Resistance selection mechanisms

Anthelmintic resistance may be inevitable, but its development can be delayed. The genes, or alleles, which allow parasites to be resistant to anthelmintics are believed to be in existence in unselected worm populations. Consequently, for all anthelmintics developed to date, it appears that the development of AR is an inevitable consequence of their use. Despite this, there are methods to delay resistance. There are several factors known to influence the rate at which AR appears in a worm population and these are summarised in the table below. Improvements in our understanding of these factors has led to the development of the SCOPS principles for anthelmintic use.

Table 2. Selection factors influencing rate of anthelmintic resistance development

Factor	Rationale
Treating at a time of low refugia (see below)	Treating when the proportion of the worm population in refugia is low can rapidly select for resistance
Over-use (see below)	Using anthelmintics more often than is necessary increases the pressure for resistance to develop and propagate
Under-dosing (see below)	Failing to ensure worms are exposed to the recommended dose of the anthelmintic can lead an increase in frequency of resistance genes in a population
Biotic potential	Worms' ability to quickly repopulate a pasture due to their innate life history traits e.g. ability to produce huge numbers of eggs/larvae, as in the case for *Haemonchus*, or a short time to patency (i.e. time to develop to mature adults from ingestion of infective larvae)
Resistant gene frequency at onset of treatment	The lower the resistant gene frequency in a worm population the longer it takes to select for resistance. See chapter 1.2 Genetics of AR, section 1.2.1 What is anthelmintic resistance?
Importation of resistant worms.	*Buyer Beware* – Returning or newly purchase animals may have worms and resistance profiles not present on the receiving farm. See chapter 2. SCOPS Principles, section 2.3 Quarantine

Refugia (also see chapter 2. SCOPS Principles, section 2.3 Preserving susceptible worms)

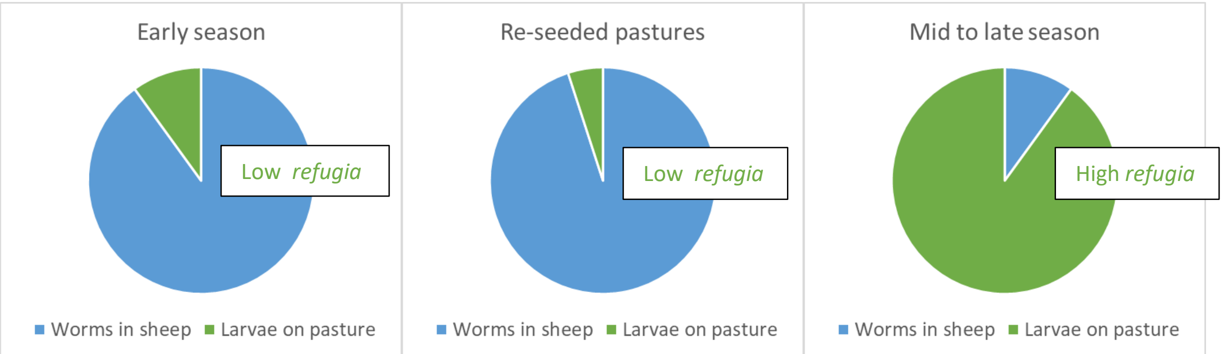
Maintaining a proportion of the worms *in refugia*, (i.e. not exposed to anthelmintic treatment – 'in refuge') is vital for the maintenance of susceptible genes in the worm population. This is because anthelmintic resistant worms that survive treatment will produce resistant offspring, which without competition will use their reproductive advantage to contaminate pastures. It is important to try and minimised this advantage period.

Harnessing the benefits of refugia management means ensuring that roundworms *in refugia* re-infect animals almost immediately after treatment. This includes ingesting larvae on pasture (assuming a long acting anthelmintic hasn't been used) and/or utilising unexposed worms in untreated animals, which will produce unselected eggs in their dung. The net effect is a rapid dilution of the resistant worms which will reduce their impact on the overall AR status of the worm population on that farm.

What is refugia?

The *in refugia* population is the proportion of the roundworm population that is unexposed to anthelmintic treatment. These may be larvae on pasture and/or worms in untreated animals. These worms and larvae are then able to contribute to maintaining genes for anthelmintic susceptibility in a population. The number of worms *in refugia* at any one time is affected by a range of climatic, environmental and managemental factors. Examples of possible proportions of worms *in refugia* on pasture, versus those in sheep are shown in the figure below:

Figure 6. Graphical representation, of the possible composition of parasite populations in the host (sheep) and free-living on pasture.



Actual numbers will vary depending on a huge number of factors including climate, pasture and grazing management.

When are levels of refugia at their lowest?

- Early in the grazing season when numbers of over wintered larvae on pasture are low.
- Re-seeded pastures that have not been grazed by sheep.
- Hay/silage aftermaths
- During periods of drought or low rainfall larvae will remain in dung pats. This reduces the numbers of infective larvae on pasture so although pastures may in theory have a high worm challenge, it is temporarily reduced until enough moisture is present to allow the larvae to escape from the faeces and migrate up the grass.
- Pastures previously grazed by another species, not sheep

Refugia can be used in a number of ways:

Harnessing the benefits of refugia is a key part of the SCOPS principles. At times when the *in refugia* population on pasture is low, management must seek to allow for a proportion of the worms in the sheep to be unexposed. For example:

- Target treatments to individuals or groups of animals to provide a source of unexposed worms when anthelmintics are used. This is very important when considering the treatment of ewes around lambing with a persistent anthelmintic (chapter 2. SCOPS Principles, section 2.7 Use Anthelmintics only when necessary)

- When moving and treating sheep to low challenge grazing or hay/silage aftermaths, they can either be treated and left on contaminated pasture for a few days before moving OR moved and treated a few days later. Either strategy ensures that worms unexposed to the anthelmintic can contaminate the low challenge pasture and subsequently re-infect the sheep.
- Following quarantine, animals should be moved to contaminated pasture to ensure that any worms surviving treatment are diluted with a significant susceptible population.

Frequency of treatment

The more frequently anthelmintics are administered, the faster AR will develop.

The underlying principle of selection for AR is that ***treatment gives resistant worms a reproductive advantage over the susceptible* worms** which lasts for 2-3 weeks after dosing. If a persistent product is used, then this reproductive advantage may be longer. This occurs because until larvae ingested after dosing mature into egg laying females, the only eggs being passed out in the faeces of dosed sheep are from resistant worms that survived treatment. If the interval between dosing shortens towards the pre-patent period of the worm, then susceptible worms have less and less opportunity to produce eggs and most, or all, pasture contamination occurs with eggs from resistant parasites. Over time this strategy means the susceptible worm population is progressively replaced with a resistant one.

However, the impact of the frequency of dosing is also influenced by other factors, particularly the proportion of the worm population *in refugia* at the time of treatment. For example, if the *in refugia* population is small, replacement of susceptible with resistant worms occurs quickly and AR may develop after relatively few treatments. Conversely, a large *'in refugia'* population will reduce the selection pressure of a similar number of treatments because the numbers of resistant worms are diluted by the numbers in the wider population.

Modelling studies suggest that a strategy of just two treatments with a class of anthelmintic, combined each time with a move to low-contamination pasture, selects for resistance as rapidly as five treatments, without using low-contamination pasture. The lessons from these studies are that not all high-frequency dosing strategies are equally bad, and that seeking to reduce dose frequency alone, is unlikely to be enough to slow the development of AR.

Under-dosing

Under-dosing is recognised as a highly significant factor in the development of resistance. In addition to failure to administer the anthelmintic correctly (chapter 2. SCOPS principles, section 2.1 Administer effectively), there are a number of other factors which can lead to under exposure of worms to the anthelmintic. These include:

- Out of date product
- Incorrect storage of the anthelmintic (e.g. too high or too low temperatures)
- Faulty equipment
- Poorly/non-calibrated equipment
- Dose rate incorrect for the target parasite