

Model predictions for anthelmintic resistance amongst *Haemonchus contortus* populations in southern Brazil

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ABSTRACT

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A computer model developed to study *Ostertagia circumcincta* resistance to anthelmintics in UK sheep flocks has been adapted for use with *Haemonchus contortus* under southern Brazilian conditions. The model simulates the effect of different anthelmintic control regimens on the year-to-year pattern of resistance in breeding ewes. The nematode control regimen most used by Brazilian sheep farmers was found to increase the frequency of genes which confer resistance from approximately 3% to 14% in an *H. contortus* population over a 20 year period. The effect of early versus late season anthelmintic treatment was investigated. This indicated that early season treatment would select for resistance rapidly, whereas late season treatments would not, owing to large numbers of untreated parasites accumulating at the beginning of the season. A model which can predict the development of anthelmintic resistance in parasites of ewes is a valuable tool in the understanding of the effect of different strategies on nematode control programmes and merits further consideration.

INTRODUCTION

Brazil has a sheep population of between 16 and 17 million, of which 55% are in the south, mainly in the state of Rio Grande do Sul where 95% of the wool sheep are grazed. The main breeds are Corriedale, Merino and Romney Marsh, which are almost entirely reared on natural pastures and on mixed grazing with beef cattle. Climatically, there are four seasons in the south: summer (December, January, February), autumn (March, April, May), winter (June, July, August) and spring (September, October, November). Annual rainfall over the period 1974-1989 has ranged from 744 to 1748 mm and

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averaged 1310 mm year⁻¹. Between October and March, evaporation normally exceeds rainfall and very occasionally droughts of up to 60 days can occur. In January, the maximum daily temperatures over the period 1974–1989 were highest and averaged 30.5°C, whereas in July the minimum temperatures averaged 8.1°C. Between May and September there were on average 19 frosts per year. Figures 1 (a) and 1 (b) illustrate the seasonal pattern of the long-term averages. The climate is suitable for many gastrointestinal nematodes of sheep of which *Haemonchus contortus* is the most important. Outbreaks of haemonchosis normally occur in autumn.

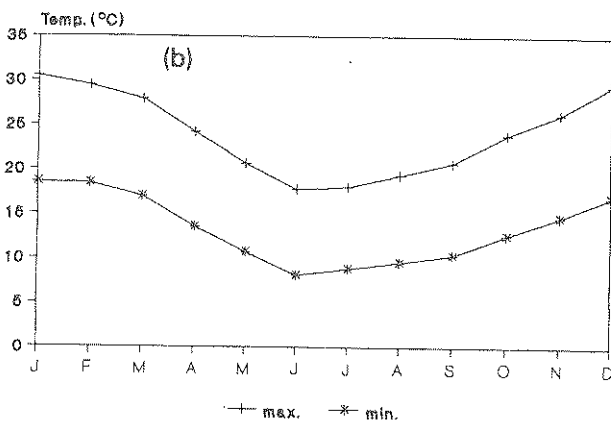
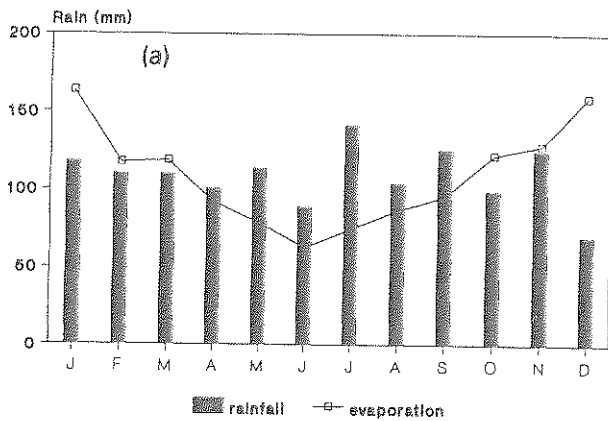


Fig. 1. (a) Mean monthly rainfall and evaporation rates at Bage, southern Brazil based on long-term averages over the years 1974–1989. (b) Mean daily maximum and minimum temperatures for each month of the year at Bage, southern Brazil based on long-term averages over the years 1974–1989.

As anthelmintic resistance in sheep nematodes continues to evolve to all broad spectrum anthelmintics in Brazil (Echevarria and Trindade, 1989; Echevarria and Pinheiro, 1990) there is a need for continuous investigation of the different contributory factors. In this respect, mathematical and statistical approaches have been used in the study of the complex process of the development of anthelmintic resistance.

Martin et al. (1984) and Waller et al. (1985) reported on the use of statistical dose response models to characterise resistant populations. Dobson et al. (1987) described a population dynamics model to predict changes in the genetic compositions of *Trichostrongylus colubriformis* populations under drug challenge, using an approach similar to that proposed by Georghiou and Taylor (1977a,b). In addition, Gettinby et al. (1989) developed a site-specific drug action model for genetic selection of anthelmintic resistance in *Ostertagia circumcincta* in sheep. This provided a method for the investigation of the effect of different anthelmintic programmes on nematode resistance under UK conditions. More recently, the same method was used by Gettinby et al. (1990) to assess the effects of parasite factors and climatic change on resistance.

In this paper, an adaptation of the model of Gettinby et al. (1989) is explored as a method of predicting the effect of weather and management factors on the development of *H. contortus* resistance to anthelmintics in a flock of breeding ewes under conditions typical of southern Brazil.

MATERIALS AND METHODS

Haemonchosis

Sheep that graze pastures of southern Brazil are infected with different gastrointestinal nematodes but *H. contortus* is by far the most important of these as it causes serious losses. This parasite is able to induce 30–40% mortality if lambs are not treated with anthelmintics. Infections reach dangerous levels during autumn when pasture larval availability reaches its highest level. Under such conditions, young susceptible lambs are treated very often. In a survey by Echevarria and Pinheiro (1990) it was shown that lambs in their first year receive on average nine treatments, ranging from six to 12 treatments per year. This in turn has led to the development of anthelmintic resistance to most of the broad spectrum compounds available.

Genetic prediction model

Investigations by Paton et al. (1984) demonstrated that good agreement could be obtained between *Ostertagia circumcincta* population levels observed on pasture over a grazing season and those predicted using a mathe-

mathematical computer simulation model. The basis of the prediction model was to simulate the effect of daily meteorological factors on the survival and development of an *O. circumcincta* population infecting ewes at lambing. Using the model as a building block, Gettinby et al. (1989) extended the simulations to take account of the various drug regimens commonly practiced for nematode control and the genetic response of the parasite population to drug control.

This model has been used to predict resistance amongst *H. contortus* populations maintained under Brazilian management conditions. It consists of an algorithm which in response to meteorological inputs determines the day-to-day development of parasites from the egg to larval third stage (L_3) on pasture. Stocking density and herbage availability entries are used to predict the daily consumption rate of infective larvae. Parasite factors relevant to the establishment rate from L_3 to adults and from adults to eggs are included. Details are as follows.

Development fractions used to determine the day-to-day development of parasites from egg to infective L_3 stage have been based on the findings of Silverman and Campbell (1959): 10°C requires 28 days, 12°C requires 16 days, 15°C requires 8 days, 18°C requires 6 days, 21°C requires 4 days, 24°C requires 3 days and 33°C requires 3 days. The daily development fractions are modified according to whether or not rain has occurred on each day. Meteorological conditions obtained for the year 1987 were considered typical and used for each year of the simulation. Annual rainfall was 1599 mm and evenly spread throughout the year. There was no drought during this year. Mean temperatures ranged from a daily maximum of 30.3°C in February to 17.8°C in May, whereas the daily minimum ranged from 18.8°C in February to 7.9°C in June.

Ewes lambing at the start of September come under challenge from contaminated pasture. The daily consumption rate of herbage is approximately 1.4 kg of dry matter (DM). Similarly, lambs under challenge consume approximately 0.1 kg DM day⁻¹ until 1 month of age when daily herbage consumption increases to approximately 0.6 kg DM. At the age of 2 months, most lambs will consume 0.7 kg DM day⁻¹. Infective L_3 larvae consumed within herbage are assumed to establish after 16 days subject to an attrition rate of 75%. The next stage of the life cycle relevant to the population dynamics is the rate at which infected animals translate eggs to pasture. During the periparturient period it is believed that egg output from infected ewes is density independent and time dependent. The egg output is estimated to be 280 eggs per gram (epg), 1270 epg and 590 epg respectively in the first 4 weeks after lambing, second 4 weeks after lambing and thereafter. Infected lambs are assumed to produce 12 000 eggs day⁻¹ for each adult female infection, i.e. eight eggs per female g⁻¹ × 1500 g of faeces day⁻¹.

Daily survival rates of parasites from the egg to L_3 and the infective stage

were estimated as 0.92 and 0.60, respectively, the latter reflecting the effect of desiccation on L_3 larvae that accumulate on pasture. Adult parasites are less vulnerable to environmental factors and are estimated to have a daily survival rate of 0.99. This is equivalent to a median life expectancy of around 70 days. Overwintered infective larvae are assumed to decay and at lambing each year the challenge levels are estimated to be $150 L_3 \text{ kg}^{-1} \text{ DM}$.

The exact mechanism of the genetic response of *H. contortus* parasites to anthelmintic treatment remains unidentified. However, using classical genetic theory, a single locus model can be used to approximate the response. Parasites with alleles RR, RS and SS which become exposed to drug treatment are assumed to survive with respective fitnesses f_1 , f_2 and f_3 . Adult worms which survive treatment are assumed to mate at random and the offspring inherit genes in Mendelian fashion, so that each new generation of offspring is in Hardy-Weinberg equilibrium. Consequently, the R and S proportions of the gene population of newly laid eggs will be equal to $(p_1 + p_2/2)$ and $(p_3 + p_2/2)$, where p_1 , p_2 and p_3 are the proportions of adult worms surviving drug treatment with genotypes RR, RS and SS respectively.

Findings from the genetic prediction model consist of the expected daily population sizes of egg, egg to L_3 and infective L_3 parasites on pasture. In addition, a day-to-day inventory is maintained of the number of adult worms per animal. Genetic predictions consist of the proportions of different genotypes expected within the infective larval population on the pasture at the end of each year. Changes in these proportions from year to year reflect the annual impact of drug control on selection for resistance.

RESULTS

The genetic prediction model has been used to predict the changing pattern of drug resistance under conditions in southern Brazil in a flock of 20 ewes and 16 lambs with four breeding ewe replacements per annum. The flock is maintained in a 20 ha paddock and the following four management regimens considered.

- (A) Dose ewes on Days 60 (marking), 110 (weaning), 194 (mating) and two later treatments on Days 244 and 314 after lambing.
- (B) Dose ewes on Days 60, 110, 194 and 254 after lambing.
- (C) Dose ewes on Days 90, 120, 150, 180 and 210 after lambing.
- (D) Dose ewes at 30 day intervals starting 90 days after lambing and ending 270 days after lambing.

Lambs are not treated but moved from the paddock at weaning 110 days after lambing.

Anthelmintic dosing affects only the immature and adult parasites and not those in refugia on pasture. The effect depends on the genotype and fitness of the parasite in response to drug treatment. In the case of incomplete domi-

nance, parasites with genotypes RR, RS and SS are assigned fitness parameters 1, 0.5 and 0. Consequently, parasites with genotypes RR, RS and SS survive with probabilities 1, 0.5 and 0, respectively. Alternatively, in the event of the expression of the R gene being recessive, fitnesses corresponding to the probability of survival of 1, 0 and 0 are assigned to parasites with genotypes RR, RS and SS.

Commencing with a worm population with gene frequencies in Hardy-Weinberg equilibrium and consisting of one in 1000 worms with genotype RR, simulations were undertaken of the changing genetic status of the parasite population on the paddock grazed by the ewe and lamb flock over a 20 year period. The simulation produces results of the expected numbers of worms of each genotype in each of the free-living stages. At the end of each

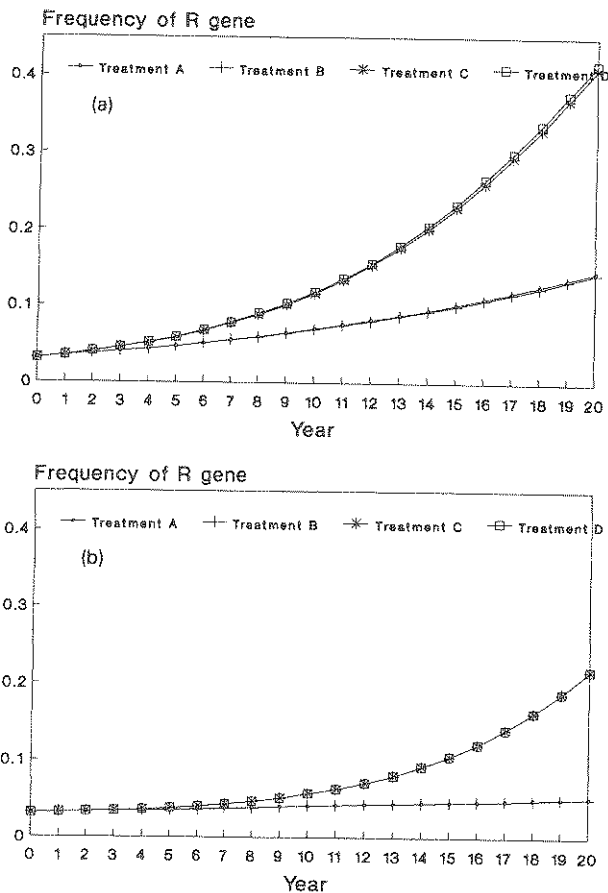


Fig. 2. The effect of various treatment regimens under southern Brazilian conditions, on the rate of selection for resistance in an *H. contortus* population over a 20 year period, when there are three parasite genotypes with fitnesses: (a) 1, 0.5 and 0; (b) 1, 0 and 0.

year the proportion of worms with genotypes RR, RS and SS found on herbage is used to calculate the distribution of the genotypes. Examination of the proportion of R genes amongst the larvae on pasture at the end of each year provides a measure of the rate at which selection for resistance occurs.

Figure 2(a) illustrates results when Regimens A, B, C and D are used under conditions of incomplete dominance whereby all worms with genotype RR and 50% with genotype RS survive drug treatment. Using Regimen A, the most widely used drug treatment programme in the region, there is a very slow but steady increase in the frequency of R genes in the infective larval population over the 20 year simulation period. The proportion of R genes in the infective larval population grew from approximately 3% to reach a maximum of 14.6%. A similar profile was obtained with Regimen B. The rate of

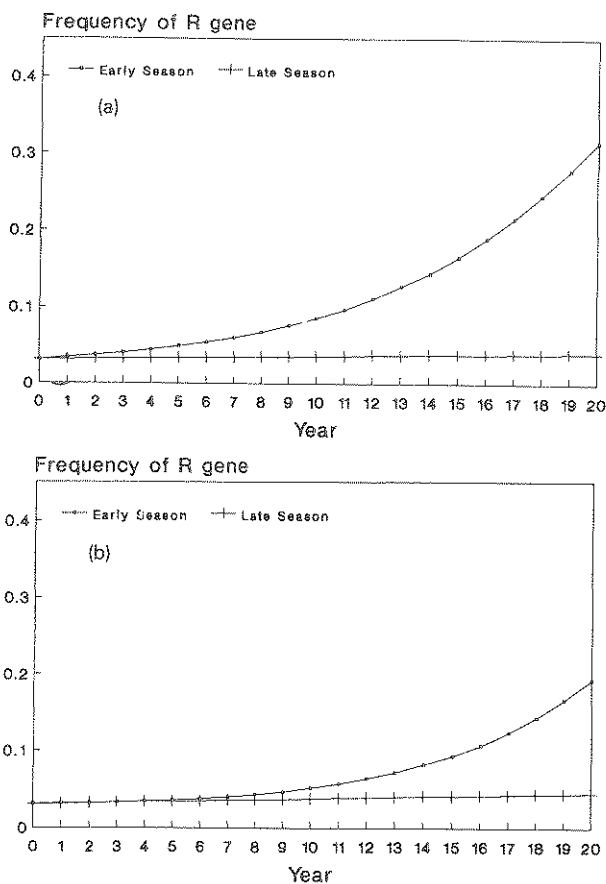


Fig. 3. The effect of early versus late season treatments under southern Brazilian conditions, on the rate of selection for resistance in a *H. contortus* population over a 20 year period, when there are three parasite genotypes with fitnesses: (a) 1, 0.5 and 0; (b) 1, 0 and 0.

TABLE 1

The effect of various treatment regimens under southern Brazilian conditions, on the selection for resistance in an *H. contortus* population over a 20 year period, maximum worm burden during a grazing season and mean daily worm burden

Control regimen	Frequency of R gene after 20 years	No. of doses per ewe year ⁻¹	Maximum worm burden	Mean worm burden
<i>(a) f1=1, f2=0.5, f3=0</i>				
A	0.146	5	5967	278
B	0.143	4	5938	280
C	0.409	5	12292	1276
D	0.415	7	12292	1290
Early ¹	0.315	4	5489	1101
Late ²	0.040	4	13712	39
<i>(b) f1=1, f2=0, f3=0</i>				
A	0.052	5	5731	23
B	0.052	4	5731	24
C	0.216	5	12292	699
D	0.216	7	12292	699
Early	0.192	4	5447	618
Late	0.045	4	13712	18

¹Treatments on Days 30, 60, 90 and 120 after lambing.

²Treatments on Days 110, 140, 170 and 210 after lambing.

development of resistance was fractionally lower, reflecting the fact that Regimen B used one fewer drug treatment than Regimen A. In contrast, when Regimen C is compared with Regimens A and B there is a clear difference in the rate of increase in the proportion of R genes. At the end of the 20 year period it is seen that the reduced interval between treatments for Regimen C produced a much higher proportion of R genes in comparison with Regimens A and B. The proportion of R genes in the infective larval population grew from approximately 3% to reach a maximum of 40.9%. However, the additional two treatments given with Regimen D and with the same 30 day interval between treatments did not produce a greater increase in the frequency of R genes in comparison with C.

When the expression of the R gene is recessive the results are shown in Fig. 2(b). Regimens A and B are indistinguishable and the R gene proportion only increases from approximately 3% to 5% over the 20 year selection period. However, more frequent administration of drug treatments associated with Regimens C and D increased the R gene proportion from approximately 3% to 21.6% over the 20 year horizon.

The effect of early versus late season treatment was compared by simulating four treatments at 30 day intervals starting 30 days after lambing with the same treatment starting at weaning. It can be seen from Figs. 3(a) and 3(b)

that early treatment selected for R genes while the same regimen starting at weaning did not produce an increase in R genes either when the R gene is recessive or incompletely dominant. In the cases of the R gene being recessive and incompletely dominant, early season treatment increased the R gene proportions from approximately 3% to 31.5% and 19.2%, respectively.

The simulation also produced results on the expected numbers of worms in each stage. From this it is possible to obtain estimates of the mean daily worm burden of a ewe throughout the grazing season and the maximum worm burden. Worm burdens, when the expression of the R gene is incomplete dominance, are given for each of the regimens in Table 1 (a). With the exception of Regimens A, B and early season treatment, all treatment regimens had very high mean worm burdens. When the R gene selection is recessive, the maximum worm burdens for each of the regimens are similar to those for incomplete dominance. However, average worm burdens are lower, reflecting the absence of parasites which have been genetically susceptible to drug treatment.

DISCUSSION

The different anthelmintic programmes used in the simulation model employed in the present studies have shown that they do not select for high levels of resistance very quickly. The model has shown the nematode control programme most used by the Brazilian farmers whereby ewes dosed on Days 60 (marking), 110 (weaning), 194 (mating) and two later treatments on Days 244 and 314 after lambing, would only increase the frequency of R genes from approximately 3% to 14% over a 20 year period. In contrast, if treatments are given at 30 day intervals between Days 90 and 210 after lambing, the frequency of the R gene increases from approximately 3% to 40%. Anthelmintic resistance in weaned lambs in Brazil has been well documented in a survey where it was shown to be highly prevalent and also that lambs harbour heavy infections and are treated very often (Echevarria and Pinheiro, 1990). The level of anthelmintic resistance in breeding ewes is not known and the output produced by the model may be correct as ewes are regarded as more refractory than lambs to nematode infections and are treated with less intensity.

The importance of parasite fitness in response to drug exposure is important in the determination of the absolute value of resistance. The comparisons carried out in this experiment have showed that the nematode control programmes select for higher frequencies of R genes when this fitness is set to 0, 0.5 and 1 instead of 0, 0 and 1 for SS, RS and RR, respectively. In this model it is assumed that the drug used is 100% effective in removing all stages of *H. contortus*. In practice, this is rarely obtained so that susceptible parasites can survive and consequently the selection for resistance can be expected to be slower.

The simulation model used in these studies has produced some interesting results. Nevertheless, they have to be interpreted with caution. Although the

model has been adapted for Brazilian conditions it still needs extensive revisions to provide a better approximation to the field situation. The findings are based on genetic parameters which remain theoretical estimates and as yet unsubstantiated by field or laboratory studies. The development periods of the parasite were based on findings reported in the 1950s and need to be verified for *H. contortus* strains found in southern Brazil. The model, in the version used, did not include treatment of lambs. As young lambs can produce high worm egg counts before they are weaned and moved away, they are an important part of the epidemiological process. Furthermore, under the conditions of southern Brazil, many farmers treat their lambs at marking, about 60 days after lambing. This is certainly an important factor and should be stressed in further studies on the improvement of the simulating facilities of the model. Other points to be considered in the future are the inclusion of facilities for treatment and movement of animals. Despite these reservations the model is a potentially powerful tool in the study of the interactions of control programme management and selection for anthelmintic resistance. Furthermore, it has drawn attention to the fact that further studies are necessary on the biological aspects of the process of anthelmintic resistance in particular, resistant and susceptible populations need to be better characterised with respect to infectivity, pathogenicity, development and survival.

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