

COMBAR combatting anthelmintic resistance in ruminants

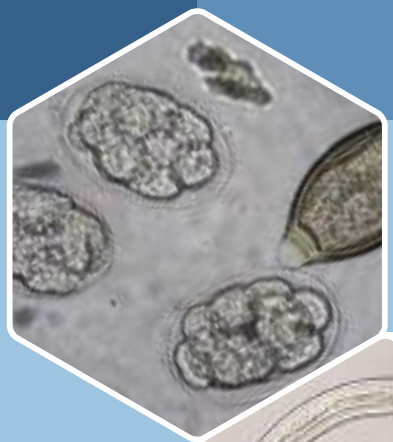
LiHRa Livestock Helminth Research Alliance



FAECAL EGG COUNT REDUCTION TEST (FECRT) PROTOCOL GASTROINTESTINAL NEMATODES



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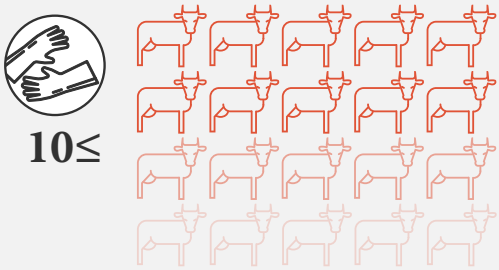






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Sample collection

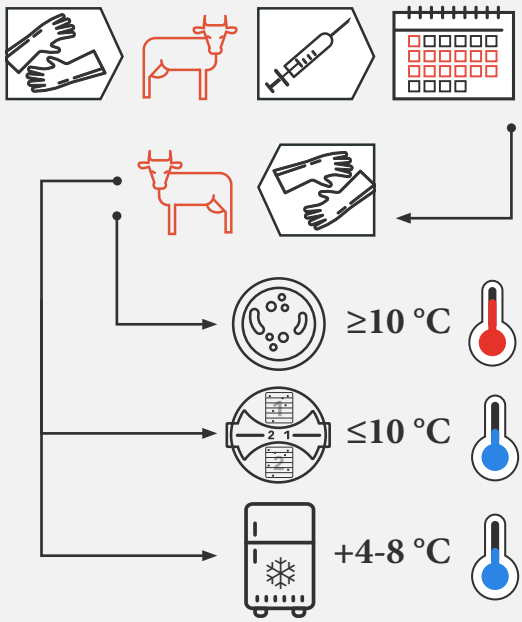


Collect individual rectal faecal samples (minimum 20g) from a minimum number of **10 animals** per group on the selected farms (Kaplan, 2020).

Higher numbers, where available, will allow a more precise result. Weaned animals at first grazing season are preferable (Kaplan, 2020).

In order to identify animals with high egg counts 15 (or more) animals can be selected pre-treatment, with post-treatment sampling focused on the 10 animals with the highest egg counts. Herds should not have received anthelmintic treatments during the past six weeks (extended periods will apply if long-acting formulations have been applied).

Sampling



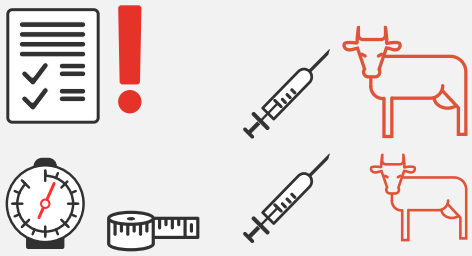
The same animals must be sampled pre- and post-treatment, i.e. **on Day 0 and between 7 to 21 days after treatment, depending on the drugs tested.**

- *levamisole: 7 to 10 days;*
- *benzimidazoles: 10 to 14 days;*
- *ivermectin and other macrocyclic lactones: 14 to 17 days;*
- *moxidectin: 17 to 21 days;*
- *when testing in parallel two or more drugs in same herd: 14 days (Coles et al., 1992; Kaplan, 2020)*

Samples should best reach the lab at the day of sampling and be cooled to below 10 °C during transport.

The samples can be stored for 5 days at +4-8 °C; if there is no possibility to analyse the samples within 5 days of sampling, keep the faecal samples in a **vacuum packed** plastic bag (Rinaldi et al., 2011) (or use anaerobic storage) and store them **up to three weeks** *in the* **fridge at +4–8 °C** (refrigeration is needed to prevent fungal growth).
Additionally, one composite sample (equal amounts from all individual samples for each drug), which should not be cooled below 10 °C before processing, will be taken to perform a larval culture.

Treatment instructions



Whichever route of administration of the respective anthelmintic drug is used, it is important to read the manufacturer's instructions carefully.

Particular attention should be paid to: avoid under dosing, dose according to individual liveweight or the heaviest animal using scales or a weigh band. Ensure that the equipment is appropriate for the product and is calibrated to deliver the dose accurately. On farms where more than one drug is tested and if one of these is a pour-on product, any contact of animals between groups should be prevented.

Faecal egg count (FEC)





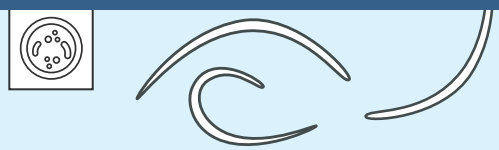
On each individual sample, FEC will be performed to obtain worm EPG (eggs per gram of faeces) data using a quantitative copro-microscopic method e.g. Mini-FLOTAC, McMaster, FECPAKG2 etc. using saturated sodium chloride (specific gravity = 1.200) as flotation solution. When low number of eggs are expected sensitive methods are recommended (e.g. Mini-FLOTAC, analytic sensitivity of 5 EPG) (Cringoli et al., 2017).

To ensure reliable conclusions on the drug efficacy, a **minimum number of 200 eggs per treatment group** need to be counted prior treatment (Kaplan, 2020). This number is the total number of eggs counted across the 10 animals sampled and is thus not the number of EPG. Therefore, if this number was not obtained following analysis of all samples the practical solution is to examine a second aliquot of each of the same samples (i.e. leading to an analytic sensitivity of 2.5 EPG if for example Mini-FLOTAC is being used).

Consequently, the same analytic sensitivity has to be employed also for examining the post-treatment samples of the same animals **(see the flowchart in Annex 1)**. The total number of eggs counted (≥200) under the microscope compensates for the variations in both study design (analytic sensitivity and sample size) and host-parasite interactions (level and aggregation of egg excretion) (Levecke et al., 2018).

Perform the egg count of gastrointestinal strongyles, *Nematodirus* and other nematodes (e.g. *Strongyloides, Trichuris*, etc.) separately.

Species identification



Can be conducted either using morphological examination of L3 from coprocultures or using molecular techniques using either L3 from coprocultures or eggs/L1.

Coprocultures



Coprocultures will be performed pre- and post-treatment per farm for **identification of nematode larvae (L3)** according to the procedures and morphological keys of van Wyk and Mayhew (2013).

Separate pooled samples need to be prepared using faecal samples which were not cooled, since cooling may affect the hatching and/or larval development. L3 should be maintained in water, at 4 °C until morphological examination.

Molecular analysis



If samples are to be analysed using molecular methods, then obtained larvae/eggs from pre- and post-treatment samples can either be stored in DNA extraction buffer at 4 °C, in 70% ethanol or in dH2O at -20 °C to -80 °C until further molecular analysis.

Anthelmintic efficacy

The recommended methods to calculate FECR and confidence intervals are:

- R package 'eggCounts' that uses a Bayesian hierarchical model (Wang et al., 2018) http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/

- Beta negative binomial method analysis tool by Matthew Denwood https://mdenwood.shinyapps.io/fecrt_bnb/

Anthelmintic treatment efficacy is interpreted according to the following table based on WAAVP guidelines (Coles et al.,1992), lastly reviewed by Levecke et al. (2018).

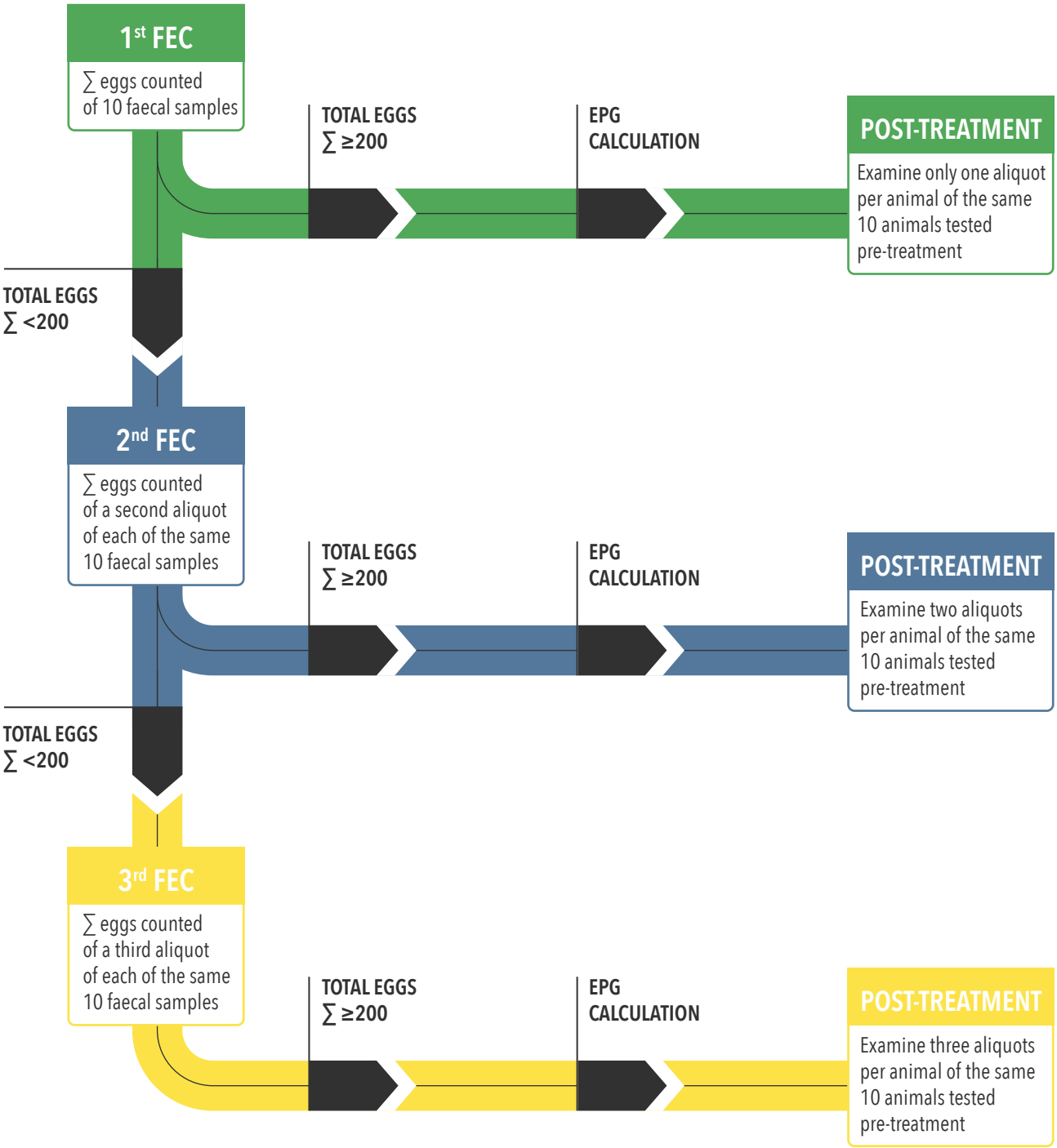
Efficacy	Results
Reduced	FECR <95% and lower limit of the 95% Confidence Interval <90%
Doubtful	Either FECR <95% or lower limit of the 95% Confidence Interval <90%
Normal	FECR ≥95% and lower limit of the 95% Confidence Interval ≥90%

Practical example using Mini-FLOTAC with a lower detection limit of 5 EPG*

Animal #	1	2	3	4	5	6	7	8	9	10	Σ	Factor	Mean EPG
1st FEC. Sum of eggs counted in two chambers (= 1 Mini-FLOTAC device):	6	7	28	0	8	13	7	26	9	9	113	5	56.6
2nd FEC. Sum of eggs counted in two chambers (= 1 Mini-FLOTAC device):	8	5	26	2	3	11	10	28	8	9	110	5	55
Counts to be entered in R package 'eggCounts'	14	12	54	2	11	24	17	54	17	18	223	2.5	55.75

*Another approach is to increase the number of animals per group

Annex 1. COMBAR Flowchart for Faecal Egg Count procedures.







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