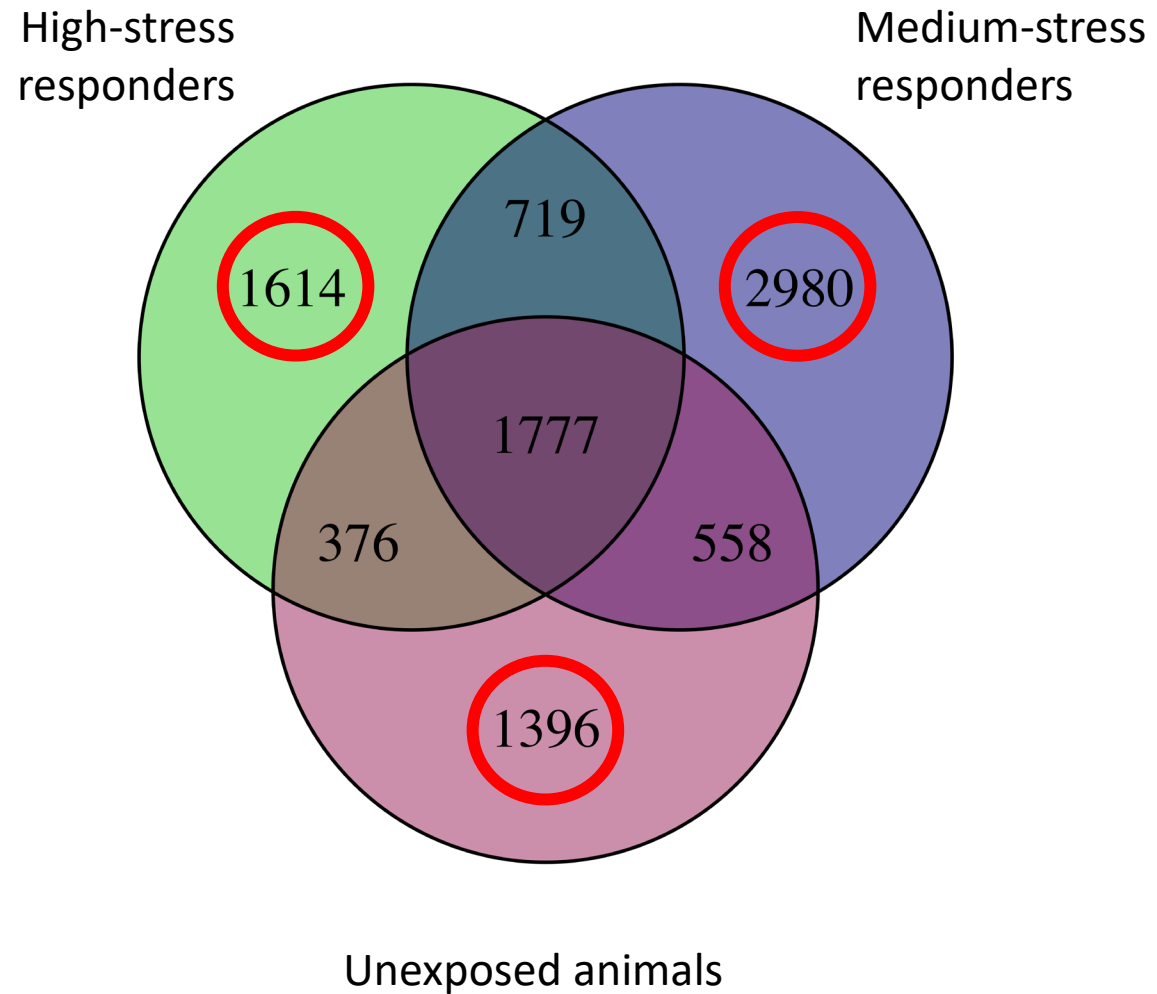
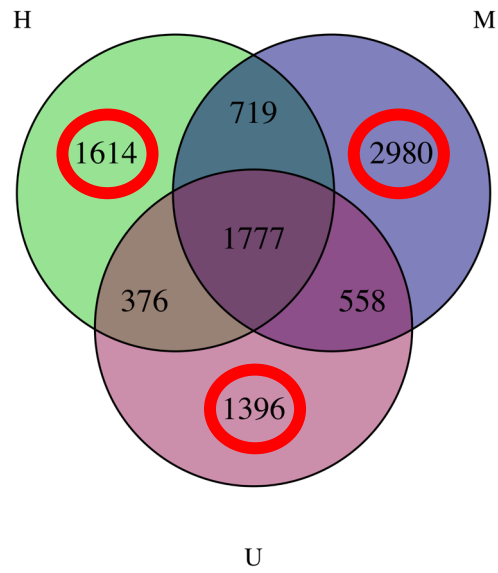
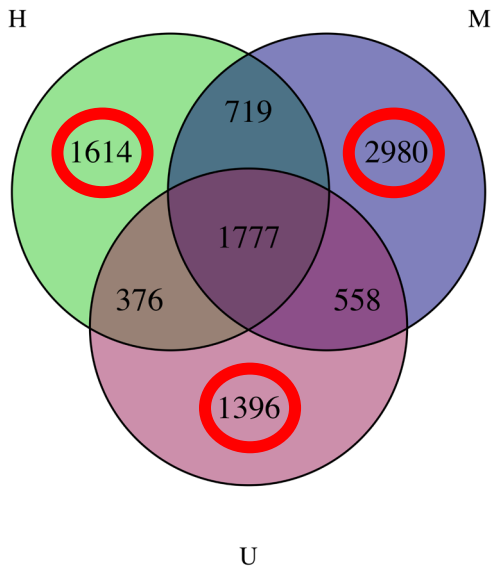


Figure 1. Number of INDELs identified

Genes

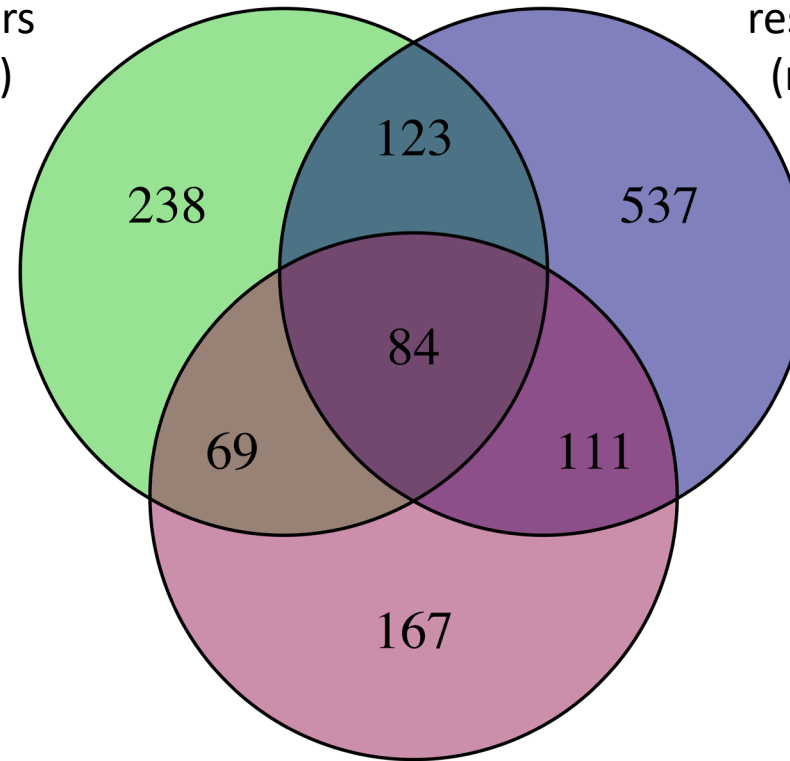


Genes



High-stress
responders
(n = 514)

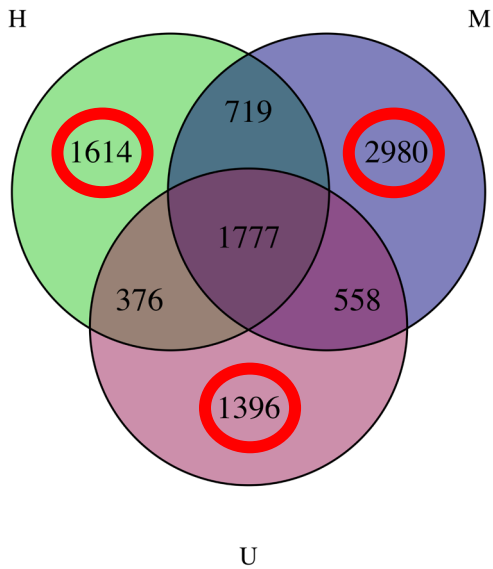
Medium-stress
responders
(n = 855)



Unexposed animals
(n = 431)

Figure 2. Number of genes identified

Genes



High-stress
responders
(n = 514)

Medium-stress
responders
(n = 855)



Unexposed animals
(n = 431)

Figure 2. Number of genes identified

Genes

CFI

- The **complement system** is one of the **first responses** to be activated in a **nematode infection**.
- **Up-regulated in resistant lambs** in different studies when compared to susceptible and control groups.
- Identified in genome-wide association studies associated with traits **measuring resistance to nematode**.

Alba-Hurtado and Muñoz-Guzmán 2013; Guo et al. 2016; Toscano et al. 2019; Zhang et al. 2019; Estrada-Reyes et al. 2019; Niciura et al. 2022

Genes

LBP

- Encodes the Lipopolysaccharide Binding Protein that plays an important role in the **acute-phase immune response to gram-negative bacteria**.
- **LBP blood concentration** was significantly different between **uninfected and infected animals**.
- **LBP** can be used as a **biomarker in *H. contortus* infection**.

Bernheiden et al. 2001 and Zhong et al. 2014

Genes

STAT6

- *STAT6* gene is responsible for several **T-cell responses**, the **development of Th2 response**, and the **release of Interleukin-4 (IL-4)**.
- **IL-4** is a major cytokine for the **activation and expansion of the Th2 response**.
- Th2 response is the **primary adaptative immune response in helminth infection in sheep**.

Goenka & Kaplan, 2011; McRae et al. 2015; Stear et al. 2023

Functional analyses

Group	Pathway name	TGV	TGP	FDR ($p < 0.05$)
M	Cross-presentation of soluble exogenous antigens (endosomes)	14	53	0.001
M	Downstream signalling events of B Cell Receptor (BCR)	18	91	0.002
M	NIK-->noncanonical NF-kB signalling	14	61	0.003
M	Activation of NF-kappaB in B cells	15	72	0.003
M	Dectin-1 mediated noncanonical NF-kB signaling	14	66	0.004
M	ER-Phagosome pathway	18	106	0.007
M	Antigen processing-Cross presentation	20	128	0.008
M	TNFR2 non-canonical NF-kB pathway	16	104	0.028
M	Interleukin-1 signaling	18	125	0.029
M	Terminal pathway of complement	4	8	0.037
M	CLEC7A (Dectin-1) signaling	17	120	0.042

Functional analyses

Nuclear Factor Kappa B (NF- κ B) related process

- **NF- κ B** is a family of transcription factors that **regulate** different processes in the **innate and adaptive immune systems**.
- Effective participation of **NF- κ B** in **mice's inflammatory response to nematode infection**.
- Playing roles in the regulation of **macrophage-killing capabilities**.

Artis et al. 2002; Baska & Norbury, 2022

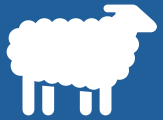
Functional analyses

Cross-presentation of antigen

- It is an immunological process **not well characterized**.
- Cross-presentation is a **mechanism** used by **antigen-presenting cells** (APCs) when they **encounter extracellular antigens**.
- **Nematode antigens** are **presented by APCs** to naïve T cells, leading to their differentiation.

Amigorena and Savina, 2010; Joffre et al. 2012; Karrow et al. 2014; McRae et al. 2015

Take Home Messages



- Several **INDELs** and **genes** were identified for **high- and medium-acute phase immune responders** and **GIN-Unexposed** groups.
- The ***CFI*, *LBP*, and *STAT6*** genes seems to play important roles in the inflammatory response to GIN infection.
- Enriched **metabolic pathways** associated with **NF-κB, cross-presentation of antigen**, and the **complement system** were identified.
- This study brought **additional information** to understand the complex **immune response to GIN in sheep**.

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Cánovas Lab [UofG] and Delma Kennedy [OMAFRA] at the Breezy Ridge Farm, April 2022.



**CANADA
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FOOD FROM THOUGHT



Thank you!

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