8 Techniques and strategies

8.1 Detecting anthelmintic resistance

Testing for anthelmintic resistance is a vital part of the SCOPS recommendations. Early detection is essential because it encourages the adoption of practices which will sustain the effectiveness of anthelmintics. However, it is also important to balance the positive advantages of early detection of AR with the danger of over-stating the situation which may deter farmers from taking appropriate action.

Although not reversible, the presence of AR is dynamic. Detection of AR on a farm will vary according to season and the worm species/ strains present at the time a test was applied and the test's specificity and sensitivity in detecting resistant alleles within the worm populations. It is important, therefore that we do not assume we have full knowledge of the situation on an individual farm on the basis of one test for AR.

The presence of anthelmintic resistance can be detected in flocks in a number of ways. These vary in terms of their cost, complexity and robustness and are outlined below in terms of this hierarchy, starting with the cheapest and most simple, the Drench Test:

8.1.1 Post-dosing faecal egg counts ("Drench Tests")

A quick indication of the efficacy of an anthelmintic can be gauged by laboratory testing faecal samples from 10 sheep following after treatment. The time after treatment depends on the anthelmintic used: **7 days after 2-LV, 10-14 after 1-BZ and 14-16 days after a 3-ML**. In practice, this means checking either 7 days for 2-LV, or 14 post treatment for 1-BZ and 3-ML products. The test is merely an indicator of anthelmintic inefficacy and not necessarily anthelmintic resistance per se, as many other factors can influence test results. The utility of this test is improved if faecal samples from 10 sheep in the dosed group are collected and submitted on the day of dosing, to provide a rough estimate of the reduction in FEC achieved (and to confirm there was a measurable epg before treatment). The advice about faecal collection in Section 8.2 should be followed.

8.1.2 Faecal Egg Count Reduction Tests (FECRT)

A more structured on-farm test can be conducted in which a number of different anthelmintics are tested against a control. Fifteen to twenty ** sheep are randomly allocated to control or treatment groups, which might include a 1-BZ, 2-LV, and 3-ML. (In Australia, ivermectin at half-dose is used in order to detect 3-ML resistance as early as possible, but this represents an off-label use of the product in the UK.). FECs are performed prior to treatment on at least 10 of the control sheep and the 2-LV sheep after 7 days, then from all sheep in the control and 1-BZ and 3-ML groups at 14 days. AR is suspected if the percentage reduction in FEC of a test group compared with treatment controls is < 95%. Results may differ according to whether arithmetic or geometric means are used in the calculations. Where necessary, the advice of an expert should be sought with interpretation of the results.

In a modification of the FECRT, pre-dose FECs are not performed, and results are based on the percentage reduction in mean FEC in the treatment groups compared to the controls.

***NB. Sheep that have not been dosed within 30 days (or longer if MOX has been previously used), and a mean FEC of 200 epg or more is recommended before starting the trial.*

8.1.3 Larval Development Tests (LDTs)

A range of *in-vitro* tests has been developed to avoid the use of animals in testing for resistance. The two most commonly used are the egg hatch assay (EHA) for the 1-BZ anthelmintics, and the larval development test (LDT) for 1-BZ and 2-LV classes. There are no *in-vitro* tests yet available for ML resistance. Farm visits are not necessarily required and the samples can be sent by post direct to the laboratory. However, currently these tests are relatively expensive, precluding their widespread use, Sensitivity is generally considered higher than with the FECRT so AR may be detected when the frequency of resistant alleles within the worm populations is still low. Interpretation is, however, not straightforward and requires expert input.

8.2 Faecal Egg Count (FEC) monitoring

To monitor FECs, you can use a suitably equipped and trained veterinary practice, a commercial service or adopt a DIY approach using the FECPAK system. (see Appendix)

FECs can be used to:

- Help determine the need to treat
- Test the efficacy of a treatment
- Give information on the amount of contamination going onto the pasture

8.2.1 Guidelines for collection of faeces

These guidelines are for the estimation of the mean FEC of a group of sheep. A 'group' in this context refers to a flock of sheep of the same age and reproductive status grazing together in the same field and with the same anthelmintic-treatment history. The easiest way to sample a group is to loosely gather them in the corner of the field for 5-10 minutes, then let them walk away. Fresh dung samples can then be collected from the pasture.

❖ At least 10 sheep in the group should be sampled. The wide variation in FEC between sheep grazing together in the same field means that random sampling effects have a significant impact on the confidence limits surrounding the estimate of the group mean FEC. Even if 10 sheep are sampled, the confidence limits are wide. This number is generally considered to be an acceptable compromise between repeatability and cost (Fig. 8.2).

❖ The sheep should be healthy and have had full access to pasture and/or feed before sampling because counts are reported as eggs per gram (epg) of faeces and variation in faecal output will affect the count. If sheep have been held off feed for more than a few hours before sampling, or if any sheep included in the sample are inappetant due to illness, the FEC will be difficult or impossible to interpret. A high count may be incorrectly assumed to reflect a high worm burden. **For this reason, FECs should not be used as a diagnostic aid when PGE is suspected in cases where sheep are profoundly ill.** A worm count as part of a post-mortem examination is a much more appropriate way to estimate worm burden in such cases.

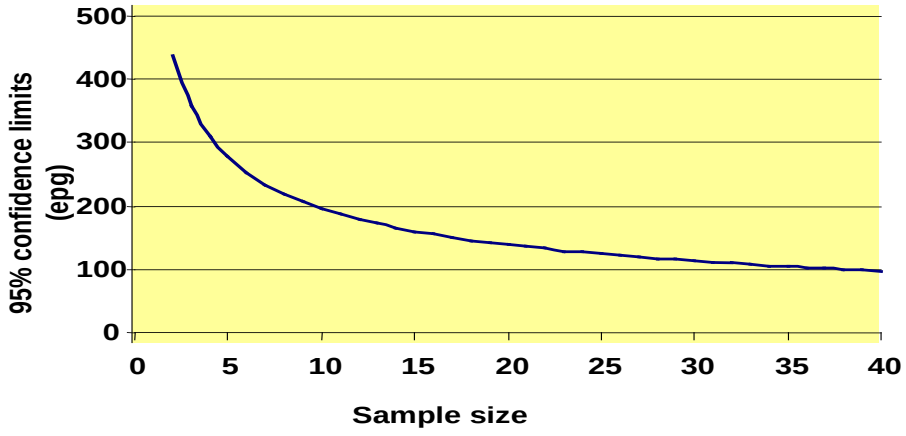

Fig. 8.2 ***Confidence limits around an estimate of mean FEC become narrower as the sample size increases***

❖ Samples should be fresh when collected (less than one hour old) and kept cool (not frozen) in an airtight container or plastic bag, before delivery to the laboratory within 48 hours. If the faeces are too old some eggs will have hatched and the reported egg count will be an underestimate.

❖ Some laboratories pool the 10 samples and report the average of the 10 animals as a single count. This is acceptable and can substantially reduce the cost, but the faecal samples should still