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RNA-Sequencing in Elucidating Immune Responses to *Haemonchus contortus* Infection in Small Ruminants: Systematic Review

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ABSTRACT

Haemonchus contortus poses a major threat to small ruminant production in subtropical regions worldwide. Unfortunately, there is growing anthelmintic resistance, and the only licensed vaccine has limitations. This paper aims to review the use of RNA-sequencing in understanding the immune responses of small ruminants to *H. contortus* infection, focusing on identifying differentially expressed genes and elucidating key immune pathways associated with resistance and susceptibility. This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement guidelines and Population, Intervention, Control, and Outcome framework covering publications from January 2015 to December 2023. RNA-seq identified the activation of key immune pathways, such as Th1/Th2, NK cell, B cell receptor signalling, MAPK, CAMs, and TNF signalling. There was upregulation of a range of PRRs, including TLRs and CLECs, in the resistant sheep, suggesting a crucial role for trained innate immune cells in resistance. However, there are no direct comparisons of TLR and CLEC expression between resistant and susceptible goats. This shows that there is a gap in understanding of the immune response mechanisms in goats. Addressing these knowledge gaps will lead to the development of more effective and sustainable control strategies.

1 | Introduction

The blood-sucking gastrointestinal nematode (GIN) *Haemonchus contortus* poses a significant threat to small ruminants, leading to severe economic losses in tropical and subtropical regions worldwide [1]. Traditionally, anthelmintics have been the primary method of controlling *H. contortus* infection. However, the incorrect and overuse of these drugs has led to increasing instances of drug resistance [2, 3]. Wirevax, also known as Barbervax in Australia and the United Kingdom, is currently the only registered vaccine for *H. contortus* in South

Africa. Despite its approval for use in sheep, the vaccine requires multiple doses for optimal efficacy (<http://www.barbervax.com.au> accessed July 19, 2024) and does not provide sufficient protection for goats [4, 5]. Therefore, there is an urgent need for additional control strategies effective for all small ruminants affected.

Many factors are involved in the *H. contortus* hosts' immune response mechanisms. *H. contortus* infections typically elicit a type 2 immune response, which is crucial for combating parasitic infections [6–8]. The follicular T helper cells (Thf) play a

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role in promoting the Th2 immune response, which is characterised by the production of interleukin (IL)-4 and IL-5. Moreover, Thf cells facilitate the formation of germinal centers (GCs) where they interact with B cells, enhancing their proliferation and class-switch recombination, allowing them to produce other classes of immunoglobulins. They migrate to B cell follicles in response to chemokines, particularly CXCL13, and express key markers such as CXCR5 and PD-1, which are important for their localization and function within GCs [9]. Thf cells are also responsible for the differentiation and activation of B cells into memory B cells as well as antibody-secreting plasma cells [10, 11].

IL-4 is essential for the differentiation of naive T cells into Th2 cells and promotes the class switching of B cells to produce immunoglobulin E (IgE) [12]. IL-5 plays a key role in the growth and differentiation of eosinophils, which are important for combating parasitic infections [13]. IL-13, similar to IL-4, contributes to B cell differentiation and mucus production, enhancing the expulsion of parasites from the gastrointestinal tract [14].

Despite these insights, the detailed mechanisms and pathways involved in disease resistance and host immune response against *H. contortus* remain poorly understood [13], especially in goats. Given the high prevalence of *H. contortus* in goats in southern Africa [15], it is concerning that most immune response studies have focused on sheep. Research has shown that goats deploy different host immune response strategies to sheep in combating parasitic infections [14, 16]. Understanding the genetic and biological mechanisms underlying these responses in goats is essential for identifying direct genetic markers that can be used in developing sustainable disease management practices.

High throughput technologies such as microarrays and RNA-sequencing (RNA-seq) are pivotal in studying these immune response mechanisms. RNA-seq has emerged as a comprehensive, and precise tool for identifying new genes and transcripts and measuring transcript expression in a single assay [17]. This technique has provided detailed insights into gene expression changes; and tissue and cell-specific events during infection using an untargeted approach over the years [18–20]. Therefore, RNA-seq offers a promising technique for understanding the immunological pathways and mechanisms responsible for triggering the immune response against *H. contortus* in goats [18]. Whole-transcriptome analysis through RNA-seq can reveal the mechanisms responsible for complex traits such as resistance to gastrointestinal nematode (GIN) infections [17, 21].

However, existing immune transcriptome studies have limitations, including the inability to ascribe the expression of immune genes to individual immune cell populations, and the primary focus is on sheep. This paper aims to systematically review the use of RNA-seq in understanding the immune responses of small ruminants, particularly goats and sheep, to *H. contortus* infection, with a focus on identifying differentially expressed genes (DEGs), elucidating key immune pathways, and uncovering potential biomarkers for resistance and susceptibility.

2 | Methodology

2.1 | Search Strategy

The study methodology was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement guidelines (Figure 1) [22]. The research question was structured using the population, intervention, control, and outcomes (PICO) framework [23, 24]. The review was designed to address the following question: What is the significance of RNA-seq in transcriptomics studies in evaluating the host immune response mechanisms against *H. contortus*?

The population referred to host used (goat or sheep); intervention (I) included any intervention [naïve/pre-infected animals during experimental infections; control (C) was defined as uninfected]; and outcome (O) being the differentially expressed genes, immune response mechanisms on resistance/susceptible and immune response pathways. Information extracted from each study included nematode genus, host species (sheep or goat), host age (months), infection period (days post-infection), sample type [Peripheral blood mononuclear cells (PBMCs), abomasal lymph nodes or tissue], infection type (artificial or antigen stimulation), number of animals per group, the total number of infective larvae, and infection method for artificial infection (single or trickle).

The literature search was done using electronic databases: Google Scholar, Web of Science, PubMed, and Science Direct, covering worldwide publications from January 2015 to December 2023. The search terms and Boolean operators (AND, OR) used across all databases were: “goat” AND “sheep” AND “RNA sequencing” AND “*Haemonchus contortus*” AND “immune response” AND “transcriptomes.”

2.2 | Screening, Data Extraction, and Risk of Bias Assessment of the Articles

The titles and abstracts of all identified articles were screened independently to determine their eligibility. Articles meeting the inclusion criteria were read in full. Disagreements were resolved through discussion or consultation with a third reviewer. Data extraction was performed using a standardised form, collecting information on nematode genus, host species, host age, infection period, sample type, infection type, number of animals per group, total number of infective larvae, and infection method (single or trickle).

2.3 | Risk of Bias Assessment

The risk of bias in the included studies was assessed using the Cochrane risk of bias tool. Each study was evaluated for potential biases in selection, performance, detection, attrition, and reporting. The risk of bias was considered in the analysis and synthesis of the findings.

2.4 | Eligibility Criteria

Articles from peer-reviewed journals published after 2015–2023 were used. Studies were included if they used RNA-seq to

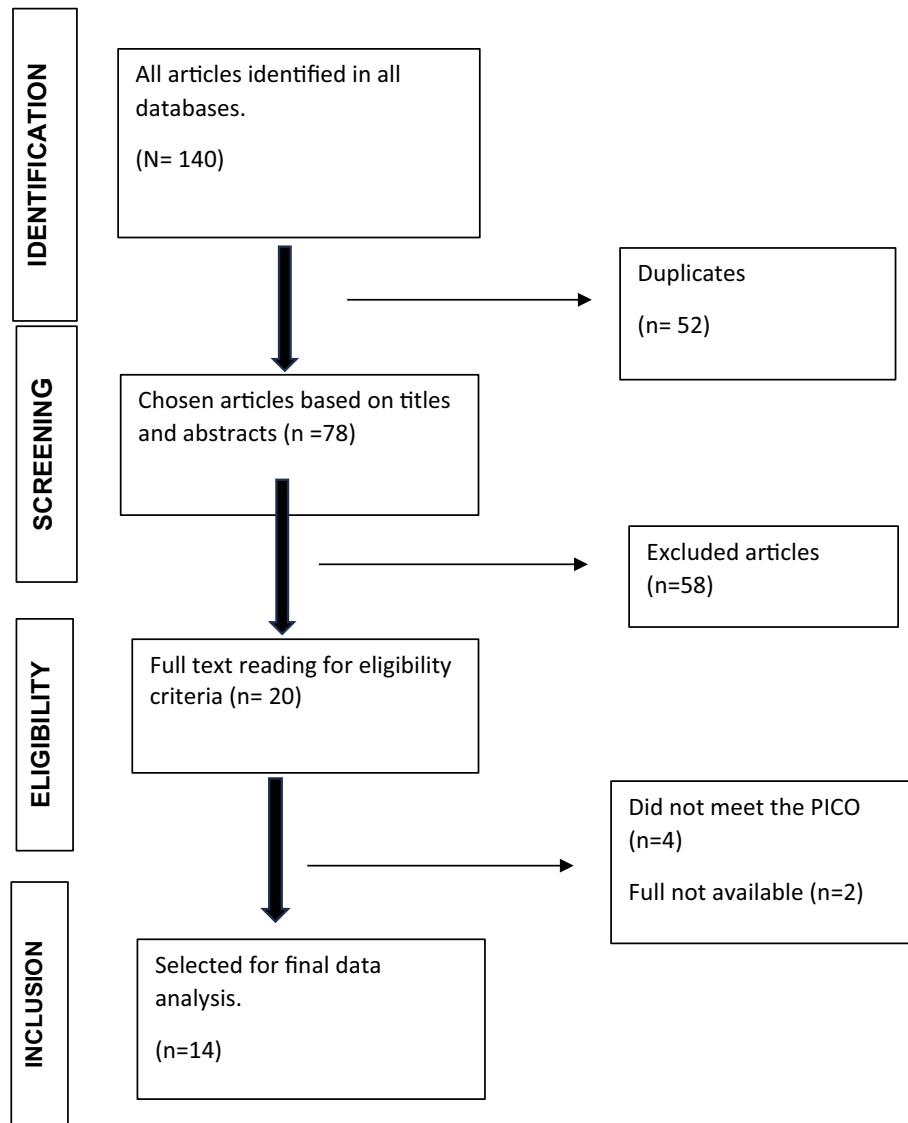


FIGURE 1 | PRISMA diagram showing the screening and selection process followed in selecting articles for this review.

evaluate immune response mechanisms and pathway activation against *H. contortus* and provided extractable data. Full-text articles were included if they met the inclusion criteria.

2.5 | Exclusion Criteria

Studies were excluded if they were: (i) duplicated in more than one database; (ii) case reports, conference papers, letters to the editor, literature reviews, systematic reviews, and overviews; (iii) dissertations or full text not available; (iv) research with a sample size less than two; and (v) not relevant to the research question.

3 | Results and Discussion

3.1 | Data Extraction

The initial literature search from the electronic databases resulted in a total of 140 articles: Science Direct ($n = 5$), Medline ($n = 2$), PubMed ($n = 45$), Scopus ($n = 8$), Web of Science ($n = 11$),

and Google Scholar ($n = 69$) (Figure 1). Of these, 14 studies met the PICO framework; after that, data extraction was possible, and full-text articles were used. The data extracted from these studies is presented in Tables 1, 2a, 2b.

3.2 | Population

Nine of the 14 studies were done on sheep, while the other five were on goats. The sample types collected across studies included abomasal mucosa, PBMCs, and abomasal lymph nodes. Each study examined at least one of these sample types. The immune response transcriptomes were evaluated during primary, secondary, and tertiary infection in three studies, primary and secondary infection in four, and primary infection alone in three. Ten of the 14 studies used animals artificially infected with *H. contortus* L3 larvae, while two studies used in vitro stimulation of naïve PBMCs with *H. contortus* antigen for 6 h [28] and abomasal tissue explants [35] respectively. The age of animals used ranged from 1 to 10 months old, and gender was not considered as a factor in all the studies. The studies compared

TABLE 1 | Studies that used RNA-seq to evaluate immune response mechanisms and pathway activation in goats and sheep against *H. contortus*.

Study no.	Host	Breed	Location	Sample size (No of animals)	Tissue sample types	Infection/Challenge	Sampling time points	qRT-PCR validated?	References
1.	Sheep	Canaria Hair – Resistant (R) Canaria Sheep— Susceptible (S)	Spain	n = 23 (11 resistant) (7 of 11 infected) and 12 susceptible (8 of 12 Infected)	Abomasal mucosa	20,000 L3 primary infection only	20 dpi	Yes	[25]
2.	Sheep	Not stated	China	n = 12 (6 infected and 6 uninfected)	Abomasal mucosa	10,000 L3 primary infection only	7 and 50 dpi (3 sheep at a time)	Yes	[26]
3.	Goats	Yichang White	China	n = 8 (4 resistant and 4 susceptible; all infected)	Peripheral blood (no re-stimulation)	5145 L3 735/ day—for 7 days	42 dpi	Yes	[27]
4.	Sheep	Not stated	China	n = 3, uninfected	PBMCs (stimulated with Hc antigen for 6 h)	0 (no infection)	n/a	Yes	[28]
5.	Sheep	Merino	South Wales	n = 48 (8x groups of 6/7 resistant and susceptible infected once or 3x) A group and I group	Abomasal mucosa	5000 L3 twice at 16 and 27 weeks old for A-group 5000 and 10,000 L3 larvae Primary once infection for I-group	and 3dpi for both groups	Yes	[29]
6.	Goats	Croele	French West Indies	n = 24 (9 resistant (R) and 9 susceptible (S)-infected; 3 resistant and 3 susceptible— uninfected)	Abomasal mucosa Lymph nodes	10,000 L3 primary and secondary infection	Week 7 post-secondary infection	Yes	[30]
7.	Goats	Croele	French West Indies	n = 8 (4 resistant and 4 susceptible; all infected)	Abomasal mucosal biopsies	10,000 L3 Primary and secondary infection	0, 8, 15, 35 d post-secondary infection	Yes	[31]
8.	Sheep	Suffoli (S) St Croix (R)	USA	n = 6 (3 resistant and 3 susceptible; all infected)	Blood (PBMC stimulated with crude L3 Ag for 6 h)	10,000 L3 larvae Primary and secondary infection	35 d post-secondary infection	No	[32]

(Continues)

TABLE 1 | (Continued)

Study no.	Host	Breed	Location	Sample size (No of animals)	Tissue sample types	Infection/Challenge	Sampling time points	qRT-PCR validated?	References
9.	Goats	Croele	French West Indies	$n = 8$ (4 resistant and 4 susceptible; all infected)	Abomasal mucosal biopsies	10,000 L3 larvae Primary and secondary infection	0, 8, 15, 35d post-secondary infection	No	[18]
10.	Sheep	Merino	Australia	$n = 36$ (18 resistant and 18 susceptible, infected once or 3x)	Abomasal mucosa	5000 L3 larvae Primary and secondary infection 10,000 L3 tertiary infection	3 dpi tertiary infection	No	[33]
11.	Sheep	Merino	Australia	$n = 36$ (18 resistant and 18 susceptible, infected once or 3X)	Abomasal mucosa	5000 L3 primary and secondary 10,000 L3 tertiary infection	3 dpi tertiary infection	Yes	[34]
12.	Goats	Croele	French West Indies	$n = 8$ (4 naïve and 4 pre-infected; all infected)	Abomasal mucosa	10,000 L3	0, 4, and 6 dpi	No	[14]
13.	Sheep	Not stated	China	$n = 20$	Abomasal mucosa (stimulated with L4 <i>H. contortus</i> for 6 h)	No infection	N/A	Yes	[35]
14.	Sheep	Santa Inness Ille de France	Brazil	$n = 8$ all naïve (4 Res and 4 Sus)	Abomasal mucosa	5400 L3 (small doses for 52 days)	54 dpi	No	[36]

^aDpi-days post infection. Abbreviation: qRT-PCR, quantitative reverse transcription polymerase reaction.

TABLE 2A | Summary of results for studies that evaluated/investigated at immune response against *H. contortus* during early infection in small ruminants in primary, secondary, and tertiary infection.

No.	Summary of results	References
1.	548 DEG were identified in infected animals with Gal-11 upregulated <i>Gal- 4, MHC Iα, MHC II α, DRα</i> downregulated pro-inflammatory cytokines TNF- α and <i>IL-6</i> were upregulated Complement protein activation: <i>C7 and C9</i> -upregulated	[26]
2.	0 dpi: <i>IL-17F</i> was three times higher in resistant kids 8dpi: <i>TGF-β1</i> upregulated, <i>IL-2RG</i> activated and Both Th1 and Th2 pathways activated	[31]
3.	32 and 21 KEGG pathways were significantly impacted in the innate and adaptive immune responses respectively, to infection between susceptible groups 19 KEGG pathways involved in innate and adaptive immunity were enriched, DEGs involved in innate immunity (<i>PRKG1, PRKACB, PRKACA, and ITGB1</i>) and adaptive immunity (<i>CALM2, MYL1, COL1A1, ITGB1, and ITGB3</i>), some of which are involved in platelet activation, smooth muscle contraction, NO signalling pathway.	[34]
4.	The 21 DEGs, including MCP1, TFF3, and IL-17RB, were expressed at a lower level in the tertiary infection <i>CD19, CD22, CD79B, CXCR5, FCRL1, FCRL3, POU2AF1, KCNN4, CD248, and CXCL11</i> , were significantly downregulated 32 genes, such as <i>TFF2, LGALS1, TNXB, COL1A1, COL1A2, and COL3A1</i> , were expressed significantly higher in the tissues during primary infection Immune-related genes, such as <i>IL-17RB, ITGAD, MCP1, CFB, IFI44, and CD320</i> , were also identified as DEG between the primary and tertiary infection	[33]
5.	18 DEGs were found for the comparison of pre-infected vs. naïve animals at 6 dpi 0 dpi pre-infected kids had already activated upstream regulators immune genes like <i>TNF, IL-1β, IL2, IL-5, IFN-γ, CD3, TCR, IL-8, IL-6, and IL-4</i> 4 dpi naïve kids had activated the expression of different immune-related genes like <i>TNFRSF12A, IL-1β, CXCL8, and OLFM4</i> At 4 and Th2 and Th1 pathways, natural killer cell, B cell receptor, <i>IL-2, and IL-15</i> signalling were activated on pre-infected Th1 and Th2 responses were activated in naïve and pre-infected animals, with earlier activation in preinfected animals compared with the naïve ones. A similar pattern was recorded for upstream regulator genes that were related to immunity like <i>TNF, IL-1β, IL-2, IL-5, TGFβ1, IFNγ, IL-18, IL-6, and IL-4</i>	[14]
6.	TFF3 gene was strongly upregulated Transcripts of <i>IL-15 (IL-15RA)</i> and <i>IL-22 (IL-22RA1)</i> were upregulated in susceptible breed 9 genes, including heat shock protein family A (<i>Hsp70</i>) member 6 (<i>HSPA6</i>), lectin, galactoside-binding, soluble, 15 (<i>LGALS15</i>), and complement factor I (<i>CFI</i>), upregulated in both resistant breeds Cytokines highly regulated in resistant breeds: C-C motif chemokine ligand 14 (<i>CCL14</i>), <i>CCL20, CCL25, CCL5, C-X-C motif chemokine ligand 10 (CXCL10), CXCL14</i> , and atypical chemokine receptor 3 (<i>ACKR3</i>) Several pathways were significantly impacted during primary infection, including leukocytes trans endothelial migration, MAPK signalling, TNF signalling, cell adhesion molecules, B cell development and receptor signalling, B cell activating factor signalling and PI3K signalling.	[29]
7.	Upregulated genes: <i>IL-6, IL-8, C1q, ACKR3, CCL2, MnSOD, ITGA-7, -8, -9, ITGB 1, ITGB 6, ICAM-1 and ACTA-1</i> <i>IL-6</i> and <i>IL-8</i> over-expressed on stimulated samples. Upregulated DEGs <i>C7</i> and <i>C1q</i> (innate response) in the stimulated	[35]
8.	Cytokines: <i>IL-4</i> upregulated; <i>IL-12</i> downregulated. Upregulation of some PRR associated genes: <i>CLRs (CLEC2L and KLRG2)</i> , two <i>NLRs</i> attenuating inflammation (<i>NLRP12 and NLRC3</i>) Upregulation of Th2 related transcription factors: <i>ATF3, IRF4, BCL3 and NFATC</i> Upregulation of negative regulators of inflammation. <i>NF-κB</i> signalling and <i>HCAR2</i>	[28]

TABLE 2B | Summary of results for studies that evaluated/investigated the immune response against *H. contortus* during late infection in small ruminants for primary, secondary, and tertiary infection.

No.	Summary of Result	References
1.	<i>Gal-15</i> gene upregulated <i>MMP-9</i> and <i>TIMP-1</i> upregulated MHC class II α chain	[26]
2.	Th2 cytokine and chemokines released- <i>IL-25</i> and <i>IL-33</i> 57 DEGs were related to 13 putative cell types (B cells), basophils, CD4 T and CD8 T cells, dendritic cells, endothelial cells, enterochromaffin-like cells, macrophages, mucous cells, neutrophils, parietal cells, pre-B-cells-, and-tuft cells DEG- <i>TLR2</i> is highly expressed in resistant breeds. First report of increased expression of <i>CSF1</i> Innate immunity (cell-mediated). The resistant breed showed a robust APP mechanism characterised by greater T cell APP, macrophage differentiation, and cytokine signalling.	[36]
3.	<i>IL4R</i> and <i>STAT6</i> at 15 dpi, <i>GATA3</i> and <i>CCR4</i> at 35 dpi Th17 pathway; showed by a positive fold change of <i>STAT3</i> and <i>RORC</i> genes in resistant kids at 15 dpi, then for <i>IL-17F</i> at 35 dpi while for <i>STAT3</i> the expression was higher in susceptible kids at 35 dpi	[31]
4.	Th2 Cytokines: Upregulation of <i>IL-6</i> , <i>IL-10</i> and <i>IL-13</i> in both breeds overexpression of <i>IL10</i> and <i>IL13</i> in resistant breed <i>IL-4</i> , <i>IL5</i> —highly expressed in susceptible breed. <i>IL-9</i> —unchanged Complement activation: 11 genes significantly impacted in res breed. Other genes: Granzyme A and B, cell adhesion molecules—upregulated in res breed. 15% of the genes significantly impacted in a resistant breed	[25]
5.	Upregulation of inflammation-associated genes (<i>NLRP3</i> , <i>IL36A</i> , <i>PTX3</i>) Upregulation of cytokines and cytokine receptors (<i>IL-18R1</i> , <i>IL-18RAP</i> , <i>IL-1A</i> , <i>IL-1B</i> , <i>IL-1RAP</i> , <i>IL-6</i> , <i>NFKB1</i> , <i>NFKB2</i> , <i>IFNGR1</i> and <i>IFNGR2</i>) Upregulation of neutrophil activation genes-neutrophil cytosolic factors-(<i>NCF2</i> and <i>NCF4</i>) Upregulation of alternatively activated macrophage markers and wound healing-associated genes Upregulation of PRRs—in res breed only <i>TLR4</i> , <i>TLR2</i> , <i>TLR6</i> ; C-type lectin receptors (<i>CLEC</i>) <i>CLEC4D</i> , <i>CLEC1A</i> , <i>CLEC5A</i> , <i>CLEC4E</i> and <i>CLEC12A</i> .	[35]
6.	Significant upregulation of genes involved in protein kinase activity, complement activation, transforming growth factor (TGF), mitogen-activated protein kinase (MAPK), and cell adhesion molecules (CAMs) pathways. Th2 cytokines upregulated on resistant goats	[27]
7.	Differential gene expression in the following biological processes: DEG was higher in lymph node tissue in the resistant breed DEG is lower in the abomasal mucosa for both breeds antigen processing and presentation of peptide antigen via MHCI; TGF- β signalling pathway	[30]
8.	T cell receptor signalling pathway was one of the top significant pathways, with 78% of the genes involved in this pathway showing genomic variants between breeds.	[18]

immune responses in resistant versus susceptible breeds and/or pre-exposed versus naïve animals.

3.3 | RNA-Seq Approaches in Studying Host Immune Response Against *H. contortus*

The study design and experimental models play a vital role in RNA-Seq studies, and it has a major impact in the interpretation of the transcriptomic data. Ten of the 14 studies reviewed in this study were focused on comparing resistant versus susceptible breeds (Table 1); these comparative studies help in the identification of DEGs and pathways associated with immunity

against *H. contortus* and other GIN as shown by McRae et al. [13]. The infection method is also critical; it is either natural or artificial infection/ex vivo models. These controlled experiments provide precise comparisons; however, they might not provide the whole picture of the host–parasite interaction in real field conditions where other parameters such as health, diet, and exposure to other pathogens come into play. This was illustrated by a comparative study by Wang et al. [37] which looked at immune response transcriptomes of cattle naturally infected with *Staphylococcus aureus* Mastitis. It showed that there is no clear tracking of transcriptional changes at different time points ex vivo. Transcriptomic studies conducted in natural field conditions monitoring *H. contortus* infection are rarely done, due

to the need for precise sampling timepoints after infection was initiated and easy tracking of time-dependent transcriptional changes. The sampling time point also varies; some studies evaluated immune responses during early infection stages, and others are focused on later stages of infection (Tables 2a and 2b). Sampling time points are very crucial in transcriptomic studies and depend on prior knowledge on when the *H. contortus* infection started. It is well known that early sampling helps detect innate immune response activation, as was shown in the study by Niedziela et al. [38], on *Fasciola hepatica*. Missing the correct sampling window may lead to crucial immune response activation events not being detected [13]. The sample type is another key consideration. PBMCs are commonly used, and three of the reviewed studies used PMBCs [27, 28, 32]. However, PBMCs reflect only systemic responses rather than localised immune responses [38, 39]. Ten of the reviewed studies used abomasal mucosa (Table 1); the tissue sample is useful to detect direct host-parasite interaction at the site of infection [40, 41]. Only one study included in this review considered studying abomasal lymph nodes [30]; since they can provide infection site specific insights into adaptive immune response [42].

3.4 | Immune Response Against *H. contortus* Infection

3.4.1 | Immune Response During Early Infection

Eight of the studies evaluated immune responses in the early stages of infections during primary, secondary, and tertiary infection (Table 2a, Figure 2). Across multiple studies, in both sheep and goats, it was consistently shown that resistant breeds display a higher number of immune specific DEGs compared to susceptible breeds. This suggests a more robust and diverse immune response in resistant animals. It was shown that cytokines including tumour necrosis factor (*TNF*), *IL-1 β* , *IL-2*, *IL-5*, *transforming growth*

factor (TGF)- β 1, *IFN- γ* , *IL-18*, *IL-6*, and *IL-4* were found to be activated in goats during early infection [14]. This study suggests that both cellular and humoral pathways are activated in goats, but the relative strength of each pathway may vary between resistant and susceptible breeds. Resistant goats seem to have a stronger humoral response, which is associated with the expulsion of parasitic worms [14]. Reinfection of goats with prior exposure to *H. contortus* showed earlier activation of multiple immune pathways, including Th1, Th2, NK cell responses, and *IL-2/IL-15* signalling [14]. This suggests that prior exposure to *H. contortus* primes the immune system for a faster and potentially more effective memory response.

Furthermore, there were common findings across the reviewed studies, such as the expression of pro-inflammatory cytokines in both sheep and goats. The *IL-6* and *IL-8* genes were found to be upregulated in sheep during early infection in multiple studies [26, 33, 34]. The upregulation of those genes suggests their role in parasite recognition, immune cell activation, and eventual parasite expulsion [43]. The *IL-10* gene was consistently found to be upregulated in both sheep and goats, suggesting a role in the regulation of the immune response to *H. contortus*. The expression of *TNF- α* during early infection was observed in sheep but not consistently in goats, which is associated with Th1 activation [14].

Studies by [25, 29, 35] indicated upregulation of *C7*, *CFI*, *C3*, and *Clq* and possibly other complement components in the immediate immune response to *H. contortus* L4 antigen in sheep. These complement components control alternative pathway activation and bridge between innate and adaptive humoral immune responses [44]. However, it is not known if complement activation occurs in goats.

Induction of innate immunity relies on the ability of the host to recognise pathogen-associated molecular patterns (PAMPs)

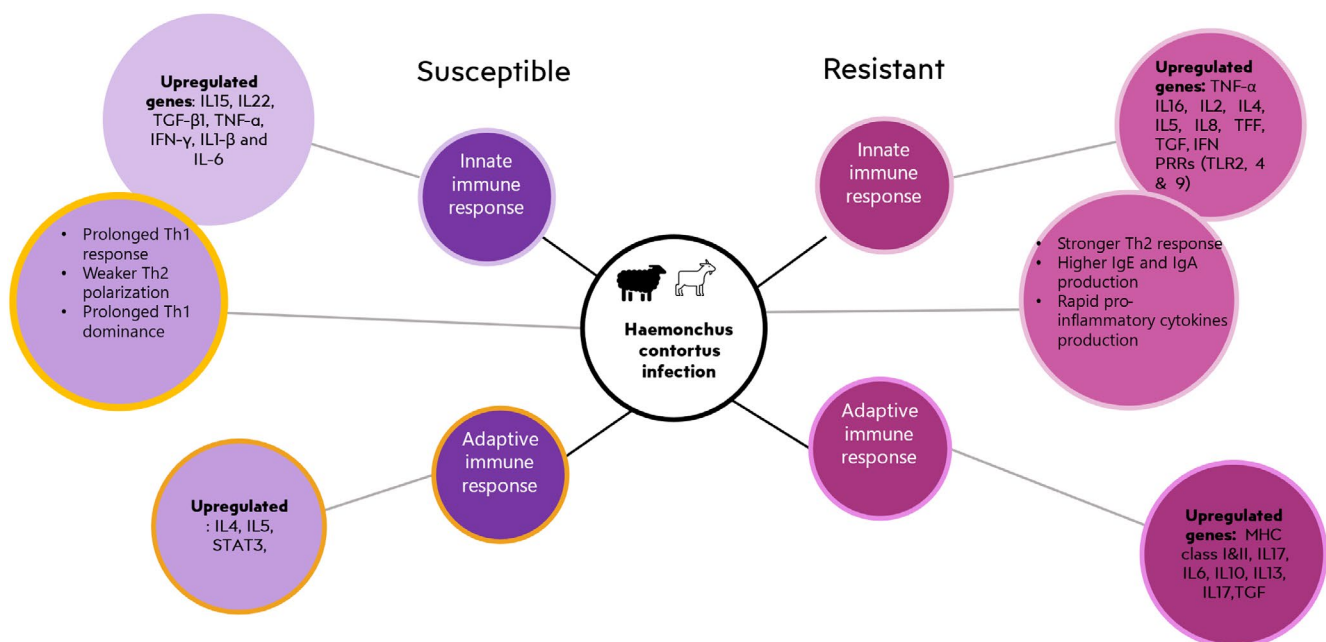


FIGURE 2 | Schematic representation of genes associated with innate and adaptive immune responses in resistant versus susceptible breeds.

through various pattern recognition receptors (PRRs). Jacobs et al. [32] reported upregulation of a range of PRRs, including TLRs and CLECs, in resistant sheep during early secondary *H. contortus* infection. These resistant sheep had upregulated PRRs including toll-like receptors (TLR) such as *TLR2*, *TLR4*, and *TLR6* as well as C-type lectin-like receptor genes (CLEC) including *CLEC4D*, *CLEC1A*, *CLEC5A*, *CLEC4E*, *CLEC12A* [32]. The upregulation of the TLR indicates activation of innate immune response pathways, which suggests early parasite recognition and promoting adaptive responses. While the CLECs trigger the pro-inflammatory response pathways [45]. The activation of PRRs can also involve macrophage markers and wound healing-associated genes in sheep and goats [29, 46]. However, another limitation was identified in that there is no direct comparison of *TLR* and *CLEC* gene expression between resistant and susceptible breeds in goats. Further research is needed to specifically investigate the expression of *TLRs* and *CLECs* in goats, especially during stages of *H. contortus* infection.

Nucleotide-binding oligomerization domain-like receptors (*NLRs*) were upregulated in sheep PBMCs after *H. contortus* antigen stimulation [28] which are responsible for coordinating an effective immune response and also regulating inflammation and cytokine maturation [47].

3.4.2 | Immune Response During Primary? Late Infection

Studies examining late primary infection stages in sheep revealed that Th2-mediated environments led to the upregulation of humoral immunity-associated cytokines (Table 2b; Figure 2). Resistant breeds displayed higher levels of *IL-10* and *IL-13*, while *IL-4* and *IL-5* were more highly expressed in susceptible breeds and induced the hyperplasia and migration of activated effector cells (such as eosinophils and mast cells) to the site of infection [25, 28]. *IL-13*, a key humoral cytokine, plays a huge role in B cell functionality and mucus production, and while *IL-10*, which is an anti-inflammatory cytokine, activates B cells and regulates T-cell activity [48]. *IL-4* facilitates the differentiation of naïve T cells into Thf cells and has been shown to inhibit proinflammatory cytokines in mice [49] while *IL-5* is responsible for eosinophil activation and enhances antibody response [8]. Additionally, goats and sheep showed evidence of B cells, basophils, CD4- and CD8 T cell activation during late infection [29, 31, 36]. The study by Zhang et al. [29] found higher levels of *IL-15* and *IL-22* receptors in susceptible sheep during late primary infection. Resistant breeds showed upregulation of wound-healing genes such as extracellular matrix, fibronectin, and multiple collagen-associated genes [25]. *IL-15* controls both innate and adaptive immune responses and is also responsible for the development of CD8+ T cells, natural killer (NK) cells, and $\gamma\delta$ T cells [29, 50–52]; *IL-22* is primarily produced by Th17 cells, and it is responsible for mucosal immunity [52].

Abomasal lymph nodes showed more DEGs than the abomasal mucosa of resistant goat breeds. More specifically, there was an activation of Thf-producing type 2 cytokines, which was shown

by the upregulation of *IL-4R* and *STAT6* at 15 dpi, and *GATA3* and *CCR4* at 35 dpi. There was a higher expression of Th17 pathway-associated genes. These were Signal Transducer and Activator of Transcription 3 (*STAT3*) and RAR-Related Orphan Receptor C (*RORC*) genes, which were upregulated in resistant kids and then *IL17F* during late infection [31].

In goats, there was consistent activation of signalling pathways such as mitogen-activated protein kinase (MAPK), cell adhesion molecules (CAMs), and transforming growth factor (TGF) signalling pathway; the CAM pathway-associated genes (*SELP*, *NLGN2*, *LICAM*, *ITGA4*) were highly expressed in resistant goats [27] CAMs play a crucial role in tissue repair and immune response modulation by recruiting immune cells to the site of infection late primary infection [29].

3.4.3 | Immune Response Pathways Activated During *H. contortus* Infection

The review studies showed that resistant breeds, both sheep and goats, consistently had an upregulation of Th2 pathway associated cytokines, which include *IL-10*, *IL-5*, and *IL-13*. The Th1-associated cytokines (*TNF- α* , *IFN- γ* , and *IL-12*) were observed in susceptible breeds. However, goats showed an activation of both Th1 and Th2 immune response pathways during early infection [14, 31]. Goats showed an earlier activation of Th2 immune response associated genes than sheep. The study by Aboshady et al. [14], showed that Th2 immune pathway-related genes were activated as early as 8 days post-infection in goats, while for sheep they were activated between 10 and 28 days, as shown by Gill et al. and Shakya et al. [8, 53]. The Th2 pathway is a driver of eosinophil activation, mast cell recruitment, and stimulates antibody production by plasma cells that facilitate parasite expulsion [48, 54, 55]. While Th2 immune responses remain the main indicator of host resistance to GIN infection, there is emerging evidence that shows the role of Th17 and Thf pathways in coordinating and modulating humoral immunity and suppressing the Th1 pathway activity [17, 55]. However, only one study investigated Th17 and Thf pathways in goats [31] and there was no direct comparison of these immune response pathways between the two species.

Three of the reviewed studies showed that RNA-seq was able to identify the activation of key immune pathways, such as Th1/Th2, NK cell, B cell receptor signalling, MAPK, CAMs, and TNF signalling [27, 29, 31] These pathways form a well-coordinated network for immune response activation, sending stress signals and proinflammatory response. This provides insights into the complex interplay of immune cells and signalling molecules that contribute to resistance and protective immunity. Identifying potential biomarkers for resistance and susceptibility is another key feature of RNA-seq [25, 31]. These biomarkers could be used to develop more effective selection criteria for breeding resistant animals or to identify individuals at risk of severe infection. RNA-Seq provides a global view of the transcriptome, allowing for the identification of a vast number of DEGs, including those not previously known to be involved in the immune response. This untargeted approach significantly expands our understanding of the host's response to infection.

3.5 | Assessment of the Methodology and Methodological Limitations of the Articles

RNA-seq was used in many studies to provide detailed gene expression information at specific time points. One study did not give detailed results on which transcriptomes or DEGs were upregulated [18], and some studies [25, 27, 30, 36] focused only on later infection stages, potentially overlooking early infection DEGs, which remain unknown.

There may be a possibility that low abundance transcripts or transient gene expression changes may have been overlooked during data analysis, potentially affecting the comprehensiveness of the findings. One study had a relatively small sample size $n=3$ [28], which can limit the statistical power of the analysis and increase the risk of false-positive or false-negative results. RNA-seq is costly since transcriptomics requires high-depth sequencing and high-performance computing resources for data analysis, which results in the use of a smaller sample size and may affect the quality of the results obtained [56]. Not all studies provided access to the raw sequencing data or detailed tables of DEGs, which limits the ability to perform secondary analyses or meta-analyses. Some studies used *ex vivo* models [28, 35], which may not fully reflect the *in vivo* immune response. Studies often focused on specific pathways or genes, potentially overlooking other important aspects of the immune response.

4 | Future Perspective

This systemic review has highlighted the transformative role of RNA-seq in improving our understanding of the complex immune responses of small ruminants, particularly goats and sheep, to the gastrointestinal parasite *H. contortus*. This technology offers several advantages over traditional methods for studying gene expression. The use of RNA-seq in the identification of the key immune response-associated genes and pathways is a key to novel approaches in vaccine development against *H. contortus*. The transcriptomic data can also be used in the identification of genetic markers for selective breeding programmes; the resistance-associated genes can be used to breed for strong natural immunity, thus reducing the use of anthelmintic drugs.

More research is required using RNA-seq to study the global immune response against *H. contortus* in small ruminants, especially goats. Single-cell RNA-seq technology provides a more specific approach to studying which specific immune cells are associated with immune response pathways; this can be very useful in future research advancing the knowledge of host immune response mechanisms. Currently, there is only one study that used scRNA-seq to study the immune response in goats [21]. The development of standardised methodologies for RNA-seq experiments, including best practices for data analysis and quality control, will improve the reliability and comparability of findings. Additionally, encouraging data sharing so that it can be used for meta-analysis of existing RNA-seq datasets can improve and strengthen conclusions drawn from transcriptomic studies. Addressing these knowledge gaps will improve our understanding of the immune responses to *H. contortus* in small ruminants and lead to the development of more effective and sustainable control strategies.

Author Contributions

This paper is the component of the PhD thesis of the first author, B.S.N., under the supervision of S.I.T., M.C.M., E.F., and A.P. B.S.N.: Developed the main concepts, structured the framework, and wrote the manuscript. The main supervisor and co-supervisors: Reviewed, provided feedback, and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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