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Effect of dexamethasone treatment on the immune response of Gulf Coast Native lambs to *Haemonchus contortus* infection

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Abstract

Neonatal and weaner Gulf Coast Native (Native) lambs were studied to determine whether an immunological basis underlies their natural resistance to *Haemonchus contortus* infection. Neonatal Native lambs ($n = 8$) and weaner Native lambs ($n = 15$) were randomly assigned to a treatment or a control group. Lambs in the treatment group received dexamethasone by intramuscular injection three times a week for 10 weeks (neonatal) and 15 weeks (weaners). All lambs were monitored for fecal egg count (FEC), blood packed cell volume (PCV), and white blood cell differential counts on a weekly basis for the duration of the studies. Neonatal lambs were kept on pasture with their dams and weaner lambs were dewormed at weaning and kept in pens where they received trickle infections. Serum antibody titers to *H. contortus* whole worm antigen (WWA) were determined using ELISA. Lymphocyte proliferation assays on peripheral blood mononuclear cells (PBMC) were done to assess lymphocyte function. All lambs were vaccinated with killed *Brucella abortus* strain 19 to assess the effect of dexamethasone treatment on antibody response. All lambs were necropsied at the end of each study to recover the contents of the gastrointestinal tract for nematode enumeration and identification. The results showed that mean FEC and mean PCV of the treatment group was significantly higher and lower, respectively, than in the control group in both neonatal and weaner lambs from weeks 6 and 5, respectively. At necropsy, total nematode count was significantly higher in treatment groups than in the control groups. Serum antibody titers to *H. contortus* WWA were significantly lower in treated groups than in control groups. Treatment groups showed a consistent depression in lymphocyte percentage being significantly lower from week 6 in both neonatal and weaner lambs. No differences were found in the response of PBMC to mitogen

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stimulation between the groups. Lambs in the control groups showed strong positive brucellosis card tests and the treatment groups did not. Dexamethasone treatment resulted in depression of the immune response and loss of natural resistance of Native lambs to *H. contortus* infection. The results of these studies suggest that some aspects of the immune response may underlie the natural resistance of Native sheep to *H. contortus* infection.

Keywords: Sheep; *Haemonchus contortus*; Dexamethasone; Immunity

1. Introduction

The evidence for genetic variation in resistance to infection with gastrointestinal nematodes parasites comes from three sources: variation within breed, variation between breeds, and the identification of genes contributing to the variation (Stear and Murray, 1994). There is a substantial body of evidence that supports variation between breeds in resistance to *Haemonchus contortus*. Breeds that have previously been shown to be relatively resistant to *H. contortus* include the Scottish Blackface (Abbott et al., 1985a,b), Red Maasai (Preston and Allonby, 1978, 1979; Bain et al., 1993), and Barbados Blackbelly, Florida Native and St. Croix (Bradley et al., 1973; Yazwinski et al., 1979, 1980; Courtney et al., 1985a,b; Gamble and Zajac, 1992). More recently, epidemiological studies have shown that the Gulf Coast Native (Native) sheep are more resistant to gastrointestinal nematode infection (specifically *H. contortus*) than Suffolk sheep (Bahirathan et al., 1996; Miller et al., 1998). Miller et al. (1998) demonstrated that resistance is characterized by significantly ($P \leq 0.05$) lower fecal egg count, 5300 EPG Suffolk lambs compared to 430 EPG Native lambs; and lower nematode burden, 9105 total *H. contortus* counts in Suffolk lambs compared to 500 total *H. contortus* counts in Natives lambs. Suffolk lambs required substantially more anthelmintic treatments, and deaths due to haemonchosis were only observed in Suffolk lambs.

Several studies have demonstrated that resistance to gastrointestinal nematode infection within breed is related to a local inflammatory reaction involving different cell types, antibodies, gastrointestinal mucus, and inflammatory mediators. This local response, known as immune exclusion, in which incoming larvae fail to establish, has been documented in sheep against *H. contortus* infection (Jackson et al., 1988; Miller, 1984; Miller et al., 1985). Several studies have demonstrated that immune exclusion can be reduced by corticosteroid treatment (Jackson et al., 1988; Miller, 1984; Matthews et al., 1979; Huntley et al., 1992). Presson et al. (1988) observed that resistance to *H. contortus* in a resistant genotype of Merino sheep was abrogated by treatment with corticosteroids and this loss of resistance was associated with a decline in mast cell and globule leukocyte density and decline in concentrations of sheep mast-cell proteinase in abomasal mucosal tissues.

T lymphocytes are an important component of the local inflammatory response to gastrointestinal nematode infection and the role of helper T lymphocytes has been demonstrated in studies where sheep became highly susceptible to *H. contortus* challenge after being depleted of these cells (Gill et al., 1993; Karanu et al., 1997).

Different effector mechanisms have been reported for between-breed variations in resistance to nematode infection. Resistant Red Maasai sheep and Scottish Blackface sheep showed strong peripheral eosinophil responses that were associated with high levels of globule leucocytes and *H. contortus*-specific mucus IgA (Stear and Murray, 1994). The mechanism(s) involved in the resistance in Native sheep have not been characterized. The objective of this study was to determine whether an immunological basis underlies the natural resistance of Native sheep to *H. contortus* infection by suppressing the immune response with dexamethasone during the initial natural exposure infection period as neonatal lambs and during an experimental challenge infection after weaning.

2. Materials and methods

2.1. Experimental design

2.1.1. Natural infection in neonatal lambs

At 4 weeks of age (week 0), eight Native lambs that were raised on pasture since birth, were randomly allocated into either a treatment or a control group ($n = 4$) and lambs in the treatment group began treatment with dexamethasone (Azium®, 1 mg/kg bodyweight, IM) three times a week for a period of 9 weeks. The lambs were maintained on naturally infected ryegrass pasture with their dams at the Central Station Sheep Farm, Louisiana Agricultural Experimental Station, Baton Rouge, LA.

Fecal and blood samples were collected on a weekly basis to monitor infection level based on FEC and blood packed cell volume (PCV). Blood was also processed for leukocyte differential determination, and serum was tested for antibody levels in response to mature *H. contortus* whole worm antigen (WWA). To test lymphocyte functionality, lymphocytes were isolated from blood at weeks 1, 6 and 9 of dexamethasone treatment and from abomasal lymph nodes at necropsy.

At week 8 of treatment, all lambs were vaccinated with a killed *Brucella abortus* strain 19 (1 mg in 1 ml of saline solution per animal, IM) vaccine. Serum samples were tested for a response on the day of vaccination and 10 days after vaccination with the brucellosis card test.

All lambs were euthanized (Beuthanasia®-D, 1 ml per 10 kg bodyweight, IV), at the end of the study (week 9) to recover abomasal lymph nodes and gastrointestinal tract nematodes.

2.1.2. Experimental challenge infection in weaner lambs

At 19 weeks of age, 15 Native lambs were removed from pasture and dewormed with doramectin (Dectomax®, 0.2 mg/kg) and albendazole (Valbazen®, 10 mg/kg) to remove existing nematode infection. Lambs were maintained under parasite-free conditions in dirt floor pens at the Central Station Sheep Farm, Louisiana Agricultural Experimental Station, Baton Rouge, LA. They were fed a growing ration and water was available at all times. Lambs were randomly allocated (week 0; 20 weeks of age) to treatment ($n = 8$) and control ($n = 7$) groups and the lambs assigned to the treatment group began treatment with dexamethasone (Azium®, 1 mg/kg, IM) three times a week for a period of 14 weeks. At week 6, all lambs began receiving trickle infections with a mixed species larval inoculum (500 L_3 composed by 90% *H. contortus*, and the remaining 10% *Trichostrongylus* spp. and *Cooperia* spp.) given orally three times a week for 9 weeks.

Fecal and blood samples were collected on a weekly basis and serum was tested for antibody levels in response to *H. contortus* WWA at weeks 7, 9, 11, 13 and 15 of treatment. To test lymphocyte functionality, lymphocytes were isolated from blood at weeks 1, 5 and 14 of treatment and from abomasal lymph nodes at necropsy.

At week 8, all lambs were vaccinated with a killed *B. abortus* strain 19 vaccine (1 mg in 1 ml of saline solution per animal, IM). Serum samples were tested for a response on the day of vaccination and 4 and 8 weeks post-vaccination with the brucellosis card test.

All lambs were necropsied at the end of the study (week 15) to recover abomasal lymph nodes and gastrointestinal tract nematodes.

2.2. FEC and necropsy

Individual fecal samples were collected from the rectum and were processed to determine FEC using the modified McMaster technique and reported as eggs per gram (EPG) (Whitlock, 1948).

Nematodes were recovered from the gastrointestinal tract at necropsy and counted and identified as described by Miller et al. (1987). The recovery procedure was modified so that organ contents were brought to a volume of 5 l and a 500 ml aliquot was taken and preserved with formalin.

2.3. Hematology

Blood PCV was obtained by an automated hematology analyzer (INC. Baker System 9110 + Plus). Differential leukocyte percentages were made on blood smears stained with modified Wright's stain.

2.4. Lymphoproliferation assays

Lymphoproliferation assays were run on peripheral blood mononuclear cells (PBMC). Lymphocytes were isolated from whole blood using Ficoll-Plaque. After three washes with PBS, 1 ml of RPMI-1640 was added, and cell concentration was determined with a haemocytometer using Trypan Blue to obtain a final suspension of 2×10^6 cells/ml. Phytohemagglutinin (PHA), Concanavalin A (ConA), and Pokeweed (PW) were used to test T lymphocyte function. ConA and PW were used at 4, 2 or 1 μg/ml and PHA was used at 8, 4 or 2 μg/ml. Mitogens were diluted in RPMI-1640 and added to respective wells in 100 μl volumes. RPMI-1640 was added to the control wells, and then 100 μl of the cell suspension was added to all wells. All cultures were done in triplicate. The plates were incubated at 39 °C in a humidified incubator with 5% CO_2 for 3 days. The plates were then pulsed with 0.5 μCi [^{3}H] thymidine per well for 4 h and then harvested for liquid scintillation counting.

2.5. Enzyme-linked immunosorbent assay (ELISA) for antibodies to H. contortus WWA

The ELISA test used was a modification of the procedure described by Smith et al. (1999). Microtiter plates were coated with 50 μl of WWA obtained from mature *H. contortus* worms at necropsy. WWA was obtained following the procedure described by Kabagambe (1997).

Briefly, nematodes were washed in water under a 500 μm filter, and rinsed in phosphate buffered saline (PBS). The nematodes were hand-homogenized in about 5 ml of PBS and the homogenate was centrifuged at $5000 \times g$. The supernatant was collected and stored at −20 °C. Total protein concentration was determined using a microtiter plate-adapted bicinchoninic acid assays (Pierce, Rockford, IL, USA). The antigen was diluted in carbonate (pH 9.6) coating buffer to 10 μg/ml. The plates were incubated at room temperature overnight and then washed with PBS containing 0.05% Tween-20 (washing buffer). Serum samples diluted 1/500 in serum diluent were added to the plates and then incubated for 2 h at room temperature (24 °C). Serum from a mature ewe with a high infection level was used as a positive control and serum from colostrum deprived lambs was used as a negative control and both were also diluted to 1/500 dilution in serum diluent and incubated for 2 h at room temperature. All serum samples (test, negative and positive controls) were done in triplicate. After another wash, 50 μl of rabbit anti-sheep IgG alkaline phosphatase conjugate (Kirkegaad and Perry, MD, USA) diluted to 1:1000 in blocking buffer was added and incubated for 2 h. The plates were then washed and dried, and 75 μl of pNPP substrate (Kirkegaad and Perry, MD, USA) was added. The plates were then incubated in the dark for 1 h and the color reaction was stopped with 75 μl of 5% EDTA. The plates were read at 405 nm with an automatic ELISA plate reader.

2.6. Statistical analysis

Statistical analysis was done with raw data except for FEC and total nematode count that were log transformed ($\log x + 1$) to stabilize variance. The means of the groups were compared using PROC MIXED and PROC GLM for repeated measures in SAS. Tukey test was used to compare differences in nematode count between groups and Scheffe's test was used for pair-wise treatment group comparisons for percent lymphocytes. Differences were considered statistically significant when $P \leq 0.05$.

3. Results

3.1. Neonatal lambs

3.1.1. Fecal egg counts

The mean FEC for both groups started increasing at week 3, peaked at week 6 and subsequently decreased (Table 1). The mean FEC of the treated group was significantly higher than the control group from week 6 to the end of the study. The overall mean FEC for the treated group (9719 EPG) was significantly higher than the control group (2792 EPG).

3.1.2. Total nematode counts at necropsy

H. contortus was the predominant nematode with *Trichostrongylus*, *Cooperia*, and *Oesophagostomum* also present (Table 2). Except for *Oesophagostomum*, the burden of the other three nematodes and the total burden of the treated group was significantly higher than the control group.

Table 1
Mean (±S.E.M.) fecal egg count and mean (±S.E.M.) blood packed cell volume for control ($n = 4$) and dexamethasone-treated ($n = 4$) Gulf Coast Native neonatal lambs

Week[a]	Fecal egg count[b]		Packed cell volume[b]	
	Control	Treated	Control	Treated
0[c]	4 ± 2	0	33.5 ± 1.6	32.8 ± 0.6
1	147 ± 59	0	34.0 ± 1.8	32.5 ± 1.2
2	195 ± 134	8 ± 5	32.0 ± 1.6	32.3 ± 1.3
3	1453 ± 401	1503 ± 586	31.3 ± 0.5	29.8 ± 1.3
4	5788 ± 1939	6338 ± 1383	30.0 ± 0.8	25.0 ± 1.7
5	2325 ± 925	9113 ± 3207	29.5 ± 0.6	24.5 ± 1.7
6	12700 ± 8843 a	41963 ± 5456 b	31.8 ± 1.4 a	23.3 ± 1.4 b
7	2250 ± 1240 a	19874 ± 7510 b	34.0 ± 1.1 a	22.3 ± 1.7 b
8	2225 ± 1026 a	9013 ± 1968 b	32.8 ± 1.3 a	23.0 ± 2.3 b
9	837 ± 480 a	9375 ± 1927 b	33.3 ± 2.5 a	22.5 ± 1.4 b
Mean	2792 ± 1003 a	9719 ± 2163 b	32.2 ± 0.5 a	26.8 ± 0.8 b

[a] Week of dexamethasone treatment (1 mg/kg three times a week).
[b] Means with unlike letters are significantly different ($P \leq 0.05$) based on log-transformed data for fecal egg count and raw data for PCV.
[c] Lambs were 4 weeks old.

3.1.3. *Blood packed cell volume*

The mean PCV of both groups was similar through week 3 and then the treated group PCV continually decreased being significantly lower from week 6 to the end of the study (Table 1).

3.1.4. *White blood cell differential*

Mean lymphocyte percentage in the treated group was significantly lower than that of the control group at week 4 and from week 6 to the end of the study (Table 3). Other leukocyte differences were not informative (data not shown).

Table 2
Mean (±S.E.M.) nematode burden in the abomasum, small intestine and large intestine of control and dexamethasone-treated Gulf Coast Native neonatal and weaner lambs

Age	Group	*n*	Abomasum[a]	Small intestine[a]		Large intestine[a]	Total[a]
			Haemonchus	*Trichostrongylus*	*Cooperia*	*Oesophagostomum*	
Neonate[b]	Control	4	238 ± 156 a	19 ± 12 a	0 a	6 ± 6	263 ± 147 a
	Treated[c]	4	2325 ± 335 b	169 ± 76 b	125 ± 75 b	0	2619 ± 447 b
Weaner[d]	Control	7	886 ± 329 a	35 ± 28	0	0	921 ± 330 a
	Treated[c]	8	1389 ± 526 b	40 ± 13	6 ± 6	0	1429 ± 192 b

[a] Means within age group with different letters are significantly different ($P \leq 0.05$) based on log-transformed data.
[b] Natural infection from grazing, necropsied at 14 weeks of age.
[c] Dexamethasone® (1 mg/kg three times a week).
[d] Experimental trickle infection over 9 weeks, necropsied at 35 weeks of age.

Table 3
Mean (±S.E.M.) percent lymphocytes for control and dexamethasone-treated Gulf Coast Native neonate and weaner lambs

Week[a]	Age group			
	Neonate[b]		Weaner[b]	
	Control ($n = 4$)	Treated ($n = 4$)	Control ($n = 7$)	Treated ($n = 8$)
0[c]	50.5 ± 4.1	48.5 ± 5.2	59.3 ± 3.4	66.0 ± 2.2
1	41.8 ± 6.4	47.0 ± 4.2	66.9 ± 3.7	63.9 ± 2.5
2	48.0 ± 3.0	48.0 ± 6.4	66.9 ± 3.4 a	52.3 ± 1.3 b
3	53.5 ± 4.4	40.0 ± 5.2	72.3 ± 2.4 a	59.2 ± 2.7 b
4	61.3 ± 2.8 a	42.0 ± 6.0 b	63.9 ± 2.6	58.3 ± 3.7
5	59.5 ± 3.3	50.5 ± 3.4	66.1 ± 3.9	62.0 ± 3.6
6[d]	67.5 ± 2.8 a	48.8 ± 2.5 b	71.3 ± 1.0 a	62.0 ± 2.7 b
7	63.8 ± 3.4 a	40.5 ± 7.2 b	68.7 ± 2.6 a	59.1 ± 2.6 b
8	64.5 ± 4.8 a	33.3 ± 7.8 b	65.0 ± 3.3 a	49.9 ± 5.9 b
9	67.0 ± 4.8 a	39.8 ± 1.9 b	66.4 ± 3.2 a	47.4 ± 6.4 b
10			70.3 ± 0.9 a	38.8 ± 7.7 b
11			69.0 ± 3.2 a	45.6 ± 5.0 b
12			64.4 ± 1.8 a	43.5 ± 4.0 b
13			69.0 ± 3.7 a	44.1 ± 4.0 b
14			65.7 ± 1.7 a	34.1 ± 2.6 b
15			63.7 ± 3.9 a	38.1 ± 3.7 b

[a] Week of dexamethasone treatment (1 mg/kg three times a week).
[b] Means with unlike letters are significantly different ($P \leq 0.05$).
[c] Neonatal and weaner lambs were 5 and 20 weeks of age, respectively.
[d] Weaner lambs started receiving trickle infections with 500 infective larvae three times a week.

3.1.5. *Lymphoproliferation assays*

Dexamethasone treatment did not affect T lymphocyte functionality as evidenced by similar lymphoproliferation responses between groups (data not shown).

3.1.6. *ELISA for H. contortus WWA*

The antibody levels started high in both groups and steadily declined through week 5 of dexamethasone treatment (Table 4). At week 6, antibody levels increased again in both groups and subsequently, remained elevated in the control group being significantly higher from week 7 and declined continually in the treated group.

3.1.7. *Brucellosis card test*

All lambs were negative prior to vaccination and the treated group remained unresponsive to the vaccination, whereas the control group had a strong response (3+) to vaccination by day 10 (Table 5).

3.2. *Weaner lambs*

3.2.1. *Fecal egg counts*

The mean FEC remained low through week 9 (week 3 after experimental infection started), increased slightly at week 10 and then increased substantially in the treated group

Table 4
Mean (±S.E.M.) OD to *H. contortus* whole worm antigen for control and dexamethasone-treated Gulf Coast Native neonate and weaner lambs

Week[a]	Age group			
	Neonate[b]		Weaner[b]	
	Control ($n = 4$)	Treated ($n = 4$)	Control ($n = 7$)	Treated ($n = 8$)
0[c]	0.178 ± 0.052	0.334 ± 0.041		
1	0.155 ± 0.046	0.280 ± 0.034		
2	0.070 ± 0.027	0.133 ± 0.019		
3	0.065 ± 0.021	0.106 ± 0.016		
4	0.028 ± 0.015	0.042 ± 0.018		
5	0.019 ± 0.002	0.017 ± 0.002		
6[d]	0.049 ± 0.004	0.046 ± 0.008		
7	0.052 ± 0.002 a	0.034 ± 0.011 b	0.064 ± 0.006	0.033 ± 0.0121
8	0.056 ± 0.006 a	0.034 ± 0.001 b		
9	0.060 ± 0.012 a	0.017 ± 0.006 b	0.108 ± 0.029 a	0.036 ± 0.007 b
10				
11			0.271 ± 0.043 a	0.076 ± 0.012 b
12				
13			0.353 ± 0.060 a	0.067 ± 0.013 b
14				
15			0.221 ± 0.028 a	0.049 ± 0.009 b

[a] Week of dexamethasone treatment (1 mg/kg three times a week).
[b] Means with unlike letters are significantly different ($P \leq 0.05$).
[c] Neonatal and weaner lambs were 5 and 20 weeks of age, respectively.
[d] Weaner lambs started receiving trickle infections with 500 infective larvae three times a week.

Table 5
Brucellosis card score of control and dexamethasone-treated Gulf Coast Native neonatal lambs exposed to natural nematode challenge

Group	Animal no.	Day[a]	
		0[b]	10
Control	9052	–	3+
	9058	–	3+
	9059	–	3+
	9081	–	3+
Treated	9064	–	–
	9080	–	+/–
	9084	–	+/–
	9085	–	+/–

[a] Post-vaccination with killed *B. abortus* strain 19 vaccine.
[b] Week 8 of dexamethasone treatment and lambs were 12 weeks of age.

Table 6
Mean (±S.E.M.) fecal egg count and mean (±S.E.M.) blood packed cell volume for control ($n = 7$) and dexamethasone-treated ($n = 8$) Gulf Coast Native weaner lambs

Week[a]	Fecal egg count[b]		Packed cell volume[b]	
	Control	Treated	Control	Treated
0[c]	93 ± 69	88 ± 64	32.8 ± 1.0	32.1 ± 1.4
1	100 ± 36	38 ± 26	32.9 ± 1.3	29.5 ± 1.7
2	150 ± 63	88 ± 74	34.2 ± 1.8	31.0 ± 2.1
3	143 ± 48	138 ± 110	34.0 ± 1.0	30.5 ± 1.6
4	114 ± 34	75 ± 62	36.2 ± 0.9	29.2 ± 1.1
5	36 ± 28	38 ± 26	36.8 ± 0.6	28.8 ± 0.8
6[d]	50 ± 50	19 ± 13	34.7 ± 0.8	27.0 ± 0.8
7	64 ± 26	13 ± 8	34.7 ± 1.2 a	24.8 ± 0.9 b
8	14 ± 9	25 ± 16	33.7 ± 1.1 a	25.5 ± 1.1 b
9	141 ± 83	155 ± 47	34.6 ± 1.1 a	26.1 ± 1.3 b
10	264 ± 82	1563 ± 192	34.4 ± 1.0 a	25.1 ± 1.4 b
11	293 ± 136 a	3219 ± 379 b	33.4 ± 0.8 a	24.1 ± 1.2 b
12	614 ± 432 a	5231 ± 816 b	34.1 ± 1.9 a	24.2 ± 0.7 b
13	1600 ± 766 a	12706 ± 4 784 b	32.1 ± 1.4 a	23.1 ± 0.9 b
14	788 ± 517 a	5988 ± 1272 b	33.5 ± 1.1 a	23.4 ± 1.5 b
15	1136 ± 639 a	8300 ± 2540 b	36.4 ± 1.3 a	26.7 ± 1.6 b
Mean	496 ± 129 a	3722 ± 703 b	34.3 ± 0.3 a	26.9 ± 0.4 b

[a] Week of dexamethasone treatment (1 mg/kg three times a week).
[b] Means with unlike letters are significantly different ($P \leq 0.05$) based on log-transformed data for fecal egg count and raw data for PCV.
[c] Lambs were 20 weeks old.
[d] Lambs started receiving trickle infections with 500 infective larvae three times a week.

being significantly higher from week 11 to the end of the study (Table 6). The overall mean FEC for the treated group (3722 EPG) was significantly higher than the control group (496 EPG).

3.2.2. *Total nematode counts at necropsy*

H. contortus was the predominant nematode with *Trichostrongylus* and *Cooperia* also present (Table 2). Only the *H. contortus* burden and the total burden of the treated group were significantly higher than the control group.

3.2.3. *Blood packed cell volume*

The mean PCV of both groups was similar at week 0 and then the treated group PCV continually decreased being significantly lower from week 7 to the end of the study (Table 6).

3.2.4. *White blood cell differential*

Mean lymphocyte percentage in the treated group was significantly lower than that of the control group at weeks 2 and 3 and from week 6 to the end of the study (Table 3). Other leukocyte differences were not informative (data not shown).

Table 7
Brucellosis card score of control and dexamethasone-treated Gulf Coast Native weaner lambs exposed to an experimental nematode challenge[a]

Group	Animal no.	Week[b]		
		0[c]	4	8
Control	9066	−	+	2+
	9068	−	2+	2+
	9071	−	−	+
	9075	−	2+	2+
	9077	−	2+	2+
	9087	−	2+	2+
	NT4	+	+	2+
Treated	9055	−	−	−
	9070	−	−	+
	9073	−	−	+
	9088	−	−	+
	9089	−	−	+
	NT1	2+	2+	2+
	NT3	−	−	−
	NT5	−	−	+

[a] 500 L3 three times a week for 9 weeks.
[b] Post-vaccination with killed *B. abortus* strain 19 vaccine.
[c] Week 8 of dexamethasone treatment and lambs were 28 weeks of age.

3.2.5. *Lymphoproliferation assays*

Dexamethasone treatment did not affect T lymphocyte functionality as evidenced by similar lymphoproliferation responses between groups (data not shown).

3.2.6. *ELISA for H. contortus WWA*

At the time experimental infection started, mean serum antibody levels were similar in both groups (Table 4). Levels remained low in the treated group and increased in the control group being significantly higher from week 3 after initial experimental infection.

3.2.7. *Brucellosis card test*

All animals were negative prior to vaccination except for one animal in the treated group with a 2+ reading and one in the control group with a + reading (Table 7). All the treated animals remained negative (except for the one 2+ animal) at week 4 post-vaccination and five had a + reading at week 8 post-vaccination. All but one control animal showed strong response (2+) at weeks 4 and 8 post-vaccination.

4. Discussion

Dexamethasone treatment resulted in naturally resistant Native (neonatal and weaner) lambs becoming more susceptible to *H. contortus* infections as evidenced by significantly higher FEC and nematode burden and lower PCV than control lambs. Previous studies demonstrated that sheep were rendered more susceptible to *H. contortus* challenge after corticosteroid treatment (Miller et al., 1985; Huntley et al., 1992; Matthews et al., 1979; Jackson et al., 1988). Presson et al. (1988) demonstrated that a resistant genotype of Merino sheep was rendered susceptible to *H. contortus* challenge after corticosteroid treatment. The reversion to susceptibility was evidenced by no differences with the susceptible genotype in FEC, nematode burdens, nematode weights, thymus weights, and globule leucocyte numbers in response to infection with *H. contortus*. In these studies, Native neonatal and weaner lambs treated with dexamethasone showed higher FEC and nematode burdens, and lower PCV and lymphocyte percentages than the control lambs. Although direct comparison with a more susceptible breed was not possible in this study, the levels of infection in dexamethasone-treated lambs was consistent with that of Suffolk lambs maintained at the study location in previous studies (Bahirathan et al., 1996; Miller et al., 1998).

Lymphocytes are involved in resistance to gastrointestinal nematode infection as has been demonstrated by other studies (Kambara and McFarlane, 1996; Pfeffer et al., 1996; Gill et al., 1992, 1993; Karanu et al., 1997). It has also been demonstrated that corticosteroid treatment affected the number and functionality of lymphocytes (Matthews et al., 1979; Cohen, 1972; Cohen and Duke, 1984; Hamid and Mohi Aldeen, 1992). Similar to those studies, Native lambs treated with dexamethasone had a consistent depression in lymphocyte percentages.

The humoral response is also associated with resistance to gastrointestinal nematode infection. Gill et al. (1993) found significantly higher anti-*H. contortus* IgA and IgG_1 antibody levels in a resistant genotype of Merino sheep compared with random-bred Merino sheep and that there was a negative correlation between FEC and the levels of IgA and IgG_1. In this study, the results of the ELISA test in neonatal lambs showed that there was a continual reduction of antibodies to *H. contortus* WWA through week 5 of the study and there were no significant differences between groups. This decline coincides with depletion of colostral transferred antibodies. After week 5, antibody production increased in each group indicating that lambs were capable of mounting an independent immune response. However, the response then consistently decreased in the treated group and remained significantly lower than the control group. Similarly, the weaner treated group showed a significantly lower production of total antibodies to *H. contortus* WWA than the control group from week 5 after experimental infection. This relative reduction in antibody response coincides with the observed lymphopenia. There was also essentially no response to *B. abortus* vaccination in the treated group for both neonatal and weaner lambs. A decrease in antibody production was associated with a higher nematode establishment in treated lambs. The higher FEC and nematode counts seen in the treated lambs may suggest that dexamethasone treatment was effective in suppressing the inflammatory response allowing greater nematode establishment in the treated lambs compared to the control lambs. These results indicated that dexamethasone treatment caused a lymphopenia and reduction in antibody production, thus suggesting that humoral antibodies are important in the resistance mechanism(s) of Native lambs to *H. contortus* infection.

The results of this study suggest that dexamethasone treatment affected some component(s) of the immune response that may be important in the natural resistance of Native neonatal lambs to *H. contortus* infection.

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