The effect of Fenbendazole on the immune system of lambs

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ABSTRACT

Measurement of lymphocyte blastogenesis and antibody production were used to determine the effect of a benzimidazole anthelmintic on the immune response. Both parasitized and parasite-naive (n=12) lambs, 6 months of age, were treated with fenbendazole (5mg/kgBW) and compared with untreated control animals. All animals (n=24) were given bovine virus diarrhoea (BVD) vaccine the day following anthelmintic treatment. Heparinised blood samples were collected 0,3,7,14 and 21 days after the anthelmintic treatments. The entire treatment regime was repeated at Day 28. No effects on immunity were apparent after the initial dosage with fenbendazole. However, peripheral lymphocytes from lambs (parasitized and parasite-naive) collected 14 days after the second administration of fenbendazole treatment, had significantly lower stimulation indices compared to control animals, when cultured *in vitro* with concanavalin A (Con A) ($P<0.01$), phytohaemagglutinin (PHA), and lipopolysacchride (LPS)($P<0.05$). In addition, stimulation to Con A was depressed 7 days after the second treatment. The primary and secondary humoral responses to BVD vaccination, as measured by serum neutralisation titre, were similar among groups. The repeated use of anthelmintics from the benzimidazole group may interfere with immune responsiveness in young sheep.

Keywords: fenbendazole; immunosuppression; lymphocyte proliferation; antibody.

INTRODUCTION

New Zealand is a temperate country with an agricultural system based around livestock grazing pasture for twelve months of the year. As a result animals are exposed to various environmental conditions, including pathogens. Gastro-intestinal parasites can be detrimental to animal health causing ill thrift and reducing animal production and performance. In sheep, carcass and wool quality and quantity suffer, primarily due to a reduction in voluntary feed intake and efficiency of feed conversion (Sykes and Poppi, 1982). To combat these potential effects livestock are frequently placed in a management system which requires regular anthelmintic administration to obtain maximum productivity.

A major function of the immune system is to protect the host from both internal and external pathogens. The immune system acts against gastro-intestinal parasites in a complex manner including the production of systemic antibodies and local hypersensitivity responses, both thought to be dependant on thymus-derived (T) lymphocytes (Miller,1984).

Recent research has indicated that anthelmintics may have a detrimental effect on the immune system. It was discovered that Ivermectin administered to parasite-free lambs decreased blood lymphocyte proliferation up to 7 and 14 days after the first and second doses, respectively (Stankiewicz *et al.,* 1995).

The aim of this experiment was to identify possible effects that anthelmintic drench may have on the immune response of lambs. This was achieved by measuring T and B lymphocyte proliferation and antibody production to bovine virus diarrhoea vaccine following treatment with fenbendazole, in parasitized and naive lambs.

MATERIAL AND METHODS

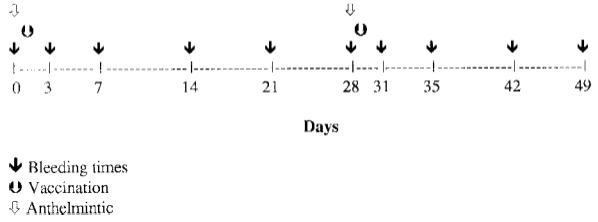
Animals

Twenty four, eight month old, lambs from the Lincoln University Farm were used in the trial. Twelve lambs were run on pasture and exposed to normal levels of parasite challenge (parasitized group); and the remaining twelve lambs (naive) were kept indoors under parasite-free conditions from birth and during the trial period.

Treatment

On days 0 and 28 of the trial six lambs from each of the naive and parasitized groups were orally drenched with fenbendazole at a rate of 5mg/kg liveweight. To determine the humoral response all 24 lambs were injected subcutaneously with 1 ml of the vaccine “Mucobovin” (Rhone Merieux) on days 1 and 29 of the trial. Blood sample collection was by jugular venipuncture on days 0,3,7,14, 21, 28, 31, 35, 42 and 49 for both lymphocytes and serum. Blood was collected aseptically into vacutainer tubes containing anticoagulant (EDTA) for lymphocyte collection and into plain tubes for serum collection.

FIGURE 1: Timeline of Trial Treatments



Lymphocyte Blastogenesis Test (LBT)

The *in vitro* LBT was performed as described by Kambara *et al.,* (1993). Lymphocytes were separated by density-gradient centrifugation with Lymphoprep (Nycomed, Norway), diluted and cultured in Dulbecco's Modified Eagle Media (DMEM) containing 10% foetal calf serum with antibiotics. Cell suspensions were cultured in triplicate with mitogen for 48hrs, and for a further 20 hrs after the addition of tritiated thymidine (Amersham, NZ). Controls were present in quadruplicate. The mitogens used included: Concanavalin A (Con A) (Sigma, USA) at 5ug/ml, Phytohaemagglutinin (PHA) (Gibco,NZ) at 12ug/ml, and Lipopolysaccharide (LPS) (Difco, USA) at 50ug/ml. Cells were stored frozen at -80°C until harvested on glass fibre filters using a cell harvester. Tritiated thymidine incorporation was measured in a Beta Scintillation Counter (Beckman, USA). Results were recorded as counts per minute (CPM) and then converted to stimulation indices (SI) by dividing treatment mean by control mean. The raw data was transformed (square root) to increase normality.

Serum Neutralisation Test

Sera was tested for neutralising antibody against the NADL cytopathic strain of BVDV at the Department of Veterinary Pathology, Massey University. Virus was propagated and serum neutralisation test conducted in the Madin-Darby bovine kidney (MDBK) cell line grown in Eagle's minimum essential medium (modified) (Flow Laboratories, UK) with the addition of 10% heat inactivated horse serum (Gibco, NZ), antibiotics and vitamins. The titre of each serum was taken as the reciprocal of the highest dilution of test serum which completely inhibited the cytopathic effect of the added virus.

Statistical Analysis

All data is presented as means ± S.E. An analysis of variance (Minitab) was used to assess any significance between treatments with transformed data. A value of $P<0.05$ was considered significant and $P<0.01$ highly significant.

RESULTS

Lymphocyte Proliferation

In general, the transformed stimulation indices were relatively low and there was a large variance in the data sets which is typical of this assay, (McFarlane *et al.*, 1993).

A) Effect of in vitro stimulation with Con A

Prior to the treatments, lymphocyte proliferation from nondrenched lambs were significantly lower than from the drenched groups (Table 1), irrespective of parasitic burden. This was thought to be due to a management effect. After secondary exposure to fenbendazole a significant reduction in the T cell proliferation occurred in the parasitized lambs on day 35 ($P<0.05$) and in the naive and parasitized lambs on day 42 ($P<0.01$).

B) Effect of in vitro stimulation with PHA

As with Con A, there was a significant difference in the T cell proliferation in response to PHA regardless of parasite infection on Day 0, prior to treatment. No differences in lymphocyte proliferation rates were apparent after the first application of anthelmintic (Table 2). However, after the second application T cell proliferation rate was higher in the drenched naive lambs on day 31. As with Con A, drenching also decreased T cell proliferation in the parasitized group on day 42, ($P<0.05$).

C) Effect of in vitro stimulation with LPS

When blood lymphocytes were stimulated by LPS, B cell proliferation was similar in all groups, except for an increased rate in drenched parasitized lambs prior to treatment. On day 42 the B cell proliferation rate of the parasitized drenched lambs was significantly less than in parasitized non drenched lambs, (1.875 and 2.684, respectively, $P<0.05$), (Table 3).

TABLE 1: Transformed stimulation indices in response to Con A.

		Day 0	Day 3	Day 7	Day 14	Day 21
Naive	NF	3.933 ± 0.39	3.109 ± 0.35	1.509 ± 0.09	1.487 ± 0.10	1.223 ± 0.10
	F	5.455 ± 0.54	3.108 ± 0.55	2.286 ± 0.42	1.807 ± 0.14	1.368 ± 0.09
		*	NS	NS	NS	NS
Para	NF	2.727 ± 0.31	2.626 ± 0.45	1.344 ± 0.06	0.833 ± 0.05	1.187 ± 0.05
	F	6.915 ± 0.38	1.767 ± 0.14	1.520 ± 0.12	0.753 ± 0.06	1.209 ± 0.02
		**	NS	NS	NS	NS
		Day 28	Day 31	Day 35	Day 42	Day 49
Naive	NF	1.952 ± 0.22	0.953 ± 0.03	1.502 ± 0.07	5.834 ± 0.26	4.179 ± 0.34
	F	1.915 ± 0.15	1.024 ± 0.04	1.659 ± 0.22	3.142 ± 0.49	3.928 ± 0.49
		NS	NS	NS	**	NS
Para	NF	1.001 ± 0.04	0.948 ± 0.02	1.199 ± 0.03	3.866 ± 0.28	1.934 ± 0.33
	F	1.126 ± 0.03	0.974 ± 0.05	1.093 ± 0.02	1.995 ± 0.29	2.262 ± 0.38
		*	NS	*	**	NS

NF non-fenbendazole
F fenbendazole
Para parasitized

* P< 0.05
** P< 0.01
NS non significant

TABLE 2: Transformed stimulation indices in response to PHA.

		Day 0	Day 3	Day 7	Day 14	Day 21
Naive	NF	2.201 ± 0.22	1.844 ± 0.22	1.335 ± 0.13	1.209 ± 0.15	0.958 ± 0.06
	F	4.368 ± 0.39	1.811 ± 0.38	1.644 ± 0.18	1.358 ± 0.15	1.036 ± 0.06
		**	NS	NS	NS	NS
Para	NF	2.484 ± 0.50	1.815 ± 0.24	1.714 ± 0.18	0.543 ± 0.03	0.946 ± 0.06
	F	6.088 ± 0.33	1.669 ± 0.09	1.286 ± 0.16	0.555 ± 0.03	1.055 ± 0.10
		**	NS	NS	NS	NS
		Day 28	Day 31	Day 35	Day 42	Day 49
Naive	NF	1.854 ± 0.14	0.961 ± 0.04	1.225 ± 0.07	3.810 ± 0.33	2.078 ± 0.45
	F	1.814 ± 0.16	1.181 ± 0.03	1.487 ± 0.19	3.065 ± 0.26	2.499 ± 0.26
		NS	**	NS	NS	NS
Para	NF	1.075 ± 0.04	0.955 ± 0.05	1.161 ± 0.06	2.684 ± 0.28	1.985 ± 0.30
	F	1.089 ± 0.03	1.082 ± 0.09	1.032 ± 0.04	1.875 ± 0.18	1.700 ± 0.21
		NS	NS	NS	*	NS

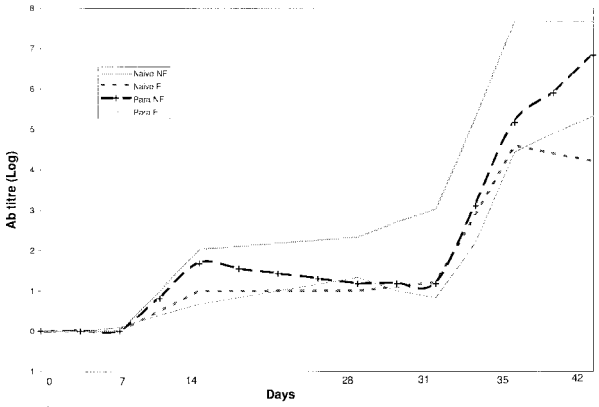
TABLE 3: Transformed stimulation indices in response to LPS.

		Day 0	Day 3	Day 7	Day 14	Day 21
Naive	NF	2.430 ± 0.31	2.793 ± 0.13	2.176 ± 0.21	1.419 ± 0.07	1.260 ± 0.14
	F	1.830 ± 0.21	2.015 ± 0.13	2.073 ± 0.25	1.520 ± 0.04	1.241 ± 0.10
	NS	NS	NS	NS	NS	
Para	NF	2.209 ± 0.35	1.772 ± 0.24	1.649 ± 0.21	1.058 ± 0.09	1.249 ± 0.08
	F	4.788 ± 0.18	1.369 ± 0.06	1.536 ± 0.17	1.086 ± 0.02	1.371 ± 0.05
		**	NS	NS	NS	NS
		Day 28	Day 31	Day 35	Day 42	Day 49
Naive	NF	1.655 ± 0.13	0.964 ± 0.05	1.201 ± 0.05	3.810 ± 0.33	1.636 ± 0.19
	F	1.529 ± 0.06	1.074 ± 0.03	1.588 ± 0.03	3.065 ± 0.26	1.593 ± 0.14
		NS	NS	NS	NS	NS
Para	NF	1.179 ± 0.08	1.115 ± 0.02	1.199 ± 0.04	2.684 ± 0.28	1.336 ± 0.14
	F	1.218 ± 0.04	1.188 ± 0.05	1.132 ± 0.06	1.875 ± 0.18	1.473 ± 0.14
		NS	NS	NS	*	NS

Humoral response

There was no significant difference between the antibody titres produced in response to bovine virus diarrhoea virus (BVD) vaccine from drenched or non drenched naive or parasitized lambs. The sample size of one of the treatments was altered leading to a large standard error, which made comparisons difficult. Drenched parasitized lambs tended to have higher antibody levels compared to other lambs by day 42, but the differences were non significant (Figure 2).

FIGURE 2: Serum neutralisation titres of antibodies to BVD virus among treatment groups; in sheep vaccinated with BVD vaccine and either drenched (F) or not (NF), and challenged with parasites (Para) or not (Naive).



DISCUSSION

Fenbendazole treatment depressed the *in vitro* T cell proliferation in both the naive (Con A) and the parasitized lambs (Con A and PHA). Decreased T cell function has been described by other researchers, including Stankiewicz *et al.*, (1995), where the affect was most evident up to 14 days following the second exposure of ivermectin. This may be of major practical importance as T lymphocytes are involved in the immune expulsion of parasites by increasing the number of mucosal mast cells or globule leukocytes causing an hypersensitivity reaction which results in an unsuitable environment for the parasites (Sykes *et al.*, 1992, Miller 1984). T lymphocytes are also responsible for producing cytokines which enhance B cell activity and therefore influence antibody production from plasma cells.

A confounding factor to explain the reduced blastogenesis in the pasture-fed (parasitized) group may have been the effect of a lower nutritional status on the lambs' immune system. The naive lambs were fed concentrates compared to the parasitized lambs which were run outside on pasture. It is possible that the absorption of protein may have been impaired in the animals infected with internal parasites reducing their protein status. Sykes and Poppi (1982) showed impairment of protein digestion occurred, as measured by nitrogen apparent digestibility, in lambs infected with abomasal parasites. Kambara *et al.*, (1993) showed that lymphocyte responsiveness to T cell mitogens was lower in lambs fed a low protein diet. Thus the effect of the second anthelmintic treatment in combination with reduced protein level of the parasitized lambs may have had a deleterious effect on the immune system approximately 14 days after the second fenbendazole treatment. The availability of dietary protein was not measured directly.

The administration of fenbendazole appeared to cause a decrease in B cell proliferation of the parasitized lambs 14 days after the second anthelmintic treatment. Therefore it is possible that the parasitized drenched lambs would not have been able to initiate a fully fledged (cell mediated and humoral) immune response to the same extent as the parasitized non-drenched lambs.

There was no significant effect of fenbendazole treatment on specific antibody production to a commercial vaccine. In general, a typical primary and secondary (anamnestic) response resulted, although it appeared that the naive lambs had lower antibody titres compared to the parasitized lambs being run on pasture, which was non significant. A possible reason for this could be the increased "priming" of the outdoor lambs' immune system.

There were significant differences between the treatment groups on day 0 due to unusually high proliferation responses, particularly in the parasitized drenched group. This was thought to be due to stress effects particularly in the outdoor group, that is a change in locality, weighing procedure and hierarchy at the beginning of the trial.

CONCLUSION

This experiment indicated that following the second administration of fenbendazole, a reduction in *in vitro* proliferation of T and B lymphocytes occurred. This indicated that fenbendazole may act in an immunosuppressive fashion. The greatest reduction in lymphocyte activity was seen in treated lambs that were parasitized. Immunosuppression, even over short periods, is undesirable as it inhibits the ability to react to pathogens.

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