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Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*

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Abstract

Two morphologically marked strains of *Haemonchus contortus*, CAVRS (smooth-macrocyclic lactone resistant) and McMaster (linguiform-macrocyclic lactone susceptible), were used to investigate the selection for anthelmintic resistance following exposure to ivermectin (IVM), a non-persistent anthelmintic, and a more persistent anthelmintic, oral moxidectin (MOX). Three types of selection were investigated: (1) selection of resident worms at the time of treatment (Head selection); (2) selection of incoming-larvae–post-treatment (Tail selection); and (3) selection of both resident population and incoming larvae (Head + Tail selection). The experimental animals were adult sheep and lambs. In the controls where there was no anthelmintic selection, the proportion of CAVRS in the adult worm population was the same as the proportion in larvae given to both adults and lambs indicating that CAVRS and McMaster *H. contortus* were equally infective. There was a significant effect of anthelmintic on total worm numbers in adult sheep with MOX treated adults having less worms, but selection type was non-significant. Anthelmintic type had a significant effect on numbers of resistant worms in adult sheep with less resistant worms in the MOX treated groups, but selection type had no effect. Analysis of variance of arcsine-transformed proportions of resistant worms found that the type of anthelmintic had a highly significant effect, with MOX treated adults having a higher proportion of resistant worms, while type of selection was not significant. In the lambs, nil treated controls and IVM Head + Tail and Tail selected groups had similar geometric mean total worm burdens while Head selected had less total worms. In the MOX treated lamb groups the worm burdens were similar within selection type but less than the IVM treated groups. In the lambs, the types of selection that resulted in more resistant worms were IVM Tail, MOX Head + Tail and MOX Tail. Resistant worm numbers were similar in both adult and lamb groups with Head selection by either MOX or IVM. Moxidectin selected out higher proportions of resistant worms than did IVM in the lambs, with Tail and Head + Tail being stronger selectors than Head. Computer simulations were used to estimate the rate at which resistance developed in the field using the information generated in the present study. The anthelmintic treatments used in the simulation followed a strategic parasite control program for *H. contortus* in which all sheep receive three Closantel (CLS) treatments in summer, all sheep receive a broad-spectrum (BS) drench or capsule at weaning and lambs receive an additional two BS drenches in

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summer or no further treatment in the case of the capsule. Moxidectin, IVM-capsule and IVM were the broad spectrum anthelmintics simulated. All simulations were run four times assuming high or low efficacy against resident resistant worms and in the presence or absence of CLS resistance. The simulations indicated that the presence of CLS resistance hastened selection for macrocyclic lactone (ML) resistance. While the IVM-capsule will select most rapidly for ML resistance, IVM oral is expected to be least selective. Moxidectin treatment is intermediate, except in simulations with no CLS resistance and when MOX is assumed to be highly effective against resident ML-resistant worms, in which case MOX can be expected to select more slowly than IVM oral treatments.

Keywords: Adult worm; Anthelmintics; *Haemonchus contortus*; Ivermectin; Larva; Mathematical model; Moxidectin; Persistent anthelmintics; Resistance; Resistance selection; Sheep

1. Introduction

Moxidectin (MOX), a newer member of the macrocyclic lactone (ML) family, has a longer half-life in host fat (13 to 15 days) [1] than that of ivermectin (IVM) (1 to 2 days) [2]. The persistent efficacy of orally administered MOX against *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Ostertagia circumcincta* was shown to be 5-, 2- and 5-weeks, respectively, while IVM efficacy lasted 1 week or less (Bairden K, et al, 1993. Anthelmintic persistency of MOX in sheep. In: *Proceedings WAAVP Cambridge*. Abstract 131). The extended presence/availability of MOX resulted in clinically resistant nematodes, originally selected by non-persistent ML anthelmintics, being susceptible to the longer drug exposure. Shoop et al. [3] described the elimination curve of MOX as a gradual decay curve extending over 40 days. During this period it would be expected that concentration of anthelmintic would decline to a level that would allow establishment of resistant infective larvae, while still eliminating susceptible larvae. This was demonstrated to be true for *O. circumcincta* when the first effect of ML resistance is a decrease in the duration of efficacy [4, 5]. Consequently, there was a period when only the resistant genotypes survived in the host [5] and the authors of the report concluded that persistent anthelmintics will select anthelmintic resistance. Persistent drugs apply two selective forces on worm populations. The first is applied when animals are first treated and the increased efficacy of the persistent drug removes established resistant worms. The second is applied as the concentration of anthelmintic gradually declines in the host to the point where resistant genotypes can establish but susceptible ones are still excluded [6].

The response of a dominant resistance gene subjected to further selection by a persistent anthelmintic, such as ML resistance, was investigated using a simulation model [7]. In this model the efficacy against incoming L3 was assumed to decline or remain high over the period of drug persistence (3 days to 4 weeks) providing an estimation of the relative importance of selecting resistant L3 on the development of resistance in the worm population. These factors were also examined against a background of initial efficacy levels against adults, and mode of inheritance. The simulations indicated that persistence period and initial efficacy were far more important in determining the rate of selection for resistance than whether efficacy against incoming resistant L3 declined or remained high during the persistence period. Therefore, we decided to measure the efficiency of a non-persistent and persistent anthelmintic in selecting a resistant strain of *H. contortus* in sheep.

2. Materials and methods

2.1. Worm strains

The macrocyclic lactone resistant strain was selected from the CAVR strain [8], with IVM at 50 μg/kg host liveweight in the first generation following its isolation and then at 400 μg/kg for

a further two generations. Then the strain was inbred for two generations by culturing the eggs from individual females with the smooth vulvar morphological type. During each of these generations the parasites were selected with 200 μg/kg IVM. This inbred strain is henceforth referred as the CAVRS strain. At the time of the present experiments 96% of the female CAVRS were of the smooth morphological type. The susceptible strain was the McMaster *H. contortus* strain, originally isolated prior to the introduction of broad spectrum anthelmintics and routinely used as a reference susceptible strain in anthelmintic resistance studies. Female worms of the McMaster strain are all of the linguiform morphological type. Thus, resistant CAVRS and McMaster susceptible females can be distinguished by examination under a dissecting microscope. The infective larvae were examined for viability, as determined by the percentage of motile larvae, at the commencement, day 35 and during the last week of infection in each experiment. In all cases the percentage of motile larvae was >95%.

2.2. Experimental design

The first experiment compared selection by a non-persistent anthelmintic and a persistent anthelmintic on ML resistance in *H. contortus* in 14-month-old adult sheep. The second experiment was similar in design except that selection by anthelmintic was tested in 6-month-old weaner lambs (lambs). In both experiments, oral dosing with 400 larvae three times per week simulated conditions of grazing a contaminated pasture. This infection rate is likely to provide high parasite establishment without eliciting a host response that would decrease worm numbers [9]. Macrocyclic lactone resistance is rare on most Australian farms so the percentage of CAVRS in the dose was kept low. The response to selection of resident worms at the time of treatment (Head selection) was determined by infecting the animals with 1% of CAVRS (smooth strain) and 99% IVM susceptible McMaster (linguiform strain) larvae until the animals were treated with either IVM or MOX. Following anthelmintic treatment, the Head groups received a trickle infection of 100% susceptible larvae. In the groups with selection of incoming larvae post-treatment (Tail selection) the process was reversed, i.e. the sheep received 100% susceptible worms until drug treatment after which time they were dosed with 1% resistant and 99% susceptible larvae. Head and Tail selection groups (Head + Tail) received 1% resistant and 99% susceptible larvae thoughout the experiment. In both the adult and lamb experiment the animals were given the trickle infections for 5 weeks after which the anthelmintic treated groups were given either oral IVM or MOX at the recommended dose of 200 μg/kg liveweight. The trickle infections were continued for a further 5 weeks when they were stopped. The animals were kept for a further 3 weeks before slaughter in order to allow the worms to mature for morph type identification. Controls in both experiments were animals that received 1% resistant and 99% susceptible larvae thoughout the experiment and were not treated with anthelmintic.

The experimental animals were taken from the Pastoral Research Laboratory flock where they had been grazing on paddocks known to be contaminated with worm larvae, treated with oxfendazole (9 mg/kg) and levamisole (14 mg/kg) to remove their worm burdens, and housed under conditions that precluded further natural infection. After a 2-week period of adjustment to animal house conditions, faecal egg counts were done using the modified McMaster technique, sensitivity at 100 eggs g^{-1} [10], to ensure that their worm burdens were removed. Experimental groups are shown in Table 1. In the first experiment, all groups contained five sheep except the Head + Tail selection groups which contained 10 sheep. In the second experiment, all groups contained five sheep. In both experiments, sheep were stratified according to liveweight then randomly allocated to treatment groups.

2.3. Worm counts

The abomasum was removed immediately after slaughter and the contents were washed and

Table 1
Anthelmintic treatments imposed on Head + Tail selection, Tail selection and Head selection larval dosing regimes

Anthelmintic	H + T selection	Head selection	Tail selection
IVM	+ (Group 1)	+ (Group 2)	+ (Group 3)
Nil	+ (Group 4)	0	0
MOX	+ (Group 5)	+ (Group 6)	+ (Group 7)

+Signifies that a group of sheep were allocated to that treatment while 0 indicates that the particular combination was not tested.Group numbers are shown in brackets ( ).

scraped into 2-L graduated cylinders. After settling, a portion of the supernatant was removed and the remaining 500–600 mL preserved with formalin. In the adult sheep experiment all the abomasum contents were examined to determine the worm burden and the proportion of CAVRS worms for most animals. In the minority of animals that had high worm burdens, differentiation was halted when 100 adult female worms had been identified by vulval flap morphology; in these cases total worm burden was estimated from a 20% sample of the abomasum contents (see Table 2Table 3). For the lamb experiment in groups 1 to 4 (IVM and Nil treated) worm burden estimates were based on a 20% sample and vulval flap differentiation of 100 adult female worms. Because of low worm numbers in the MOX treated groups (five to seven) the entire abomasum contents were examined to determine worm populations. No larvae were observed in any sheep.

2.4. Statistical analysis

Analysis of variance was performed separately for each experiment, on total worm burden, CAVRS worm burden and proportion CAVRS after transforming worm counts by log(-count + 1) and proportion CAVRS by arcsin(sqrt(proportion)). The geometric means presented in this paper are the back-transformed log mean worm counts.

Table 2
The geometric mean number of total worms, resistant worms and proportion of resistant worms found in adult sheep following treatment with ivermectin or moxidectin. The back transformed two standard deviations on either side of the geometric mean worms numbers are shown in brackets (approximate 95% confidence intervals). The proportion resistant was obtained by back transforming the arcsine

Group (Table 1)	Treatment	Selection	Geometric mean total worm burden	Geometricmean resistantworm burden	Arcsine-transformed proportion		Percentage resistant worms
					Mean	1 S.D.	
1	IVM	H + T	137* (69–271)	12 (9–17)	0.317	0.074	9.7
2	IVM	Head	234 (186–292)	3 (1–8)	0.105	0.105	1.1
3	IVM	Tail	117(98–140)	10 (7–12)	0.295	0.105	8.4
4	Nil	None	214(120–379)	5 (2–8)	0.117	0.074	1.4
5	MOX	H + T	3 (0.6–16)	2 (1–5)	0.647	0.136	36.3
6	MOX	Head	2 (0.8–5)	2 (0.8–5)	1.290	0.235	92.3
7	MOX	Tail	8 (2–24)	4 (2–9)	0.683	0.118	39.8

The arcsine transform was made on proportions obtained from the untransformed data for individual animals and not the geometric means shown in the tables. Hence, the proportions shown cannot be obtained from the geometric means shown in above or in Table 3.

Table 3
The geometric mean number of total worms, resistant worms and proportion of resistant worms found in lambs following treatment with ivermectin or moxidectin. The back transformed two standard deviations on either side of the geometric mean worms numbers are shown in brackets (approximate 95% confidence intervals). The proportion resistant was obtained by back transforming the arcsine

Group (Table 1)	Treatment	Selection	Geometric mean total worm burden		Geometricmean resistant worm burden		Arcsine-transformed proportion		Percentage resistant worms
							Mean	1 S.D.	
1	IVM	H + T	465	(440–491)	9	(7–10)	0.132	0.032	1.7
2	IVM	Head	293	(279–308)	4	(2–5)	0.097	0.058	0.9
3	IVM	Tail	700	(681–719)	23	(18–30)	0.191	0.073	3.6
4	Nil	None	611	(528–707)	10	(9–11)	0.123	0.034	1.5
5	MOX	H + T	27	(22–32)	15	(12–19)	0.828	0.073	54.3
6	MOX	Head	18	(15–22)	6	(4–8)	0.568	0.231	28.9
7	MOX	Tail	24	(20–28)	20	(18–22)	1.182	0.230	85.6

2.5. Computer simulations

The computer simulations were performed using a model similar to the *T. colubriformis* model used by Barnes et al. [11] but modified to account for the population dynamics of *H. contortus* in sheep and on pasture in a summer rainfall environment (Barnes, Dobson and Barger, unpublished). The simulations were run for 20 years and the treatments used followed the 'WormKill' recommendations for controlling *H. contortus*. That is: all sheep receive a Closantel (CLS) treatment on 1 November, 21 December and 22 February, all sheep receive a broad-spectrum (BS) drench at weaning on 21 December, and lambs receive additional BS drenches on 22 February and 1 May. The four treatments simulated were: (A) IVM-Controlled Release Capsule, in which a single capsule was given to ewes and lambs at weaning and no further BS treatments; (B) MOX-persistent oral, in which MOX was used as the BS drench; (C) MOX-persistent oral, treatment same as B except the second BS treatment given to lambs was not applied; and (D) IVM was used as the BS drench. Each simulation was run four times, with or without CLS resistance and with high or low BS efficacy, as follows. Closantel resistance was assumed to be low (99% CLS efficacy against resident worms, i.e. resistance was not detectable) or approximately 20% (80% efficacy, i.e. present at a detectable level). Efficacies for IVM and MOX, which influence selection for IVM resistance, were varied also, assuming: low (L) efficacy where both drugs removed few IVM resistant genotypes, or high (H) efficacy where MOX and the IVM-capsule killed all resident worms at the time of treatment regardless of their IVM resistance genotype. Under the latter assumption (H efficacy), because all resistant genotypes are removed at the time of treatment, resistance can only arise as a result of Tail selection. However, L efficacy, assuming 99, 4 and 2% efficacy against homozygote susceptible (SS), heterozygote (RS) and homozygote resistant (RR) genotypes, respectively, allows for almost maximum contribution to selection for resistance at the time of treatment. For Tail selection the assumptions made were: (1) MOX and IVM-capsule excluded 99% of SS larvae (L3s) for 32 days and 92 days post-drench, respectively, (RR and RS L3 genotypes were not affected); (2) IVM excluded 99% of susceptible L3s (SS) for 4 days post-drench; (3) neither MOX nor IVM excluded any IVM resistant L3s after anthelmintic treatment. That is, 'Tail' selection is high as no R alleles are removed during the post-drench phase, consistent with the results in this study and other studies on the residual effect of MOX [4, 5]. (4) CLS efficacy after treatment against incoming L3 was broken into three 10-day phases: excluding

99, 90 and 60% of L3 for days 1–10, 11–20 and 21–30, respectively, after treatment when low CLS resistance was assumed. Because CLS resistance has been confirmed in Australia, all simulations were re-run assuming 80% CLS efficacy against resident worms and excluding 80, 70 and 50% of L3 for days 1–10, 11–20 and 21–30, respectively, after treatment. The level of resistance to CLS was fixed throughout the simulations, that is, the evolution of CLS resistance was not simulated.

3. Results

There was no difference in faecal egg counts between Head + Tail, Tail and Head selection larval dosing regimes within anthelmintic treatments in either the adult or lamb experiments. Therefore, the selection types were pooled within treatment (IVM, MOX and Nil) and age groups. The geometric mean faecal egg counts for these groups are shown for adults in Fig. 1 and for lambs in Fig. 2. It can be seen that following treatment with IVM the egg counts fell to low levels in both lambs and adults. A geometric mean egg count of >100 was regained 4 weeks after treatment in the lambs but pretreatment level of faecal egg count was not achieved until the eighth week in the adults. At the end of the experiment all of the IVM treated lambs had positive faecal egg counts while few of the IVM treated adults developed an egg count. Moxidectin treatment reduced the egg count in most lambs and adults to below 100 epg. The geometric mean epg in the MOX treated lambs at the end of the experiment was 5 epg. This mean was due to six lambs having 100 epg and the remainder with no eggs being detected. On post mortem it was found that all of the lambs were infected with *H. contortus*.

In these experiments the control groups were used to determine whether the two strains, CAVRS and McMaster, were equally infective and to estimate total accumulated worm burdens. The control groups were deleted from the analysis of variance to allow a balanced comparison of anthelmintic (MOX and IVM) and selection (Head, Tail and Head + Tail) and to further allow testing for a significant interaction between anthelmintic and selection. In the following text, *F*-values for the interaction will only be given where they achieve a *P*-value of 0.05 or less. Pairwise comparisons were performed, where appropriate, using the Tukey method.

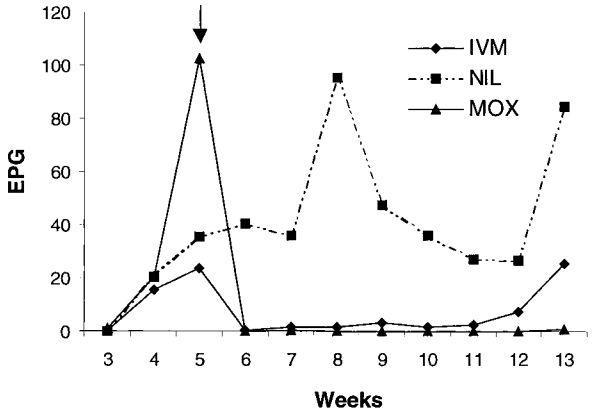

Fig. 1. The geometric mean faecal egg counts for adult sheep divided into groups according to anthelmintic treatment. Arrow indicates anthelmintic treatment of IVM and MOX groups. Graph commences at 3 weeks after larval dosing began.

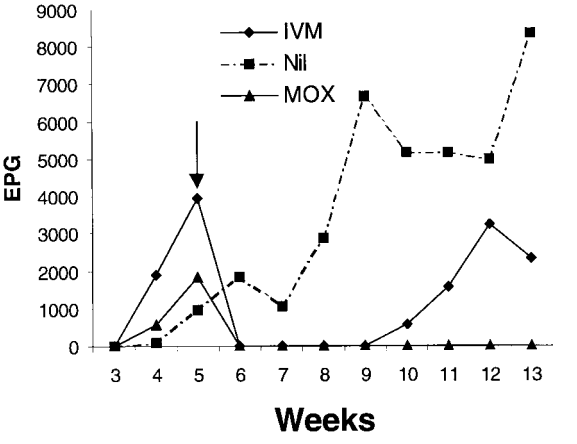

Fig. 2. The geometric mean faecal egg counts for lambs divided into groups according to anthelmintic treatment. Arrow indicates anthelmintic treatment of IVM and MOX groups. Graph commences at 3 weeks after larval dosing began.

The geometric mean worm counts and proportion of resistant worms are shown in Tables 2 and 3 for the adult sheep and lambs, respectively. In the controls (Group 4) where there was no anthelmintic selection CAVRS was about 1% in both adults and lambs indicating that CAVRS and McMaster larvae had similar overall establishment.

For the adult sheep, there was a highly significant effect of anthelmintic on total worm burdens ($F = 60$, $P < 0.0005$), with IVM-treated groups having much higher worms burdens than MOX-treated groups, but selection type and interaction between selection type and anthelmintic were not significant. There was also a significant effect of anthelmintic on CAVRS worm burdens ($F = 7.3$, $P = 0.01$) with MOX-treated groups having fewer CAVRS worms, but selection type and interaction were not significant. In the MOX Head selected group only one adult sheep had any worms and they were 92% resistant type. There was a highly significant effect of anthelmintic on the proportion of CAVRS worms ($F = 23$, $P = 0.0005$), with IVM-treated groups having much lower proportions of CAVRS than MOX-treated groups. Selection type was not significant but the interaction was almost significant ($F = 3.2$, $P = 0.06$).

For the lambs, there were significant effects of anthelmintic ($F = 285$, $P < 0.0005$) and of selection type ($F = 3.7$, $P = 0.04$) on total worm burdens, but the interaction was not significant. Ivermectin-treated groups had much higher worm burdens than MOX-treated groups, and Tail selected groups had higher worm burdens than Head selected groups. The only significant effect on CAVRS worm burdens was of selection type ($F = 15$, $P < 0.0005$), with Head selected groups having the lowest CAVRS worm burdens. There was a significant interaction effect on the proportion of CAVRS worms in lambs ($F = 8.4$, $P = 0.002$). Ivermectin-treated groups had a lower proportion of CAVRS worms than the MOX-treated groups. In the IVM-treated groups there were no differences between the three selection types, but in the MOX-treated groups the Tail selected lambs had the highest proportion of CAVRS worms. It can be seen in Tables 2 and 3 that the percentages of resistant worms in adults and lambs were greater in the MOX selected groups so that the possibility of mating between resistant individuals is higher. The highest proportion of resistant worms in the lamb group was due to Tail selection by MOX. Within the IVM selected groups, the percentage of resistant worms was higher in the adult sheep than in the lambs.

The results of the computer simulations are presented in Table 4 that displays the relative time to resistance for each treatment. This was obtained by averaging for each treatment the years to 10 and 70% R allele frequency on ewe paddocks, which are grazed by ewes and their lambs, and lamb paddocks onto which lambs are weaned. The years to resistance was then divided by the shortest time to resistance that was for the

Table 4
Relative time to anthelmintic resistance for different treatment frequency and drugs with different persistency. Efficacy against resident worms was assumed to be low (L: negligible removal of worms with R alleles) and high (H: 100% removal of all resident worms regardless of genotype) to yield the range of relative times for each treatment

Efficacy against resident worms Treatment type	No. given *	L–H 0% CLS**	L–H 20% CLS
A. Capsule	1	1.3–1.4	1.0–1.2
B. Persistent oral	3	1.6–2.7	1.5–1.6
C. Persistent oral	2	1.7–2.9	1.5–1.9
D. Short half-life oral	3	2.6	2.3

*Number of broad spectrum treatments given to lambs. Ewes received only one broad spectrum treatment in all simulations.
**0% CLS indicates resistance to Closantel not detected, 20% CLS indicates resistance to Closantel reducing its efficacy by approximately 20%.

capsule treatment in the presence of CLS resistance (approximately 3 years). This is consistent with the recent field observations of Chick et al. (Chick BF, Woodgate RG, Wooster MJ. Macrocyclic lactone resistance in field strains of *Haemonchus contortus*. Australian Sheep Veterinary Society Conference, Sydney, May, 1998) who, 2 years after the release of IVM-capsules in Australia, observed that sheep had positive faecal egg counts 80 days after administration of a 100-day IVM controlled release capsule. Ivermectin resistance developed more rapidly in simulations where CLS resistance was assumed to be present. If no CLS resistance and high efficacy against resident worms was assumed then selection for resistance was delayed in the case of persistent oral MOX simulations, however, this advantage was lost when CLS resistance was present. In this case, resistance was slowest to develop when short half-life oral IVM use was simulated.

4. Discussion

Tail selection is stronger with a persistent compared with a non-persistent anthelmintic in younger animals because young animals are normally re-infected more quickly. When young animals are treated with a persistent anthelmintic only the resistant worms are able to re-establish. A similar effect on Tail selection was noted in the older animals, however the difference between the drugs was not as large. These results are supported by previous studies that found ML resistant parasites could establish in MOX treated sheep while ML susceptible strain were still excluded [4, 5]. However, The total numbers of ML resistant worms per animal were higher in lamb groups MOX Tail, MOX Head + Tail, and IVM Tail when compared with the other lamb groups (Table 3) suggesting that the competition with susceptible worms was eliminated due to the persistent effect of MOX. Even the shorter half life of IVM was enough to give the resistant worms an advantage in re-establishing. The low numbers in both MOX and IVM Head selection could be expected as MOX has 95% efficacy against the CAVR strain, from which the CAVRS strain was obtained by further selection. Furthermore, the survivors of the anthelmintic had to survive in the host 8 weeks before the animals were slaughtered for worm counts. Previous data indicated MOX was more effective than IVM against CAVR [8], however, numbers of CAVRS resistant worms were similar in the Head selection groups. The reason resistant worm numbers were similar could reside in the increased efficacy of MOX against CAVRS being offset by the persistent action of MOX eliminating competition from incoming susceptible larvae and thereby reducing the death rate of established worms. Consequently, a major finding of the present study is that selection of resident worms was less important than selection of incoming larvae after treatment. Previous models that simulate the development of resistance [11–13] have generally ignored selection post-treatment and should be re-examined in the light of this result.

Studies in *H. contortus* indicate that ML resistance is inherited as a completely dominant trait [7, 14]. At low frequencies of resistance genes, a dominant trait will increase in frequency more quickly than resistance that is inherited as a recessive or incompletely dominant trait, such as BZ resistance [7]. This indicates that under the same conditions ML resistance in *H. contortus* will evolve more quickly than did BZ resistance. Furthermore, increasing the drug dose rate to make the heterozygote susceptible is not an option when dealing with completely dominant resistance genes as described in CAVRS. An alternative to increasing the dose rate in combating resistance [15] could be to increase the persistence of the anthelmintic. In the case of the MLs this increased persistency could be achieved by using MOX. However, the results of the present experiment provide a clear warning against uncritically following this path. Use of persistent anthelmintics in lambs leads to a screening out of susceptible genotypes and provides an excellent opportunity for recombination among the resistant worms to develop the next stage of ML resistance. This has been referred to as a sequential development of resistance [16] and has been

reported in insects and in BZ resistant worms. We are now witnessing a sequence of anthelmintic introductions into Australia with the macrocyclic lactone group (ML) that is similar to the succession of increased persistency in BZ formulations up to controlled release capsules. Resistance has already developed to the relatively short half-life IVM and is inherited as a complete dominant trait so we predict that resistance will develop in a sequential fashion in the same manner as did BZ resistance. Therefore, resistance will probably remain dominant during each step of its development as it adapts to more persistent (or otherwise more effective) MLs.

The results presented in this study indicate that the greater effect of anthelmintic treatment is not on the numbers of resistant worms in an adult sheep or lamb, but on the proportions of resistant worms. Treatment removes susceptible worms, and although the absolute numbers of resistant worms may be the same as before, the proportion of resistant worms has increased. When the anthelmintic is not persistent and the host is not immune to reinfection, the resistant worms are soon diluted with susceptibles. When the anthelmintic is a persistent one, the resistant genotypes enjoy a considerable period of advantage. Barger et al. [9] found that the development of immunity against *H. contortus* was first manifested by a decrease in establishment of infective larvae while established adult worms remained. The present study demonstrates that when adult sheep that have a degree of resistance to infection are treated with a persistent anthelmintic the adult resistant worms survive but there is little replacement of the balance of the pre-treatment population due to host resistance acting as a brake on further establishment. In lambs treated with a persistent anthelmintic, continual exposure to infective larvae results in establishment of only those worms that are resistant. Furthermore, in the absence of competition from other genotypes, the numbers of resistant worms are actually greater than in lambs receiving no anthelmintic.

The present experiment and associated computer simulations provide some insights on likely outcomes of various nematode control strategies on sheep in a summer rainfall environment. From the point of view of the farmer, who is trying to control parasitism in lambs grazing a pasture with high levels of contamination, the best option in the short term is to use a persistent anthelmintic that achieves a substantial reduction in worm burden. However, this strategy is not sustainable, as a high percentage of those worms accumulating in the lambs are resistant. The simulations predict that the greater the persistency of the anthelmintic, the less sustainable the strategy. Preparations with the greatest persistence such as the controlled release IVM capsule, select for resistance at an even greater rate than the long lasting MOX. Consequently, treating sheep with a persistent ML while grazing on a contaminated paddock should be seen as an emergency procedure when there are no alternatives. The simulations also show that ML resistance in *H. contortus* can emerge more rapidly on farms where resistance to CLS is present. This highlights the need for assessment of drug resistance levels and management practices on individual farms before advocating a worm control strategy. Through planning, farmers may be able to prepare a paddock that has very low levels of contamination. In this case, which would be the better strategy: to use a persistent ML or a non-persistent one before moving their sheep to the low contamination paddock? This situation would be somewhat analogous to Head selection described in the present paper, in that similar numbers of resistant worms were in both MOX and IVM Head selection groups and, therefore, both are options before moving animals to a 'clean' pasture. However, since Head selection resulted in fewer resistant worms than either Head + Tail or Tail, the strategy of moving animals to a 'clean' pasture following treatment has a greater degree of sustainability than does using a persistent anthelmintic and leaving the animals on a contaminated pasture. A more sustainable method of utilising a contaminated pasture would be to graze it with adult sheep that have a level of resistance and have been treated with MOX. Such usage would take advantage of the host's resistance working in conjunction with the action of the anthelmintic to limit reinfection.

The strategic use of adult hosts, with increased resistance to infection as an adjunct to the action of an anthelmintic, has previously been advocated in sustainable parasite control systems [17]. The aim of incorporating older sheep into control systems would be to reduce the necessity of frequent anthelmintic treatment and thus reduce selection. Another method of increasing host resistance is through vaccination. Despite extensive research [18] there is no killed or synthetic vaccine on the market for gastrointestinal parasitic nematodes of small ruminants. However, one method of vaccinating ruminants against gastrointestinal parasites is with radiation-attenuated larvae. A recent review of the data suggests that vaccination of adult animals with irradiated larvae could be very successful if results from experimental studies can be extrapolated to the field [19]. The summarised data in this review shows that efficacy of the irradiated larval vaccine is approximately 90% in six-month-old sheep. Likewise, it is possible to select sheep for increased immunity to parasite infection that results in early development of resistance to infection [20]. Host genetic and vaccination-induced immunity are likely to reduce selection for anthelmintic resistance provided that frequency of drug treatments is reduced when such methods are part of sustainable parasite control systems.

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