DETERMINING THE LEVEL OF EFFICACY OF ANTHELMINTICS

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Introduction

Given the high levels and spectrum of resistance to worm remedies(anthelmintics) that have been documented, before developing an effective control program for *H. contortus* or any other gastrointestinal nematode (GIN) parasite on a farm, it is extremely important to know the resistance status of worms on that property. Presently, this can be done only 2 ways: 1) by performing a fecal egg count reduction test (FECRT); or 2) by performing an *in vitro* larval development assay (LDA). The FECRT is presently the most commonly used means of determining whether a worm remedy is effective on a particular property, and has the advantage that it can be done on any farm with any drug. An alternative to the FECRT is the DrenchRite[i] LDA; however, the test is not suited for on-farm or in-clinic use and can only be performed in a specialized parasitology diagnostic laboratory. Unfortunately, very few laboratories around the world provide this test. Thusmost often, the only practical means to determine if you have resistance to worm remedies on your farm is with a FECRT.

In a FECRT, the numbers of worm eggs per gram of feces are measured in each animal before treatment and again after treatment by performing a fecal egg count (FEC) procedure. The percent reduction in the number of eggs seen before vs after treatment is then calculated. The FECRT provides a direct measurement of the effectiveness of the anthelmintic, but really should only be used to indicate whether resistance is present of not. This is because the observed efficacy is subject to high variability once it falls below 95%. And the fewer animals tested and lower the FEC in the pre-treatment measurement, the high the variability will be. Furthermore, the FECRT is performed only at a single dose (the label dose [sheep] or 1.5-2X the label dose [goats]), thus the results will only tell if you the drug is effective or not at that dose; it provides no warning of emerging resistance until the drug fails. The FECRT also requires significant time and effort by the veterinarian or livestock specialist, as fecal samples must be collected from individually identified animals, FEC performed, treatments applied accurately, treatment records kept and entered into a spreadsheet or other analysis program, and data analyzed and interpreted. Clearly, the FECRT does require an investment of time, effort and cost; but it is the only practical means to determine if you have worms that are resistant to your worm remedies. Given the economic cost of wasted drug, wasted labor, reduced productivity and animal death due to failed treatments, performing a FECRT is almost always a good investment for a farm.

How to perform a FECRT: old guidelines

When performing a FECRT in sheep or goats, it is suggested that proper standardized guidelines be followed. For the past 20+ years, guidelines published by the World Association for the Advancement of Veterinary Parasitology (WAAVP)(Coles et al., 1992)[ii]have been recommended, with additional practical modifications to fit the situation on the farm. However, much has been learned over this time, and new improved recommendations are in development by WAAVP that will supersede the previous recommendations. Using the current guidelines outlines by Coles (92), groups of 15 animals that have not been treated with a worm remedy within the past 8-12 weeks are randomly allocated to either a treatment group (worm remedy to be tested) or a non-treated control group (15 per group). Of course you can only test the animals you have so if you have less than 30 animals available to test, then you will need to have less than 15 per group. However, the fewer animals you have the less confidence you can have in the results from a statistical perspective (more on this below). Because it is recommended that the average FEC of the group be at least 200 eggs per gram (EPG), lambs or kids are the preferred age group to test because they tend to have higher FEC. Adult ewes, unless around the time of lambing tend to have FEC that are too low. Adult does often have sufficient FEC to use. Ultimately, in a FECRT you are measuring a reduction in FEC; so if you don't start out with very many eggs in the pre-treatment FEC, it is difficult to accurately measure the level of reduction after treatment.

Because the treated group is being compared to the control group, FEC are not needed on the day of treatment, but are performed on all animals (usually using the modified McMaster technique) 10-14 days after treatment. If enough animals are present on the farm, multiple drugs can be tested simultaneously. Calculations for percent reduction in FEC are then performed using the following formula (FECR% = 100[1-Xt/Xc]), where Xt and Xc are the arithmetic mean (average) EPG in the treated (t) and nontreated control (c) groups, respectively. Software is available for free that performs all calculations using this approach and gives data interpretation.[iii] If this FECR calculator program is used, the assignment of resistance status is based both on observed percent reduction and the 95% confidence intervals. Interpretation is then as follows: 'Resistant' when FEC reduction (FECR) is less than

95% and lower limit of the confidence interval less than 90%; as 'Suspected Resistant' when either FECR is less than 95% or lower limit of the confidence interval is less than 90%, and as 'Susceptible' when FECR is ≥95% and lower limit ≥90%. If a FECR calculator program is not used, the following guidelines can be applied: reductions of greater than 95% indicate sensitivity, reductions of 90-95% indicate low or suspected resistance, and reductions of <90% indicate resistance.

Changes recommended in the new guidelines

As mentioned above, new guidelines are in preparation but are not yet available. However, several changes that will appear in the new guidelines will be highlighted here. First, recent studies have shown that it is better to compare the pre-treatment and post-treatment FEC of each animal, rather than using treated and control groups. Thus, there is no need to have anontreated control group, but FEC must be performed twice on each animal. If a FEC cannot be obtained from an animal posttreatment, then the pre-treatment FEC from this animal should be omitted from the data set and not used in the calculation of FECR.

It is still recommended that 15 animals be tested, but fewer can still yield good results if the requirements cited below are met. There is no minimum level of EPG that is required under the new guidelines, but the number of eggs counted during the FEC is important. To increase the diagnostic power of the FECRT result, the total number of eggs counted pre-treatment under the microscope across these animals should exceed 140 eggs,though anything >100 should yield fairly good results. Thus, if mean FEC of the animals being tested are low then the modified McMaster method may not be appropriate and an alternative egg counting technique with greater detection sensitivity should be used. If fewer eggs are counted pre-treatment, then a second FEC (or additional chamber) from each animal should be counted so the egg tally exceeds 140 eggs. Also, the post-treatment FEC must be conducted at the same level of sensitivity as the pre-treatment count (i.e. the same detection level and same number of chambers counted). It also is necessary to ensure that FECRT results are not based on one or two animals contributing the majority of the eggs, consequently the 3 highest egg counts should not account for more than 50% of the sum of all individual egg counts.

With regard to analysis, it is recommended to use a hierarchal Bayesian framework. This is a very complicated analysis and is difficult to set up properly. To address this issue, an analysis program has recently been developed by the University of Zurich that uses this approach(Torgerson et al., 2014).[iv] This program provides the mean FECR and the 95% confidence intervals. This program does not provide a clinical interpretation, but the same criteria as cited above should be followed.

Other things that can improve the diagnostic result of the FECRT

Because of the over-dispersed nature of parasitic infections where approximately 20% of the animals harbor 80% of the parasites, FEC vary widely between animals, and a small percentage of animals have much higher FEC than the rest. Thus there is a strong possibility of a biased or erroneous result if too few animals are used. For reasonable accuracy in the FECRT at least 6 and preferably 10 to 15 animals should be tested for each drug. If >10 animals are included in each group, it is probable that random allocation will produce treatment groups sufficiently balanced to obtain accurate results. However, if less than 10 animals are used per group it is recommended to balance groups by level of infection. This can be achieved by performing a FEC prior to the start of the FECRT, and then allocating animals to group based on their FEC. But this requires a great deal of additional work and expense, consequently, it is not practical at the farm level. However, when *H. contortus*(wireworm) is the primary parasite present (often this is the case) we have found that treatment groups can be reliably balanced if animals are assigned to treatment group based on FAMACHA© score (see paper on FAMACHA©). Therefore, if this method is used a pretreatment FEC is not needed; assignment to treatment group can be made on the spot based on the FAMACHA© score. For example, if 3 drugs are being tested, of the first 3 animals to come through the chute with the same FAMACHA© score, each of the 3 will be assigned randomly to 1 of the 3 treatment groups (4 if a control group is included). The process then repeats itself for the next 3 with the same FAMACHA© score, and so on.

Practical hints regarding interpretation of FECRT results

If drugs are highly effective (>97%) or poorly effective (<60%), the results will be pretty clear even with relatively few animals and/or relatively few eggs being counted pretreatment. In such cases you can be fairly confident the drug was either highly effective (no resistance) or poorly effective (worms are resistant). But whenresults fall into the gray area (80-95%), if too few animals are tested and/or too few eggs counted pretreatment, inherent variability may lead to an erroneous conclusion. This is especially important when resistance is first emerging, since efficacy is only modestly reduced. If using the University of Zurich analysis software, you can look at the 95% confidence intervals to assist in making your interpretation. In general, the more animals tested and the more eggs counted, the more accurate the results will be.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology44, 35-44.

Torgerson, P.R., Paul, M., Furrer, R., 2014. Evaluating faecal egg count reduction using a specifically designed package "eggCounts" in R and a user friendly web interface. International Journal for Parasitology44, 299-303.

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[i] Dr Jennifer Gill, Microbial Screening Technologies, Smithfield, Australia

[ii] New guidelines for FECRT are currently under development by a WAAVP subcommittee, and are expected to be published in 2016. These will then supersede the recommendations referenced in Coles et al. (1992)

[iii] A. Cameron, RESO fecal egg count reduction analysis spreadsheet. AusVet Animal Health Services, University of Sydney, Sydney, Australia 2000. Based on calculations developed by Martin, P.J., Wursthorn, L., 1991. RESO faecal egg count reduction test calculator, CSIRO, Animal Health, Melbourne, Australia.

[iv] University of Zurich, http://www.math.uzh.ch/as/index.php?id=254