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Microarray analysis reveals difference in gene expression profiles of hair and wool sheep infected with *Haemonchus contortus*

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ABSTRACT

Sheep infected with the abomasal parasite, *Haemonchus contortus*, have reduced growth rates, decreased wool production, and anemia, and heavy infections may result in death. Anthelmintic treatment can remove worms, but the cost of treatment and prevalence of drug-resistant worms has led to greater focus on genetic resistance of the host to parasitism. Variation in parasite resistance exists within and among sheep breeds, and Caribbean hair sheep have greater resistance than most conventional wool breeds. Our objective was to investigate differences in gene expression between 24 parasite-resistant hair and 24 susceptible wool lambs to determine genetic mechanisms involved in resistance to *H. contortus*. Half of the animals of each breed were infected and sacrificed at 3 or 27 days post-infection; the remaining animals were uninfected controls. Breed differences in abomasum and abomasal lymph node tissue gene expression were assessed using bovine cDNA microarrays. Over 60 transcripts differed between breeds for each tissue and infection status. Genes differentially expressed between hair and wool sheep 3 days PI were assessed for gene function and mechanisms for greater immune cell infiltration, abomasal tissue repair, Th17 response, and anticoagulation were present in parasite-resistant hair sheep. By 27 days PI, hair sheep had greater expression of genes involved in gut motility, inflammatory cytokines, and cell proliferation and differentiation compared to wool sheep. Changes in these processes indicate Caribbean hair sheep have a stronger inflammatory response when infected with *H. contortus* which may facilitate the increased parasite resistance observed in these sheep.

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1. Introduction

Ruminants and internal parasites have co-existed for thousands of years, but an increase in stocking density has contributed to greater parasite burdens for the host and decreased revenue for livestock producers. Parasitism by gastrointestinal worms is the primary animal health concern of 62% of US sheep producers (NAHMS, 1996). The parasite of greatest concern in warm, humid areas is the blood-feeding nematode *Haemonchus contortus*. Infection with *H. contortus* causes severe anemia, anorexia, loss of condition, reduced growth rate, and possibly death. Health problems associated with gastrointestinal parasites result in an estimated loss of $369 million (Australian dollars) per year to the Australian sheep industry (Australian Wool Innovation, 1999) with additional losses occurring in other countries. This problem is expected to worsen as the incidence of parasites that are resistant to anthelmintics continues to increase (Jackson and Coop, 2000).

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An alternative to use of chemical anthelmintics would be to obtain or select sheep that are resistant to gastrointestinal nematodes. Selection within existing wool breeds has resulted in lines of sheep with up to a 35-fold difference in fecal egg counts (FEC), a common measure of parasite burden (Woolaston, 1992; Bisset et al., 1996, 2001). Unfortunately, selection to obtain meaningful reductions in FEC has taken many years and in some cases resulted in decreased wool production and carcass merit (Morris et al., 1997, 2001; Bisset et al., 2001). Variation in parasite resistance also exists among breeds of sheep. For example, Caribbean hair sheep develop resistance sooner and at higher levels than wool sheep under the same conditions (Gamble and Zajac, 1992; Vanimisetti et al., 2004).

An overall synthesis of published results on the difference in immune response to parasitism of resistant and susceptible sheep is difficult to achieve. The immune response is a dynamic process and variation in breed, sheep age, infective parasite, and post-infection (PI) time point complicate interpretations. Typically, extracellular parasite infection in sheep leads to increased production of local and systemic antibodies, globule leukocytes/mucosal mast cells, and tissue and circulating eosinophils in more resistant animals (Zajac et al., 1990; Stankiewicz et al., 1993; Gill et al., 2000; Amarante et al., 2005; Bricarello et al., 2005; Balic et al., 2006). Other studies suggest that the timing of cell infiltration is critical, with resistant animals responding more quickly (Lacroux et al., 2006). A rapid immune response to parasitic larvae is desirable to prevent development of the larvae into adults that then move into the lumen of the abomasum. Tissue eosinophils can surround larvae in abomasal crypts within 24 h PI (Balic et al., 2006), and tissue eosinophil counts normally peak 3–5 days after artificial infection with *H. contortus*, corresponding to the time when larvae occupy their tissue niche (Balic et al., 2000, 2003). *In vitro*, antigen-specific antibodies contribute to the immune response by interaction with activated eosinophils, leading to a significant increase in larval immobilization and damage (Rainbird et al., 1998).

Immune cells from sheep infected with gastrointestinal parasites such as *H. contortus* exhibit differentiation of $CD4^+$ T-helper lymphocytes into Th2 cells and production of cytokines IL-4, IL-5, and IL-13 in abomasal lymph nodes and mucosa (Balic et al., 2000; Lacroux et al., 2006). It was originally thought that a typical Th2 immune response was induced during *H. contortus* infection (Gill et al., 2000; Schallig, 2000). However, more recent studies involving lines of Romney sheep that are resistant or susceptible to *Trichostrongylus colubriformis* show a slightly modified Th2 response to infection, with greater IL-13, IL-5, and TNF-α, but not IL-4 or IL-10 in resistant animals (Meeusen et al., 2005; Pernthaner et al., 2006). Resistant sheep clearly have a different immune repertoire than their susceptible counterparts. However, it is not clear what other immune parameters or non-immune factors are involved in induction of these enhanced levels of resistance.

Three previous studies have used microarray technology to attempt to describe genome-wide expression differences in parasite-resistant and susceptible sheep (Diez-Tascon et al., 2005; Keane et al., 2006; Rowe et al., 2008). The first ovine cDNA microarray was developed by Diez-Tascon et al. (2005) and was used to measure differences in mRNA expression of pasture-infected resistant and susceptible Perendale sheep. The authors report mixed-parasite infection results in greater expression of genes typically involved in acquired immune responses and genes related to intestinal smooth muscle. Unchallenged, parasite-naïve animals from these same lines of Perendale sheep also show susceptible animals have increased expression of genes associated with inflammation, oxidative stress, and apoptosis, possibly indicating an inappropriate induction of stress response genes within the intestine of susceptible sheep (Keane et al., 2006). Both of these studies indicate that there are immune and non-immune gene expression differences associated with increased resistance to gastrointestinal parasites. A more recent study measured expression difference associated with *H. contortus* infection in Merino crossbred sheep (Rowe et al., 2008). However, the focus of this study was to assess changes in mRNA expression resulting from abomasal fistulation.

Although reports on the general characterization of the immune response of sheep to *H. contortus* are available, a genome-wide characterization of genes and gene functions involved in resistance to *H. contortus* in Caribbean hair sheep has not been conducted. Thus, our objective was to compare levels of gene expression in abomasum and abomasal lymph node tissues associated with acquired resistance to parasite infection in hair and wool sheep.

2. Materials and methods

2.1. Animals and tissue collection

This experiment was conducted at the Virginia Polytechnic Institute and State University Sheep Center, Blacksburg, VA and utilized 24 hair and 24 wool lambs. Wool lambs were from a composite line of 50% Dorset, 25% Rambouillet, and 25% Finnsheep breeding established in 1983. Hair lambs were derived from St. Croix and Barbados Blackbelly ancestors and were at least 87.5% St. Croix. Lambs were born in January, raised under field conditions with no effort to prevent parasite infection, and were 4–5 months of age at the start of the study. Equal numbers of female and castrated male lambs were represented, and body weights ranged from 14 to 35 kg. Levels of parasitemia were not quantified before the start of the study, but signs of clinical haemonchosis had not been observed for any of the experimental animals.

The experimental design is shown in Fig. 1. All procedures were approved and carried out in accordance with the Animal Care Committee of Virginia Tech. Pastures surrounding the Sheep Center are known to be infected with *H. contortus*, and prior exposure of experimental animals to the parasite was assumed. However, to further standardize prior exposure levels, all lambs were drenched weekly with 3000 infective third-stage larvae (L_3) of *H. contortus* for 4 consecutive weeks before initiation of the study. The experiment was thus designed to compare breed differences in acquired immunity, as previously