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# A multi-species model to assess the impact of refugia on worm control and anthelmintic resistance in sheep grazing systems.

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**Objective** Develop a computer simulation model that uses daily meteorological data and farm management practices to predict populations of *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Teladorsagia (Ostertagia) circumcincta* and the evolution of anthelmintic resistance within a sheep flock. Use the model to explore if increased refugia, provided by leaving some adult sheep untreated, would delay development of anthelmintic resistance without compromising nematode control.

**Procedures** Compare model predictions with field observations from a breeding flock in Armidale, NSW. Simulate the impact of leaving 1–10% of adult sheep untreated in diverse sheep grazing systems.

**Results** Predicted populations of *T. colubriformis* and *T. circumcincta* were less than those observed in the field. Attributed to nutritional stress experienced by sheep during drought, and not accounted for by the model. Observed variation in faecal egg count explained by the model ( $R^2$ ) for these species was 40–50%. *H. contortus* populations and  $R^2$  were both low. Leaving some sheep untreated worked best in situations where animals were already grazing or were moved on to pastures with low populations of infective larvae. In these cases anthelmintic resistance was delayed and nematode control was maintained when 1–4% of adult stock remained untreated.

**Conclusions** In general, the model predicted that leaving more than 4% of adults untreated did not sufficiently delay the development of anthelmintic resistance to justify the increased

production risk from such a strategy. The choice of a drug rotation strategy had an equal or larger impact on nematode control, and selection for resistance, than leaving 1–10% of adults untreated.

**Keywords** anthelmintic resistance; *Haemonchus contortus*; monepantel; refugia; simulation model; *Trichostrongylus colubriformis*; *Teladorsagia (Ostertagia) circumcincta*; un-targeted treatments

**Abbreviations** AAD, Amino-acetonitrile derivative; ABA, Abamectin — non-persistent ML; BZLV, Combination treatment of BZ+LEV; BZ, White drenches or benzimidazoles — e.g. albendazole, oxfendazole; COM, Combination treatment of BZ+LEV+ABA; epg, eggs per gram faeces; WEC, faecal worm egg count; L3, third-stage larvae; LEV, Clear drenches or imidazothiazoles — levamisole; ML, Macrocyclic lactones — abamectin, doramectin, ivermectin, moxidectin; MOX, Moxidectin — persistent ML; MPL, Monepantel (an AAD); UT, untreated;

## Introduction

A number of computer models have been developed to simulate populations of the common gastro-intestinal parasites affecting grazing ruminants.<sup>1–5</sup> These models simulate either ‘general’ nematode disease or infections with one nematode species, although recently Learmount et al<sup>6</sup> developed a model for three species on farms in the United Kingdom. The advantage of multi-species models is that they better predict disease in the host by considering concurrent infections. This paper describes how previously published models were modified and combined to predict concurrent populations of *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Teladorsagia (Ostertagia) circumcincta* in sheep and on pasture under Australian grazing systems.

Interactions between these three nematode species have been studied by many groups.<sup>7–14</sup> The major interaction found is that *Teladorsagia* infections reduce *Haemonchus* burdens. The presence of *T. circumcincta* in the abomasum causes the pH to increase with an associated reduction in establishment of *H. contortus*, while removal of infection restores normal establishment.<sup>7</sup> Barger<sup>15</sup> observed this phenomenon in the field and Simpson et al<sup>16</sup> demonstrated that *H. contortus* was also capable of causing an elevation in abomasum pH. These findings suggest that two processes cause reduction in establishment of *H. contortus* larvae: one due to physiological changes in the abomasum and the other due to acquired immunity. Anthelmintic treatment that removes *Haemonchus* and *Teladorsagia* infections, and restores normal abomasal function, results in increased establishment of *Haemonchus* larvae.

The multi-species model described here allows for these phenomena and estimates host mortalities and inappetence based on the total worm populations. To determine the utility of the model, worm populations were monitored in flocks of breeding ewes and weaners that were managed under typical grazing systems in north-eastern Australia. Daily meteorological and management records were kept so that worm populations could be predicted using the multi-species model.

In this simulation study, the model was used to: a) estimate the consequences of leaving a proportion of adult stock untreated (as a source of refugia) on the development of anthelmintic resistance and effectiveness of parasite control; b) determine how best to use a new anthelmintic class (amino-acetonitrile derivatives (AAD), represented by monepantel; Zolvix<sup>®</sup>, Novartis Animal Health Inc., Switzerland) to control gastro-intestinal nematodes while minimising selection for drug resistance; and c) examine these issues in four environments to determine if there are common principles that can be applied and/or how they need to be modified to suit local environments. The aim was to fully explore the interaction between the fundamental elements of helminth control; refugia, anthelmintic use protocols, environment and grazing management.

## **Materials and methods**

### *Field study*

A breeding flock of approximately 500 fine-wool Merinos ewes and their lambs were regularly monitored for faecal worm egg counts (WEC) and total worm burdens for the calendar years 2001 and 2002. The ewes were maintained at Chiswick Research Station (CSIRO Armidale, New South Wales, Australia) and were assigned to this study on their second lambing. Decisions on flock management and anthelmintic treatment were in accordance with common commercial practices in the area and animal welfare principles. Lambs were born in September 2000 and weaned in early January 2001. At this time the ewe lambs were moved to a separate paddock and combined with ewe lambs of the same age from other flocks at Chiswick to make a total flock of approximately 650 ewe-weaners. Male lambs were pooled in another flock and not observed in this study. All weaners were fine-wool Merinos and had received the same management and anthelmintic treatments, despite being reared in different flocks.

The two flocks utilised five paddocks, numbered 1–5, which were 51, 19, 17, 39 and 17 hectares in area, respectively. Paddock 4 was used for lambing and was not used after weaning. The weaners grazed paddocks 2 and 5, and the adults grazed paddocks 1 and 3. However, because of feed shortages the adults briefly used paddock 2 in August 2001. Paddocks 1 and 2 consisted of white clover, phalaris, kangaroo grass, ryegrass and fescue; paddock 3 contained fescue and ryegrass; and paddocks 4 and 5 had clover, phalaris and ryegrass. All paddocks had been fertilised annually in October with superphosphate at the rate of 100 kg/acre, but this was not done in 2001 due to the dry weather conditions.

It was also necessary to give supplementary feed (wheat and lupins) to the ewes between mid-July and late September 2001. In mid-September 2001 ewes lambed onto paddock 1 and these lambs were weaned in early February 2002. The mobs were moved seven times throughout the year, generally as a result of food shortages on the paddocks. Adult ewes were treated with broad-spectrum anthelmintics on 3 January 2001, 22 August 2001 and 6 November 2001, and treated with closantel (37.5 g/L; Seponver Se Sustained Action Oral Anthelmintic and Flukicide for Sheep; Schering-Plough Animal Health Limited) on 6 November 2001. The ewe-weaners that were born in 2000 were treated with broad-spectrum anthelmintics on 3 January 2001, 5 April 2001, 7 July 2001, 4 September 2001 and 25 October 2001. The broad-spectrum drugs used were moxidectin (1 g/L; Cydectin Oral Drench for Sheep; Fort Dodge Australia Pty Limited) for the weaning treatment on 3 January 2001 and combination benzimidazole and levamisole

(oxfendazole 45.3 g/L and levamisole hydrochloride 80 g/L; Coopers Scanda Mineralised with Selenium, Cobalt and Zinc Broad Spectrum Oral Anthelmintic Solution; Schering-Plough Animal Health Limited) on all other occasions.

The liveweights of 30 animals from each flock were recorded every two weeks except when sheep were unable to be mustered because of lambing. In 2002, year 2 of the study, approximately the same management as 2001 was continued with weaning on 4 February 2002. Four hundred and twenty ewes and their lambs grazed the five paddocks; ewes and lambs were moved eight and 10 times (generally due to feed shortages) and treated four and five times, respectively. Again, oral moxidectin was used to treat the ewes and lambs at weaning, and combination treatments were given to ewes on 23 May 2002, 16 August 2002 and 7 November 2002, and to weaners on 9 April 2002, 23 May 2002, 16 August 2002 and 21 November 2002. The drought that began in 2001 became more severe in 2002, requiring a continuation of supplementary feeding.

#### *Parasitological observations*

Thirty sheep from each of the ewe and weaner flocks were ear-tagged at the beginning of the study. These sheep were faecal sampled at two-weekly intervals and liveweights recorded. At times when these animals were not able to be mustered, for example at lambing, nine samples of fresh faeces were collected directly from the paddock. Faecal egg counts were carried out on individual samples using a modified McMaster method where each egg counted represented 100 eggs per gram faeces (epg). Bulk cultures of faeces were prepared and incubated for one week at 25°C, and larvae harvested and differentiated according to the morphological characteristics of 100 larvae or all larvae if less than 100 were recovered. On five occasions each year, five ewes and weaners were removed from pasture and held in pens for three weeks prior to being euthanased to determine worm burdens. The abomasal and small intestine contents were washed and collected separately before the clean tissue from each organ was digested separately in 0.17% hydrochloric acid at 37°C for 2 h. The contents and digests were then combined for each organ, the fluid volume reduced to 1L and then three 28 mL sub-samples of the fluid of each organ frozen for counting at a later date. Two samples from each organ were counted to estimate total worm burdens. The field study and observations were performed with approval from the CSIRO Livestock Industries Armidale Animal Ethics Committee (permit number 00/22).

#### *Single species models*

Daily weather data for use in the models were obtained from the University of New England, which is approximately 15 km from the experimental site. The *T. colubriformis* models described by Barnes et al<sup>2</sup> and modified by Barnes & Dobson,<sup>3</sup> provided the theoretical framework for developing the models to predict *H. contortus* and *T. circumcincta* host worm populations and survival of infective third-stage larvae (L3) on pasture.

Models were developed from previously published studies.<sup>17-29</sup> The methods for the modelling are shown in Appendix 1 in the online version, or are available from the senior author.

#### *Application of the multi-species model*

Simulations were defined by anthelmintic treatment, lambing dates, sheep movements from paddock to paddock and historical weather data for four regions in Australia – Kojonup (Western Australia, WA), Hamilton (Victoria, VIC), Glen Innes and Armidale (New South Wales, NSW). The first two regions represent winter rainfall zones, with WA having the more severe hot dry summers. The two NSW sites are summer rainfall zones with Glen Innes and Armidale selected for years having medium and high risks for haemonchosis, respectively. The multi-species model was used to predict worm burdens and sheep deaths for self-replacing Merino flocks at the four sites. The grazing management and nematode control program is described in detail by Dobson et al.<sup>29</sup> but a brief description is given here. At all sites, lambs were moved off the lambing paddock at weaning to a second paddock and treated with an anthelmintic. In WA, ewes received anthelmintic treatments in June (pre-lambing) and April; lambs received treatments in October (weaning), December and June; lambs were moved again in May to a paddock previously grazed by untreated adult stock. In VIC, ewes received anthelmintic treatments in July (pre-lambing), November (weaning) and February; lambs received treatments in November (weaning), February and May; lambs were moved again in February to a paddock previously grazed by adult stock. The same management was applied at both NSW sites: ewes received anthelmintic treatments in August (pre-lambing), December (weaning), February, March and April; lambs were treated in October, December (weaning), February, March, April and June. At the WA and VIC sites *H. contortus* populations were assumed to be absent. At the two NSW sites *T. circumcincta* was not included in the simulations because of their ability to reduce *H. contortus* numbers in the host<sup>7,10,14,15</sup>, which may have masked the negative impact of leaving some animals untreated. *H. contortus* populations only were modelled at the NSW-Armidale site because the greatest risk to worm control from leaving animals untreated is likely to occur in *Haemonchus* endemic environments. These management assumptions are typical for Merino wool-producing systems but not for prime lamb enterprises, which may require some modification to the treatment times and risk weights for the output variables.<sup>29</sup>

#### *Selection for anthelmintic resistance*

Selection for anthelmintic resistance from a treatment with benzimidazole (BZ) plus levamisole (LEV) was modelled as a single gene (denoted BZLV) and initial R-allele frequency was set at 40% as resistance to this combination was assumed to be common. Macrocytic lactone (ML) resistance was assumed to be incomplete recessive, selected for by the drugs abamectin (ABA) and moxidectin (MOX). Initial ML-R allele frequency was set at 3%, denoting an emerging resistance problem. Resistance to a new class of drugs, the AADs represented by monepantel (MPL), was modelled by assuming resistance was co-dominant and R-allele frequency was set at 0.001% for *H. contortus* and *T. colubriformis*, and 0.003% for *T. circumcincta*. These R-allele frequency levels were chosen so that MPL resistance would purposely emerge within 10 years of exclusive use of MPL. In the *H. contortus* high risk area, where only *H. contortus* populations were simulated, it was necessary to increase the initial MPL R-allele frequency to 0.01% as development and survival of larvae on pasture was high, leading to high levels of refugia on all paddocks. The high refugia prevented MPL resistance from developing within the total 20 year simulation period if initial R-allele frequency remained at 0.001%. More details on treatment timing, drug efficacy, anthelmintic resistance selection coefficients, genetic assumptions and initial R-allele frequencies for each drug and worm species are given by Dobson et al.<sup>29</sup>

### *Drug management options*

At all sites, four control and four drug management options were simulated. Control simulations against which the success of delaying selection for resistance and worm control could be measured were: Untreated; and exclusive use of MPL; MOX; or COM (combination treatment with BZ, LEV and ABA). The four drug management options simulated were: 1. MPL+COM; 2. MPL/COM rotation; 3. MPL/MOX rotation; 4. MPL/COM/MOX rotation. Drug rotations were within the treatment cycle and not annually (e.g. for option 2 animals would receive MPL, COM, MPL, COM ... for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> ... treatment, respectively). For option 1 all scheduled anthelmintic treatments were MPL and COM applied in combination.

### *Maintaining refugia by leaving 1-10% of adult stock untreated*

In addition to the normal practice of treating all sheep, the simulations investigated the predicted consequences of leaving 1%, 4%, 7% or 10% of adult stock untreated under the four drug management options. It was assumed that leaving animals less than a year old untreated would be unacceptable to sheep farmers and their veterinary advisors, and so all young sheep received all scheduled treatments. The unselected animals were chosen at random, i.e. not assumed to be any fitter or more or less refractory to worms than their treated counterparts, however, once selected these animals did not receive any anthelmintic treatment.

### *Scoring effectiveness to control worms and delaying selection for anthelmintic resistance*

A risk-based scoring system was developed to rank the relative effectiveness of each treatment option.<sup>29</sup> This system attempts to quantify, as a single pooled measure, the efficacy of nematode control and the impediment of drug resistance, which in practice are conflicting objectives. These ranks are given in the results (summarized in Figures 1–4 and described in detail in Tables A2–A5 (Appendix 1)) and are determined from the sum of the ‘mean worm score’ and the ‘mean resistance score’ for each option. The tables also provide results expressed as percentage effectiveness to control nematodes and to delay resistance.<sup>29</sup> ‘Effectiveness of nematode control’ is based on a death rate score and a nematode burden score for each option expressed as a percentage reduction of the equivalent score for the untreated-controls, i.e. equivalent to ‘efficacy’. ‘Effectiveness to delay resistance’ is the resistance score for each option expressed as a percentage reduction from the highest resistance score from the treatment-controls where each drug was used exclusively.

## **Results**

### *Field study*

The mean liveweights of ewes fluctuated between 39 and 54 kg. Weaners grew to 32 and 33 kg in year 2001 and 2002, respectively. Because of drought-associated food shortages, supplementary feeding of ewes began on 16 July 2001 and ceased on 21 September 2001. In year 2, supplementary feeding of ewes continued from 17 June 2002 until 12 September 2002 and recommenced 8 October 2002. In early December 2001, 12 lambs died of a protozoan infection, the remainder recovered without treatment.

### *Multi-species model*

For *T. circumcineta* and *T. colubriformis* the combined species model predicts the same result as that given for the single species predictions. Because *T. circumcineta* infection reduces the establishment of *H. contortus* L3, *H. contortus* populations predicted by the combined species model were markedly reduced by comparison with those predicted when *H. contortus* was modelled alone (data not shown). Figures A1–A6 (Appendix 1), show the observed and predicted (from the multi-species model) worm counts and WEC data for each species. The predicted results were generally lower than the observed counts except for *H. contortus* in year 1, where observed counts were very low (Figure A4). A possible explanation is host immune responses of grazing animals were compromised by nutritional stress because of the drought that occurred during the study. In the field study it would have been best to leave the animals set stocked, however, the drought compelled regular movement of animals between paddocks and this was less than optimum for model validation.

Table A1 (Appendix 1) gives the  $R^2$  values estimated from log transformed WEC. In year 1  $R^2$  values were low for *H. contortus* but reasonable for the other two species accounting for approximately 50% of the observed variation. In year 2, when *H. contortus* was common, the  $R^2$  improved, accounting for 37–54% of the observed variation. For *T. circumcineta* the  $R^2$  values in year 2 were similar to those in year 1 while *T. colubriformis* values declined accounting for approximately 30% of the observed variability. Pooling WEC data for *T. circumcineta* and *T. colubriformis* improved  $R^2$  values for year 1 but had little effect in year 2. For *H. contortus* ewe predictions were better than those for lambs accounting for approximately 20% more of the variation in both years. For *T. circumcineta* the  $R^2$  values in lambs were higher than those in the ewes and for *T. colubriformis* there was no trend in the  $R^2$  values between ewes and lambs. The only non-significant  $R^2$  values were associated with *H. contortus* predictions for lambs in year 1 (Table A1, Appendix 1).

#### *Application of the multi-species model*

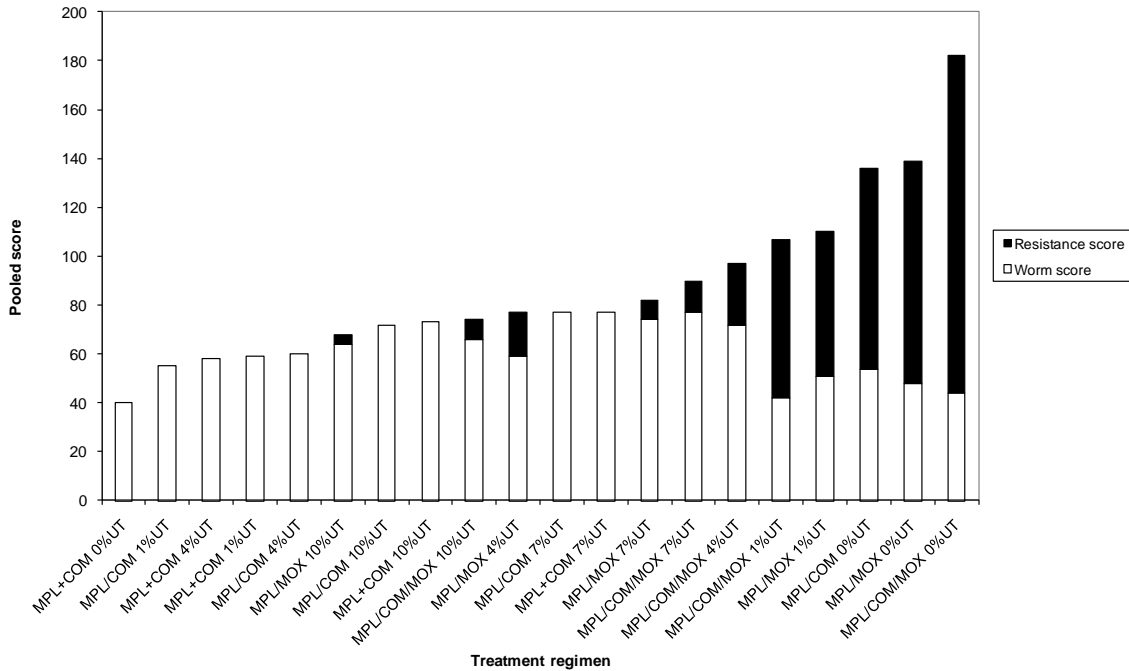
Tables A2–A5 (Appendix 1) show the results of treating all animals or leaving 1–10% of adult sheep untreated for all options in each environment. These tables show the derived scores and effectiveness of each option,<sup>29</sup> and they also give the death rates of ewes and lambs as this was considered a key indicator of risk to the strategy of leaving some sheep untreated. These results are summarized in Figures 1–4, which provide the worm and resistance scores for each simulation.



*Western Australia*

Table A2 (Appendix 1) gives the results for all treatment options in WA, which are summarized in Figure 1. Maximum mean ‘worm’ and ‘resistance’ scores (including controls) were 614 and 167, respectively. Within an option the lowest worm score was generally in regimens where all animals were treated. Based on mean rank, the best and second best options were MPL+COM and MPL/COM, respectively. Ignoring MPL+COM where no resistance developed, on average an additional 53% delay in resistance was achieved by leaving 4% of adults untreated. However, only an additional 9% delay was achieved if another 6% (total 10%) of adults were left untreated. The mean death rate for the untreated ewes, in the 16 options where refugia were created (denoted 1–10%UT in Table A2), was 3.4% (range 1.1–6.6%).

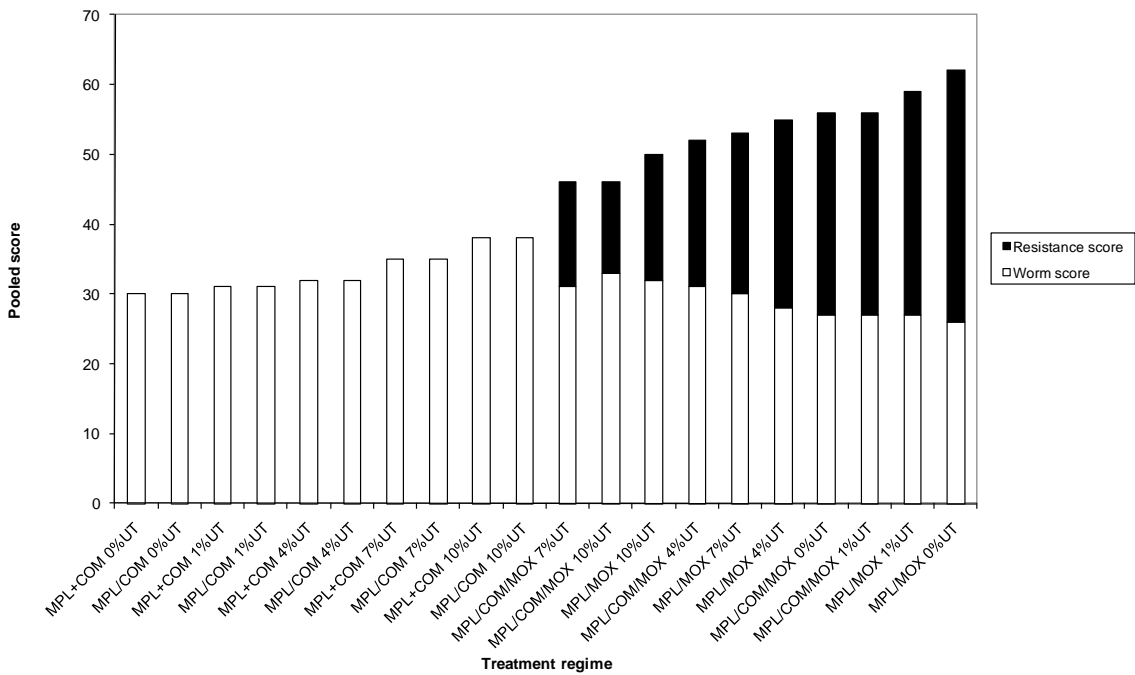
**Figure 1.** Western Australia simulation results (summarised). ‘Pooled score’ = worm score (weighted mean of nematode burden and death rate from each nematode species) + resistance score (weighted mean of resistance to each drug and nematode species). Note: The lower each score, the more favourable the outcome for that variable.



Victoria

Table A3 (Appendix 1) gives the results for all treatment options in VIC (summarised in Figure 2). Maximum mean ‘worm’ and ‘resistance’ scores (including controls) were 419 and 167, respectively. Like WA, within the options the lowest worm score was generally in regimens where all animals were treated. Based on mean rank, the best and second best options were MPL+COM and MPL/COM, respectively. Ignoring the first two options, where no resistance developed, on average an additional 5% delay in resistance was achieved by leaving 4% of adults untreated and an additional 5% delay was achieved if another 6% (total 10%) of adults were left untreated (Figure 2, Table A3). No deaths from nematodes were predicted in the four treatment options for any adult stock; however, in the untreated control simulations death rates for ewes and dry adult stock were 15% and 9%, respectively.

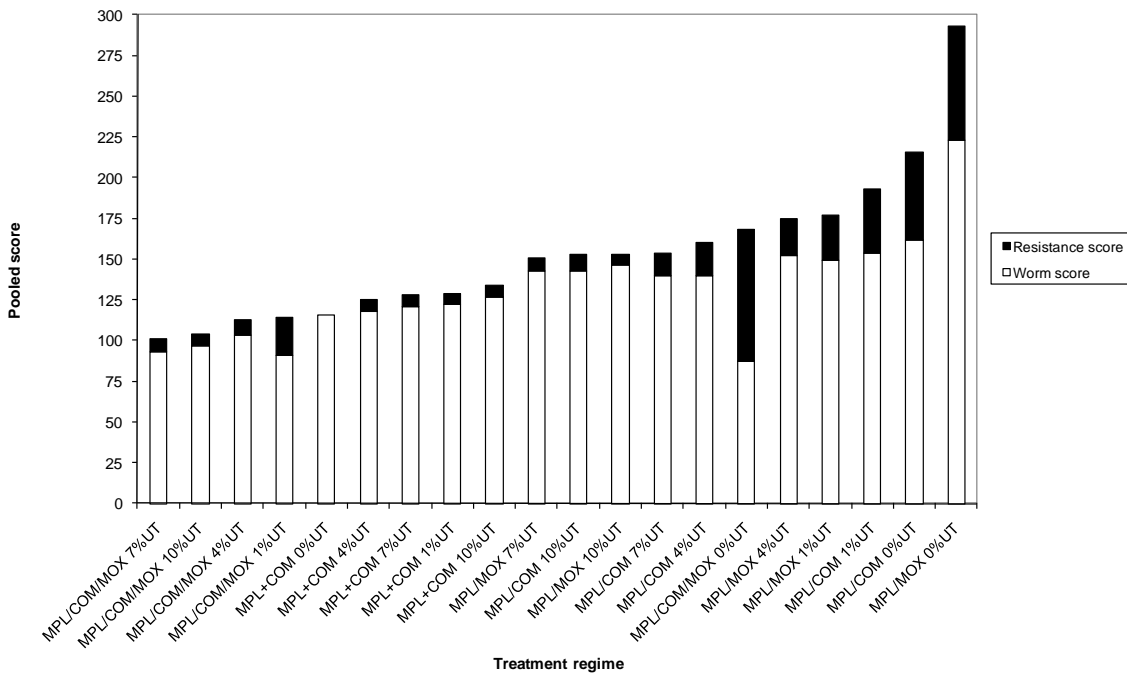
**Figure 2.** Victoria simulation results (summarised). ‘Pooled score’ = worm score (weighted mean of nematode burden and death rate from each nematode species) + resistance score (weighted mean of resistance to each drug and nematode species). Note: The lower each score, the more favourable the outcome for that variable.



*New South Wales – H. contortus and T. colubriformis*

Table A4 gives the results for all treatment options in NSW for a low-moderate risk *H. contortus* environment (summarised in Figure 3). Maximum mean ‘worm’ and ‘resistance’ scores (including controls) were 509 and 167, respectively. Unlike WA and VIC, the best and next best options in this environment were MPL/COM/MOX and MPL+COM respectively, based on the mean ranks from Table A4. There was no consistent pattern within options for the lowest worm score. Again ignoring MPL+COM where no resistance developed, on average an additional 30% delay in resistance was achieved by leaving 4% of adults untreated. However, only an additional 6% delay was achieved if another 6% (total 10%) of adults were left untreated. The mean death rate for the untreated ewes, in the 16 options denoted 1–10%UT in Table A4, was 2.1% (range 1.6–3.8%).

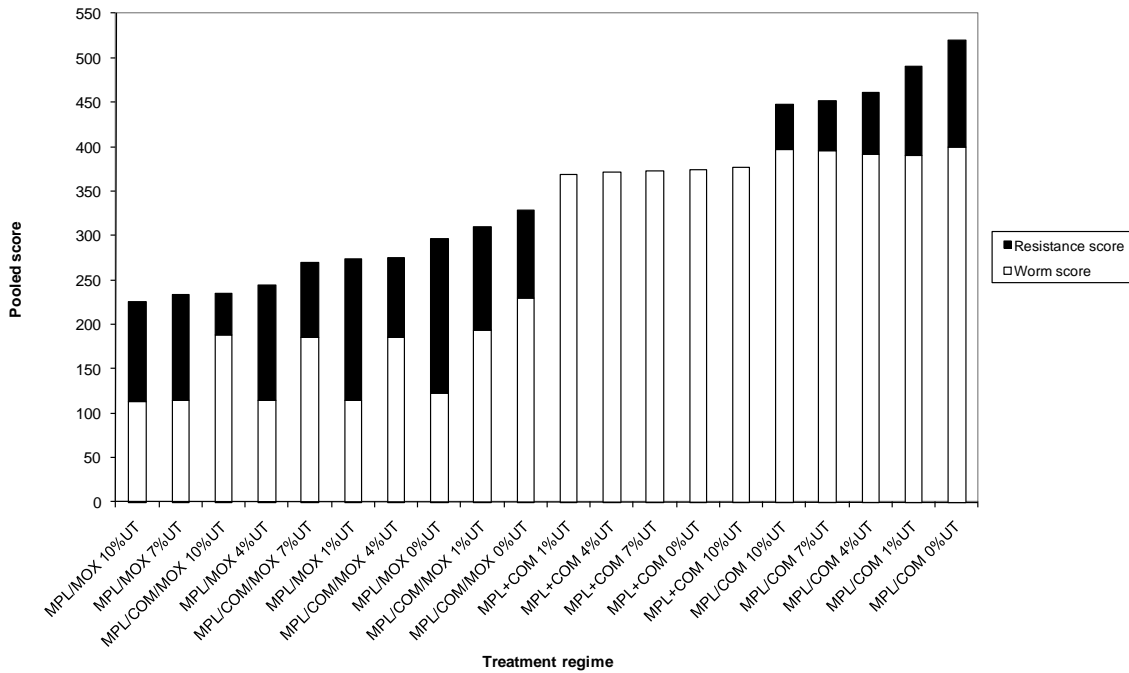
**Figure 3.** New South Wales – *H. contortus* and *T. colubriformis* simulation results (summarised). ‘Pooled score’ = worm score (weighted mean of nematode burden and death rate from each nematode species) + resistance score (weighted mean of resistance to each drug and nematode species). Note: The lower each score, the more favourable the outcome for that variable.



*New South Wales – H. contortus*

Table A5 gives the results for all treatment options in NSW for a high risk *H. contortus* environment (summarised in Figure 4). Maximum worm and resistance scores (including controls) were 564 and 173, respectively. The mean death rate for the untreated ewes, in the 16 options denoted 1–10%UT in Table A5, was 8.8% (range 8.4–9.4%). Only the MPL/MOX option reduced death rates to an acceptable level (Table A5) and the next best option was MPL/COM/MOX. For this high risk environment the management regimen for all options except MPL/MOX would require additional stock rotations and/or additional short-acting anthelmintic treatments to provide effective control. However, for all options worm control was not made worse by leaving some adult stock untreated. Ignoring MPL+COM, where no resistance developed, on average an additional 20% delay in resistance was achieved by leaving 4% of adults untreated and an additional 15% delay was achieved if another 6% (total 10%) of adults were left untreated.

**Figure 4.** New South Wales – *H. contortus* simulation results (summarised). ‘Pooled score’ = worm score (weighted mean of nematode burden and death rate for *H. contortus*) + resistance score (weighted mean of resistance to each drug by *H. contortus*). Note: The lower each score, the more favourable the outcome for that variable.



## Discussion

Although field observations were taken for comparison with model predictions, modelling primarily remains a theoretical exercise, as it is almost impossible to validate an epidemiological model over a range of climatic conditions, population sizes, species compositions, management practices and levels of drug resistance. Modelling serves to give insights into the long-term impact of management practices and how they might be varied to control pest populations or delay selection for drug resistance. Model predictions of R-allele frequency and L3 populations on pasture were not able to be tested against the routine observations made in the field study (egg and nematode counts). However, WEC are a useful measure for testing predictions as they are easy to collect and represent an integrated measure of L3 acquisition, establishment, adult populations and fecundity. They are also the only measure of parasite abundance that is routinely collected by farmers. The  $R^2$  values in Table A1 indicate the proportion of observed variation explained by the model for WEC and show that the model generally accounts for 40–50% of the variation, except for *H. contortus* in year 1 when few *H. contortus* were found. The drought that began in New England (NSW) in 2001 and became more severe in 2002 caused increased nutritional stress of ewes and lambs although supplementary feeding was provided to minimise this impact. Currently, this cannot be adequately accounted for in the model and substantial enhancement would be required to allow for multiple stresses that cause a decline in acquired immunity. It is also possible that the model predicts lower worm survival on pasture than actually occurred during the drought. Models for larval survival on pasture were fitted to data obtained from pasture plot studies of only a few years duration at three sites and thus may not be able to predict the broad range of climatic conditions that occur in Australian sheep-growing regions. Both these factors could contribute to underestimating worm populations.

In WA and VIC (winter rainfall areas), leaving adult animals untreated did not change the ranking order of the best options for rotating or combining anthelmintic groups,<sup>29</sup> i.e. the best treatment option was a combination of all drug actives (MPL+COM) and MPL/COM was generally the next best option. In the summer rainfall zones for the moderate risk *H. contortus*/*T. colubriformis* environment, MPL/COM/MOX was the best option if some animals were left untreated while MPL+COM remained the best option when all animals were treated. However, in the high risk *Haemonchus* simulation, only MPL/MOX achieved effective control. Because *Teladorsagia* infections reduce *Haemonchus* burdens and *Haemonchus* was seen as the greatest risk to this strategy, *Teladorsagia* was not included in the simulations for the summer rainfall areas. This gave the greatest opportunity for *Haemonchus* burdens to escalate from contamination provided by untreated animals and thus present the most severe test for this tactic. Despite this, Tables A4–A5 show that generally within options (i.e. where the proportion of untreated animals varied) nematode control was less variable than between the options. Thus, the choice of anthelmintic treatment option was the more critical decision than simply leaving 1–10% of stock untreated, although the latter remains important.

Simulations by Barnes et al<sup>30</sup> suggested that leaving a portion of animals in a flock untreated could delay selection for resistance. Hoste et al<sup>31</sup> was able to maintain nematode control in alpine dairy goats in France by only treating animals that were either high milk producers or in their first lactation. Haemonchosis can be controlled by treating animals exhibiting clinical signs of

anaemia,<sup>32</sup> however this is unlikely to be applied in Australia because it is labour intensive. On four farms in Southern Italy, Cringoli et al<sup>33</sup> left 40–60% of dairy sheep untreated without jeopardising the control of mixed infections of mainly *Trichostrongylus*, *Haemonchus* and/or *Nematodirus*.

Using the multi-species model, leaving 1–10% of traditionally managed adult sheep untreated was examined in climatic zones where it was considered feasible to introduce such a practice. Results suggest that this would partially slow the development of drug resistance without increasing overall flock mortality, though the untreated animals did suffer higher death rates than their treated counterparts. Generally most of the benefit to delay resistance was achieved by leaving up to 4% untreated, beyond this there were diminishing returns that would not be justified by the potential associated risk. Given that drug efficacies greater than 95% are considered adequate to control worms in Australia and not indicative of anthelmintic resistance<sup>34</sup> the results here are consistent with this convention. That is, worm control could be maintained when all young sheep and 96% or more of adult sheep were treated with a highly effective product or combination of drugs. The release of a new anthelmintic is the ideal time for veterinarians and professional sheep advisors to seriously consider this strategy. In practice, unpredictable climatic events will greatly influence the outcome of providing increased refugia populations, but it is an additional tactic to help reduce the risk of selecting for anthelmintic resistance.

The proportion of adult stock left untreated, and choice of anthelmintic groups, will be influenced by the levels of ‘natural’ refugia and anthelmintic resistance on individual farms. If there are very few larvae on pasture, as in summer in WA, then leaving a small number (1%) of animals untreated throughout the year can have a large effect on delaying anthelmintic resistance (Figure 1 and Table A2, MPL/COM). In contrast to WA, leaving 10% of adults untreated in VIC did not sufficiently enhance delaying resistance to warrant its use. This result is consistent with observations in flocks in the high winter rainfall environment of western VIC which, compared to WA, has milder summers, a much longer growing season (7–9 months v 4–6 months), and pastures dominated by perennial rather than annual pasture species. For example, Merino lambs inadvertently or deliberately left untreated in flocks in this area often have higher worm egg counts and deaths from nematodiasis than their treated counterparts (J. Webb Ware, pers. comm.). Estimates were recently summarised<sup>35</sup> from two separate two-year studies in this region of the proportion of nematodes in winter that were derived from either the previous spring (i.e. before the most recent strategic or summer anthelmintic treatments, and so, by definition, in ‘refugia’ from those treatments), or from late summer and autumn deposition of eggs (the autumn generation, by definition not in ‘refugia’ as they are deposited after the summer treatments). These results, plus those of Anderson,<sup>36</sup> show that the proportion of spring derived ‘refugia’ population is, on average, from 30–50% of the total larval populations the following winter, as judged by tracer worm counts. It would appear that this proportion is high enough to have prevented the rapid selection for ML resistance in *T. circumcincta*, unlike many areas in WA that have a more pronounced Mediterranean climate and where ML resistance developed more rapidly and is considered common.<sup>37</sup> Unfortunately, similar estimates for populations in ‘refugia’ in other climatic regions do not appear to have been published. The proportion of a nematode population in ‘refugia’ that is sufficient to impede the development of anthelmintic resistance, but still permit

reasonable worm control and productivity will vary depending on the initial resistance level and environment. However, a study on the impact of refugia on the development of thiabendazole resistance in *H. contortus* provides a clue.<sup>38</sup> When 35–70% of an experimentally manipulated infection was in refugia, composite worm populations were susceptible, as judged by log-dose probit lines for the third to fifth generation of worms.

The strategy of leaving a fixed percentage of the same adult stock untreated for all treatments was expedient for modelling and should be viewed as one way to demonstrate the importance of refugia. Other options based on local epidemiological knowledge are less simple to model but easily applied in the field, would be: leaving a high percentage (say 10%) untreated, selected at random or the healthiest stock, when larval numbers on pasture are very low but then treating all animals when refugia on pasture is high; leaving animals untreated based on production or disease criteria.<sup>31–33</sup> As drug resistance increases many more worms survive routine flock treatment and the number of untreated animals required to substantially dilute resistant genotypes (from treated animals) becomes too large and would compromise worm control. In this situation a combination drug treatment strategy is necessary to reduce surviving resistant genotypes to very low numbers so that they can be well diluted by parasites from untreated animals. To be a successful strategy, a highly efficacious anthelmintic is required for most of the flock while the remainder are left untreated.

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## Appendix 1

Briefly, within the *T. colubriformis* model, the function  $f(t)$  defines the probability that an egg deposited on pasture at time zero is a L3 at time  $t$ . The three parameters of this function,  $p$ ,  $E(X_1)$  and  $E(X_2)$ , were derived from combinations of standard weather data (daily maximum and minimum air temperatures, rainfall and evaporation). The parameter  $p$  is the probability that an egg will develop to a L3 and migrate to the herbage,  $E(X_1)$  is the average time it takes to do so and  $E(X_2)$  is the average lifetime for a L3 on herbage.

Only the functions that were adapted or developed for the *H. contortus* and *T. circumcincta* models are set out below, otherwise the models for each worm species remain as previously described for *T. colubriformis*.<sup>3</sup> To optimise the various function parameters the simplex method of Nelder & Mead<sup>17</sup> was used, as outlined by Dobson et al.<sup>18</sup> The parameters were adjusted by this method to fit the functions to mostly peer reviewed published data; the references and data sources are given below with each function. For a small number of model parameters no relevant previous data were found and in this situation estimates were based on expert opinion. For example in Section 2 threshold numbers of *T. circumcincta* were required because the presence of *T. circumcincta* reduces the establishment of *H. contortus*, but suitable quantitative data were not available. All model parameters were fixed prior to the commencement of the field study.

### *The Haemonchus contortus* model

The survival and development of *H. contortus* eggs to L3 and their migration to the herbage was predicted by adjusting the model of Barnes et al<sup>2</sup> to fit *H. contortus* L3 on pasture observed at two sites in Western Australia.<sup>19</sup> To predict  $p$ , three faecal deposition conditions were defined as: *dry*, *wet-cool* and *wet-warm*. If total evaporation minus total rainfall (mm) in the first two days after faeces were deposited on pasture was greater than -2.5 mm then conditions were classed as *dry* and  $p = 0.000075$ , otherwise conditions were classed as *wet*. Note that an evaporation-rainfall greater than -2.5 indicates rain exceeds evaporation by less than 2.5 mm. For *wet* conditions, if the mean maximum air temperature was less than 16°C for the first 21 days after deposition then the conditions were classified as *wet-cool* and  $p = 0.000095$ , otherwise  $p = 0.000940$  for *wet-warm* conditions.  $E(X_1)$  was set to 0.5 weeks, that is independent of weather, and  $E(X_2)$  remained a function of weather variables, the same as those used to predict *T. colubriformis* L3 survival on pasture.

Establishment, development and death rates of *H. contortus* parasitic stages were predicted by fitting an immigration-death model to previously observed worm burdens.<sup>20,21</sup> The sigmoidal function to describe the proportion of incoming L3 that establish (*estab*) in the host was similar to that used by Dobson et al<sup>18</sup> and Smith,<sup>22</sup> and was fitted to the radio-labelled worm populations found by Barger et al.<sup>20</sup> Establishment was assumed to be a function of duration of continuous infection ( $t$ ) in weeks, i.e.  $estab(t)$  was given by:

$$estab(t) = d + ax/(1+x) \quad \text{equation (1)}$$

where  $x = \exp(c(b-t))$  and  $t$  is duration of infection in weeks.

A description of the parameters and the fitted values were:

lower limit	=	d	= 0.01338
upper limit	=	d + a	= 0.43094
slope	=	c	= 4.01784
ed50	=	b	= 6.47627 (weeks)
a	=	upper limit - lower limit	= 0.41756.

This function (1) defines establishment for a sheep aged 30 weeks and was modified to account for host age (*age*) using data provided by IA Barger (pers. comm.) so that final establishment is a function of *t* and *age*:

$$\begin{aligned}
 estab(t, age) &= (2.08 - 0.036age) * estab(t) && \text{for } 6 \text{ weeks} < age < 52 \text{ weeks} \\
 &= 1.864 * estab(t) && \text{for } age \leq 6 \text{ weeks} \\
 &= 0.208 * estab(t) && \text{for } age \geq 52 \text{ weeks,}
 \end{aligned}$$

note *estab(t)* is obtained from equation (1). This provides maximum establishment of approximately 80% and 10% for 6- and 52- weeks old animals, respectively.

The proportion of establishing L3 whose development is halted (*arrest*) at the fourth larval stage (EL4) was defined as:

$$\begin{aligned}
 arrest(t) &= 0.0 && \text{if } t < 3.22954 \\
 &= 0.613969(1 - \exp(-0.574918(t - 3.22954))) && \text{if } t \geq 3.22954.
 \end{aligned}$$

The three parameters for this model were obtained by fitting the model to the proportion of labelled EL4 stages found by Barger et al.<sup>20</sup>

The death rate of adult worms (*drw*) was assumed to be 0.0054 until total worm burden exceeded 4613 at which point *drw* = 0.0530. The value of these three parameters was obtained by fitting an immigration death model to the data of Barger et al<sup>20</sup> and Barger & Le Jambre.<sup>21</sup> After worms are removed (e.g. by anthelmintic treatment) *drw* returns to the initial level for an uninfected animal (0.0054) over two weeks.

Faecal egg counts were predicted using the method of Roberts and Swan<sup>1</sup> where *epg* is predicted by a simple function of total adult worm burden, i.e.

$$epg = 4.57(\text{total adult worm burden})^{0.84}$$

#### *The Teladorsagia circumcincta* model

The survival, development and migration of the free-living stages of *T. circumcincta* were predicted using data from Callinan.<sup>23</sup> Using the *T. colubriformis* model,<sup>2</sup> the size and shape of the predicted curve gave a reasonable approximation of Callinan's observed data, although the predicted larval populations occurred several weeks later than the observed data. Better agreement with the observed data were obtained by setting  $E(X_1)$  to one week, independent of weather, while *p* and  $E(X_2)$  remained functions of weather identical to those used to predict *T. colubriformis* larval development and survival.

The parasitic stages of *T. circumcincta* were predicted from the data of Seaton et al<sup>24</sup> (for L3 establishment rates) and Hong et al<sup>25</sup> (for death rates of adult worms). For infection rates (*ir*) of

50 L3/day and above, percentage establishment (*%estab*) of L3 as parasitic worms was assumed to be a function of duration (*d*) of infection, given by:

$$\begin{aligned} \%estab(d) &= 35 - 5d/28 && \text{for } 0 \leq d \leq 28 \text{ days,} \\ &= 30 - 23(d-28)/28 && \text{for } 28 < d \leq 56 \text{ days,} \\ &= 7 - 4(d-56)/28 && \text{for } 56 < d \leq 84 \text{ days,} \\ &= 3 && \text{for } d > 84 \text{ days.} \end{aligned}$$

This was obtained by linear interpolation of the mean establishment of irradiated challenge L3 observed by Seaton et al.<sup>24</sup> For infection rates less than 50 L3/day establishment was assumed to: (a) Decline to a minimum of 3% if adult worm burden was greater than 2000; (b) Remain static if the adult burden was between 400 and 2000 worms; (c) Increase to a maximum of 25% if adult worm burden was less than 400 and *%estab* was less than 25%, or remain at its current level if *%estab* was greater than 25%. Under these circumstances, where *%estab* changed (a or c above), establishment was assumed to change linearly over 34 days until it reached 3% or 25%.

The death rate of adult worms (*drw*) was assumed to be zero until the duration of infection exceeded *minE* days (minimum worm exposure in days). *MinE* was assumed to depend on infection rate and was defined as:

$$\begin{aligned} minE(ir) &= 103 && \text{if } ir < 250 \text{ L3/day,} \\ &= 103 - 24((ir/250) - 1) && \text{for } 250 \leq ir < 500 \text{ L3/day,} \\ &= 79 - 7((ir/500) - 1) && \text{for } 500 \leq ir \leq 1000 \text{ L3/day,} \\ &= 72 && \text{for } ir > 1000 \text{ L3/day.} \end{aligned}$$

Thus for low (< 250 L3/day) and high (> 1000 L3/day) infection rates, *drw* remains zero till 103 and 72 days of infection, respectively. Once *minE* was exceeded *drw* was assumed to be a function of infection rate. The function *drw* is defined as a proportion of the adult worms that die per day, and was given by:

$$\begin{aligned} drw(ir) &= 0.041 && \text{if } ir < 250 \text{ L3/day,} \\ &= 0.041 - 0.012((ir/250) - 1) && \text{for } 250 \leq ir < 500 \text{ L3/day,} \\ &= 0.029 - 0.009((ir/500) - 1) && \text{for } 500 \leq ir \leq 1000 \text{ L3/day,} \\ &= 0.020 && \text{for } ir > 1000 \text{ L3/day.} \end{aligned}$$

Together these can be summarised as follows: at high infection rates worm death commences after a shorter worm exposure but at a reduced death rate than those observed at lower infection rates. This is consistent with the data of Hong et al<sup>25</sup> and provided a better fit to the data than an alternative linear model fitted to each infection rate in which *drw* increased with time.

Arrested development (*arrest*) and fecundity (*fec*) are also described as functions of infection rate and are derived from the data of Hong et al.<sup>25</sup> The proportion of establishing L3 (*estab*) whose development is halted at the fourth larval stage was defined as:

$$\begin{aligned} arrest(ir) &= 0.36 && \text{if } ir < 250 \text{ L3/day,} \\ &= 0.36 + 0.2((ir/250) - 1) && \text{for } 250 \leq ir \leq 500 \text{ L3/day,} \\ &= 0.56 && \text{for } ir > 500 \text{ L3/day.} \end{aligned}$$

These break points were derived from Hong et al<sup>25</sup> where the arrested development of the proportion of establishing worms can be calculated as 0.36, 0.59 and 0.50 for infection rates of

250, 500 and 1000 L3/day, respectively. The mean of the latter two proportions was chosen as the maximum proportion for arrested development of fourth-stage larvae at infection rates above 250 L3/day. The egg per adult worm, including males (i.e. multiply *fec* by the total adult worm burden to determine egg), was determined by:

$$\begin{aligned}
 fec(ir) &= 0.13 && \text{if } ir < 250 \text{ L3/day,} \\
 &= 0.13 - (ir - 250)/6250 && \text{for } 250 \leq ir < 500 \text{ L3/day,} \\
 &= 0.09 - (ir - 500)/25000 && \text{for } 500 \leq ir \leq 1000 \text{ L3/day,} \\
 &= 0.07 && \text{for } ir > 1000 \text{ L3/day.}
 \end{aligned}$$

The break points in this model were the mean *fec* for each infection rate calculated from Hong et al.<sup>25</sup>

### *Multi-species model*

The effect of single versus concurrent infections was examined for the impact on parasite establishment and death rates. Unpublished work by Barger (pers. comm.) showed no interaction between *H. contortus* and *T. colubriformis* for establishment and worm death rates in animals that received single or concurrent infections. The same conclusion can be drawn for *T. circumcincta* and *T. colubriformis* from some studies.<sup>8,9,11,26-28</sup> However, two studies,<sup>11,28</sup> reported some interaction between the two species. It should be noted that Coop et al<sup>8</sup> and Jackson et al<sup>11</sup> used *T. vitrinus* and not *T. colubriformis*. Jackson et al<sup>11</sup> observed significantly lower *T. vitrinus* establishment at week 4 in concurrently infected sheep than in sheep that received only *T. vitrinus*, but at weeks 8 and 12 there was no difference in establishment between the infection groups. Based on a covariate analysis, Sykes et al<sup>28</sup> concluded that infection with *T. colubriformis* reduced the number of adult *T. circumcincta*. However, this conclusion is not supported by the worm burdens, which show the highest *T. circumcincta* burdens occurred at the highest *T. colubriformis* infection rate, while at lower *T. colubriformis* infection rates there was little if any effect on *T. circumcincta* burdens. On balance, from these six studies, we concluded there is little interaction between *T. circumcincta* and *T. colubriformis*. Because final worm burdens for each species in these studies show no differences between single and concurrent infections, we also concluded that concurrent infection did not change worm death rates.

There is substantial evidence to indicate that the presence of *T. circumcincta* reduces the establishment of *H. contortus*.<sup>7,10,12,13</sup> From these studies the average reduction in *H. contortus* caused by the presence of *T. circumcincta* was 55%. Because there was no obvious trend over time and the effect of *T. circumcincta* on *H. contortus* was physiologically mediated, we assumed that if sheep harboured >3000 *T. circumcincta* worms or if >20% of incoming larvae were *T. circumcincta* then *H. contortus* establishment would be reduced by 55% of that predicted by the single species model. No data exist to test if these *T. circumcincta* thresholds are valid, however, the model can be changed to use different thresholds.

For estimating host deaths from concurrent infections it was necessary to calculate *T. circumcincta* and *H. contortus* as *T. colubriformis* ‘equivalents’. The values for lethal worm burdens in the single species models are 50,000, 25,000 and 15,000 for *T. colubriformis*, *T.*

*circumcincta* and *H. contortus*, respectively, in adult sheep and the user can change these values for each flock if desired. From the lethal burdens it can be seen that approximately:

$$1 T. colubriformis = 1/2 T. circumcincta = 1/3 H. contortus$$

so total mixed species worm burdens can be converted to approximate *T. colubriformis* equivalents, i.e.:

$$\text{total } T. colubriformis \text{ equivalents} = T. colubriformis + 2 T. circumcincta + 3 H. contortus.$$

The *T. colubriformis* equivalent worm burden can be compared with the *T. colubriformis* lethal burden to determine host mortalities. Because the user defines the various lethal worm burdens, the factors actually used for converting *T. circumcincta* and *H. contortus* to *T. colubriformis* are:

$$(T. colubriformis \text{ lethal burden} / T. circumcincta \text{ lethal burden}) \quad \text{and}$$

$$(T. colubriformis \text{ lethal burden} / H. contortus \text{ lethal burden})$$

respectively, rather than using the factors 2 and 3 as shown above.

The following steps were used to predict host deaths: (1) The mean predicted worm burden of each species (from the multi-species model) was converted to *T. colubriformis* equivalents, using the relative lethal worm burdens of each species; (2) The predicted worm burden and dispersion parameter (k) of the negative binomial distribution<sup>3</sup> for each species were used to estimate the ranked burdens of each worm species (as *T. colubriformis* equivalents) in an arbitrary flock of 100 sheep (generated only for the purpose of determining percentage mortalities); (3) The total worm burden of each sheep was calculated as the sum of the ranked *T. colubriformis* equivalent worm burdens of each species. This assumes positive correlation between worm burdens of the three species, but other more complex relationships could be substituted; (4) The proportion of the flock with total (*T. colubriformis* equivalent) worm burden exceeding the *T. colubriformis* lethal worm burden was calculated and this proportion was 'killed' and the simulated flock size reduced accordingly; and (5) Mean predicted worm burdens of each species were adjusted to account for the loss of worms due to the deaths of the more heavily infected individuals.

#### *Validation of the multi-species model*

The predicted and observed mean nematode WEC were log transformed by  $\log(\text{count}+50)$  prior to estimating coefficient of determination ( $R^2$ ) values for each nematode species by host type and year. The  $R^2$  values are a measure of the proportion of observed variation explained by the model. Additional  $R^2$  values were estimated for data pooled over years. Egg counts of *T. colubriformis* plus *T. circumcincta* were also pooled to estimate  $R^2$  because the L3 of these two species are difficult to differentiate and some misclassification can occur. The model predicts L3 populations on pasture and resistance (R-) allele frequency in the nematode populations, although, these aspects of the model cannot be tested against the observations made in the field study.

## **Results**

Table A1. Coefficient of determination ( $R^2$ ) values that shows the proportion of the egg count variability (see Figure A2, A4 and A6) accounted for by the multi-species model.

Species	Sheep	Year 1 (2001)		Year 2 (2002)		Year 1 and 2	
		$R^2$	P-value	$R^2$	P-value	$R^2$	P-value
<i>Haemonchus</i>	Ewe	0.2377	0.0134	0.5376	<0.0001	0.4976	<0.0001
<i>Haemonchus</i>	Lamb	0.0061	0.7042	0.3712	0.0010	0.0409	0.1503
<i>Teladorsagia</i>	Ewe	0.5015	0.0001	0.4150	0.0003	0.4705	<0.0001
<i>Teladorsagia</i>	Lamb	0.5570	<0.0001	0.6324	<0.0001	0.5565	<0.0001
<i>Trichostrongylus</i>	Ewe	0.5113	0.0001	0.2860	0.0041	0.3611	<0.0001
<i>Trichostrongylus</i>	Lamb	0.4921	0.0001	0.3611	0.0012	0.4209	<0.0001
<i>Telad. + Trich.</i>	Ewe	0.6250	<0.0001	0.3197	0.0021	0.4307	<0.0001
<i>Telad. + Trich.</i>	Lamb	0.5900	<0.0001	0.4218	0.0003	0.4760	<0.0001

Table A2. Western Australia simulation results, death rates are averaged over 20 years, ‘worm score’ is a weighted mean of nematode burden and death rate from each nematode species, ‘resistance score’ is a weighted mean of resistance to each drug and nematode species.

Treatment options <sup>b</sup>	Effectiveness			Ewe <sup>a</sup> death rate (%)	Lamb death rate (%)	Mean worm score	Mean resistance score	Score rank
	Effectiveness of worm control (%)	to delay resistance (%)						
1.MPL+COM	94	100		0.0	0.2	40	0	1
1%UT	90	100		2.9	0.5	59	0	4
4%UT	91	100		2.0	0.3	58	0	3
7%UT	87	100		5.5	0.4	77	0	12
10%UT	88	100		2.3	0.3	73	0	8
<i>Mean</i>	<i>90.0</i>	<i>100.0</i>		<i>2.5</i>	<i>0.3</i>	<i>61.3</i>	<i>0.0</i>	<i>5.6</i>
2.MPL/COM	91	51		2.0	0.4	54	82	18
1%UT	91	100		2.8	0.3	55	0	2
4%UT	90	100		2.0	0.6	60	0	5
7%UT	87	100		5.5	0.2	77	0	11
10%UT	88	100		2.3	0.2	72	0	7
<i>Mean</i>	<i>89.6</i>	<i>90.2</i>		<i>2.9</i>	<i>0.3</i>	<i>63.6</i>	<i>16.4</i>	<i>8.6</i>
3.MPL/MOX	92	46		2.4	0.5	48	91	19
1%UT	92	65		3.3	0.4	51	59	17
4%UT	90	89		3.2	0.2	59	18	10
7%UT	88	95		3.6	0.3	74	8	13
10%UT	90	98		2.0	0.2	64	4	6
<i>Mean</i>	<i>90.3</i>	<i>78.4</i>		<i>2.9</i>	<i>0.3</i>	<i>59.3</i>	<i>36.0</i>	<i>13.0</i>
4.MPL/COM/MOX	93	17		1.0	0.1	44	138	20
1%UT	93	61		2.6	0.1	42	65	16
4%UT	88	85		5.6	0.3	72	25	15
7%UT	88	92		5.2	0.2	77	13	14
10%UT	89	95		2.1	0.1	66	8	9
<i>Mean</i>	<i>90.2</i>	<i>70.1</i>		<i>3.3</i>	<i>0.1</i>	<i>60.1</i>	<i>49.8</i>	<i>14.8</i>

<sup>a</sup> Ewe death rate is the flock average including treated and untreated ewes.

<sup>b</sup> UT represents the percentage of adult stock left untreated.

**Table A3.** Victoria simulation results, death rates are averaged over 20 years, ‘worm score’ is a weighted mean of nematode burden and death rate from each nematode species, ‘resistance score’ is a weighted mean of resistance to each drug and nematode species.

Treatment options <sup>b</sup>	Effectiveness		Ewe <sup>a</sup> death rate (%)	Lamb death rate (%)	Mean worm score	Mean resistance score	Score rank
	Effectiveness of worm control (%)	to delay resistance (%)					
1.MPL+COM	93	100	0.0	0.7	30	0	1
1%UT	93	100	0.0	0.8	31	0	3
4%UT	92	100	0.0	0.7	32	0	5
7%UT	92	100	0.0	0.8	35	0	7
10%UT	91	100	0.0	1.0	38	0	9
<i>Mean</i>	<i>92.0</i>	<i>100.0</i>	<i>0.0</i>	<i>0.8</i>	<i>33.3</i>	<i>0.0</i>	<i>5.0</i>
2.MPL/COM	93	100	0.0	0.7	30	0	2
1%UT	93	100	0.0	0.8	31	0	4
4%UT	92	100	0.0	0.7	32	0	6
7%UT	92	100	0.0	0.8	35	0	8
10%UT	91	100	0.0	1.1	38	0	10
<i>Mean</i>	<i>92.0</i>	<i>100.0</i>	<i>0.0</i>	<i>0.8</i>	<i>33.4</i>	<i>0.0</i>	<i>6.0</i>
3.MPL/MOX	94	78	0.0	0.4	26	36	20
1%UT	94	81	0.0	0.4	27	32	19
4%UT	93	84	0.0	0.4	28	27	16
7%UT	93	86	0.0	0.6	30	23	15
10%UT	92	89	0.0	0.6	32	18	13
<i>Mean</i>	<i>93.2</i>	<i>83.6</i>	<i>0.0</i>	<i>0.5</i>	<i>28.6</i>	<i>27.3</i>	<i>16.6</i>
4.MPL/COM/MOX	94	83	0.0	0.4	27	29	17
1%UT	94	83	0.0	0.4	27	29	18
4%UT	93	87	0.0	0.4	31	21	14
7%UT	93	91	0.0	0.5	31	15	11
10%UT	92	92	0.0	0.6	33	13	12
<i>Mean</i>	<i>92.9</i>	<i>87.1</i>	<i>0.0</i>	<i>0.5</i>	<i>29.6</i>	<i>21.6</i>	<i>14.4</i>

<sup>a</sup> Ewe death rate is the flock average including treated and untreated ewes.

<sup>b</sup> UT represents the percentage of adult stock left untreated.



**Table A4.** New South Wales – *H. contortus* and *T. colubriformis* simulation results, death rates are averaged over 20 years, ‘worm score’ is a weighted mean of nematode burden and death rate from each nematode species, ‘resistance score’ is a weighted mean of resistance to each drug and nematode species.

Treatment options <sup>b</sup>	Effectiveness		Ewe <sup>a</sup> death rate (%)	Lamb death rate (%)	Mean worm score	Mean resistance score	Score Rank
	Effectiveness of worm control (%)	to delay resistance (%)					
1.MPL+COM	77	100	1.4	2.2	116	0	5
1%UT	76	96	1.0	2.3	122	7	8
4%UT	77	96	0.6	2.3	118	7	6
7%UT	76	96	0.5	2.3	121	7	7
10%UT	75	96	0.7	2.3	127	7	9
<i>Mean</i>	<i>76.3</i>	<i>96.5</i>	<i>0.9</i>	<i>2.3</i>	<i>120.7</i>	<i>5.8</i>	<i>7.0</i>
2.MPL/COM	68	68	1.8	3.4	162	54	19
1%UT	70	77	1.5	3.4	154	39	18
4%UT	72	88	0.6	3.4	140	20	14
7%UT	72	92	0.6	3.3	140	14	13
10%UT	72	94	0.7	3.4	143	10	11
<i>Mean</i>	<i>71.0</i>	<i>83.6</i>	<i>1.1</i>	<i>3.4</i>	<i>147.8</i>	<i>27.3</i>	<i>15.0</i>
3.MPL/MOX	56	58	2.8	4.7	223	70	20
1%UT	71	83	1.9	3.4	149	28	17
4%UT	70	86	1.8	3.4	152	23	16
7%UT	72	95	1.4	3.4	143	8	10
10%UT	71	96	1.2	3.4	146	7	12
<i>Mean</i>	<i>68.1</i>	<i>83.6</i>	<i>1.8</i>	<i>3.6</i>	<i>162.6</i>	<i>27.4</i>	<i>15.0</i>
4.MPL/COM/MOX	83	52	0.9	0.9	87	81	15
1%UT	82	86	1.0	0.9	91	23	4
4%UT	80	94	1.2	0.8	103	10	3
7%UT	82	95	0.7	0.9	93	8	1
10%UT	81	96	0.7	0.8	97	7	2
<i>Mean</i>	<i>81.5</i>	<i>84.6</i>	<i>0.9</i>	<i>0.9</i>	<i>94.2</i>	<i>25.7</i>	<i>5.0</i>

<sup>a</sup> Ewe death rate is the flock average including treated and untreated ewes.

<sup>b</sup> UT represents the percentage of adult stock left untreated.

**Table A5.** New South Wales – *H. contortus* simulation results, death rates are averaged over 20 years, ‘worm score’ is a weighted mean of burden and death rate for *H. contortus*, ‘resistance score’ is a weighted mean of *H. contortus* resistance to each drug.

Treatment options <sup>b</sup>	Effectiveness		Ewe <sup>a</sup> death rate (%)	Lamb death rate (%)	Mean worm score	Mean resistance score	Score Rank
	Effectiveness of worm control (%)	to delay resistance (%)					
1.MPL+COM	34	100	8.1	31.1	374	0	14
1%UT	35	100	8.2	31.4	369	0	11
4%UT	34	100	8.3	31.3	371	0	12
7%UT	34	100	8.4	31.5	373	0	13
10%UT	33	100	8.5	31.8	377	0	15
<i>Mean</i>	<i>34.0</i>	<i>100</i>	<i>8.3</i>	<i>31.4</i>	<i>373</i>	<i>0</i>	<i>13.0</i>
2.MPL/COM	29	31	8.5	37.9	400	120	20
1%UT	31	41	8.4	37.3	390	101	19
4%UT	30	60	8.4	37.4	392	69	18
7%UT	30	68	8.5	37.2	396	56	17
10%UT	30	71	8.5	35.8	397	51	16
<i>Mean</i>	<i>30.0</i>	<i>54.2</i>	<i>8.5</i>	<i>37.1</i>	<i>395</i>	<i>79.4</i>	<i>18.0</i>
3.MPL/MOX	78	0	3.5	2.6	123	173	8
1%UT	80	9	3.2	2.6	115	158	6
4%UT	80	25	3.3	2.2	114	130	4
7%UT	80	31	3.5	2.0	114	119	2
10%UT	80	35	3.7	1.7	113	113	1
<i>Mean</i>	<i>79.6</i>	<i>20.0</i>	<i>3.4</i>	<i>2.2</i>	<i>116</i>	<i>139</i>	<i>4.2</i>
4.MPL/COM/MOX	59	42	5.0	18.7	229	100	10
1%UT	66	33	3.7	18.9	194	116	9
4%UT	67	49	3.7	18.6	186	89	7
7%UT	67	51	3.7	18.8	185	85	5
10%UT	67	73	3.8	19.0	188	47	3
<i>Mean</i>	<i>65.2</i>	<i>49.6</i>	<i>4.0</i>	<i>18.8</i>	<i>196</i>	<i>87.4</i>	<i>6.8</i>

<sup>a</sup> Ewe death rate is the flock average including treated and untreated ewes.

<sup>b</sup> UT represents the percentage of adult stock left untreated.

Figure A1. Observed individual (-x-) and predicted (—) *Teladorsagia circumcincta* worm burdens for ewes and weaners between 2001 and 2002.

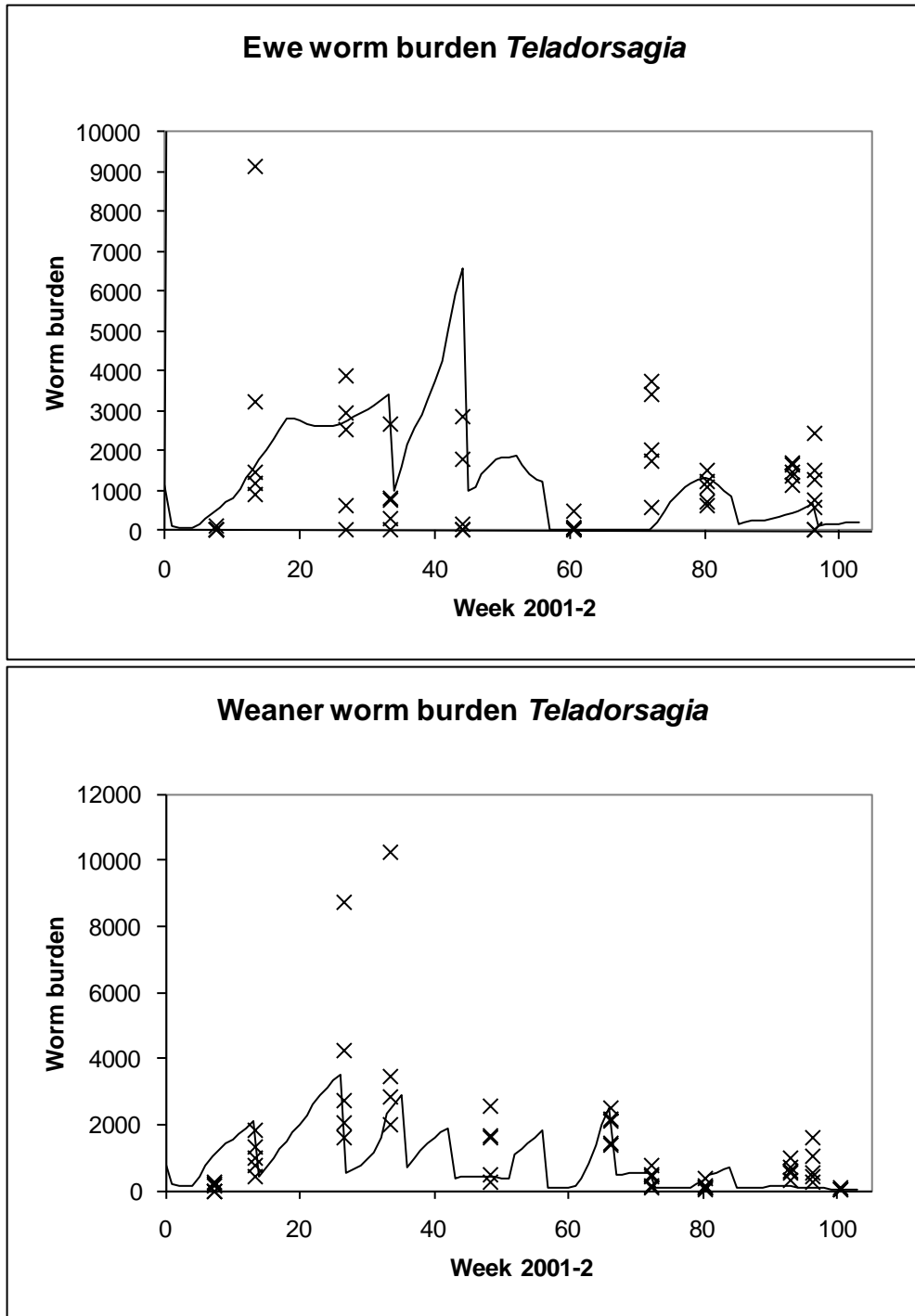


Figure A2. Observed mean (-x-) and predicted (—) *Teladorsagia circumcincta* egg counts for ewes and weaners between 2001 and 2002.

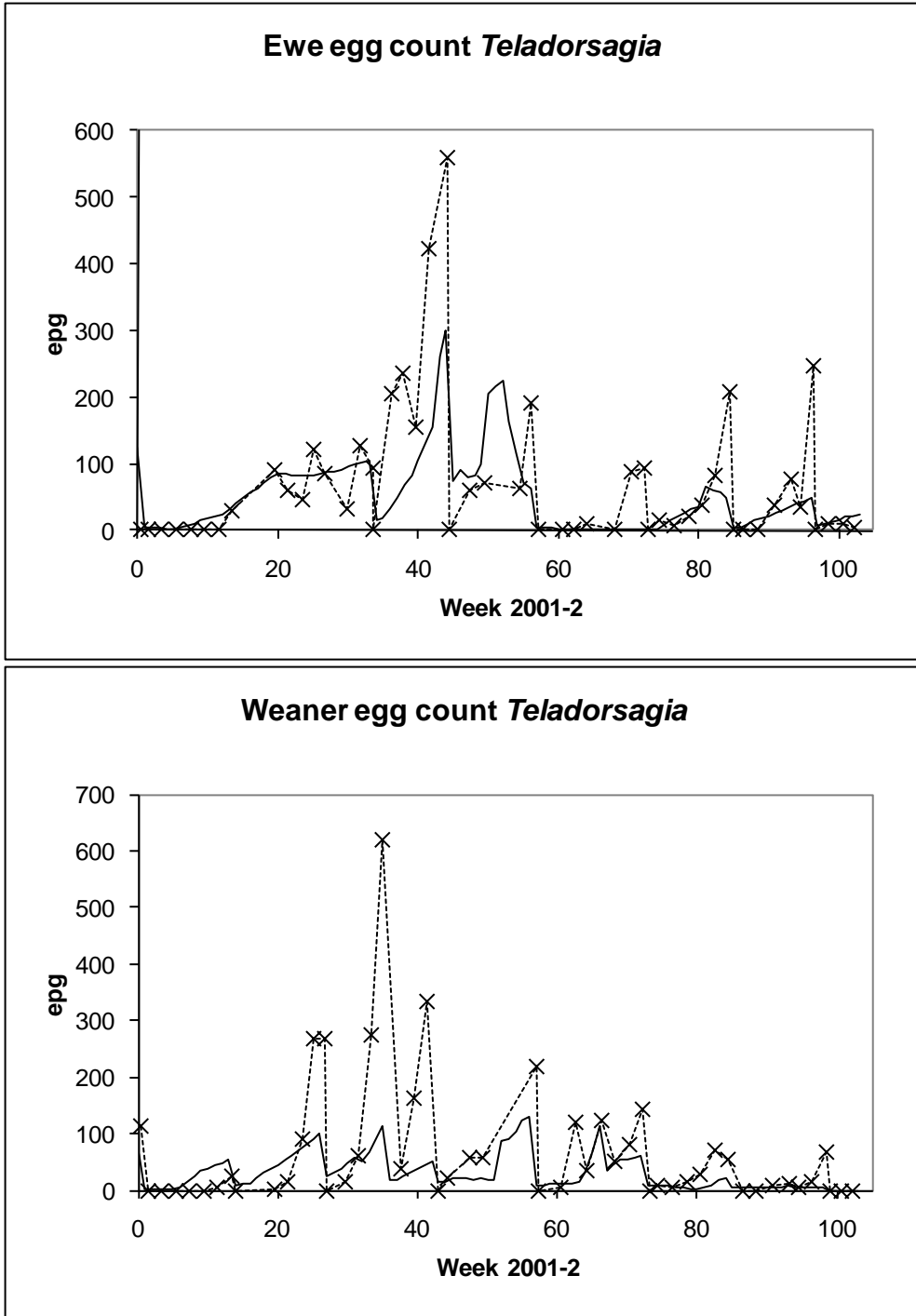


Figure A3. Observed individual (-x-) and predicted (—) *Haemonchus contortus* worm burdens for ewes and weaners between 2001 and 2002.

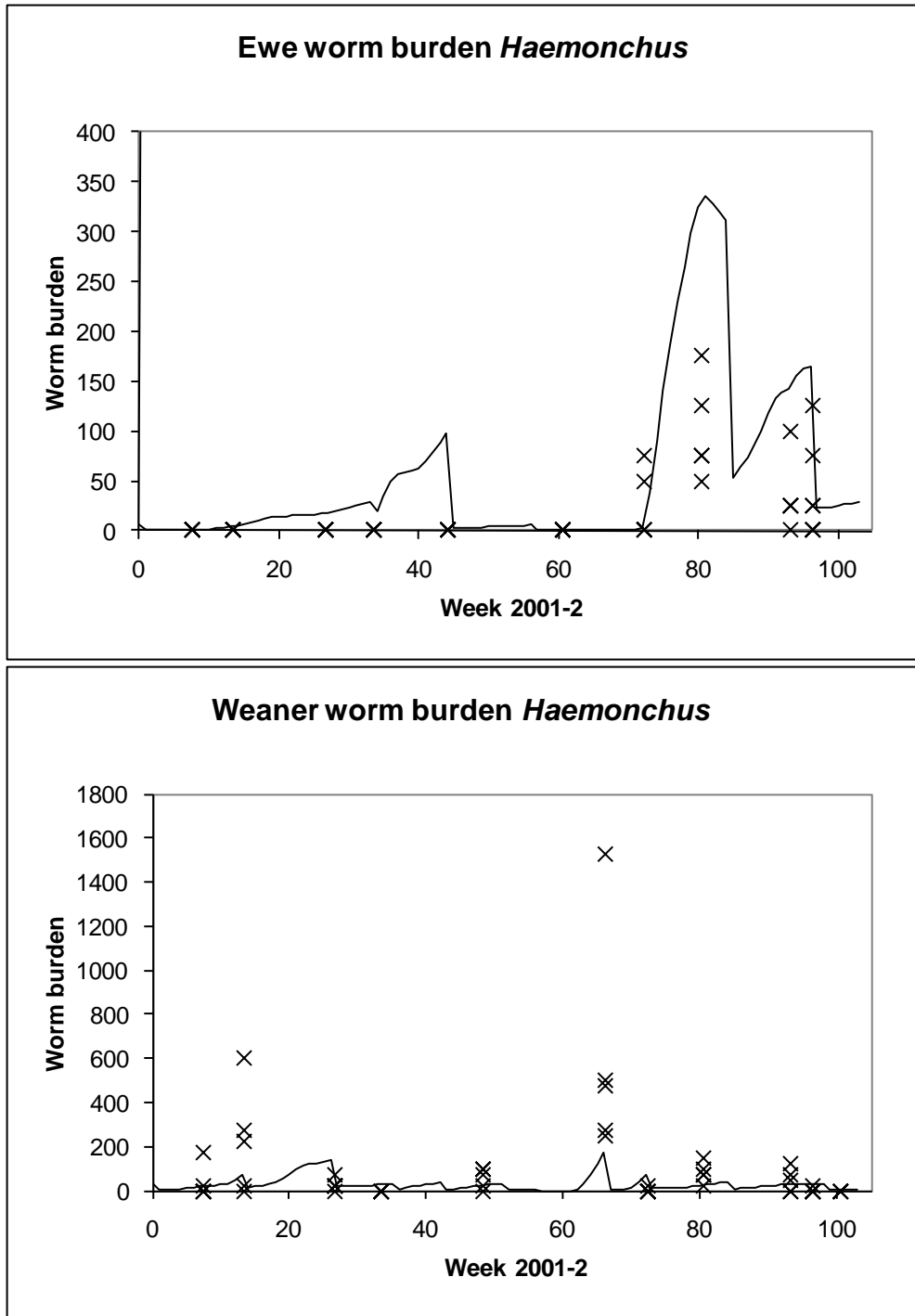


Figure A4. Observed mean (-x-) and predicted (—) *Haemonchus contortus* egg counts for ewes and weaners between 2001 and 2002.

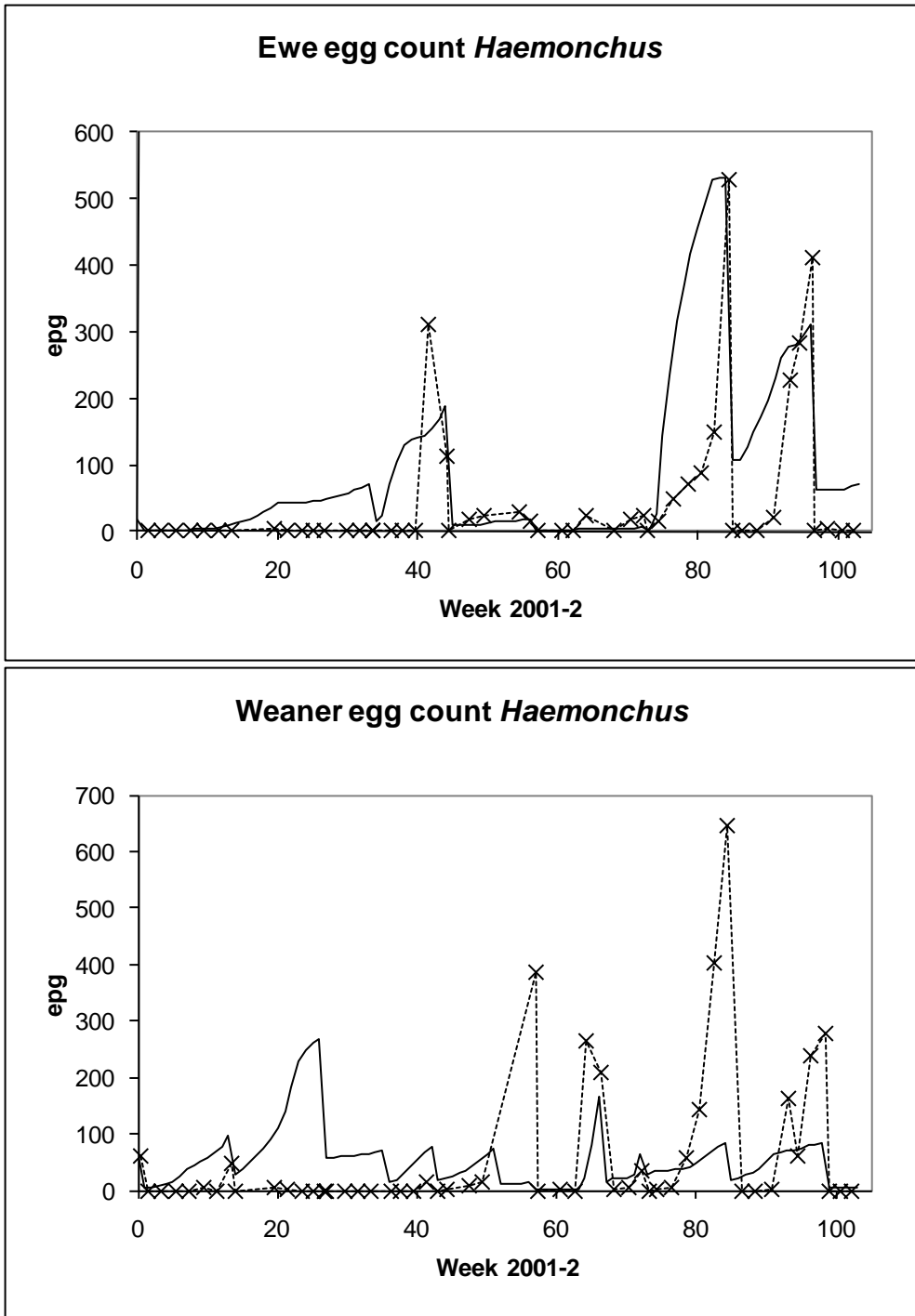


Figure A5. Observed individual (-x-) and predicted (—) *Trichostrongylus colubriformis* worm burdens for ewes and weaners between 2001 and 2002.

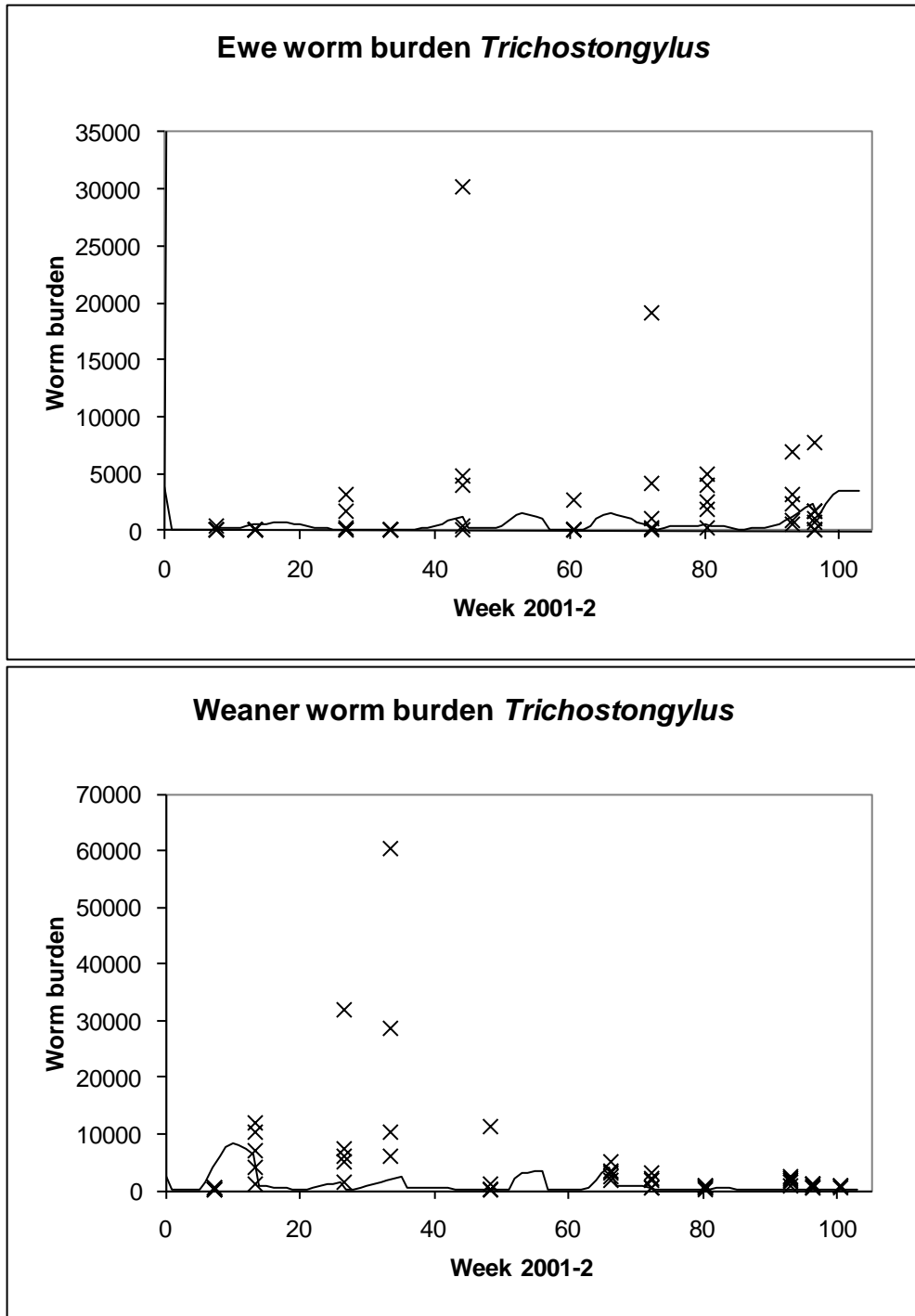


Figure A6. Observed mean (-x-) and predicted (—) *Trichostrongylus colubriformis* egg counts for ewes and weaners between 2001 and 2002.

