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Chapter 4. Testing for anthelmintic resistance

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Overview

As there are many reports of anthelmintic resistance in equine parasites, it is important that the effectiveness of anthelmintics on parasite populations be measured regularly to assess whether the administered products have the expected efficacy. This provides valuable information, because timely intervention in a situation with signs of resistance allows for management strategies to be put in place (for example, reduced administration frequency, use of a different anthelmintic) which can better manage the parasite population in the long term. It is important to report all cases where there is a suspected lack of expected efficacy (SLEE) as consistent reporting of SLEEs can enable intervention at an individual, local and country level (see Chapter 5. Reporting lack of efficacy).

Estimation of efficacy can be achieved by examining levels of helminth egg shedding in faeces at the time of, and 14 days after, anthelmintic administration, known as a Faecal Egg Count Reduction Test (FECRT). In recent years, guidelines for anthelmintic resistance testing using FECRT have been thoroughly revised by a committee of experts appointed by the World Association for the Advancement of Veterinary Parasitology (WAAVP) and prescribers can read these for detailed guidance (Kaplan *et al*., 2023). A general description of this new approach for anthelmintic resistance screening is outlined below.

In addition, determination of strongylid egg reappearance periods (ERPs) following an effective treatment is another performance metric for evaluation of anthelmintic performance, especially for the macrocyclic lactone class. This relates to the reappearance of strongyle eggs post treatment and is measured by calculating faecal egg count reduction at regular intervals in the weeks following anthelmintic treatment and determining when the measure falls below a pre-defined threshold. This approach is outlined below.

There are three new principles introduced within the revised World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines:

Thresholds for expected treatment efficacy are now based on historic efficacy data recorded for the anthelmintics when the compounds were first introduced to the equine market. This is to establish the expected efficacy for each anthelmintic, which is necessary for assessing if there has been a reduction in efficacy indicating that resistance might have developed. This new approach to efficacy thresholds in the revised guidelines reflects the fact that different anthelmintic actives had different efficacy levels against different types of parasites when first authorised, i.e. before resistance developed. These thresholds differ slightly between the different anthelmintic compounds and parasites.

Recommendations are based on an 'eggs counted' principle, which refers to the number of eggs counted under the microscope when performing faecal egg counts (FEC). All FEC procedures involve a dilution step, therefore, to obtain the value for eggs per gram (EPG) of faeces, the numbers of eggs counted under the microscope are converted to a total EPG value using a multiplication factor. The more eggs that are counted under the microscope when performing a FEC, the more reliable the resistance test will be. For example, the commonly performed McMaster FEC technique has a multiplication factor of 50 and thus may not count sufficient numbers of eggs for the resistance test. However, a technique with a lower multiplication factor will count more eggs (at the same FEC level) and can be used instead, keeping in mind that the same technique should always be used pre- and post-treatment.

The conclusions are no longer based on calculating average percent egg count reductions, but instead on **statistical confidence limits**, which consider the variation observed in the levels of egg count reduction seen between horses in a group.

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Chapter 4.1. The faecal egg count reduction test

The FECRT is a measure of the effectiveness (efficacy) of a given anthelmintic against a parasite population that is shared by herd members grazing together. The test requires two FECs determined from the same horses sharing the same pasture; one on the day of anthelmintic administration and a follow-up sample collected at 14 days post-treatment.

The WAAVP guidelines have two efficacy thresholds interpreting the results from the anthelmintic treatment:

- **Upper threshold**: represents the expected level of efficacy of the product based on historic expected treatment efficacy information.
- **Lower threshold**: below which the efficacy can be interpreted as significantly reduced and *indicative of resistance*.

The interval between the two thresholds is considered the **grey zone**, where more information is needed before a conclusion of resistance or susceptibility can be drawn.

The WAAVP efficacy threshold principles for interpreting results from anthelmintic treatment are described in Figure 1.

Egg counting technique

For good reliability of the FECRT, it is recommended to count an average of 40 eggs/horse in the group. An egg counting technique with a low multiplication factor will yield more eggs counted under the microscope than one with a higher multiplication factor and will therefore ensure a higher number of eggs counted. This can be especially useful in scenarios where FECs are generally low.

Uncertainty in the group mean faecal egg count reduction (FECR) should be calculated with 90% Confidence Intervals, which quantify the variation of the data and is a measure of the precision of the efficacy estimate. An online interface for analysis of FECRT data is available from the University of Copenhagen here. With this calculation

each FECR will have an upper and a lower confidence interval limit. The interpretation of the FECRT result is based on these confidence limits.

The interpretation of the FECRT is described in Figure 1 and is as follows:

1. **Evidence of good efficacy**: The lower confidence interval limit is above the lower threshold.
2. **Evidence of resistance:** The upper confidence interval limit is below the upper threshold.
3. **Inconclusive:** Both upper and lower confidence interval limits fall outside the thresholds.

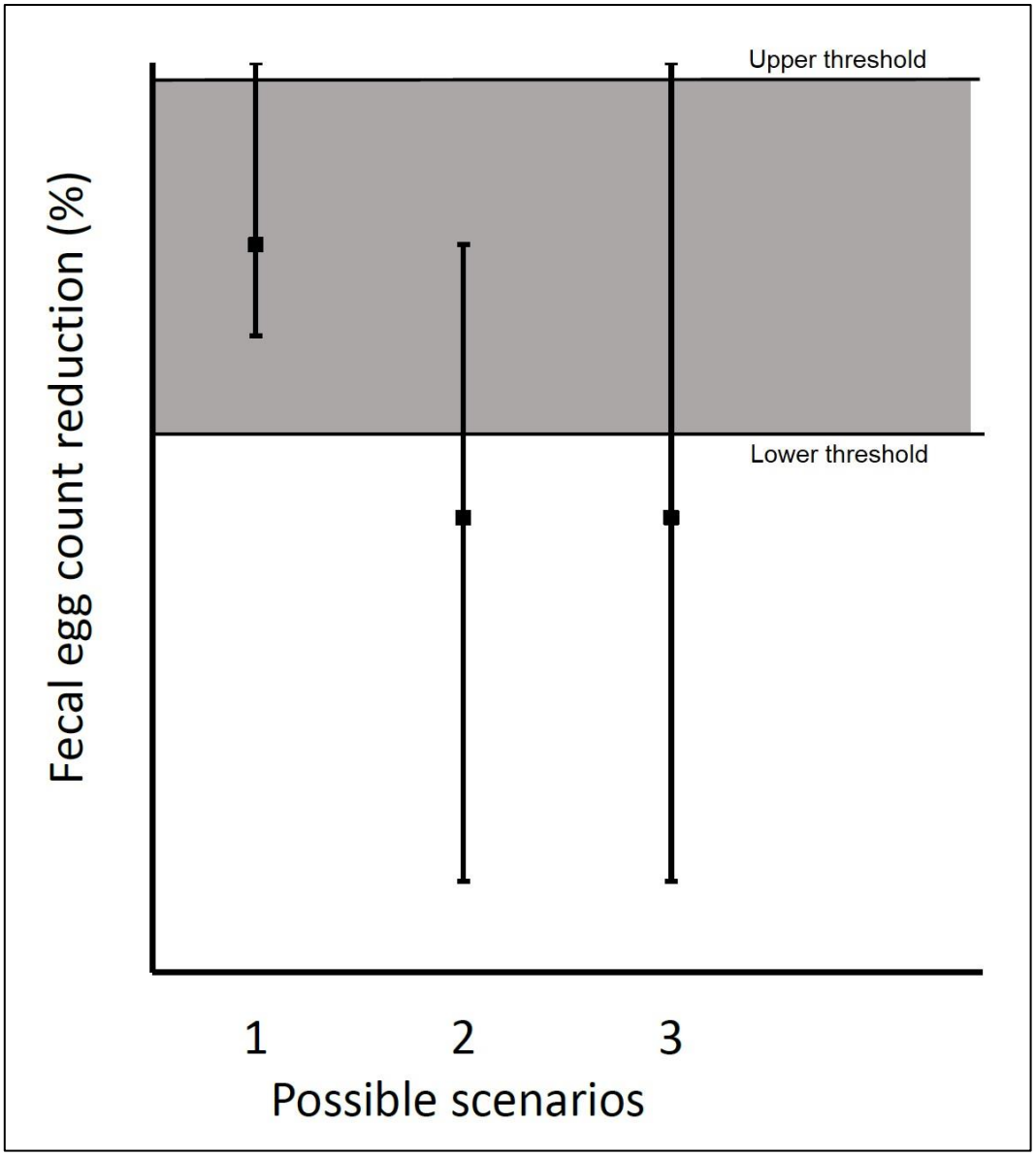

Figure 1. Thresholds and interpretation of the FECRT. The grey area represents the interval between the upper and lower thresholds. Scenario 1: Evidence of good efficacy - the lower confidence interval limit is above the lower threshold. Scenario 2: Evidence of resistance - the upper confidence limit is below the upper threshold. Scenario 3: Inconclusive result as both confidence limits fall outside the thresholds.

Guideline thresholds and group sizes

The full guidelines for FECRT testing can be found in the WAAVP guideline paper and include protocols for research studies as well as clinical on-site testing. This includes details on recommended group sizes depending on the number of eggs counted for each anthelmintic tested. A summary of these guidelines is presented Table 1 and Table 2 for scenarios, where the minimum number of eggs counted is ≥40 eggs/horse for small strongyles and ascarids, respectively. If fewer eggs are counted in the pre-

treatment samples, the recommended horse group sizes will be larger, and more details can be found in the WAAVP guidelines (Kaplan *et al.*, 2023).

Table 1. Efficacy thresholds and recommended group sizes for FECRT testing of equine small strongyles

	Ivermectin/Moxidectin	Fenbendazole	Pyrantel embonate
Upper threshold	99.9%	99%	98%
Lower threshold	92%	90%	80%
Group size[1]	5	7	7

[1] Based on counting a minimum of 40 eggs/horse pre-treatment

Table 2. Efficacy thresholds and recommended group sizes for FECRT testing of equine ascarids

	Ivermectin/Moxidectin	Fenbendazole	Pyrantel embonate
Upper threshold	99.9%	99.9%	99.9%
Lower threshold	90%	90%	90%
Group size[1]	5	5	5

[1] Based on counting a minimum of 40 eggs/horse pre-treatment

Post-treatment check with fewer than five horses

While a FECRT should follow the guidelines summarised above, it can still be very meaningful to evaluate anthelmintic treatments in scenarios with fewer than the recommended number of horses or with fewer eggs counted pre-treatment.

FECRT with fewer than five horses:

- Results should be interpreted with more caution due to a higher degree of uncertainty.
- Results are more likely to fall in the inconclusive category when group sizes are small.

- Horses should always be sharing the same pastures to be included in a FECRT.

Post-treatment check on a single horse

The FECRT is a group test and requires testing of several horses grazing the same pasture treated with the same anthelmintic. However, it can still be useful to perform a post-treatment check on a single horse in situations where this is the only animal available for testing, such as a newly arrived horse in quarantine.

FECRT on a single horse

$$FECRT(\%) = \frac{FEC\ in\ EPG\ (pre\text{-}treatment) - FEC\ in\ EPG\ (14\ days\ post\text{-}treatment)}{FEC\ in\ EPG\ (pre\text{-}treatment)} \times 100$$

- Any anthelmintic should reduce both ascarid and strongylid FECs by more than 95% at 14 days post-treatment.
- The number of eggs counted pre-treatment will largely affect the reliability of the result.
- More than 40 counted eggs can provide a reasonably meaningful test.
- 20-40 counted eggs can be expected to be moderately useful.
- Less than 20 counted eggs will provide less reliable results.

Interpretation

Testing for FECR provides useful information and test results should be reported (see Chapter 5. Reporting lack of efficacy). It is likely that an individual programme would be required if reduced FECR is detected, and it is therefore challenging to provide specific guidance for these situations. Advice should be sought from the appropriate prescriber when test results show reduced FECR, with the main emphasis on reviewing horse management practices (see Chapter 6. Reducing dependence on anthelmintics) and continued surveillance (see Chapter 1.2. Using monitoring tools effectively to determine the need for anthelmintic treatment). If practically possible, it is generally advised to repeat the FECRT to confirm the findings and rule out failure of administration and mis-dosage. If a reduced FECR is confirmed, changing to a different anthelmintic class could be considered.

Chapter 4.2. Egg reappearance periods

When asking 'Has my anthelmintic worked?' drug resistance is not the only consideration. The term Egg Reappearance Period (ERP) describes the amount of time it takes, from the date of administering an anthelmintic to a group of horses with positive strongylid faecal egg counts (FECs), until parasite eggs can be detected in the faeces again. In other words, this is a measure of how long the treatment can suppress parasite egg shedding for, and this is a meaningful measure of effectiveness of the anthelmintic. Estimating ERPs and reporting when test results show that ERPs are shorter than at original authorisation is also encouraged for continued monitoring (see Chapter 5. Reporting lack of efficacy).

While ERP can be measured for all anthelmintics, it is most relevant to the two macrocyclic lactones, ivermectin and moxidectin. This is because resistance is widespread to the two other anthelmintic classes, which means that they often do not effectively reduce strongylid FECs in the first 14 days after treatment (when an FECRT is generally performed). As a general rule, parasite eggs first have to disappear before they can reappear.

Over the past three decades, ERPs have been observed to be substantially shorter following both ivermectin and moxidectin treatment, when compared to data from when these compounds were first introduced (Table 3). Approximately 30 years ago, moxidectin treatments led to ERPs in the 12-16 week range (Nielsen, 2022a), which was substantially longer than any other anthelmintic, including ivermectin (ERPs originally in the 8-10 week range, Nielsen, 2022a). Since 2017, several studies have reported ERPs to be just 4-5 weeks for both these drugs (Nielsen, 2022a). Recently, the ERP of moxidectin was reported to be 4 weeks in a group of Thoroughbred yearlings in the UK (Bull *et al.*, 2023). The majority of these shortened strongylid ERPs have been reported from yearlings or young horses aged 2-5 years old, which suggests that age could be influencing these estimates as well. There is one recent publication in mature horses in the UK reporting acceptable efficacy of moxidectin at 14 days post-treatment; however, the ERP pattern suggests this anthelmintic has a considerably shorter suppressive effect than at original authorisation (Mair *et al.*, 2024).

Table 3. Strongylid egg reappearance periods reported for ivermectin and moxidectin in the 1990s compared to several studies conducted since 2017 (Nielsen, 2022a).

Active substance	1990s	Now
Ivermectin	8-10 weeks	4-5 weeks
Moxidectin	12-16 weeks	4-5 weeks

ERPs in this table does not indicate that the treatment frequency should increase.

Interpretation

The aim of anthelmintic treatments is to reduce the output of parasite eggs in the faeces, which, in turn, will reduce pasture contamination. One of the most important aspects of a shortened ERP is that egg suppression is not maintained, and this can lead to rapid increases in pasture contamination, therefore compromising parasite control. This has been demonstrated in a computer simulation study, which suggested that parasite burdens may increase by several thousand percent when ERPs are reduced to 4-5 weeks, depending on climatic conditions and treatment protocols (Nielsen *et al*, 2023). However, a shortened ERP does not fulfil the criteria for drug resistance, because the treatment initially reduces FECs at the 14-day post-treatment timepoint. It is unclear whether a shortened ERP is an indication of emerging drug resistance or not. Recent preliminary data suggests that selection of parasites with shorter duration of life cycles could have occurred (Nielsen *et al*., 2022a).

Shortened strongylid ERPs following macrocyclic lactone administration are very common, so it should not be surprising to encounter this. Current ERPs for a given equine operation have implications for interpreting FEC monitoring data as well as setting expectations for the impact of anthelmintic treatment on pasture strongylid infectivity. It should be emphasised that shortened ERPs do not justify increasing anthelmintic treatment frequency, but that changing other management procedures, such as pasture rotation (e.g. follow-on grazing with non-competent hosts such as sheep or cattle), resting for sufficient periods, and pasture hygiene should be considered. Advice should be sought from the appropriate prescriber when test results

show a shortened ERP and involve a review of current management practices (see Chapter 6. Reducing dependence on anthelmintics) and continued surveillance (see Chapter 1.2. Using monitoring tools effectively to determine the need for anthelmintic treatment).

Determining egg reappearance periods

The WAAVP has recently published guidelines for determining ERP (Nielsen *et al.*, 2022b). The determination is based on FEC reductions (FECRs), so many of the principles outlined for the FECR test (FECRT) detailed above apply to ERP determination as well. In general, ERP should be determined for a group of strongylid egg count positive horses sharing the same pasture(s).

Best practice

- FECs should be determined for the day of treatment and 2-weeks post-treatment (when FECRT is calculated) and at weekly intervals thereafter.
- For each post-treatment sampling time point, the group mean FECR and 90% confidence intervals should be calculated (see earlier section on egg counting technique).
- The ERP is then defined as the post-treatment week where the upper confidence limit falls below a threshold defined as [FECR at 2 weeks post-treatment minus 10]. For example, if the 2-week FECR is 98.7%, the ERP threshold becomes 88.7%.

Pragmatic approach

In many cases it is not possible to perform FEC weekly from 14-days post treatment, but it is still very important to determine if many eggs are being shed for several weeks after treatment. In this case it is advised to 'spot-check' ERP by determining FECRs at a few strategically chosen time intervals, especially at 4-6 weeks post treatment. This will provide useful information about the performance of ivermectin and moxidectin and subsequent horse and pasture management.