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Which is the best phenotypic trait for use in a targeted selective treatment strategy for growing lambs in temperate climates?

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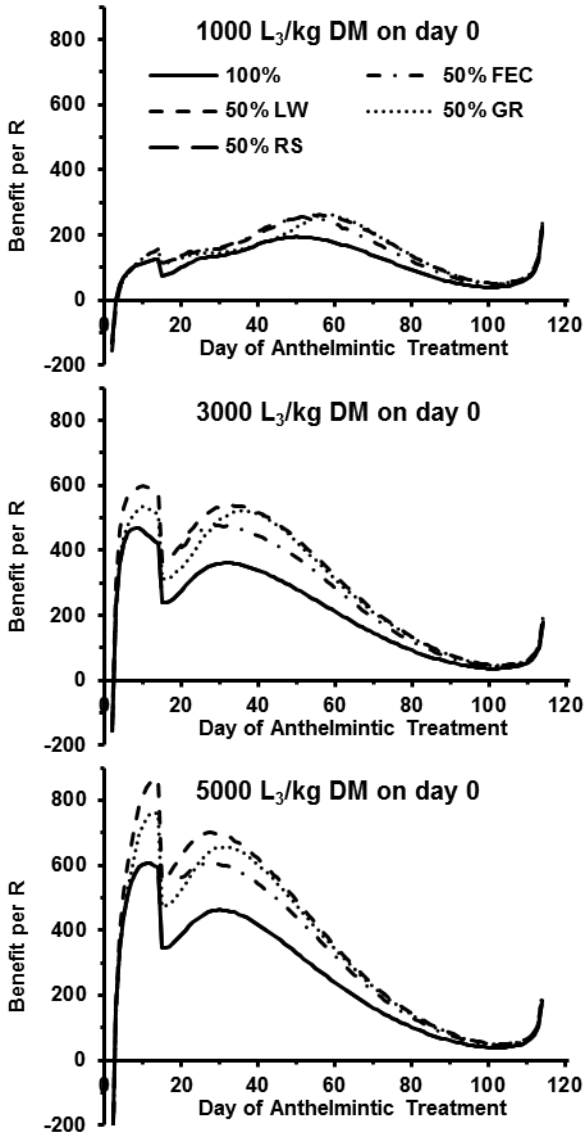
Graphical Abstract



Highlights

- Simulated evaluation of phenotypic traits determining targeted selective treatment
- Investigated the impact of nematode challenge and day of anthelmintic treatment
- Best criteria was live weight (productive benefit per increase in drug resistance)
- Identified the long-term productive benefit of targeted selective treatment

Abstract

Targeted selective treatment (TST) requires the ability to identify the animals for which anthelmintic treatment will result in the greatest benefit to the entire flock. Various phenotypic traits have previously been suggested as determinant criteria for TST; however, the weight gain benefit and impact on anthelmintic efficacy for each determinant criterion is expected to be dependent upon the level of nematode challenge and the timing of anthelmintic treatment. A mathematical model was used to simulate a population of 10,000 parasitologically naïve Scottish Blackface lambs (with heritable variation in host-parasite interactions) grazing on medium-quality pasture (grazing density = 30 lambs/ha, crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM) with an initial larval contamination of 1000, 3000 or 5000 *Teladorsagia circumcincta* L_3/kg DM. Anthelmintic drenches were administered to 0, 50 or 100% of the population on a single occasion. The day of anthelmintic treatment was independently modelled for every day within the 121 day simulation. Where TST scenarios were simulated (50% treated), lambs were either chosen by

random selection or according to highest faecal egg count (FEC, eggs/g DM faeces), lowest live weight (LW, kg) or lowest growth rate (kg/day). Average lamb empty body weight (kg) and the resistance (R) allele frequency amongst the parasite population on pasture were recorded at slaughter (day 121) for each scenario. Average weight gain benefit and increase in R allele frequency for each determinant criterion, level of initial larval contamination and day of anthelmintic treatment were calculated by comparison to a non-treated population. Determinant criteria were evaluated according to average weight gain benefit divided by increase in R allele frequency to determine the benefit per R. Whilst positive phenotypic correlations were predicted between worm burden and FEC; using LW as the determinant criterion provided the greatest benefit per R for all levels of initial larval contamination and day of anthelmintic treatment. Hence, LW was identified as the best determinant criterion for use in a TST regime. This study supports the use of TST strategies as benefit per R predictions for all determinant criteria were greater than those predicted for the 100% treatment group, representing an increased long-term productive benefit resulting from the maintenance of anthelmintic efficacy. Whilst not included in this study, the model could be extended to consider other parasite species and host breed parameters, variation in climatic influences on larval availability and grass growth, repeated anthelmintic treatments and variable proportional flock treatments.

Keywords: Sheep, Nematodes, *Teladorsagia circumcincta*, Modelling, Anthelmintic resistance, Targeted selective treatment.

1. Introduction

Reduced drug efficacy due to the emergence of anthelmintic resistance (Kaplan, 2004; Wolstenholme et al., 2004; Jabbar et al., 2006; Papadopoulos et al., 2012) threatens the sustainability of livestock systems (van Wyk et al., 1997; Waller, 2006a; Besier, 2007; Papadopoulos, 2008), prompting the proposition of a variety of non-chemotherapeutic control options (Besier and Love, 2003; Waller, 2003; Sayers and Sweeney, 2005; Jackson and Miller, 2006; Stear et al., 2007; Torres-Acosta and Hoste, 2008). Unfortunately, these do not provide sufficiently effective parasite control without chemotherapeutic support (van Wyk et al., 2006). Thus, anthelmintics remain an indispensable component of nematode control programs, further supported by their low cost and ease of use (Kenyon and Jackson, 2012). As such, integrated parasite control programs must deliver efficacious control whilst minimising negative effects on drug efficacy in a manner that meets the risk profile of sheep producers.

Anthelmintic treatments are directed towards the infra-population of nematodes (parasitic stages within the host) thereby leaving the supra-population (pre-parasitic stages on pasture) *in refugia* (unexposed to anthelmintics) (van Wyk, 2001; Soulsby, 2007; Besier, 2012), providing a reservoir of more susceptible genotypes which dilute the progeny of resistant nematodes surviving anthelmintic treatment (van Wyk et al., 2006). Anthelmintic treatment is known to select for resistance in proportion to the nematode population *in refugia* (Martin et al., 1981; Michel, 1985), and consequently practices such as administering anthelmintics prior to a move to a low worm-risk pasture should be carefully managed (Martin et al., 1985; Waghorn et al., 2009). Whilst grazing management (Waller, 2006b) and the host immune response (Laurenson et al., 2012a) affect nematode epidemiology, environmental conditions experienced by the supra-population are the predominant factor impacting upon the nematode population *in refugia* (Stromberg, 1997; O'Connor et al., 2006;

van Dijk et al., 2010; Morgan and van Dijk, 2012; Rose et al., 2015). Consequent fluctuations in the level of pasture contamination have led to the proposal of targeting whole flock treatments (TT, targeted treatment) at times when the supra-population provides a large *refugia* pool (Kenyon et al., 2009; Kenyon and Jackson, 2012), coinciding with periods in which the hosts will be exposed to a high level of infective larval challenge.

Further, the infra-population is known to be over-dispersed, such that the majority of the parasitic burden is aggregated within a small percentage of the host population (Barger, 1985; Sréter et al., 1994; Gaba et al., 2005). In practical terms, anthelmintic treatment can therefore be directed towards the individuals within a flock with the heaviest parasitic burden (Gallidis et al., 2009; Stafford et al., 2009; Gaba et al., 2010). Targeted selective treatment (TST) can thereby reduce the number of anthelmintic treatments administered to a flock and increase the nematode population *in refugia* by leaving a proportion of the infra-population untreated (van Wyk et al., 2006; Kenyon et al., 2009). As such, TST strategies require the ability to identify individuals for anthelmintic treatment. Genetic and genomic approaches have previously been proposed; however, in the absence of reliable genetic markers across populations, phenotypic traits were found to be preferable to estimated breeding values as the larval challenge experienced by an individual may be dis-similar to its family members due to large environmental effects (Laurenson et al., 2013a). A variety of clinical pathophysiological indicators have also been proposed and implemented; including various diarrhoea scores (Larsen et al., 1994; Larsen and Anderson, 2000; Cabaret et al., 2006; Broughan and Wall, 2007; Ouzir et al., 2011; Bentounsi et al., 2012), body condition score (Russel, 1984; Besier et al., 2010; Cornelius et al., 2014), anaemia score/FAMACHA© (Malan et al., 2001; Vatta et al., 2001; van Wyk and Bath, 2002; Kaplan et al., 2004; Molento et al., 2009; Ouzir et al., 2011; Bentounsi et al., 2012), and combinational indexes such as The Five Point Check© (Bath and van Wyk, 2009). However, these clinical indicators rely on visual assessment and

are therefore prone to subjective errors (Greer et al., 2009). Additionally, anaemia is indicative of haematophagous nematodes (i.e. *H. contortus*) or liver fluke infections, and thus not suitable for use as an indicator of the *T. circumcincta* and *Trichostrongylus* spp. infections predominant in temperate climates (van Wyk et al., 2006). Further, it may be considered preferable to treat animals prior to overt clinical signs becoming apparent.

Subclinical indicators of gastrointestinal parasitism can identify between-animal variation in host resistance (suppression of nematode challenges via an immune response; Bishop and Morris, 2007) and resilience (ability to withstand the pathogenic effects of nematode challenge; Bisset and Morris, 1996). Faecal egg count (FEC) has previously been used as a measure of host resistance (Woolaston and Baker, 1996; Morris et al., 1997; Kemper et al., 2010). TST based on treating animals with the highest FEC thereby reduces egg deposition and consequently the larval challenge experienced by the grazing flock (Kenyon et al., 2009; Kenyon and Jackson, 2012) and the associated impacts on performance (Coop et al., 1982, 1985; Holmes, 1987; Fox, 1997; Stear et al., 2003). Hence, FEC has previously been implemented as a determinant criterion for TST (Cringoli et al., 2009; Gallidis et al., 2009). In contrast, measures of host resilience provide a more direct welfare approach as only the least resilient animals are treated (Cabaret et al., 2009; Berrag et al., 2009; Kenyon et al., 2009; Kenyon and Jackson, 2012). Indicators of host resilience previously implemented as determinant criteria for TST include live weight (Leathwick et al., 2006a,b), weight gain (Stafford et al., 2009; Gaba et al., 2010; Bentounsi et al., 2012), production efficiency/HappyFactor^{TM} (Greer et al., 2009; Busin et al., 2013, 2014; Kenyon et al., 2013), and milk production (Hoste et al., 2002; Cringoli et al., 2009; Gallidis et al., 2009; Gaba et al., 2010).

Evaluation of these determinant criteria has predominantly focussed on the productive impact of implementing TST regimes (e.g. Busin et al., 2014). Few studies have explored the

impact upon anthelmintic efficacy (Leathwick et al., 2006b; Kenyon et al., 2013), due to the difficulty in measuring changes in anthelmintic resistance over short time periods (Gilleard, 2006; Besier, 2012). As such, computer simulation modelling has been suggested as a method of developing appropriate *refugia* strategies (Besier, 2012; Kenyon and Jackson, 2012), thereby reducing the necessity for expensive and time-consuming experimental trials.

The aim of this study was to use a mathematical model to compare the subclinical phenotypic traits proposed as determinant criteria for TST regimes, and investigate the impact of initial pasture larval contamination (*T. circumcincta* L_3/kg DM) and day of anthelmintic treatment on sheep performance and the emergence of anthelmintic resistance.

2. Materials and Methods

2.1 Mathematical model

The mathematical model of Laurenson et al. (2013b) describes the impact of host nutrition, genotype and *T. circumcincta* gastro-intestinal parasitism on a population of growing lambs, and includes the impact of anthelmintic treatment on host performance, nematode epidemiology and the emergence of anthelmintic resistance.

2.1.1 Individual lamb module

A schematic diagram describing the structure of the individual lamb module is provided in Figure 1. In brief, each lamb attempted to ingest sufficient nutrients to meet protein and energy requirements for desired growth and maintenance, as defined by its genotype (Emmans, 1997; Wellock et al., 2004). However, if the nutritional quality of available herbage was poor then resource intake may be constrained by the maximum gut capacity (Lewis et al., 2004). Grazing led to the concurrent ingestion of infective larvae (L_3)

from pasture, modelled as a function of herbage intake and pasture larval contamination (Laurenson et al., 2012b). Within the host, ingested larvae matured (following a pre-patent period; Coop et al., 1982), established, produced eggs and died, as determined by rates for establishment, density-dependent fecundity and mortality (Bishop and Stear, 1997; Louie et al., 2005). Parasitic burdens within the host were assumed to result in an endogenous protein loss (Yakoob et al., 1983), modelled as a function of larval challenge and worm mass (Vagenas et al., 2007a). To counteract this, the host was assumed to invest in an immune response causing decreased rates of establishment and fecundity, and an increased rate of nematode mortality. Thus, the host was capable of decreasing parasitic burden and the protein losses associated with parasitism. The acquisition of immunity was assumed to follow a sigmoidal pattern (thereby intrinsically accounting for antigen exposure thresholds; González et al., 2003) as a function of the cumulative daily larval population resident within the host (Laurenson et al., 2012b). However, components of the host immune response (e.g. cytokines, gastrin) are associated with inappetence causing a reduction in herbage intake (Fox et al., 1989; Greer et al., 2005), commonly known as (parasite-induced) anorexia (Kyriazakis, 2014). Reductions in desired herbage intake were modelled as a function of the rate of acquisition of immunity (Laurenson et al., 2011). The combination of protein losses associated with parasitic burden and reductions in herbage intake resulted in insufficient nutrient resources being available to fulfil requirements for maintenance and optimal growth. Thus, ingested resources, after losses due to parasitism, were assumed to be first allocated to meet the maintenance requirements and remaining resources allocated to growth and immunity proportional to requirements (Kahn et al., 2000; Doeschl-Wilson et al., 2008). If insufficient resources were available to meet maintenance requirements then the lamb catabolized its protein and lipid reserves. If the requirements from catabolism were greater than 20% of the current protein reserve (Sykes, 2000; Houdijk et al., 2001) or if lipid reserves

fell below 20% of the current protein reserve (the minimum protein to lipid ratio required for survival; Vagenas et al., 2007a) then the lamb was assumed to die.

Outputs from the individual lamb module were calculated on a daily basis and included performance and parasitological traits. Performance traits included live weight (LW, kg), herbage intake (kg DM) and empty body weight (EBW, kg); where EBW was given as LW minus gut-fill. Parasitological traits included worm burden (WB), total daily egg output, and faecal egg count (FEC, eggs/g DM); where FEC was given as the total daily egg output divided by faecal output (undigested dry matter; AFRC, 1993).

2.1.2 Population module

Between-animal variation was added to a variety of genetically controlled input traits including lamb growth attributes (optimal growth rate and body composition at maturity), maintenance requirements, and the ability to mount an immune response (rate of acquisition, as well as initial and final rates for host-controlled establishment, mortality and fecundity) (Vagenas et al., 2007b; Laurenson et al., 2012b). Initially all traits were assumed to be normally distributed, and parameters describing lamb growth attributes and maintenance requirements were assumed to be uncorrelated (Doeschl-Wilson et al., 2008). In contrast, the acquisition of immunity was assumed to be a function of overlapping effector mechanisms for establishment, mortality and fecundity (components of Th2 immune response; Jenkins and Allen, 2010). Thus, the rate-determining parameters involved in the acquisition of immunity were assumed to be strongly genetically and phenotypically correlated ($r = +0.5$). Further, random environmental variation in daily herbage intake was assumed to reflect the influence of external factors not explicitly accounted for by the model (Doeschl-Wilson et al., 2008), and were added to achieve a genetic correlation between herbage intake and growth rate of approximately 0.8 (Cammack et al., 2005).

The simulated lamb population was assumed to arise from a founder population of unrelated sires and dams with a pre-determined mating structure. Each founder animal had a breeding value (A_i) for each genetically controlled input trait sampled from a $N(0, \sigma_A^2)$ distribution, where the genetic variance $(\sigma_A^2 = h^2 \cdot \sigma_P^2)$ was determined by inputs for heritability (h^2) and phenotypic variation (σ_P^2). The trait breeding values for each offspring were constructed as $\frac{A_{Sire}+A_{Dam}}{2}$ plus a Mendelian sampling term drawn from a $N(0, 0.5\sigma_A^2)$ distribution (Falconer and Mackay, 1996). A Cholesky decomposition of the variance-covariance matrix for correlated traits was used to generate covariances between the breeding values of the animals, as well as their residual components. The phenotypic value (P_i) for each of the underlying traits was given by:

$$P_i = \mu + A_i + E_i$$

where μ is the population mean for the trait, A_i is the additive genetic deviation of the i^{th} individual, and E_i is the corresponding environmental deviation sampled from a $N[0, \sigma_P^2 \cdot (1-h^2)]$ distribution.

Outputs of the population module included the mean and coefficient of variation for each of the individual lamb model outputs. Further, genetic and phenotypic correlations between traits were calculated alongside trait heritabilities (Laurenson et al., 2012b).

2.1.3 Epidemiological module

In accordance with Laurenson et al. (2012b), the grazing pasture was defined by the area available for grazing (ha) and the initial herbage mass (kg DM/ha). The herbage available for grazing (kg DM) was updated on a daily basis to take into account herbage consumption by the lamb population and herbage growth. An initial pasture contamination of infective larvae (L_3/kg DM) was assumed to arise from the egg deposition of a ewe

population removed from pasture at lamb weaning. For simplicity, the initial egg contamination of the pasture was modelled such that the number of L_3 developing on pasture was equal to the number of larvae consumed by the lamb population for an initial period defined by the time taken for eggs to develop to L_3. Following this, L_3 only arose from eggs excreted onto pasture by the lamb population. A constant proportion of eggs were assumed to develop to L_3 following a constant developmental period. Further, L_3 were assumed to have a constant mortality rate. As such, the total L_3 population on pasture was updated on a daily basis to take into account the L_3 ingested by the lamb population, the mortality rate of L_3, and new L_3 developing from eggs. Lambs were assumed to graze randomly across the pasture, leading to an equal expected larval intake linked to herbage intake. The larval intake of any lamb was determined by its herbage intake, the herbage available for grazing and the total population of L_3 on pasture. As such, the parasitic challenge experienced by the lamb population could be summarized as the concentration of L_3 within the herbage available for grazing (L_3/kg DM), and was provided as an output of the epidemiological model.

2.1.4 Anthelmintic resistance module

Anthelmintic resistance was assumed to be conferred by 2 alleles, resistant (R) and susceptible (S) (Barnes et al., 1995; Leathwick et al., 1995; Learmount et al., 2006), in agreement with the monogenic mechanism for benzimidazole resistance (Elard and Humbert, 1999). The resistance genotypes of the initial population of infective larvae on pasture were calculated assuming Hardy-Weinberg equilibrium and an initial R allele frequency of 0.01 (Barnes et al., 1995). All genotypes were assumed to be equally fit (Barrett et al., 1998; Elard et al., 1998), such that in the absence of anthelmintic drenching the frequency of R remained the same throughout the simulated grazing season. The allele conferring anthelmintic resistance (R) was modelled to be recessive (Elard and Humbert, 1999; Silvestre and Cabaret, 2002). Anthelmintic drenching was assumed to reduce the population of L_3 and adult

nematodes resident within a host by 99% for heterozygous (RS) and homozygous susceptible genotypes (SS), and 1% for homozygous resistant genotypes (RR). Further, the oral administration of anthelmintic was assumed to be effective on the day of administration only, with no residual effects (Borgsteede, 1993). Thus in the first instance, anthelmintic drenching caused a 99% reduction in parasitic burden and, with the imposition of density-dependent effects on parasite fecundity, a 96.9% reduction in FEC, similar to the post-treatment efficacies reported by Sargison et al. (2007). Outputs of the anthelmintic resistance model included the frequency of R, and the nematode population *in refugia* (unexposed to anthelmintic) calculated on a daily basis according to Laurenson et al. (2013b).

2.2 Model parameterization

Whilst host and parasite descriptions within the model could be parameterized to represent different hosts (e.g. different sheep breeds) as well as nematode species, in this instance host growth characteristics were parameterized such that trait means and between-animal variance matched those of Bishop et al. (1996) and Bishop and Stear (1997) for Scottish Blackface lambs, and parasitological parameters matched those of Coop et al. (1982, 1985) for lambs infected with *T. circumcincta*.

A total simulated population of 10,000 parasitologically naïve lambs (arising from 250 sires and 5,000 dams) was chosen to ensure that trait means at each time point would be estimated with precision, avoiding the need for statistical analyses of the outputs. With this large population size, the expected standard error of each mean value within the simulation is $\sigma/100$, where σ is the trait standard deviation at that time point. Therefore, even with extremely variable traits such as FEC, which have a coefficient of variation close to 100%, the standard error of the mean will only be 1% of the mean value.

Lambs were grazed on a medium-quality pasture (crude protein = 140g/kg DM,

metabolisable energy = 10MJ/kg DM; AFRC, 1993), at a stocking density of 30 lambs/ha, for a duration of 4 months from weaning to 6 months of age (slaughter). The pasture was parameterized such that the initial herbage mass was 1,500 kg DM/ha (Sibbald et al., 2000), and daily herbage growth was assumed to be 60 kg DM/ha (Grass Check, 2011). The free-living stages of the nematode parasite life-cycle were parameterized to represent *T. circumcincta* such that the developmental time from eggs to L_3 was 7 days (Young et al., 1980), the proportion of eggs developing to $L_3$was 0.11 (Boag et al., 1975), and the mortality rate of L_3 was 0.035 (Gibson and Everett, 1972). As such, this provided a parameterization representative of late spring and summer for northern England and southern Scotland.

2.3 Simulation procedure and in silico experimental design

Anthelmintic treatment was administered to 0%, 50% or 100% of the population on a single occasion. For the 50% (TST) treatment group, lambs were either chosen by random selection (Gaba et al., 2012) or according to highest FEC (eggs/g DM faeces), lowest LW (kg) or lowest growth rate (GR, kg/day). Random selection was achieved using a pseudo-random number generator to identify lamb IDs to be drenched. FEC measurements were assumed to be taken 5 days prior to treatment to allow time for the samples to be processed and analyzed, and included a 20% random sampling error (Bishop et al., 1996; Stear et al., 2009). LW was assumed to be measured the day prior to treatment. GR was calculated assuming that lambs were weighed at the start of the grazing season and on the day prior to treatment. Production efficiency was not evaluated due to its inherent similarity to GR. Milk production was also not evaluated as it is only relevant to a very small percentage of the global sheep industry and because the mathematical model used in this study simulates growing lambs rather than lactating ewes.

The pasture was initially contaminated with either 1000, 3000 or 5000 *T.*

circumcincta larvae (L_3/kg DM). Lambs initially ingested around 1kg DM/day and thus these contaminations correspond to the trickle challenge levels chosen by Coop et al. (1982) that led to subclinical infections. Further, this provides a range of nematode challenges and *refugia* which can be considered to mimic variation arising from environmental conditions without accounting for it explicitly. The day of anthelmintic treatment was modelled independently for every day within the 121 day simulation (i.e. anthelmintic treatments occurring on day 1, 2, 3…119, 120, 121). As such, this encompasses the timing of any targeted treatment (TT) strategy. Importantly, all scenarios were modelled such that each treatment group grazed independently on separate pastures and thereby incorporated the epidemiological benefit of anthelmintic treatment strategies (Laurenson et al., 2012a).

2.4 Simulation outputs

2.4.1 Distribution of output traits

Genetically controlled input traits exhibiting between-animal variation (growth attributes, maintenance requirements and immune response) were assumed to be normally distributed. However, the distribution of WBs and FECs within the host population are known to be over-dispersed (Barger, 1985; Sréter et al., 1994; Gaba et al., 2005), and is one of the key characteristics of nematode epidemiology supporting the use of TST strategies. This phenomenon of over-dispersion has previously been attributed to genetically determined differences in the immune responsiveness of hosts to parasitic infections (Wakelin, 1984; Wassom et al., 1986). As such, as immunity is acquired the distribution of FEC and WB becomes increasingly over-dispersed (Stear et al., 1995; Bishop et al., 1996). Therefore, given that between-animal genetic variation in immune responses were included within the model, changes in the distribution of output traits were assumed to occur as a consequence of the functions underlying the model rather than as a result of direct input (Barnes and Dobson,

1990).

To provide a summary of the distribution of WB and FEC predictions, the mean average and coefficient of variation were recorded on each day of the simulated grazing season. Consequently, the distribution skew was quantified as:

$$\text{skew} = \frac{n}{(n-1)(n-2)} \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{\bar{x} \cdot cv} \right)^3$$

where n = 10,000 lambs, x_i = trait value for the i^{th} individual, $\bar{x}$ = trait mean, cv = coefficient of variation.

For anthelmintic treatments administered on a single occasion, as simulated in this study, the mean, coefficient of variation and distribution skew for WB and FEC on the day of anthelmintic treatment will be equal to those predicted for the non-treated (0%) control group for each level of initial pasture larval contamination. Consequently, only predictions arising for lamb populations given no anthelmintic treatments (0% treatment groups) are presented for initial pasture larval contaminations of 1000, 3000 or 5000 *T. circumcincta* L_3/kg DM.

2.4.2 Phenotypic correlations

The aggregation of parasitic burdens within the host population (Barger, 1985; Sréter et al., 1994; Gaba et al., 2005) supports the strategy of targeting anthelmintic treatments towards those individuals with the heaviest parasitic burden (Gallidis et al., 2009; Stafford et al., 2009; Gaba et al., 2010). However, time dependent effects in between animal variation for resistance (Bishop and Morris, 2007) and resilience (Bisset and Morris, 1996) may impact upon the ability of phenotypic determinant criteria to identify those individuals with the heaviest parasitic burden. In the absence of a prior anthelmintic treatment, phenotypic correlations indicate the ability of each phenotypic determinant criterion to identify those

animals with the highest WBs at any given time-point. Hence, predicted phenotypic correlations between each determinant criterion (FEC, LW and GR) and WB are only provided for lamb populations given no anthelmintic treatments (0% treatment groups) and grazed on pasture with an initial pasture larval contamination of 1000, 3000 or 5000 *T. circumcincta* L_3/kg DM. Whilst WB can be easily quantified within simulation studies, experimental studies often use FEC as a proxy for WB due to the difficulty (and requirement for slaughter) of measuring WB. Consequently, predicted phenotypic correlations between FEC and LW or GR are also provided to allow for comparison with experimental estimates.

2.4.3 Determinant criteria evaluation

The aim of a TST strategy is to maintain effective nematode control whilst minimizing negative impacts on anthelmintic efficacy by reducing the total number of anthelmintic treatments administered to a flock. Consequently, for each scenario (% treated, determinant criterion, initial pasture larval contamination, and day of anthelmintic treatment) the average EBW (to remove variation in gut-fill and provide a productive output similar to carcass weight) and the R allele frequency amongst the parasite population on pasture were recorded at slaughter (day 121). To quantify the productive implications (as the focus of the sheep industry) of anthelmintic treatment strategies (full flock treatment vs TST), the average weight gain benefit (AWGB, kg) arising from anthelmintic treatments administered on day t was calculated by comparison to a non-treated control group, such that:

$$\mathrm{AWGB_t} = \mathrm{EBW}_{121}^{\mathrm{AH}} - \mathrm{EBW}_{121}^{\mathrm{C}}$$

where $\mathrm{EBW}_{121}^{\mathrm{AH}}$ is the average EBW at slaughter for a group receiving anthelmintic treatments on day t, and $\mathrm{EBW}_{121}^{\mathrm{C}}$ is the average EBW at slaughter for a control group receiving no anthelmintic treatments.

Similarly, to quantify the impact of anthelmintic treatment strategies upon anthelmintic efficacy, the increase in R allele frequency (IRAF) arising from anthelmintic treatments administered on day t was calculated as:

$$\mathrm{IRAF_t} = \mathrm{RAF}_{121}^{\mathrm{AH}} - \mathrm{RAF}_{121}^{\mathrm{C}}$$

where $\mathrm{RAF}_{121}^{\mathrm{AH}}$ is the R allele frequency of day 121 for a group receiving anthelmintic treatments on day t, and $\mathrm{RAF}_{121}^{\mathrm{C}}$ is the R allele frequency of day 121 for a control group receiving no anthelmintic treatments.

As previously stated, integrated parasite control programs (including TST) aim to deliver efficacious control whilst minimizing negative effects on drug efficacy. Hence, in order to evaluate the use of differing determinant criteria within TST regimes, a single trait was required to account for the productive benefit of anthelmintic treatments as well as their impact upon anthelmintic resistance. Consequently, the first derivative of the relationship between average EBW and R allele frequency on pasture at slaughter was used to generate a single trait (giving equal weighting to AWGB and IRAF), such that the benefit per R (BPR) arising from anthelmintic treatments administered on day t was calculated as:

$$\mathrm{BPR_t} = \frac{\mathrm{AWGB_t}}{\mathrm{IRAF_t}}$$

As such, for the 50% (TST) treatment groups, the best determinant criterion for any level of initial pasture larval contamination or day of anthelmintic treatment will be the phenotypic trait which is predicted to result in the greatest benefit per R.

3. Results

3.1 Distribution of output traits

Figure 2 provides the mean, coefficient of variation and distribution skews for WB and FEC predictions arising from 10,000 lambs given no anthelmintic treatments and grazed on medium quality pasture with an initial larval contamination of 0, 1000, 3000 or 5000 *T. circumcincta* L_3/kg DM herbage. The distribution skew for both WB and FEC were predicted to be initially positive (right-skewed) followed by a rapid decline towards a normal distribution and then a gradual linear increase as the simulated grazing season progressed. The gradual increase in skew represents both traits becoming progressively right-skewed over time. Notably, the FEC distribution skew (Figure 2f) was predicted to be greater than the WB distribution skew (Figure 2e).

3.2 Phenotypic correlations

Figure 3 provides the time-dependent phenotypic correlations between WB and LW, GR or FEC; as well as between FEC and LW or GR; in a lamb population given no anthelmintic treatment for an initial pasture larval contamination of 1000, 3000 or 5000 *T. circumcincta* larvae (L_3/kg DM). No phenotypic correlations are presented prior to day 14, corresponding with the pre-patent period required for ingested L_3 to mature and establish as adult nematodes within the host (Coop et al., 1982).

Phenotypic correlations with WB for LW (Figure 3a) and GR (Figure 3b) became increasingly positive during the initial period of the grazing season. For the phenotypic correlation between WB and LW the maximum positive correlation (and day of maximum) was predicted to be +0.59 (32), +0.55 (21), and +0.51 (20) for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively. For the phenotypic correlation between WB and GR the maximum positive correlation (and day of maximum) was predicted to be +0.60 (32), +0.45 (23), and +0.36 (23) for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively; after which, these

phenotypic correlations tended towards zero. The day at which the phenotypic correlation between WB and LW reached zero was predicted to be day 67, 47, and 42 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively. For the phenotypic correlation between WB and GR the day at which the correlation reached zero was predicted to be day 65, 41, and 36 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively. Subsequently, the phenotypic correlations with WB for both LW and GR became increasingly negative before plateauing by the end of the simulated grazing season. The phenotypic correlation between WB and LW on day 121 was predicted to be -0.38, -0.49, and -0.54 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively. For the phenotypic correlation between WB and GR the predicted correlation on day 121 was -0.42, -0.52, and -0.57 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively.

The predicted phenotypic correlation between WB and FEC (Figure 3c) was +0.15 on day 14 and increased gradually over the grazing season to +0.76 on day 121 for all levels of initial pasture larval contamination. Minor differences were predicted between days 14 and 60, where phenotypic correlations for an initial pasture larval contamination of 1000 L_3/kg DM were lower than those for 3000 and 5000 L_3/kg DM.

The predicted phenotypic correlations between FEC and LW (Figure 3d) or GR (Figure 3e) follow expectations derived from the interaction of predicted phenotypic correlations between WB and LW, GR or FEC. For the phenotypic correlation between FEC and LW, the maximum predicted correlation (and day of maximum) was +0.14 (42) for an initial pasture larval contamination of 1000 L_3/kg DM, and the minimum predicted correlation (and day of minimum) was -0.64 (99) for an initial pasture larval contamination of 5000 L_3/kg DM. For the phenotypic correlation between FEC and GR, the maximum predicted correlation (and day of maximum) was +0.23 (42) for an initial pasture larval

contamination of 1000 L_3/kg DM, and the minimum predicted correlation (and day of minimum) was -0.66 (91) for an initial pasture larval contamination of 5000 L_3/kg DM.

3.3 Determinant criteria evaluation

Figure 4 provides the average weight gain benefit and increase in R allele frequency derived from differing determinant criteria and day of anthelmintic treatment for an initial pasture larval contamination of 1000 (Figure 4a), 3000 (Figure 4c) or 5000 (Figure 4e) L_3/kg DM herbage. For all levels of initial pasture larval contamination and treatment groups (whole flock treatment and differing determinant criterion), anthelmintic treatments occurring during the first 3 days of the simulated grazing period resulted in negative average weight gain benefits. Notably, when FEC was used as the determinant criterion to treat 50% of the grazing flock, predictions were provided for anthelmintic treatments occurring from day 19 onwards due to the assumed 14 day pre-patent period and 5 day laboratory processing delay. Following these initial negative average weight gain benefit predictions, anthelmintic treatments occurring after the third simulated day resulted in positive average weight gain benefits for all levels of initial pasture larval contamination, determinant criteria and the full flock treatment group (100% treated).

The maximum predicted average weight gain benefit and the day of anthelmintic treatment at which the maximum occurred for all levels of initial pasture larval contamination and determinant criteria are provided in Table 1. The maximum average weight gain benefit increased, and the day of anthelmintic treatment at which the maximum occurred decreased, with increasing initial pasture larval contamination for all treatment groups (100% treated, and 50% treated according to random selection, FEC, LW or GR). Whilst predictions of average weight gain benefit when using FEC as the determinant criterion to treat 50% of the lamb population were always greater than those predicted when selecting animals at random,

average weight gain benefit predictions when using LW or GR as the determinant criterion were initially less than those predicted for animals selected at random for all levels of initial pasture larval contamination (Figure 4). When using LW or GR as the determinant criterion, the day of anthelmintic treatment at which average weight gain benefit predictions were equal to those for random selection were day 60, 32 and 29 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively. Following maximum average weight gain benefit predictions, the average weight gain benefit for all determinant criteria and levels of initial pasture larval contamination became increasing similar to the 100% treatment group as the day of anthelmintic treatment increased. As the day of anthelmintic treatment approached simulation day 121, average weight gain benefit predictions for all treatment groups (100% treated, and 50% treated according to random selection, FEC, LW or GR) and levels of initial pasture larval contamination reduced in line with the reduced duration in which the weight gain benefit of anthelmintic treatment could become apparent before the end of the simulated grazing season. As such, the average weight gain benefit for anthelmintic treatments occurring on day 121 were zero for all levels of initial pasture larval contamination and treatment groups.

In contrast to the average weight gain benefit predictions, increasing the initial pasture larval contamination had little to no impact upon the increase in R allele frequency predictions (Figure 4), which is also evident within the maximum increase in R allele frequency predictions detailed in Table 1. However, increasing the initial pasture larval contamination was predicted to result in a decreasing day of anthelmintic treatment for which the maximum increase in R allele frequency occurred for the treatment groups where 50% of the population was treated according to FEC, LW or GR (Table 1). Notably, whilst average weight gain benefit predictions for the 50% treatment groups using FEC, LW or GR as the determinant criteria became increasingly similar to the full flock treatment group (100%

treated) as the day of anthelmintic treatment increased, the increase in R allele frequency remained considerably reduced in comparison with the full flock treatment until the final days of simulation where differences did not have sufficient time to become apparent.

Figure 5 provides the benefit per R derived from differing determinant criteria and day of anthelmintic treatment for an initial pasture larval contamination of 1000 (Figure 5a), 3000 (Figure 5b) or 5000 (Figure 5c) L_3/kg DM herbage. Increasing the initial pasture larval contamination resulted in an increasing benefit per R (Figure 5), which is also evident from the maximum benefit per R predictions detailed in Table 1. For all levels of initial pasture larval contamination and day of anthelmintic treatment, benefit per R predictions when treating 100% of the grazing lamb population or 50% according to random selection were equal. In comparison, all other treatment groups (50% treated according to FEC, LW or GR) resulted in an increased benefit per R (Figure 5, Table 1). Of the determinant criteria evaluated in this study, LW was predicted to result in the greatest benefit per R for all levels of initial pasture larval contamination and day of anthelmintic treatment. For FEC, benefit per R predictions were initially better than GR until anthelmintic treatments administered on day 54, 29 and 24 for initial pasture larval contaminations of 1000, 3000 and 5000 L_3/kg DM, respectively; after which GR provided a better determinant criterion than FEC. The optimum day of anthelmintic treatment (day of anthelmintic treatment on which the maximum benefit per R occurred) decreased with increasing initial pasture larval contamination (Table 1). For all determinant criteria and level of initial pasture larval contamination, anthelmintic treatments administered during the first 60 days post weaning were predicted to lead to the greatest benefit per R (Figure 5, Table 1).

4. Discussion

4.1 Distribution of output traits

Initial positive (right-skew) predictions for both WB and FEC resulted from the link between GR and herbage intake (Cammack et al., 2005). Lambs growing at a faster rate had a greater nutritional requirement (Emmans, 1997; Wellock et al., 2004) leading to an increased herbage intake, associated larval intake and consequent WB. Subsequent reductions in distribution skew arose from the relationship between the acquisition of immunity and herbage intake (Laurenson et al., 2011). Individuals with an increased larval intake acquired immunity at a faster rate than the remainder of the lamb population. Consequent reductions in herbage intake resulted in a reduced larval intake thereby counteracting the initial positive distribution skew arising from the link between GR and herbage intake. As such, distribution skews for WB and FEC reduced toward zero (normal distribution). Following this and in agreement with expectations, WB and FEC predictions became progressively right-skewed (increasing distribution skew, Figure 2) over time as a consequence of between-animal variation in immune responses (Wakelin, 1984; Wassom et al., 1986). The relationship between distribution skew and immune response is evidenced by the increased distribution skew for FEC in comparison to WB. The WB distribution skew resulted from the impact of the acquisition of immunity on nematode establishment and mortality, whilst the increased FEC distribution skew was due to the added impacts of density dependence and the acquisition of immunity on fecundity and herbage intake. Reductions in herbage intake as a consequence of the acquisition of immunity caused reductions in faecal output and hence an increased FEC (eggs/g DM faeces).

4.2 Phenotypic correlations

The time-dependent nature of the phenotypic correlations predicted between WB and LW or GR (Figure 3) was a consequence of: 1) the link between herbage intake, LW and GR;

2) the impact of the acquisition of immunity upon WB, herbage intake, GR and LW; and 3) the absolute level of acquired immunity. Initially, lambs with the highest LW or desired GR had a higher herbage intake, and hence a larger larval intake, leading to an increased WB (and positive correlation). Ingestion of L_3 prompted the acquisition of immunity, with increasing levels of initial pasture larval contamination leading to an increased rate of acquisition. The acquisition of immunity is associated with reductions in herbage intake (Greer et al., 2005) and consequent reductions in GR and LW (Dever et al., 2016). As such, lambs acquiring immunity and thereby reducing WB also have a reduced GR and LW, further reinforcing the predicted positive correlations. However, as immunity is acquired, the ability to suppress WB increases. Thus, despite the negative impact of the acquisition of immunity on GR, the increasing control of parasitic burdens reduced the associated endogenous protein losses leading to reduced maximum positive phenotypic correlations (and day of maximum) for increasing levels of initial pasture larval contamination. These negative correlations reflect the efficacy of acquired immunity in reducing resident WBs, and hence a reduced impact of parasitism on LW and GR. As such, the longer it takes for immunity to be acquired, the longer it takes to reduce WBs and their detrimental impact; as evident from the phenotypic correlations of WB with LW and GR on day 121, where increasing the initial pasture larval contamination resulted in increasingly negative phenotypic correlations. Differences between the predicted phenotypic correlations for LW and WB, or GR and WB, arose because correlations with LW accounted for the impact of current LW and desired GR on herbage intake and hence larval intake, whilst correlations with GR only account for the impact of desired GR on herbage intake.

The gradual increase in the predicted phenotypic correlation between WB and FEC over the course of the simulated grazing season (Figure 3c) was in agreement with the *T. circumcincta* trickle challenge experiments conducted by Beraldi et al. (2008), where the

phenotypic correlation between WB and FEC increased from +0.17 on day 21 post-infection to +0.83 on day 91 post-infection. The predicted time-dependent phenotypic correlations between WB and FEC arose as a consequence of the average WB and FEC predictions provided in Figure 2. Whilst the impact of immune acquisition on nematode establishment, mortality and fecundity was assumed to occur at slightly different rates, alterations in each occurred concurrently. As such, this led to the increasing phenotypic correlations predicted between WB and FEC. Minor differences in the predicted phenotypic correlations for the differing levels of initial pasture larval contamination between days 14 and 60 occurred as a consequence of strong density-dependent effects on nematode fecundity for low WBs resulting from lower challenge levels. These strong density-dependent effects on nematode fecundity only resulted in minor differences due to the counteracting impact of the acquisition of immunity on faecal output as discussed in section 4.1. Despite speculation about poor phenotypic correlations between WB and FEC, values derived from experimental studies remain strongly positive across nematode species [r = +0.74 (McKenna, 1981), r = +0.82 (Douch et al., 1984), r = +0.63 (Stear et al., 1995), r = +0.91 (Bisset et al., 1996), r = +0.91 (Amarante, 2000) etc.]. Notably, Amarante (2000) investigated the relationship between WB and FEC in mixed infections and reported a large range of species specific correlations. This study concluded that poor correlations were the result of laboratory procedures (group composite) to identify specific nematode species in mixed infections, and could be resolved by determining nematode species composition on an individual lamb basis rather than using a group composite. As such, ignoring poor species specific correlations derived from mixed infection studies, it is safe to say that WB and FEC are strongly correlated. This conclusion thereby supports the use of the FEC reduction test (Coles et al., 1992) as a measure of anthelmintic efficacy.

Predicted phenotypic correlations between FEC and LW or GR were broadly similar

to those predicted for WB and LW or GR, with differences being attributable to the time-dependent phenotypic correlation predicted between WB and FEC. A comparison of predicted genetic correlations between LW and FEC against point estimates derived from experimental studies can be found in Laurenson et al. (2012b).

4.3 Determinant criteria evaluation

Negative average weight gain benefit predictions occurring for anthelmintic treatments administered during the first 3 days of the simulated grazing season (Figure 4) arose as a consequence of reduced antigen exposure during an early stage in immune acquisition. As such, the delayed acquisition of immunity led to increased losses associated with parasitism in comparison to a non-treated (0%) lamb population. Positive average weight gain benefit predictions for anthelmintic treatments occurring after day 3, which increased with increasing initial pasture larval contamination (Figure 4, Table 1), arose as a consequence of the increasing impact upon the EBW of the non-treated lamb population (in agreement with Coop et al., 1982) against which the average weight gain benefit was calculated. The day of anthelmintic treatment at which the maximum average weight gain benefit occurred decreased with increasing levels of initial pasture larval contamination (Table 1) due to the relationship between larval exposure and the acquisition of immunity (*section 2.1.1*). As such, as initial pasture larval contamination increased, the rate at which immunity acquired also increased thereby reducing the day of anthelmintic treatment at which the maximum average weight gain benefit occurred.

For the group where 50% of the lambs where treated according to random selection, average weight gain benefit and increase in R allele frequency predictions were roughly half those predicted for the 100% treatment group with minor deviations arising from errors associated with the use of a pseudo-random number generator to identify which lambs were

to be drenched. In contrast, when using FEC as the determinant criterion, average weight gain benefit predictions were greater than those predicted when selecting animals at random for all levels of initial pasture larval contamination and day of anthelmintic treatment. These predictions are in agreement with expectations arising from the favourable positive phenotypic correlations predicted between FEC and WB (Figure 3c). Similarly, the predictions for average weight gain benefit when treating 50% of the grazing population according to LW or GR follow expectations derived from the phenotypic correlations with WB (Figure 3a,b). For anthelmintic treatments occurring during the first 30 to 60 days (dependent upon initial pasture larval contamination; see *section 3.3*), average weight gain benefit predictions were less than those predicted using random selection due to unfavourable positive correlations between WB and LW or GR. As such, treating individuals with the lowest LW or GR identified those lambs with the lowest WBs leading to reduced average weight gain benefits. Following this initial period, average weight gain benefit predictions when using LW or GR as the determinant criteria became better than when using random selection as a consequence of the time-dependent shift in phenotypic correlations with WB (from unfavourable positive to favourable negative correlations). For all determinant criteria (FEC, LW or GR) and level of initial pasture larval contamination, average weight gain benefit predictions became increasingly similar to the 100% treatment group as the day of anthelmintic treatment approached day 121 as a consequence of the increasing distribution skew in FEC and WB predictions (Figure 2) which resulted in anthelmintic treatments being directed towards an increasing proportion of the parasitic burden within the host population over time.

Notably, average weight gain benefit and increase in R allele frequency predictions were not directly proportional. In contrast to average weight gain benefit, increase in R allele frequency predictions did not increase with increasing initial pasture larval contamination.

Whilst increasing initial pasture larval contamination resulted in increasing average weight gain benefit predictions, it also resulted in an increased nematode supra-population *in refugia* on pasture and hence a lack of impact upon increase in R allele frequency predictions. This prediction is in agreement with the assertion that anthelmintic treatment selects for resistance in proportion to the nematode population *in refugia* (Martin et al., 1981; Michel, 1985). The day of anthelmintic treatment for which the maximum increase in R allele frequency prediction occurred decreased with increasing initial pasture larval contamination, corresponding with periods in which there was a low level of pasture larval contamination and hence a low level of *refugia* on pasture. This is most evident in the increase in R allele frequency peaks predicted for anthelmintic treatments occurring around day 21 (Figure 4) where L_3 on pasture reduced via L_3 mortality and ingestion prior to L_3 contributions arising from eggs deposited by the lamb population. Further, whilst average weight gain benefit predictions for each phenotypic determinant criterion (FEC, LW or GR) became increasingly similar to the full flock (100%) treatment group as the day of anthelmintic treatment increased, the increase in R allele frequency remained considerably reduced in comparison to the 100% treatment group. Whilst an increasing day of anthelmintic treatment resulted in drenches being directed towards an increasing proportion of the parasitic burden within the host population (via the WB distribution skew; Figure 2), density-dependent effects on nematode fecundity impacted upon the quantity of eggs deposited on pasture. As such, the number of eggs produced per female nematode increased for decreasing WBs (Bishop and Stear, 1997). Consequently, the egg output arising from low WBs in non-treated individuals provided sufficient *refugia* to maintain a reduced increase in R allele frequency in comparison to the 100% treatment group.

For the benefit per R predictions (Figure 5), increasing the initial pasture larval contamination resulted in an increased benefit per R for all treatment groups and day of

anthelmintic treatment because of the impact of initial pasture larval contamination on average weight gain benefit and the lack of impact of initial pasture larval contamination on the increase in R allele frequency. Benefit per R predictions when treating 100% of the grazing population or 50% by random selection were equal as a consequence of the inability of random selection to identify individuals with the highest WB and therefore exploit the over-dispersion of WBs within the host population. For all other treatment groups (50% treated according to FEC, LW or GR), disproportionate impacts upon average weight gain benefit and increase in R allele frequency predictions resulted in increased benefit per R predictions in comparison to the 100% and 50% randomly selected treatment groups. Of the determinant criteria evaluated in this study, LW was predicted to result in the greatest benefit per R for all levels of initial pasture larval contamination and day of anthelmintic treatment. As such, LW provides the best phenotypic trait to use as a determinant criterion for TST strategies, providing the greatest productive benefit across the efficacious life of an anthelmintic drug. Notably, the calculation of benefit per R in this study gave equal weighting to average weight gain benefit and increase in R allele frequency predictions; however, in practice sheep producers may be expected to give average weight gain benefit greater consideration. Hence, identification of the best determinant criterion may be expected to change from LW to FEC as the weighting of average weight gain benefit approaches 100% (e.g. Figure 4). Notably, anthelmintic treatments administered during the first 60 days post weaning were predicted to lead to the greatest benefit per R, and hence occur before the required 3 week withdrawal period (prior to slaughter) that is typical of many non-persistent anthelmintics.

4.4 Implementation of TST strategies

Within the sheep industry, the adoption of integrated parasite control programs is heavily dependent upon the productive benefit of implementation. The justification for TST

regimes is therefore constrained by potential reductions in productivity resulting from reducing the use of anthelmintics. As such, a number of experimental studies have focussed on the impact of TST on performance traits. In comparison to a suppressive full flock treatment regime, the implementation of TST strategies for *T. circumcincta* infections have previously been shown to have non-significant impacts upon weight gain (Leathwick et al., 2006a; Greer et al., 2009; Stafford et al., 2009; Besier et al., 2010; Busin et al., 2013; Valcárcel et al., 2015). Predicted average weight gain benefits within this simulation study were very similar to the full flock treatment group for a single anthelmintic drenching occasion occurring within the later stages of the grazing season. However, for treatments occurring during the early stages of the simulated grazing season, the predicted average weight gain benefits for the TST groups were reduced in comparison to the full flock treatment group (Figure 4). The maximum reduction for predicted average weight gain benefit in comparison to the full flock treatment group was -4.56kg for an initial pasture larval contamination of 5000 L_3/kg DM and anthelmintic treatments occurring on day 18 when GR was used as the determinant criterion. Differences between these predictions and observations from experimental studies may arise from differences in drenching frequency (the number of anthelmintic drenching occasions), and the determination of significance for experimental studies utilising small group sizes. Further to their potential impact (or lack thereof) on average weight gain, TST strategies require increased financial, logistical and labour costs associated with the collection of phenotypic data and determination of which animals to treat. Using FEC as the determinant criterion requires the collection of individual faecal samples, laboratory analysis, and then a secondary flock round up to administer the appropriate anthelmintic treatments; which comes at a considerable financial and diuturnal cost. When using either LW or GR as determinant criteria, on-farm automated weighing systems may be utilised, however, these also have a financial cost. Financial investment in an

automated weighing system may be considered a one-off expense (plus maintenance costs), and is therefore more desirable than the ongoing cost of FEC sampling and analysis. In labour and logistical terms, LW may be considered preferable as a determinant criterion as weighing would only be required on a single occasion in comparison to the calculation of GR which requires two weight measurements. However, the determination of appropriate treatment times would require the continual monitoring of LW, and as such GR could easily be monitored.

The implementation of TST strategies on farms will depend on the extra profit realized by producers (Kahn and Woodgate, 2012). In the short-term, a TST strategy may be considered undesirable to sheep producers as the potential reductions in weight gain alongside the cost of implementation would result in reduced economic returns in comparison to full flock treatments. However, the long-term productive benefits of maintaining anthelmintic efficacy (as illustrated by benefit per R predictions; Figure 5) support the use of TST strategies and consequently further analysis would be required to determine whether these off-set the costs of implementation. In this study, LW was predicted to provide the greatest benefit per R as a consequence of imperfect identification of individuals harbouring the greatest WB. As previously discussed, LW also provides the determinant criterion for which the cost of TST implementation is expected to be the least. Thus, TST strategies utilising LW as the determinant criterion may potentially provide long-term economic benefits, and hence support the implementation of TST strategies within populations of growing lambs.

4.4 Unaccounted factors affecting anthelmintic resistance

Predictions reported within this manuscript were for a single drenching occasion administered to a flock of growing lambs during a simulated grazing season. Drenching

frequency is also associated with the emergence of anthelmintic resistance (Jackson and Coop, 2000; Coles, 2005; van Wyk et al., 2006). A previous simulation study investigated the short- and long-term impact of drenching frequency upon weight gain and anthelmintic efficacy (Laurenson et al., 2013b). That study predicted that drenching early in the grazing season resulted in an early suppression of L_3 pasture contamination thereby reducing the need for subsequent drenches. As such, in the short-term, each consecutive drench was predicted to have a decreased impact upon average EBW and an increased impact upon R allele frequency. Consequently, in the long-term, increasing drenching frequency led to decreased productive benefits as a result of a reduced duration of anthelmintic efficacy. Further, whilst LW was predicted to be the best determinant criterion in this study where a single drenching occasion was simulated, anthelmintic treatment may be expected to break the correlation between LW and WB causing it to tend towards zero (Laurenson et al., 2012b). As such, for secondary or tertiary drenching occasions, LW may not provide the best determinant criterion for TST strategies.

Further factors affecting the emergence of anthelmintic resistance include: the proportion of the host population treated (Laurenson et al., 2013b); the genetic mechanism of resistance to differing anthelmintic classes (monogenic vs. multigenic and mode of inheritance; Barnes et al., 1995); potential reversion to susceptibility (Leathwick, 2013); and the influence of climate on L_3 pasture contamination (Rose et al., 2015) and hence *refugia*. The proportion of a lamb population treated within a TST strategy has previously been investigated using a mathematical model (Laurenson et al., 2013b). In the short-term (a single grazing season), increasing the proportion of lambs treated on any given occasion increased both the average EBW and the R allele frequency on pasture. In the long-term, the proportion treated resulted in no major differences in weight gains over the efficacious life of an anthelmintic. However, reducing the proportion of the lamb population drenched increased

the duration of anthelmintic efficacy and reduced the total number of drenches administered before resistance was reported (i.e. a reduction in the number of unnecessary drenches). For the genetics of anthelmintic resistance this study simulated a monogenic mechanism and a recessive mode of inheritance akin to benzimidazole (Elard and Humbert, 1999; Silvestre and Cabaret, 2002). For multigenic mechanisms of anthelmintic resistance, simulation studies have previously demonstrated that for an increasing number of genes there is an associated reduction in the rate at which anthelmintic resistance develops (Barnes et al., 1995). Further, the mode of inheritance (dominant, recessive or neutral) has also been shown to impact upon the selection for anthelmintic resistance. Dominant modes of inheritance increase the rate at which anthelmintic resistance develops, whilst recessive modes of inheritance decrease the rate of development of anthelmintic resistance (Barnes et al., 1995). Reversion to susceptibility was not simulated within this study as all nematode genotypes were assumed to be equally fit (Barrett et al., 1998; Elard et al., 1998). However, previous simulation studies have included a decreased fitness for resistant genotypes (Leathwick et al., 2013). That study illustrated that decreasing the fitness of resistant nematode genotypes resulted in a reduced rate at which anthelmintic resistance develops. Further, the influence of climatic variables on L_3 pasture contamination and hence *refugia* was not simulated in this study but will be a focus of future work. As such, whilst this study did not investigate differences in the proportion of the flock treated, the genetic mechanism of resistance, the mode of inheritance, nematode genotype fitness, or the influence of climatic variables on L_3 pasture contamination; these factors would be expected to impact upon the average weight gain benefit and increase in R allele frequency predictions. However, as this study simulated a single active administered on a single occasion under identical environmental conditions, these factors would equally impact on the predictions for all the treatment groups simulated. Hence, whilst the numeric value of predictions may be expected to change, the ranking of

determinant criteria would be expected to remain the same with LW still performing as the best determinant in terms of benefit per R.

In practice, sheep producers may not use anthelmintic drugs independently but rather sequentially, rotationally or in combination (Barnes et al., 1995; Leathwick, 2012). Further, treatment would not be expected to be confined to growing lambs but also used to control parasitism within ewes. Whilst cross-resistance is a potential concern for practices utilizing multiple anthelmintic actives (Bjørn et al., 1990), the general aim of these strategies is to maintain efficacy. TST may therefore be considered a complementary strategy as its implementation would reduce the rate at which anthelmintic resistance develops and thereby maintain effective control of parasitism. In this study, each determinant criterion was evaluated by its impact upon weight gain in growing lambs, however, for ewes the primary focus would be on reproductive traits and as such recommendations for which determinant criteria to use in a TST regime would be expected to differ.

5. Conclusion

Live weight was predicted to be the best trait to use as a determinant criterion (across all levels of initial pasture larval contamination and day of anthelmintic treatment) for TST strategies aimed at growing lambs in a temperate climate. This study supports the use of TST strategies as implementation was predicted to result in a decreased negative impact upon anthelmintic resistance in comparison to full flock treatments. As such, the long-term productive benefits of anthelmintic treatment were predicted to be greater when a TST regime was utilized due to the maintenance of drug efficacy. However, to convince sheep producers of the merits of TST strategies, the long-term productive benefits would need to exceed the financial, logistical and labour costs of implementation.

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Conflict of interest statement

The authors declare no conflicts of interest.

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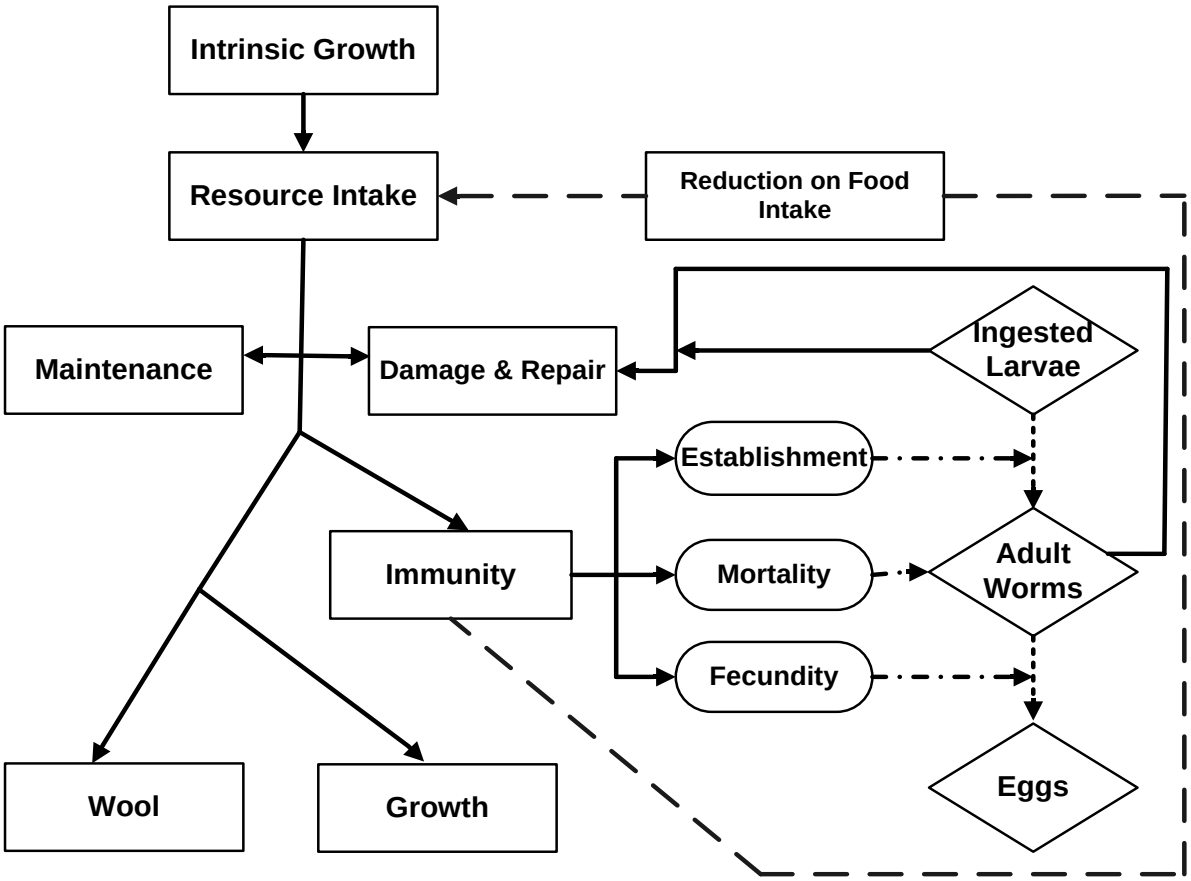

Figure 1. A schematic description of the host-parasite interactions in sheep infected with gastrointestinal nematodes. Rectangular boxes indicate the flow of nutrient resources, rounded boxes indicate host-parasite interactions and diamond boxes indicate key quantifiable parasite life-cycle stages.

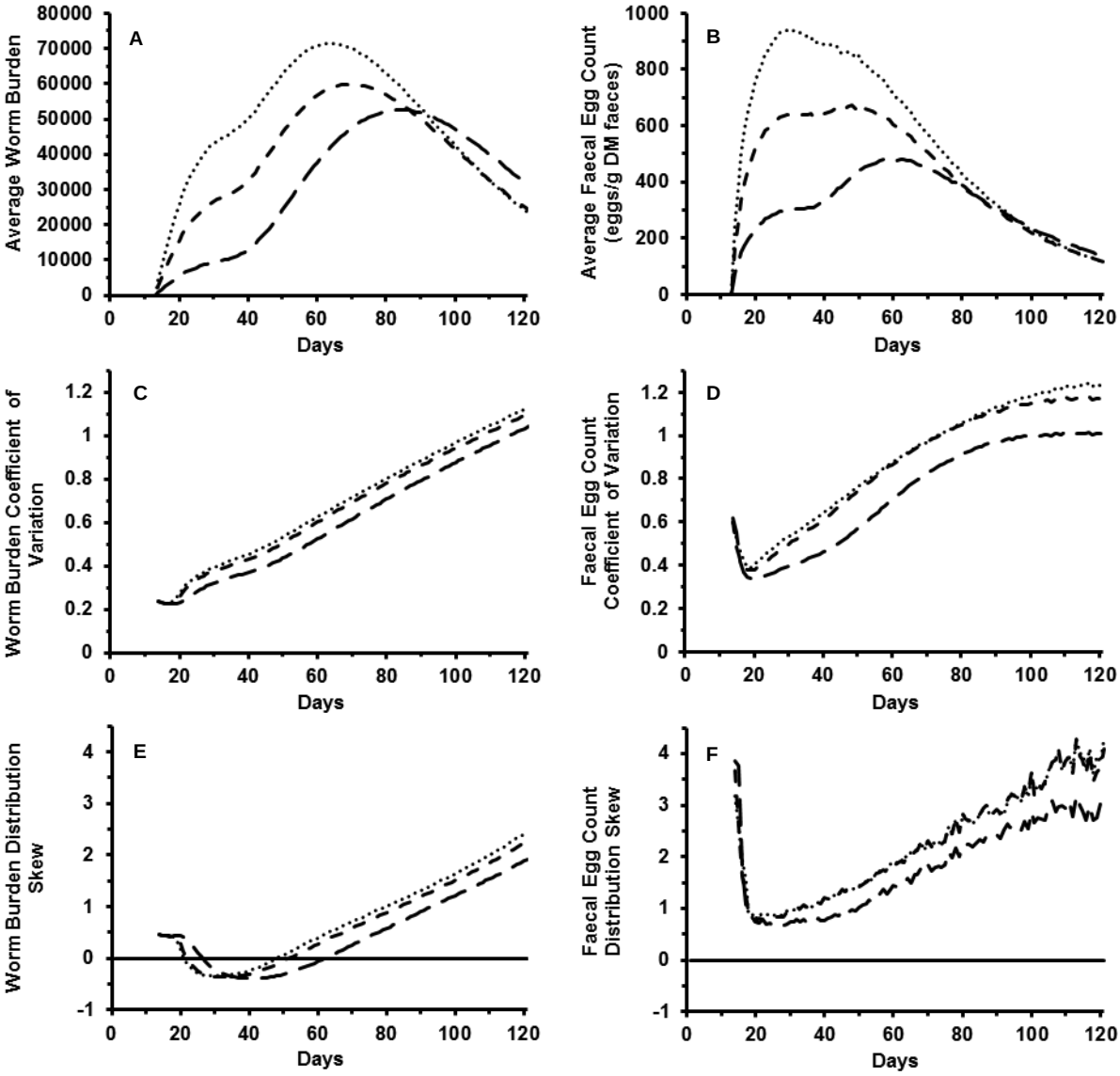

Figure 2. (A) Average worm burden, (B) average faecal egg count (eggs/g DM faeces), (C) worm burden coefficient of variation, (D) faecal egg count coefficient of variation, (E) worm burden distribution skew, (F) skew in faecal egg count distribution skew; for 10,000 lambs given no anthelmintic treatments and grazed on medium quality pasture initially contaminated with 0 (solid line), 1000 (long dash), 3000 (short dash) or 5000 (dotted) *Teladorsagia circumcincta* L_3/kg DM herbage.

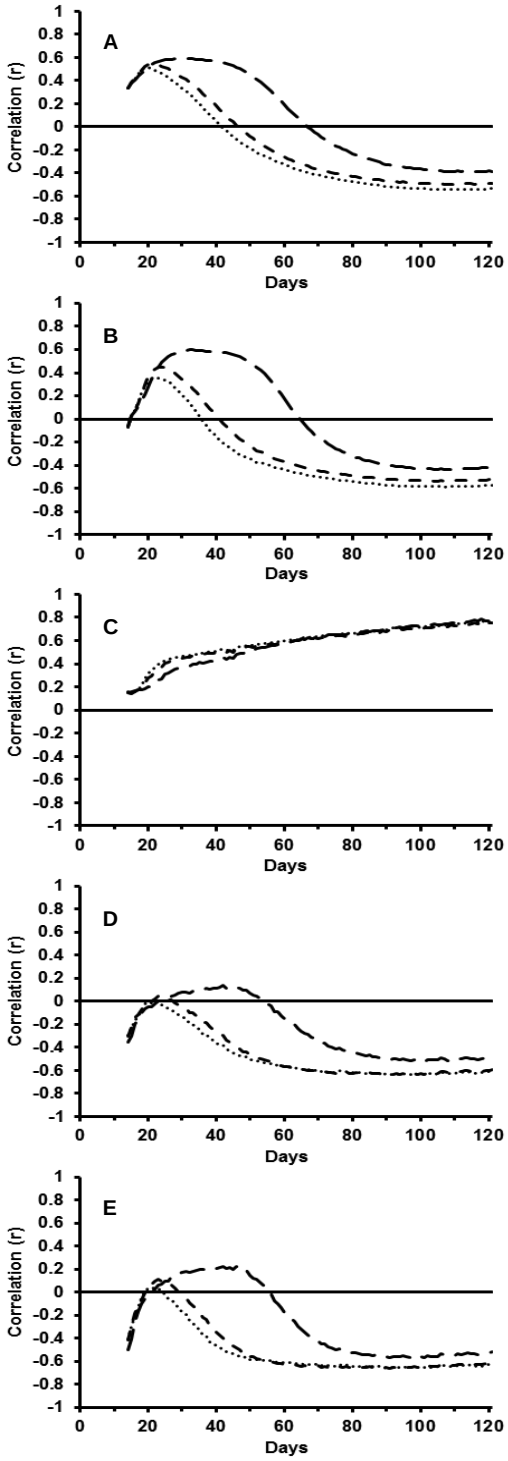

Figure 3. Phenotypic correlation between (A) worm burden and live weight (kg), (B) worm burden and growth rate (kg/day), (C) worm burden and faecal egg count (eggs/g DM faeces), (D) faecal egg count (eggs/g DM faeces) and live weight (kg), (E) faecal egg count (eggs/g DM faeces) and growth rate (kg/day); for 10,000 lambs given no anthelmintic treatments and grazed on medium quality pasture initially contaminated with 0 (solid line), 1000 (long dash), 3000 (short dash) or 5000 (dotted) *Teladorsagia circumcincta* L_3/kg DM herbage.

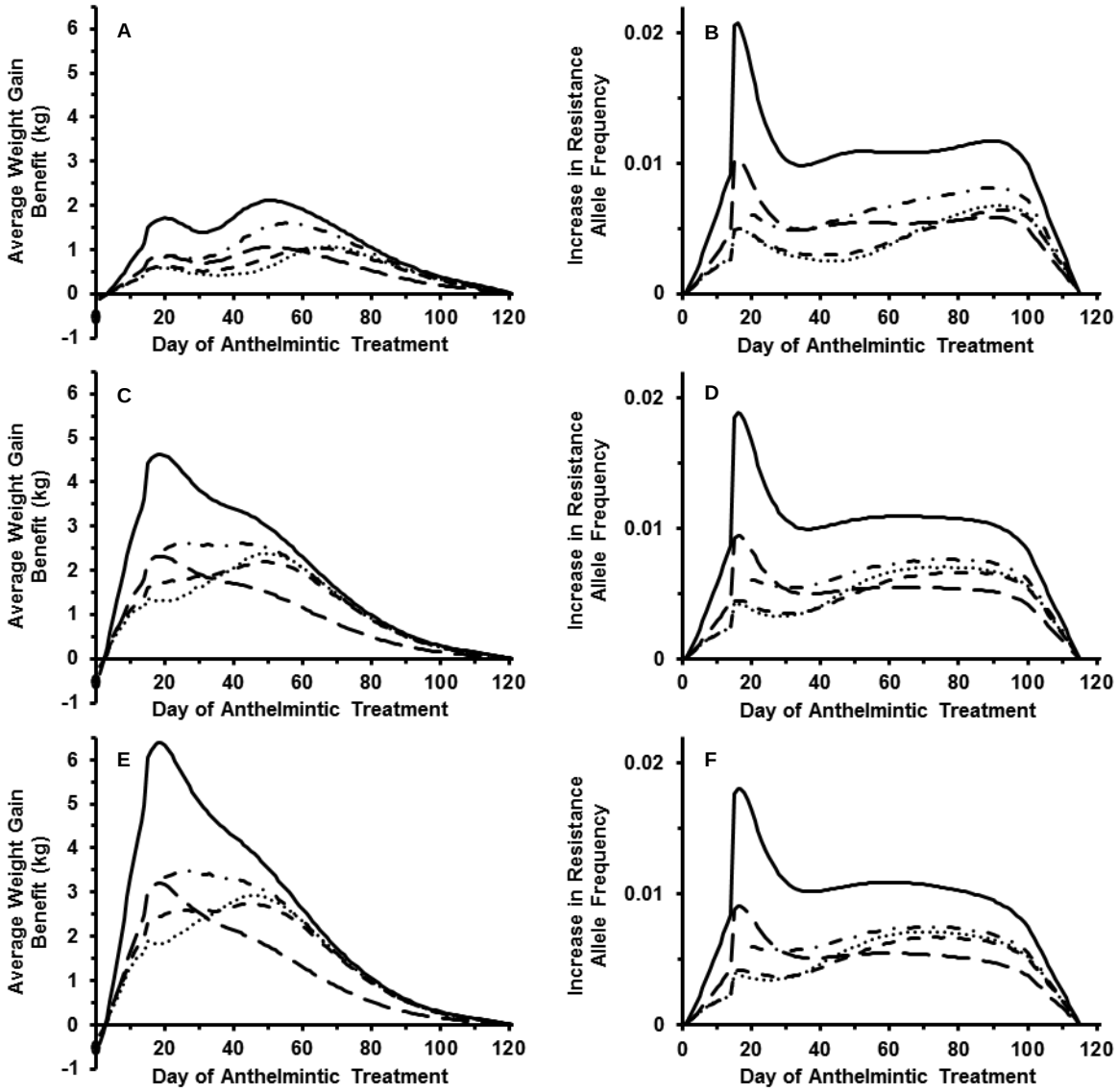

Figure 4. Average weight gain benefit (kg) for an initial pasture larval contamination of 1000 (A), 3000 (C) and 5000 (E) L_3/kg DM herbage and the increase in resistance allele frequency for an initial pasture larval contamination of 1000 (B), 3000 (D) and 5000 (E) L_3/kg DM herbage, for 10,000 lambs grazing on a medium quality pasture where anthelmintic treatment was administered to 100% of the lamb population (solid line), or to 50% of the lamb population according to faecal egg count (dash dot), random selection (long dash), live weight (short dash) or growth rate (dotted).

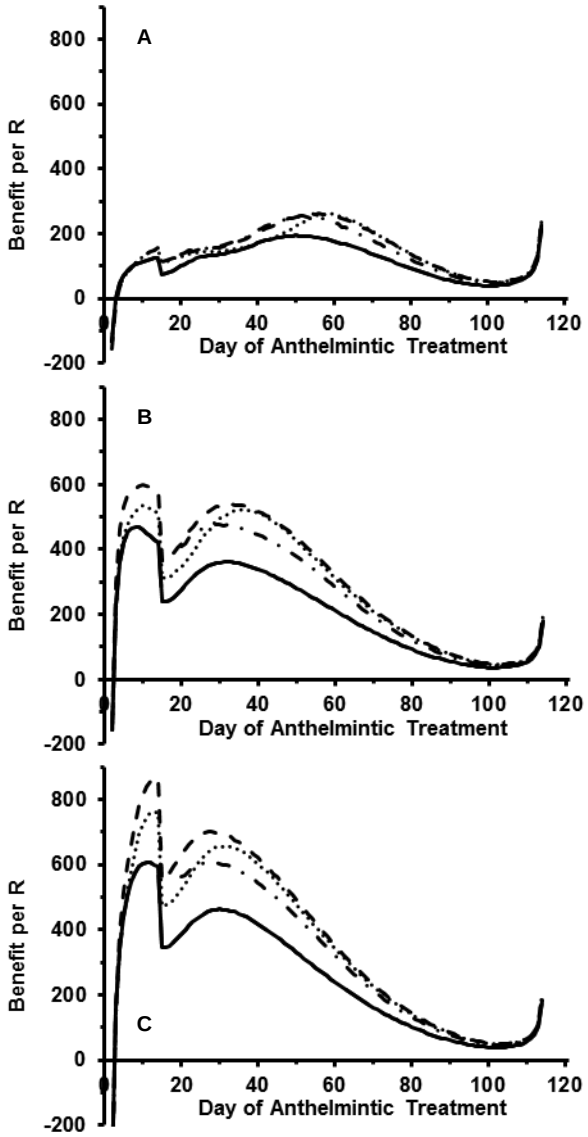

Figure 5. Benefit per R for 10,000 lambs grazing on a medium quality pasture initially contaminated with (A) 1000, (B) 3000, or (C) 5000 L_3/kg DM herbage; where anthelmintic treatment was administered to 100% of the lamb population (solid line), or to 50% of the lamb population according to faecal egg count (dash dot), random selection (long dash), live weight (short dash) or growth rate (dotted). Please note that benefit per R predictions for anthelmintic treatments administered to 100% of the lamb population (solid line) and 50% of the lamb population according to random selection (long dash) are overlapped.

Table 1. Maximum average weight gain benefit (AWGB, kg), maximum increase in R allele frequency on pasture (IRAF, proportion) and maximum benefit per R (BPR) for 10,000 lambs grazed on medium quality pasture initially contaminated (IL_0) with 1000, 3000 or 5000 *Teladorsagia circumcincta* L_3/kg DM herbage; where anthelmintic treatments were administered to the whole flock (100%), or 50% of the flock according to random selection ($50\%_{RS}$), faecal egg count ($50\%_{FEC}$), live weight ($50\%_{LW}$) or growth rate ($50\%_{GR}$). The day of anthelmintic treatment at which maximum predictions occurred are provided in brackets.

IL_0 (L_3/kg DM)		**100%**	**$50\%_{RS}$**	**$50\%_{FEC}$**	**$50\%_{LW}$**	**$50\%_{GR}$**
1000	**AWGB**	2.12 (51)	1.06 (51)	1.60 (55)	1.02 (63)	1.07 (68)
	IRAF	0.021 (16)	0.010 (16)	0.008 (89)	0.006 (92)	0.007 (91)
	BPR	194.2 (50)	194.2 (50)	256.8 (51)	263.1 (56)	261.7 (59)
3000	**AWGB**	4.63 (18)	2.32 (18)	2.63 (28)	2.20 (49)	2.38 (49)
	IRAF	0.019 (16)	0.009 (16)	0.008 (76)	0.007 (78)	0.007 (77)
	BPR	469.2 (8)	469.2 (8)	475.9 (28)	598.7 (10)	534.4 (10)
5000	**AWGB**	6.38 (18)	3.19 (18)	3.50 (28)	2.76 (45)	2.93 (45)
	IRAF	0.018 (17)	0.009 (17)	0.008 (70)	0.007 (74)	0.007 (68)
	BPR	607.9 (11)	607.9 (11)	608.3 (29)	866.4 (14)	760.3 (14)