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Immunisation of lambs with drug-abbreviated *Haemonchus contortus* infections: protection against homologous and heterologous challenge

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Abstract *Haemonchus contortus* is known as a prolific parasite, producing high numbers of eggs. It could therefore be used as a cheap source of larvae for immunisation of lambs. The value of immunisation would be improved if the immunity produced gave protection against not only homologous but also heterologous infections. Because antibody cross-reactivity between *Haemonchus* and *Ostertagia* has been well established, we wanted to know whether drug-abbreviated infections of *H. contortus* would stimulate production of antibodies that would cross-react with *Trichostrongylus colubriformis*. The results obtained from these trials indicated that *H. contortus* drug-abbreviated infections produced significant immunity against not only *Haemonchus* but also *O. circumcincta*. Immunisation increased the level of immunoglobulin E (IgE) and IgE-specific antibodies against *T. colubriformis*, but the differences between experimental and control animals were not statistically significant. Significantly higher levels of IgG-specific antibodies against *T. colubriformis* were observed.

Introduction

We have previously demonstrated that sheep and lambs can be immunised against gastrointestinal nematodes by drug-abbreviated infections (Cabaj et al. 1996; Stankiewicz et al. 1996a). As economically important parasites reside in both the abomasum and the small

intestine, practically it would be desirable to target both sites simultaneously. We have shown that lambs immunised against *Ostertagia circumcincta* (abomasum) and *Trichostrongylus colubriformis* (small intestine) display a significant reduction in parasite burden at both of these sites after a challenge on pasture (Stankiewicz and Hadas 1996; Stankiewicz et al. 1996b). This immunity in sheep and lambs also led to a decrease in the contamination of pasture with parasite eggs and an increase in live-weight gains for the lambs.

If our immunisation procedure is to be used by farmers, the costs of larvae used for immunisation should be economically justified. We have been successful in producing large numbers of *T. colubriformis* infective larvae, but we have experienced some difficulties in producing large numbers of *O. circumcincta* larvae. However, due to its prodigious egg production and to the excellent productivity of faecal cultures, *Haemonchus contortus* could be a good candidate species to replace *O. circumcincta* in our procedure. Moreover, the available data from the literature show that antibodies to *H. contortus* antigens cross-react with *O. ostertagi* (Jasmer and McGuire 1991). More importantly, some cross protection between *H. contortus* and *O. circumcincta* has recently been reported (Dobson and Barnes 1995).

There is, however, some evidence from Australia that drug-abbreviated *H. contortus* infections may not produce immunity in lambs (Barger 1988; McClure et al. 1998). It was therefore very important to establish whether oxfendazole-abbreviated infections of *H. contortus* could produce immunity against endoparasitic challenge in New Zealand lambs.

If immunity were produced, the next question would be whether that immunity could be used against the heterologous parasite *O. circumcincta*. Since antibody cross-reactivity between *Haemonchus* and *Ostertagia* has been reported (Jasmer and McGuire 1991), we wanted to know whether there was any cross-reactivity between *H. contortus* and *T. colubriformis*, the parasite that lives in another organ, the small intestine.

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Materials and methods

Experiment 1

A total of 14 Romney lambs were born on pasture and moved to pens when weaned. They were treated with levamisole and kept in a parasite-free environment until used at the age of 8 months. All lambs were treated again with levamisole at 5 days before commencement of the experiment. Animals were fed a standard ration of chaffed hay and pellets (concentrate).

Lambs were randomly allocated to two equal ($n = 7$) groups. Lambs in group 1 were immunised by drug-abbreviated infections of *Haemonchus contortus* as described by Stankiewicz et al. (1996b). In brief, on day 0, lambs in group 1 were given a dose of 20,000 infective larvae (L3) of *H. contortus*. Both groups were treated with oxfendazole (OFZ) 15 days later. At 6 days after OFZ treatment (day 21), group 1 lambs were given a second dose of 40,000 L3 of *H. contortus*. Both groups were treated with OFZ 15 days later (day 36). After an additional 6 days, group 1 lambs were dosed with 60,000 L3 of *H. contortus*. Both groups were treated with OFZ 15 days later (day 57). On day 63, all animals were challenged with 60,000 L3 of *H. contortus*.

Both levamisole (Nilverm oral drench; Coopers Pitman-Moore, New Zealand Limited) and OFZ (Systemex oral drench; Coopers Pitman-Moore, New Zealand Limited) were given orally to sheep at the manufacturer's recommended dose rates, which were calculated on the basis of individual live weights. During the entire experiment, lambs received standard husbandry care.

Faecal egg counts and blood sampling

At 3 weeks after challenge (day 84) and at weekly intervals thereafter for 3 consecutive weeks, jugular blood samples and faeces were collected from all lambs and the eggs were counted using a modified McMaster technique in which each egg counted represented 100 worm eggs/g of faeces. Serum was separated from the blood samples for serology tests (see below).

Experiment 2

A total of 14 lambs were obtained and treated (immunised and control, $n = 7$) as described for experiment 1. After immunisation, all lambs were challenged with 60,000 *H. contortus* L3 and 20,000 *Ostertagia circumcincta* L3. All lambs were killed at 21 days after challenge.

Post-mortem processing and worm-counting procedures

At the end of the experiments, sheep were humanely slaughtered and the abomasums were removed and processed using the method described by Brunson (1972). In brief, the contents of the abomasum were thoroughly washed out and the respective washings were made up to 8 l. Next, 800 ml of this material was passed through 45- μ m mesh sieve and the retained parasites were preserved in 10% formalin solution. All worms present in a 1/10 subsample of the preserved material from each organ of each lamb were counted. Worm counts for each animal were estimated by

multiplication of the numbers of each species of parasite counted in each organ by 100.

Serology tests

The IgG-specific test for *Trichostrongylus colubriformis* was used as described by Douch et al. (1994). Total IgE was quantitated as units per millilitre of serum using the enzyme-linked immunosorbent assay (ELISA) described by Shaw et al. (1997). Levels of *T. colubriformis* larval excretory/secretory antigen-specific IgE were determined as optical density (OD) values using the ELISA described by Shaw et al. (1998).

Statistical analysis

Effects of immunisation were determined by analysis of variance of the untransformed and log transformed data. All data are presented as arithmetic mean values $\pm$ SEM. $P < 0.05$ was taken to indicate a significant difference.

Results

Experiment 1

Parasites

Faecal egg production and numbers of adult *Haemonchus contortus* parasites were significantly lower in immunised lambs (group 1) than in non-immunised controls (Table 1).

Levels of Igs and antibodies

A very high variability in the level of total IgE was observed. The level of total IgE varied from 1.9 to 58.7 units for the 14 lambs. Therefore, the increased level of IgE observed at 3 weeks post-challenge is not significant (Table 2).

The immunisation procedure had no effect on the expression of IgE-specific antibodies against *Trichostrongylus colubriformis* (Table 3). Lambs immunised with *H. contortus* had significantly higher levels of IgG antibodies that reacted with *T. colubriformis* antigen at the time of and at 5 weeks after challenge (Table 4).

Experiment 2

Lambs immunised against *H. contortus* and then challenged with *H. contortus* plus *Ostertagia circumcincta*

Table 1 Faecal egg counts (eggs per gram of faeces) and total numbers of adult parasites determined in *Haemonchus contortus*-immunised lambs as compared with non-immunised controls after *H. contortus* challenge (experiment 1)

Group ($n = 7$)		Weeks after challenge			
		3	4	5	Adult <i>H. contortus</i>
Immunised	Mean	14.3*	200*	786*	1,500*
	SEM	14.3	153	547	675
Control	Mean	1,443	13,757	39,643	3,971
	SEM	777	7,447	14,740	619

* $P < 0.05$ versus the control value

Table 2 Total IgE (units per millilitre of serum) measured in *H. contortus*-immunised and control lambs after *H. contortus* challenge (experiment 1)

Group (<i>n</i> = 7)		Weeks after challenge			
		0	3	4	5
Immunised*	Mean	6.83	15.70	9.05	4.80
	SEM	2.43	7.37	2.53	1.10
Control*	Mean	4.82	9.19	6.76	4.75
	SEM	1.06	1.98	1.43	0.94

P* > 0.05 (not significantly different)Table 3** Levels (OD) of specific IgE antibodies for *Trichostrongylus colubriformis* determined in *H. contortus*-immunised and control lambs after *H. contortus* challenge (experiment 1)

Group (<i>n</i> = 7)		Weeks after challenge			
		0	3	4	5
Immunised*	Mean value	0.3840	0.4850	0.3360	0.2670
	SEM	0.1830	0.1590	0.1260	0.0941
Control*	Mean value	0.0635	0.4010	0.1865	0.1095
	SEM	0.0210	0.0970	0.0310	0.0201

P* > 0.05 (not significantly different)Table 4** Levels (OD) of specific IgG antibodies for *T. colubriformis* determined in *H. contortus*-immunised and control lambs after *H. contortus* challenge (experiment 1)

Group (<i>n</i> = 7)		Weeks after challenge			
		0	3	4	5
Immunised	Mean value	0.8358*	0.8153	1.1098	0.9219*
	SEM	0.0424	0.0461	0.0264	0.0455
Control	Mean value	0.6105	0.7882	0.9993	0.7803
	SEM	0.0488	0.0379	0.0659	0.0480

**P* < 0.05 versus the control value

had lower (*P* < 0.05) numbers of adult worms of *O. circumcincta* (4026 ± 730) than did non-immunised lambs (10,620 ± 1500).

Discussion

Our results demonstrate that experimental OFZ-abbreviated *Haemonchus contortus* infection produced a significant level of protection against homologous challenge. Although this observation contrasts with the findings in Australia, the difference between our results and those obtained by Barger (1988) and McClure et al. (1998) could be explained by the use of different breeds of lambs as well as the age at which the lambs were immunised. The older Romney lambs that were used in our experiment gave a protective response to the abbreviated infections. Another reason for the discrepancy could be the anthelmintic used, as OFZ has some stimulatory ability that could have helped the development of protective immunity in lambs (Stankiewicz et al. 1994, 1996b).

The protective response obtained seems primarily to operate at reducing the fecundity of *H. contortus*. Although a significant reduction in the burden of adult parasites was obtained in immunised lambs, the magnitude of protection expressed in the reduction of egg production was much higher (50–100 times for faecal egg counts and ~2.6 times for adult parasites; Table 1).

More importantly, the heterologous *Ostertagia circumcincta* challenge given to animals immunised with *H. contortus* resulted in a significant reduction in the numbers of adult *O. circumcincta* (experiment 2). The difference observed between controls and immunised lambs with regard to the numbers of *O. circumcincta* parasites recovered could have primarily been due to specific and non-specific mechanisms triggered by the mixed challenge of *H. contortus* and *O. circumcincta*. We exclude a disruption in abomasal physiology (Dobson and Barnes 1995) as the major factor because the same challenge was given to immunised and control lambs. Although the results we obtained with penned animals are encouraging, the final test should be done on pasture to determine finally the usefulness of *H. contortus* in immunisation procedures for practical purposes.

As discussed by Huntley et al. (1998, 1999), helminth infections in both humans and rodents are associated with a pronounced increase in total IgE levels. Only a proportion of that Ig could be specific IgE antibody. Studies investigating ovine IgE antibody responses have been hampered by a lack of specific antibodies to ovine IgE. Only recently have systems been developed that allow us to measure ovine IgE responses (Shaw et al. 1996, 1997, 1998; Kooyman et al. 1997; Huntley et al. 1998, 1999).

Our results concerning *H. contortus* show that the total IgE level can be significantly elevated in some

animals but not in others. A large variation between sheep in response to *Trichostrongylus colubriformis* infection has also been described by Shaw et al. (1998). At present it is not known why certain animals respond and some do not. If polyclonal activation is indeed responsible for the elevation of total IgE (Jarret and Bazin 1974), then one would expect that it should occur in every animal, unless the response in sheep is genetically regulated. Since anti-IgE reagents are now available, this and other questions can be answered. The total IgE levels measured in this experiment are rather low as compared with those previously determined in challenged animals on pasture (Shaw, unpublished observation) but are similar to those recorded by Shaw et al. (1998) in penned animals. This may reflect the influence of environmental conditions.

It is interesting that despite the elevated levels of IgE stimulated by *H. contortus*, the specific reaction of IgE with *T. colubriformis* antigen (Table 3) was not influenced by immunisation. This finding contrasts with the specificity of IgG antibodies for *T. colubriformis* observed in the same lambs. Therefore, the mechanism of IgE stimulation by *H. contortus* differs from that underlying the stimulation of IgG. The IgG stimulated by *H. contortus* has some cross-reactivity with *T. colubriformis*. Because immunised animals had a higher level of specific antibodies against *T. colubriformis* than did non-immunised lambs at challenge and 5 weeks later, one could argue that immunological memory was involved. To our knowledge this has not been reported before. This observation supports the idea of using drug-abbreviated *H. contortus* infections in field trials aimed at reducing parasite contamination of pasture. If parasite contamination is significantly reduced, then very young lambs, which are known to be difficult to immunise (Hadas and Stankiewicz 1998), may need only minimal anthelmintic treatment.

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