

Version adopted 15 March 2021

Faecal egg count reduction test (FECRT) protocol Gastrointestinal nematodes - SHEEP and GOATS

Sample collection

Collect individual rectal faecal samples (minimum 5 g, preferably 10 g) 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. For sheep and goats, animals of all ages may be used, as long as egg counts meet the inclusion criteria (see below) (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. Flocks 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 flock: 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. It is important to apply specific dose rates for goats (usually 1.5 to 2 times the sheep dose) (Hoste et al., 2011). Particular attention should be paid to: avoid under dosing, dose according to individual liveweight or the heaviest animal using scales. 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 a 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 flow-chart 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).

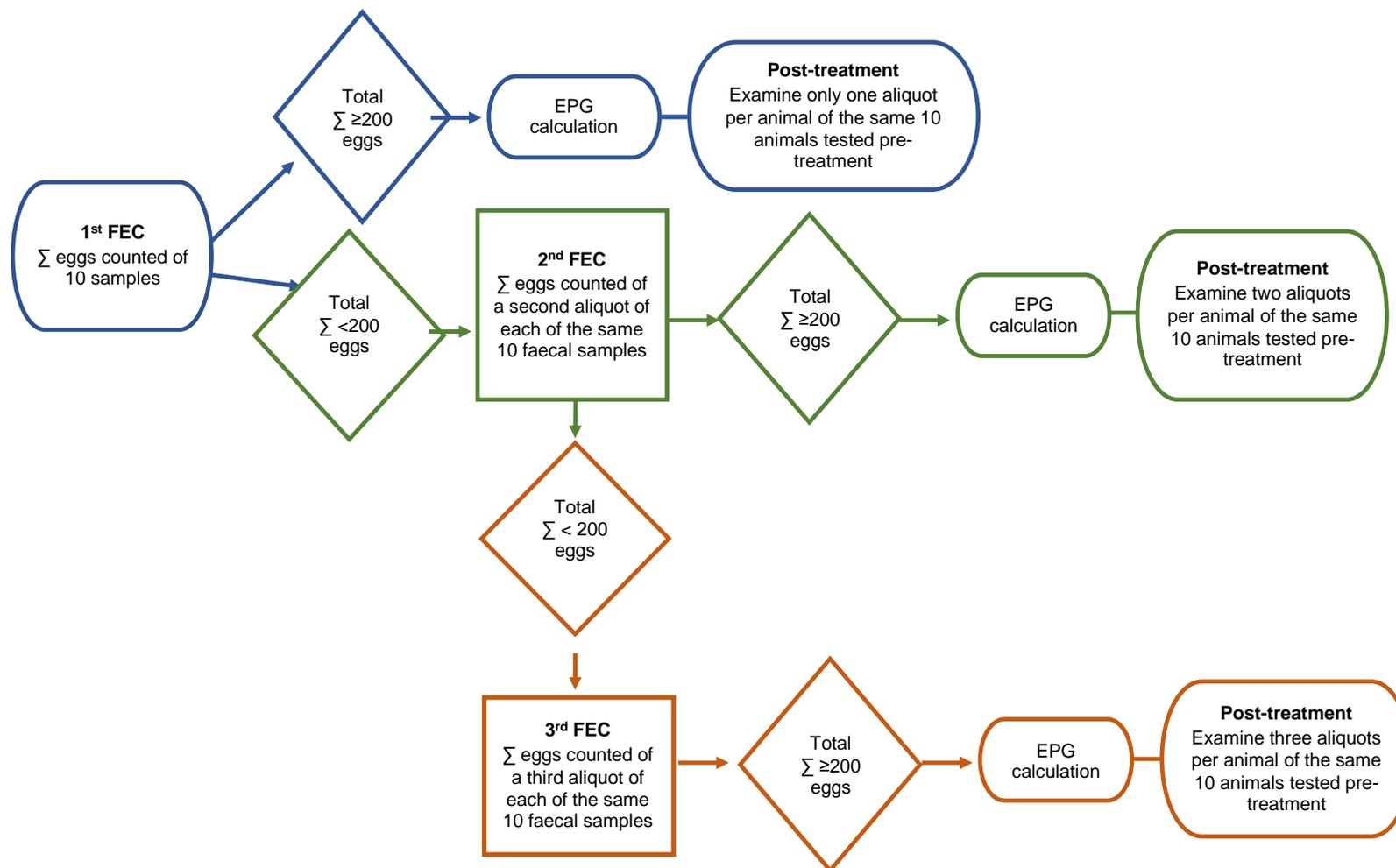
Efficacy	Results
Reduced	FECR < 95% and lower limit of the 95% Confidence Interval < 90%
Doubtful	Either FECR < 95% or lower limit of the 95% Confidence Interval < 90%
Normal	FECR \geq 95% and lower limit of the 95% Confidence Interval \geq 90%

References

- Coles G.C., Bauer C., Borgsteede F.H., Geerts S., Klei T.R., Taylor M.A., Waller P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* 44, 35-44.
- Cringoli G., Maurelli M.P., Levecke B., Bosco A., Vercruysse J., Utzinger J., Rinaldi L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nat. Protoc.* 5, 503-515.
- Hoste H., Sotiraki S., Torres-Acosta J.F.J., 2011. Control of endoparasitic nematode infections in goats. *Vet. Clin. North Am. Food Anim. Pract.* 27, 163-173.
- Kaplan R.M., 2020. Biology, Epidemiology, Diagnosis, and Management of Anthelmintic Resistance in Gastrointestinal Nematodes of Livestock. *Vet Clin North Am Food Anim Pract.* 36(1):17-30.
- Levecke B., Kaplan R.M., Thamsborg S.M., Torgerson P.R., Vercruysse J., Dobson R.J., 2018. How to improve the standardization and the diagnostic performance of the fecal egg count reduction test? *Vet. Parasitol.*, 253, 71-78.
- Rinaldi L., Coles G.C., Maurelli M.P., Musella V., Cringoli G., 2011. Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. *Vet. Parasitol.* 177, 345-352.
- Van Wyk J.A. & Mayhew E., 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal Vet. Res.* 80, 14 pp.
- Wang C, Torgerson PR, Kaplan RM, George MM, Furrer R., 2018. Modelling anthelmintic resistance by extending eggCounts package to allow individual efficacy. *Int J Parasitol Drugs Drug Resist.* 8, 386-393.



Annex 1. COMBAR Flowchart for Faecal Egg Count procedures.





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
1 st 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
2 nd 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