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Evaluation of immune response to artificial infections of *Haemonchus contortus* in Gulf Coast Native compared with Suffolk lambs

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ABSTRACT

The Gulf Coast Native (Native) breed of sheep among many others is identified as being relatively resistant to *Haemonchus contortus*, an abomasal nematode parasite of small ruminants. Understanding the mode of immune response that helps these breeds of sheep control infection could help design and implement appropriate control programs. In this experiment, the components of the immune response during the early infection period in resistant Native lambs were evaluated and compared with susceptible Suffolk breed of sheep. Groups ($n = 5$) of six month old Native and Suffolk lambs were given infective larvae as one time (single) or trickle experimental infections. Fecal, blood, and serum samples were collected on days 0, 2, 7, 14 and 21 post-infection. Abomasal mucosa and regional lymph node samples were collected at the time of necropsy on days 14 and 21. There was no significant difference in number of worms recovered at necropsy but the ratio of adult versus larvae was significantly greater in single infected Suffolk than Native lambs. Native lambs had significantly greater numbers of mast cells and eosinophils in the abomasal mucosa and serum IgG production was significantly greater compared to Suffolk lambs. Native lambs also showed a trend of increased level of serum IgA and IgE compared to Suffolk lambs.

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1. Introduction

Development of acquired immunity plays an important role in resistance against *Haemonchus contortus*, an economically important nematode parasite of small ruminants worldwide (Barger et al., 1985). In general, a Th2 type of immune response that results from recruitment of effector cells such as mast cells and eosinophils accompanied by production of IgA, IgG1, IgG2 and IgE (Schallig et al., 1994; Schallig, 2000; Gill et al., 2000; Lacroux et al., 2006; Terefe et al., 2007; Shakya et al., 2009) is responsible for resistance against *H. contortus* in sheep. Protracted prepatent periods, lowered fecal egg counts (FEC) and lowered worm burdens that have been observed in various resistant breeds of sheep infected with *H. contortus* may be attributed to

the adaptive immune response (Gomez-Munoz et al., 1999; Schallig et al., 1995; Shakya et al., 2009). Increased IgG1, IgG2, IgE specific to *H. contortus* were also demonstrated in these studies. Contrary to those observations, no differences including globule leukocytes in Florida Native lambs, a resistant breed was demonstrated when compared with Rambouillet, a susceptible breed (Amarante et al., 1999). However, a negative correlation between cell count versus worm burden and FEC was reported. These findings indicate that there is a complex immune response and variable components are involved in resistance against *H. contortus*.

Gulf Coast Native (Native) sheep, a native breed of Southeast USA has been shown to be relatively resistant to *H. contortus* infection (Bahirathan et al., 1996; Miller et al., 1998; Shakya et al., 2009). Resistance was observed in suckling lambs and was maintained through adulthood (Bahirathan et al., 1996). Pena et al. (2004, 2006) reported resistance was due to genetically controlled immunologic components and Miller et al. (2006) found it highly variable

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among individuals within the breed. In our previous experiment, pasture infected Native lambs demonstrated increased resistance compared to susceptible Suffolk lambs with peripheral and abomasal mucosal eosinophilia, mastocytosis and globule leukocytosis on day 35 post exposure or later (Shakya et al., 2009). Serum IgE was elevated on day 7 post exposure and remained significantly higher until the end of the experiment on day 42 post exposure. By the end of the experiment, adult worm recovery was significantly lower than in the susceptible Suffolk breed indicating the elimination of worms occurred earlier during the infection. In the present experiment, serum immunoglobulins, abomasal mucosal cellular response and worm burden during the early phase of infection in resistant Native and susceptible Suffolk lambs artificially infected with *H. contortus* are studied. The results indicated that there is a difference in abomasal cellular composition and immunoglobulin profile between the breeds accompanied by an adverse effect on growth and development of *H. contortus* larvae in Native lambs.

2. Materials and methods

2.1. Experimental design

Five to six month old Suffolk ($n = 30$) and Native ($n = 30$) lambs were treated with levamisole (12 mg/kg, Levasol®, AgriLabs, St. Joseph, MO) and albendazole (20 mg/kg, Valbazen®, Pfizer, New York, NY) for two consecutive days each week for three weeks to eliminate infection as much as possible. The animals were reared in worm free conditions after the anthelmintic treatment at the School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803. Hay and water was provided *ad libitum*. The experiment protocol was approved by the Louisiana State University, Institutional Animal Care and Use Committee.

Lambs were randomly allocated to six groups of five animals each within breed. Three groups from each breed were designated as 'Day 14' and three as 'Day 21' infection and were necropsied on day 14 and day 21 post infection (PI), respectively. Two infection protocols, a "single infection" and a "trickle infection" administered over time, were employed. For the day 14 PI necropsy animals, one group from each breed was given a single infection dose of 18,000 freshly cultured *H. contortus* infective third stage larvae (L3) on day 0 PI, and one group from each breed was given a trickle infection of 6000 L3 on day 0 PI and 2000 L3 on days 2, 4 and 6 PI for a total 12,000 L3. The remaining two groups were untreated control groups. For the day 21 PI necropsy animals, a similar infection protocol was followed except the trickle infected lambs received three additional doses of 2000 L3 on days 8, 10 and 12 PI for a total of 18,000 L3. The groups were identified by dose they received, day they were necropsied and the breed: Single 14 Suffolk and Native (S14S and S14N), Trickle 14 Suffolk and Native (T14S and T14N), Control 14 Suffolk and Native (C14S and C14N), Single 21 Suffolk and Native (S21S and S21N), Trickle 21 Suffolk and Native (T21S and T21N) and Control 21 Suffolk and Native (C21S and C21N). Weekly FEC, blood packed cell volume (PCV), serum immunoglobulins, differential leuko-

cyte count, parasite establishment and cellular distribution in the abomasal mucosa were monitored.

2.2. Infective larval culture

Fecal material collected per rectum from a sheep infected with *H. contortus* was cultured at room temperature for 14 days and L3s were harvested by baermannization. L3s were identified microscopically to be 98% *H. contortus* and the remainders were *Cooperia* and *Trichostrongylus* spp. The appropriate numbers of L3 for all experimental infections were given in 12 ml of tap water in plastic syringes with a metal nozzle extender attached.

2.3. Fecal egg count (FEC)

Feces were collected directly per rectum and a modified McMaster technique was used to determine FEC (Whitlock, 1948). Fecal samples with a zero FEC on McMaster were subjected to sugar flotation technique (sugar solution specific gravity 1.2). The egg counts are presented as eggs per gram (EPG) of feces.

2.4. Blood packed cell volume (PCV) and leukocyte differential count

Blood was collected by jugular venipuncture into EDTA vacutainer tubes. PCV was determined using a microhematocrit centrifuge. Thin blood smears were prepared and Giemsa–Wright stained. The first 100 leukocytes were morphologically classified as neutrophils, eosinophils, basophils, monocytes and lymphocytes and enumerated.

2.5. Necropsy and worm recovery

Necropsy of the lambs and worm recovery were carried out as described by Shakya et al. (2009). Briefly, after euthanasia the abomasal and prescapular lymph nodes were collected and weighed. The abomasa, small and large intestines were opened and washed and diluted to 5 l with tap water. The abomasum was then soaked overnight in warm water. Aliquots of 500 ml of wash and soak were collected into pre-labeled plastic bottles and preserved (10% formalin). Worms were recovered from 100 ml aliquots of the abomasum wash and soak, small intestine wash and all from the large intestine wash. A second aliquot of 100 ml was examined for samples that yielded less than 10 worms. The worms were identified to species, sex, and stage of development. The number recovered was extrapolated to estimate the total number present in each lamb.

2.6. Histopathology

Histopathological examination of abomasal tissue samples was carried out as described by Shakya et al. (2009). Briefly, the samples from cardiac, fundic and pyloric regions were collected and fixed in 10% buffered formalin solutions. They were trimmed, embedded in paraffin and sectioned as 3 μ m thick sections. Separate sets of sections were stained with hematoxylin and eosin and toluidine blue stains for

enumeration of eosinophils and neutrophils and mast cells, respectively. The number of cells in ten 0.3 mm × 0.3 mm fields were counted in the lamina propria of the three respective regions and presented as the mean number per mm².

2.7. Enzyme linked immunosorbent assay (ELISA)

Optical densities (OD) for serum IgG were determined in individual serum samples while for all other isotypes, pooled serum samples for the group were analyzed by ELISA as described by Shakya et al. (2009). Briefly, for pooled samples, 500 µl of thawed sera samples from each individual in the group were mixed in a tube for each sampling time point and 1% sodium azide solution (Sigma–Aldrich Co., St. Louis, MO, USA) was added as a preservative. The samples were refrigerated until used.

High binding polystyrene plates (Costar EIA/RIA, Corning, Inc.) were coated with 100 µl of 5 µg/ml of *H. contortus* whole worm antigen (WWA) in 0.05 M carbonate bicarbonate buffer, pH 9.6 (Sigma–Aldrich Co., St. Louis, MO, USA), sealed with a microtiter plate sealer (SealPlate Microplate Adhesive Film, E & K Scientific, Campbell, CA, USA) and refrigerated overnight. The plates were washed four times with phosphate buffered saline (PBS, Sigma–Aldrich Co., St. Louis, MO, USA) and patted dry. The wells were blocked with 200 µl of blocking buffer (Starting Block (PBS) Blocking Buffer, Pierce Biotechnology, Rockford, IL, USA) for 5 min then emptied. Dilution of sera samples used were 1:10 for isotypes IgA, IgG1 and IgE, 1:25 for IgG, and 1:50 for IgM and IgG2 in Starting Block T20 (PBS) Blocking Buffer (Pierce Biotechnology, Rockford, IL, USA). 100 µl of diluted serum, appropriate positive and negative control sera samples were added in duplicate wells, sealed and incubated at room temperature for 1 h. Plates were washed four times with PBS and patted dry. For total IgG, 100 µl of 1:25,000 rabbit anti-goat IgG horseradish peroxidase (Sigma–Aldrich Co., St. Louis, MO, USA) in Starting Block T20 blocking buffer was added per well and incubated for 1 h before adding chromagen, 1-Step Ultra TMB-ELISA (Pierce Biotechnology, Rockford, IL, USA) substrate. For other isotypes, before the addition of 100 µl of 1:25,000 conjugate, 100 µl of 1:5 mouse anti-sheep IgG1, IgA and IgE and for IgG2 and IgM, 1:10 dilution of their respective monoclonal antibodies (provided by CSIRO, Armidale, NSW, Australia) were added and incubated at room temperature for 1 h. Goat anti-mouse IgG conjugated with horseradish peroxidase was diluted at 1:25,000 and 100 µl was added to each well after washing four times with PBS. Plates were washed four times again with PBS and patted dry and 100 µl/well of the chromagen substrate, 1-Step Ultra TMB-ELISA (Pierce Biotechnology, Rockford, IL, USA) was added and incubated without a seal for 30 min in dark at room temperature. The reaction was stopped with addition of 100 µl/well of 2 M sulfuric acid. Absorbencies were read at 450–550 nm by an ELISA plate reader (ELX800 Universal Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT) and are presented as mean optical density for each experimental group.

Table 1

Weekly mean fecal egg counts per gram in single and trickle infected and control groups of Native and Suffolk lambs necropsied on days 14 and 21 post infection.

Group	0 DPI	7 DPI	14 DPI	21 DPI	SEM (21 DPI)
Single 14 Suffolk	1	7	1		
Single 14 Native	2	4	2		
Trickle 14 Suffolk	5	6	3		
Trickle 14 Native	0	0	2		
Single 21 Suffolk	10	5	4	4310 ^a	1710
Single 21 Native	3	3	2	305 ^b	237
Trickle 21 Suffolk	6	8	1	5930 ^a	3052
Trickle 21 Native	0	0	0	1930 ^a	720
Control Suffolk	1	1	2	0 ^c	
Control Native	4	3	3	0 ^c	

Note: Significantly different group means are indicated by different super-scripted letters ($p \leq 0.05$). DPI = days post infection ($n = 5$ /group except in control groups 0–14 DPI, where $n = 10$).

2.8. Statistical analysis

All the data were analyzed by using SAS statistical package version 9.1.3. FEC and worm counts were log transformed and repeated measures ANOVA was used to test FEC, PCV reduction percentages, peripheral eosinophil percentages, and ELISA optical density. Split-plot arrangement of treatments with treatment groups and animals within treatment groups were main plot effects and time by group interactions were subplot effects. A p value of ≤ 0.05 was considered to be significant. When an overall significant difference was detected, a Scheffé's test was used for main effect with pair wise comparison. Worm recovery, adult to immature worm ratios and cell counts in abomasal mucosa were compared by unpaired student t test with the significant p value set as ≤ 0.05 .

3. Results

3.1. Fecal egg count

All the groups indicated incomplete clearance of trichostrongyle type parasites in spite of repeated anthelmintic treatment before initiation of the experiment (Table 1). FEC did not increase above basal level in any of the infected groups until day 21 PI. The S21N group demonstrated significantly ($p < 0.05$) lower FEC compared with their corresponding Suffolk group. Among other groups, there was no significant ($p > 0.05$) difference observed regardless of infection regime, status and breed characters.

3.2. Blood PCV reduction percentage

Mean PCV reduction percentage was determined as ((Day 0 PCV – Sample Day PCV/Day 0 PCV) × 100) (Table 2). Although no significant ($p > 0.05$) difference between corresponding infection groups of both the breeds was detected, there was a tendency for the Suffolk groups to have greater PCV reduction percentages than the Native groups on both days 14 and 21 PI for both protocols. The reduction percentages were greater on day 21 PI compared with day 14 PI for both breeds.

Table 2

Mean blood packed cell volume (PCV) reduction percentage and standard error of the mean (SEM) by sampling time points for single and trickle infected and control groups of Native and Suffolk lambs necropsied on days 14 and 21 post infection.

Group	PCV reduction percentage and standard error of the mean							
	2 DPI	SEM	7 DPI	SEM	14 DPI	SEM	21 DPI	SEM
Single 14 Suffolk	1.9	3.1	4.2	3.4	20.5	5.1		
Single 14 Native	6.8	5.2	12.2	2.1	15.5	5.8		
Trickle 14 Suffolk	8.6	5.8	19.4	5.6	6.4	7.1		
Trickle 14 Native	−5.9	2.8	0.9	2.8	−4.8	4.5		
Single 21 Suffolk	1.1	4.8	−1.9	3.5	25.8	3.6	59.8	6.3
Single 21 Native	2.5	4.9	1.6	5.1	17	7.6	46.8	0.8
Trickle 21 Suffolk	11.4	7.5	15.6	6.8	12.7	5.1	21.1	6.1
Trickle 21 Native	2.3	6.9	2.4	11.9	15.6	3.7	41.6	4.3
Control Suffolk	7.9	3.8	13.7	4.4	8.1	3.7	9.6	2.8
Control Native	−3.7	2.5	3.3	4.1	−4.5	2.6	1.8	1.9

Note: DPI = days post infection, SEM = standard error of the mean ($n = 5$ /group except control groups where 0–14 DPI, $n = 10$).

3.3. Lymph node hypertrophy

There was no significant ($p > 0.05$) difference in mean prescapular lymph node weights of uninfected control, single and trickle infected lambs necropsied on day 14 PI within or between breeds (Table 3). Prescapular lymph nodes were significantly ($p < 0.05$) larger in S21S lambs compared with their uninfected control group and other single and trickle infected groups. Uninfected control and trickle infected groups did not have any significant differences in their prescapular lymph node weights.

Mean abomasal lymph node weights were not significantly different ($p > 0.05$) between the corresponding infected groups regardless of the infection regimes. However, they were significantly ($p < 0.05$) greater in infected groups compared with the uninfected control groups of their corresponding breeds except between trickle infected Native and uninfected control lambs.

3.4. Peripheral eosinophil percentage

Mean peripheral eosinophil percentages for all the single infected Native groups were significantly ($p < 0.05$) greater than their corresponding Suffolk groups after day 7 PI. This difference was observed only on day 7 between T14N and T14S groups and on days 14 and 21 PI between T21N and T21S groups (Figs. 1 and 2). Regardless of the infection regimen, Suffolk groups remained near the basal level throughout the study. There were no differences ($p > 0.05$) in peripheral eosinophil percentages among uninfected control groups.

3.5. Worm recovery

No worms were recovered from any of the uninfected control groups on either day 14 or 21 PI (Figs. 3 and 4). There was no significant ($p > 0.05$) difference in total worm burden between the corresponding infected groups of the two breeds regardless of the infection regimen. However, there was a trend for fewer *H. contortus* recovered from infected Native groups compared with Suffolk groups. Of particular note, the number of immature worms in relation to adults recovered was significantly ($p < 0.05$) greater in the S21N

group (Fig. 4). Although, no other groups demonstrated significant ($p > 0.05$) differences in adult to immature ratio, the Suffolk groups tended to have a greater adult to immature ratio. Male to female ratios were not significantly ($p > 0.05$) different among groups except in the 21 day trickle infection groups ($p < 0.05$).

3.6. Eosinophil, neutrophil and mast cell numbers in abomasal mucosa

Eosinophil, neutrophil and mast cell numbers in abomasal mucosa were significantly ($p < 0.05$) greater in all infected groups compared with their respective uninfected control groups (Table 4). Overall, eosinophil, neutrophil and mast cell numbers were greater for Native groups compared with Suffolk groups regardless of their infection regimes; however, differences between breeds were not always significant ($p > 0.05$). Neutrophils were observed to be in greater numbers in groups necropsied on day 14 PI while they were fewer in day 21 PI groups regardless of the breed and regime of infection. Among corresponding groups infected with similar regimes, significantly ($p < 0.05$) greater numbers of eosinophils were observed in the cardiac and fundic regions of S14N and T14N groups. Similarly, neutrophil numbers were significantly ($p < 0.05$) greater in the cardiac region of T14N, fundic region in S14N, T14N and S21N, and pyloric region of S14N groups than from their corresponding Suffolk groups. Likewise, mast cell numbers were significantly ($p < 0.05$) greater in the cardiac region of T14N and S21N, fundic region of S21N, and pyloric region of T14N, S14N and T21N groups than their corresponding Suffolk groups. For the remaining groups, regardless of infection regime, the counts for all cell types tended to be greater in Native groups compared with Suffolk groups.

3.7. Serum immunoglobulin profile

Optical density for serum total IgG on day 14 PI necropsy groups, regardless of infection regimen and breed, were not significantly ($p < 0.05$) different from each other, while it was significantly ($p < 0.05$) increased in S21N group compared with all other groups including the corresponding S21S group (Fig. 5) on days 14 and 21 PI. The response for

Table 3

Mean prescapular and abomasal lymph node weight (g) for single and trickle infected and control groups of Native and Suffolk lambs necropsied on days 14 and 21 post infection.

Group	Prescapular LN	SEM	Abomasal LN	SEM
Single 14 Suffolk	3.52 ^a	0.58	1.8 ^a	0.42
Single 14 Native	2.48 ^a	0.34	1.88 ^a	0.57
Control 14 Suffolk	3.0 ^a	0.36	0.5 ^b	0.06
Control 14 Native	2.3 ^a	0.22	0.74 ^{ab}	0.07
Trickle 14 Suffolk	3.1 ^a	0.47	2.0 ^a	0.26
Trickle 14 Native	1.9 ^a	0.07	1.4 ^a	0.54
Single 21 Suffolk	5.92 ^b	0.59	2.64 ^b	0.60
Single 21 Native	4.22 ^a	0.85	2.4 ^b	0.42
Control 21 Suffolk	4.2 ^a	0.19	0.94 ^a	0.13
Control 21 Native	4.42 ^a	0.88	0.98 ^a	0.17
Trickle 21 Suffolk	4.08 ^a	1.08	3.14 ^a	0.39
Trickle 21 Native	3.52 ^a	0.70	2.08 ^a	0.36

Note: Significant differences are indicated by different letters ($p \leq 0.05$). LN = lymph node, SEM = standard error of the mean ($n = 5/\text{group}$).

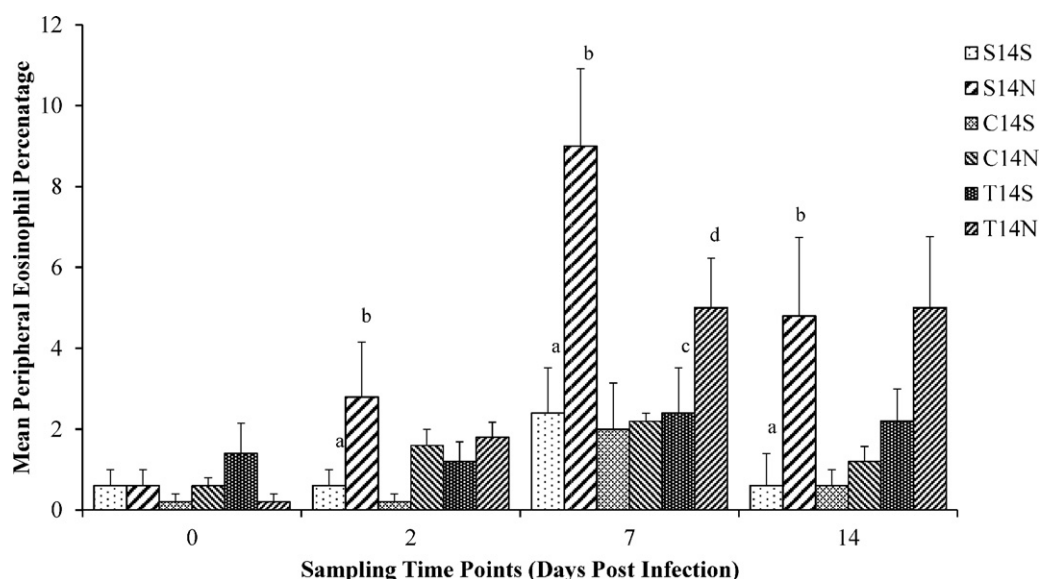


Fig. 1. Mean peripheral eosinophil percentages in uninfected and single and trickle infected groups ($n = 5/\text{group}$) of Suffolk and Native lambs necropsied on day 14 post infection. Significantly different values ($p \leq 0.05$) are indicated by different letters. S14S = single infected Suffolk group, S14N = single infected Native group, C14S = uninfected control Suffolk group, C14N = uninfected control Native group, T14S = trickle infected Suffolk group, and T14N = trickle infected Native group.

Table 4

Mean eosinophil (E), neutrophil (N) and mast cell (M) number per mm^2 of cardiac, fundic and pyloric regions of the abomasum of single and trickle infected and control groups of Native and Suffolk lambs necropsied on days 14 and 21 post infection.

Treatment groups	Cardiac abomasum Mean count/mm			Fundic abomasum Mean count/mm			Pyloric abomasum Mean count/mm		
	E	N	M	E	N	M	E	N	M
Single 14 Suffolk	16 ^a	5 ^a	4 ^a	27 ^a	8 ^a	4 ^a	13 ^a	3 ^a	6 ^a
Single 14 Native	68 ^b	17 ^a	9 ^a	63 ^b	16 ^b	5 ^a	47 ^a	1 ^b	10 ^a
Trickle 14 Suffolk	27 ^a	9 ^a	3 ^a	30 ^a	7 ^a	4 ^a	20 ^a	4 ^a	5 ^a
Trickle 14 Native	55 ^b	15 ^b	13 ^b	95 ^b	20 ^b	10 ^b	46 ^a	7 ^a	14 ^b
Control 14 Suffolk	0 ^c	2 ^c	1 ^c	1 ^c	2 ^c	0 ^c	2 ^c	1 ^c	1 ^c
Control 14 Native	0 ^c	1 ^c	0 ^c	0 ^c	1 ^c	1 ^c	0 ^c	0 ^c	0 ^c
Single 21 Suffolk	8 ^a	1 ^a	4 ^a	4 ^a	1 ^a	6 ^a	18 ^a	2 ^a	8 ^a
Single 21 Native	13 ^a	2 ^a	13 ^b	22 ^a	2 ^b	13 ^b	24 ^a	2 ^a	23 ^b
Trickle 21 Suffolk	7 ^a	1 ^a	5 ^a	11 ^a	1 ^a	3 ^a	21 ^a	11 ^a	4 ^a
Trickle 21 Native	18 ^a	2 ^a	10 ^a	26 ^a	3 ^a	7 ^a	15 ^a	1 ^a	19 ^b
Control 21 Suffolk	0 ^c	0 ^c	1 ^c	0 ^c	0 ^c	0 ^c	1 ^c	0 ^c	1 ^c
Control 21 Native	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	1 ^c	1 ^c	0 ^c	2 ^c

Note: Significant differences are indicated by different superscripted letters ($p \leq 0.05$) ($n = 5/\text{group}$).

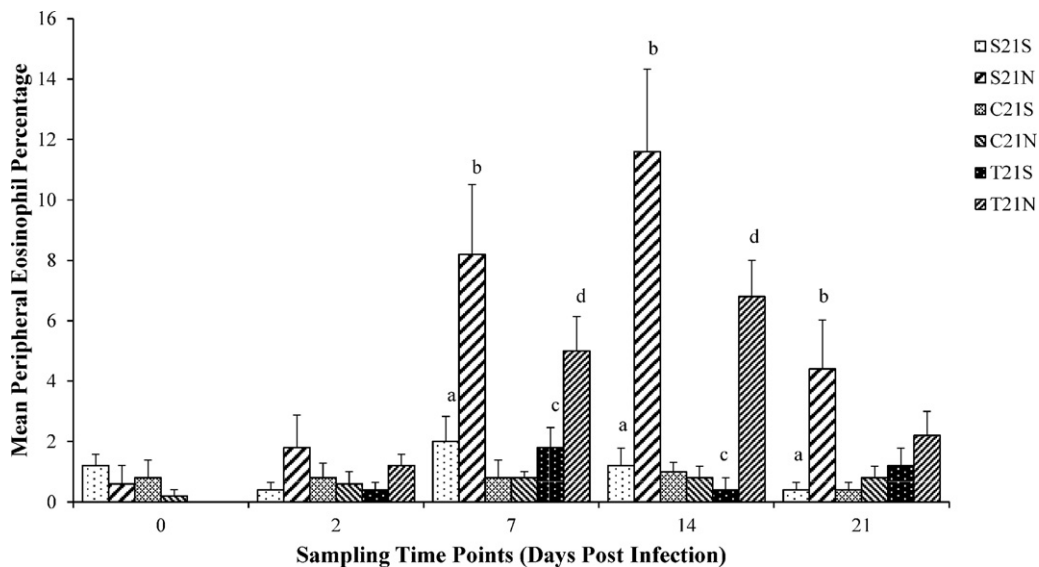


Fig. 2. Mean peripheral eosinophil percentages in uninfected, and single and trickle infected groups ($n=5/\text{group}$) of Suffolk and Native lambs that were necropsied on day 21 post infection. Significantly different values ($p \leq 0.05$) are indicated by different letters. S21S = single infected Suffolk group, S21N = single infected Native group, C21S = uninfected control Suffolk group, C21N = uninfected control Native group, T21S = trickle infected Suffolk group, and T21N = trickle infected Native group.

the T21N group was also greater than the other groups, but the difference was not as obvious. A steady increase in IgE optical density was observed in pooled serum samples from the S21N and T21N groups compared to their corresponding Suffolk groups (data not presented). All other groups regardless of their breed characteristics and infection regimes failed to demonstrate any trend in optical densities for any of the immunoglobulin isotypes (data not presented).

4. Discussion

Severity of *H. contortus* infection is generally estimated based on the FEC in small ruminants. However, PCV reduction and the worm burden depict a more accurate picture of severity. The results from this study demonstrated a substantially severe infection in Suffolk compared to native lambs. Although a trend for higher worm burdens in Suffolk lambs, similar recovery rate regardless of infection

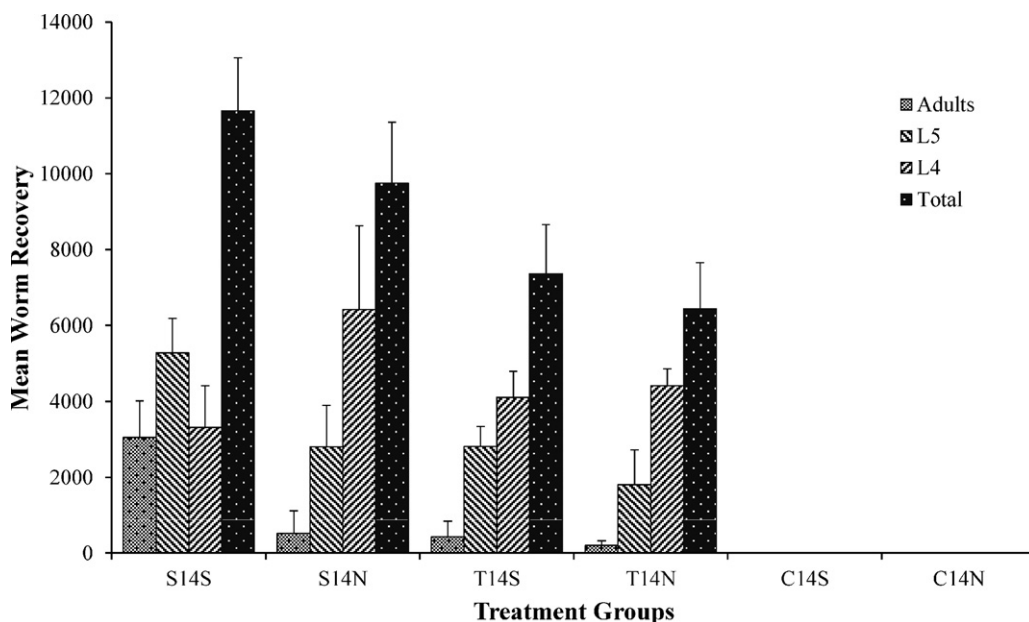


Fig. 3. Mean recovery of *H. contortus* developmental stages from uninfected and single and trickle infected groups ($n=5/\text{group}$) of Suffolk and Native lambs necropsied on day 14 post infection. S14S = single infected Suffolk group, S14N = single infected Native group, C14S = uninfected control Suffolk group, C14N = uninfected control Native group, T14S = trickle infected Suffolk group, and T14N = trickle infected Native group.

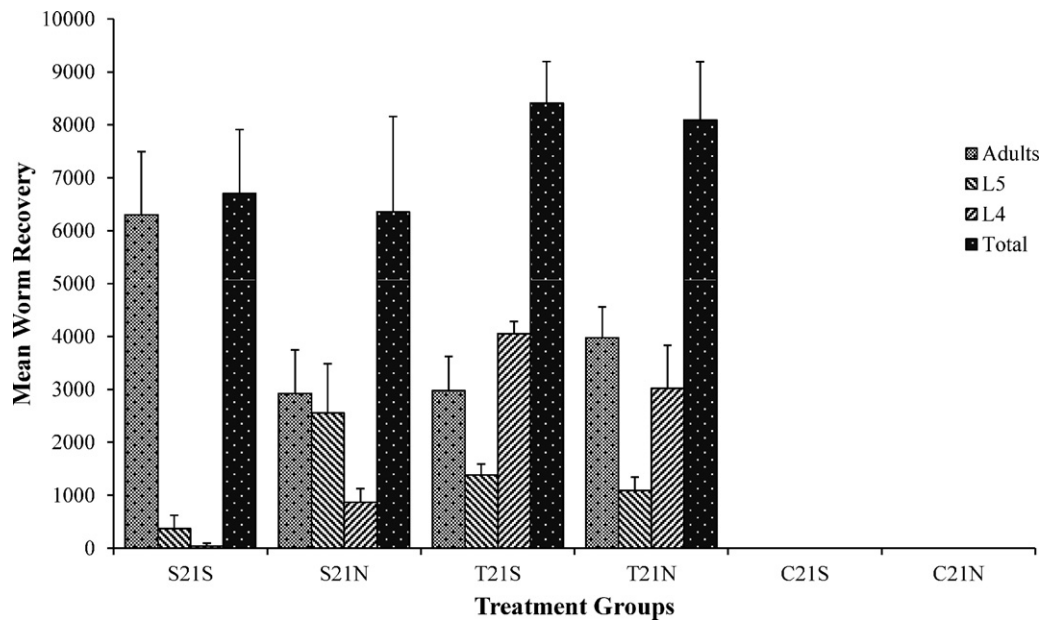


Fig. 4. Mean recovery of *H. contortus* developmental stages from uninfected and single and trickle infected groups ($n=5$ /group) of Suffolk and Native lambs necropsied on day 21 post infection. S21S= single infected Suffolk group, S21N= single infected Native group, C21S= uninfected control Suffolk group, C21N= uninfected control Native group, T21S= trickle infected Suffolk group, and T21N= trickle infected Native group.

regimen in Native lambs was in contrast to our expectations. Furthermore, a higher proportion of worms in native lambs were immature. This may be indicative of the time it takes to eliminate the larvae. It should be noted that the previous studies with Native sheep were conducted under natural grazing conditions over periods of 4–16 weeks and not short term experimental infections (Miller et al., 1998; Shakya et al., 2009). Also, worms recovered were predominantly adults with no appreciable larvae as was

found in this study. It is possible that the larvae observed in this study may eventually have been eliminated before reaching maturity or developed slowly to replace adults which would result in lower FEC. Lower worm burdens were a common feature in several resistant breeds including Native lambs (Amarante et al., 1999, 2005; Woolaston et al., 1990, 1996; Miller et al., 1998; Aumont et al., 2003; Gruner et al., 2003; Shakya et al., 2009). If that is the case, then the adult population in the Native lambs would not

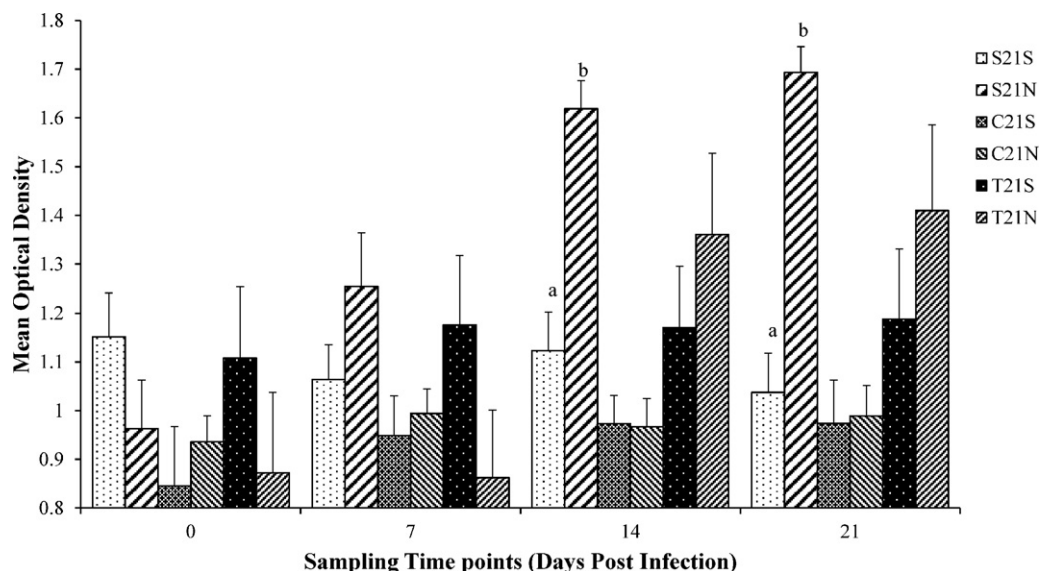


Fig. 5. Mean optical densities for serum IgG in uninfected and single and trickle infected groups ($n=5$ /group) of Suffolk and Native lambs necropsied on day 21 post infection. Significantly different p values (≤ 0.05) are indicated by different letters. S21S= single infected Suffolk group, S21N= single infected Native group, C21S= uninfected control Suffolk group, C21N= uninfected control Native group, T21S= trickle infected Suffolk group, and T21N= trickle infected Native group.

increase substantially, thus supporting previous observations. Also, if these larvae had eventually matured adding to the adult population, there would have been equivalent worm burdens in each breed. Therefore, higher number of immature worms in Native groups may be an indication that anti-*Haemonchus* effects are exerted early in infection.

There are contradictory reports on post infection lymph node weights. Gasbarre (1997) reported an increase by 20 fold in helminth infections and Balic et al. (2000) reported no significant difference with uninfected control lambs. In this study, both the S14S and T14S lambs had hypertrophied abomasal lymph nodes. For Native lambs on the other hand, a significant difference was found only between the S21N and the uninfected controls.

Increased peripheral eosinophilia was reported as an indicator of a greater responsiveness of hosts to *Trichostrongylus colubriformis* infection (Dawkins et al., 1989), and Terefe et al. (2005) reported eosinophilia to be correlated with protection against *H. contortus*. Our findings showed induction of peripheral eosinophilia as early as two days PI in Native lambs regardless of infection regimen. At the peak, the peripheral eosinophil percentage was as much as 15–25 fold greater in Native lambs compared with 2–4 folds in Suffolk lambs. Findings of this study therefore conform to the conclusions made by Dawkins et al. (1989).

Higher numbers of abomasal mucosal eosinophils, mast cells and neutrophils were observed in infected animals compared to controls, and for the most part Natives were higher than Suffolk lambs. Hunter and MacKenzie (1982) reported a few eosinophils present by the seventh day of *H. contortus* infection in lambs, and by day 12 there were no mast cells or globule leukocytes noted. In lambs infected for the first time, eosinophils were observed in the abomasal mucosa where they did not seem to increase later in adult worm infected lambs, but greater numbers of mucosal mast cells (MMC) were reported in such lambs (Balic et al., 2000). In another experiment, Balic et al. (2002) did not find any difference in eosinophils or mast cells after 12 weeks of immunizing infection, however there was a rapid eosinophil recruitment around L4 sin-flicting considerable damage to *H. contortus* larval surface especially in gastric pits of immunized sheep (Balic et al., 2006). Eosinophils from *H. contortus* sensitized sheep were seen to immobilize *H. contortus* larvae in the presence of anti-*Haemonchus* antibodies in an *in vitro* study (Rainbird et al., 1998). According to Pennock and Grencis (2006), post infection mastocytosis supported a close association with nematode infection and an important role in parasite elimination in lambs. A greater number of eosinophils, and MMC in Native lambs in general was associated with an increased immature population in our study, which indicated a possible contribution to inhibition of growth and development of the nematode population. In contrast, lower numbers of these cells in Suffolk lambs may not have provided the conditions for inhibition of growth and development. Presence of more neutrophils in the day 14 necropsy Native groups than the day 21 necropsy groups indicated that they may have a role during the early phase of *H. contortus* infection. Pesce et al. (2008) reported that neutrophils are involved in clearance of bacteria that co-infect with nematodes.

Among all the immunoglobulin isotypes, IgE has been shown to be consistently associated with nematode infection (Lacroux et al., 2006; Terefe et al., 2007; Shakya et al., 2009). Results of this study indicated that the level of serum IgE increased up to 39 fold in Native lambs whereas it was at the basal level in Suffolk lambs. Most of the IgE produced is tissue bound to the Fcε receptors on MMC, eosinophils and basophils. Upon subsequent exposure to the antigen, degranulation of the granulocytes is triggered and a variety of mediators like histamine, leukatrienes, prostaglandins and enzymes like chymase are released that bring about changes in gut physiology.

Although individual animal serum immunoglobulin isotypes were not evaluated, pooled samples showed all five immunoglobulin isotypes specific for *H. contortus* WWA were present in the serum. S21N lambs demonstrated a trend where IgE increased by 13 fold at its peak after 2 weeks, which was maintained at about the same level. Similarly, serum IgA increased in the S21N lambs which peaked at about 2 weeks (data not presented). IgA is the major immunoglobulin isotype in mucosal immunity and is found in abomasal mucus in nematode infections (Harrison et al., 2003; Amarante et al., 2005). Unfortunately, mucosal IgA level was not measured in this study. In contrast to Native lambs, serum IgE and IgA levels in infected Suffolk lambs remained around the baseline throughout the study. Therefore, production of IgE and IgA may be important in development of resistance against *H. contortus*.

In sheep vaccinated against *H. contortus*, the production of IgG2 was reported to be at a greater level (Knox et al., 2005), but Gill et al. (1994) reported a lower number of IgG2 bearing cells in *H. contortus* infected sheep abomasal. In this study, IgG2 showed an increase in Native and a decrease in Suffolk lambs. Serum IgM also showed a different trend, it decreased in Suffolk lambs, while in Native lambs it was maintained at the basal level. IgG1 level remained about the same until 14 DPI in both the single infected groups however it declined in Suffolk but showed an increased trend in Natives which is in agreement with the findings reported by Gill et al. (1994). The IgG level in Native lambs, whether single or trickle infected, increased but in Suffolk lambs it remained around the same level. Gomez-Munoz et al. (1999), have found resistant animals to produce greater levels of all the isotypes. The level of these immunoglobulins was found to be associated with decreased FEC and worm load. From the results of this study and reported by others, it is plausible that increased serum levels of immunoglobulin isotypes IgE, IgA, and IgG1 are indicative of their proportionately increased production and recruitment of inflammatory cells, or secretions such as abomasal mucus. The histological observation of increased cellular infiltration of eosinophils and mastocytosis in the resistant Native breed of sheep accompanied by greater number of delayed development of *H. contortus* larvae compared with susceptible lambs is indicative of their protective role. Secondary exposure of the parasitic antigens may have triggered the degranulation of sensitized MMC and eosinophils that have IgE bound to their surfaces which resulted in release of mediators earlier in the Native lambs than in the Suffolk lambs, thus the growth and development of *H. contortus* in Native lambs was adversely

affected.

In conclusion, our findings indicated that immune components such as mast cell hyperplasia, peripheral and tissue eosinophilia, increased serum IgE, IgA, IgG1 are associated with and may be responsible for increased resistance of Native lambs against *H. contortus* infection indicated by inhibition of its growth and development in the early infection period.

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