Practical application of fecal testing for internal parasites

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Abstract

The article reviews the most important helminth parasites of cattle, sheep, and goats in the United States and briefly describes the situation with regard to anthelmintic resistance. Reasons for fecal testing are proposed, and which groups of animals should be tested and when they should be tested is discussed. The limitations of fecal egg counts are summarized. Recommendations for fecal sampling are given. Thepros and cons of the McMaster, Mini-FLOTAC, and centrifugation fecal flotation methods are discussed. The use of a Baermann sedimentation for lungworms and tests for *Fasciola hepatica* are covered. Available methods for the identification of strongylid (trichostrongyle/strongyle) nematodes are summarized. The new guidelines for the conduct of a fecal egg count reduction test are discussed.

Keywords: Centrifugation fecal flotation, FECRT, McMaster, Mini-FLOTAC, ruminants,

Why is fecal testing needed?

The widespread emergence of anthelmintic resistance in both small ruminant and cattle nematodes, as well as exotic hoofstock in zoological collections, has prompted a re-evaluation of the way we need to be using these drugs, to preserve the efficacy of products that are still effective, for as long as possible. We can test whether an anthelmintic, or combination of anthelmintics, is effective by conducting a fecal egg count reduction test (FECRT). In animals

that are high-value patients or livestock kept as pets, we can evaluate the effectiveness of treatments of individual animals. Fecal egg counts give us an indication of the level of egg shedding occurring on pasture and may be used as an indication of when to administer treatment to the individual or the herd. They are often also useful to help us make a decision not to treat, especially in cattle, if egg counts are low or eggs are not detected during the fecal examination. While this article is focused predominantly on gastrointestinal nematodes, it is important to be aware of *Fasciola hepatica*, the common liver fluke, because while no surveys have been done in the in the past 25 years, anecdotal reports from practitioners indicate that its distribution and prevalence appear to have shifted. We can no longer accept that infections with the fluke are confined to the Pacific Northwest and the Gulf Coast regions and should be undertaking surveillance for the presence of the parasite in areas that may be suitable for the intermediate snail hosts to survive.

What are the most important helminth parasites of cattle in the United States?

Ostertagiaostertagi, the brown stomach worm, is the most pathogenic nematode infecting cattle and it affects cattle in a wide age range. Both *Haemonchuscontortus* and *Haemonchusplacei*, the barber pole worms, infect cattle and are especially pathogenic in weanling and yearling cattle. *Cooperia* spp., including *Cooperiapunctata*, *Cooperiaoncophora*, and *Cooperiapectinata*, are among the least pathogenic but under warm, wet condition, they may occur in very large numbers and present an economic and clinically significant risk. Anthelmintic resistance to the macrocyclic lactones almost always first manifests as a decreased efficacy against *Cooperia* spp.[16] As a consequence, the proportion of the worm burden made up of these species has increased substantially compared with other nematode species. As mentioned above, *F.*

*hepatica*constitutes a threat, not only because it causes liver condemnations but also because it has subclinical effects on the growth and development of cattle.[11]

What are the most important helminth parasites of sheep and goats in the United States?

By far the most pathogenic and prevalent parasite is *Haemonchuscontortus*, the barber pole worm, and is of particular concern during the warmer months of the year. Infections with *Teladorsagiacircumcincta*, the brown stomach worm, and *Trichostrongylus* spp. may play a role in causing parasitic disease during the spring and fall. *Fasciola hepatica* may exacerbate the anemia caused by *H. contortus* but the parasite may in and of itself cause ill health and even death in sheep and goats.

Anthelmintic resistance in livestock helminths

All FDA-approved anthelmintics for treating gastrointestinal nematode infections in the United States belong to three drug classes, namely the benzimidazoles (e.g., fenbendazole and albendazole), the macrocyclic lactones (e.g., ivermectin, doramectin, eprinomectin, and moxidectin), and the imidazothiazoles (e.g., levamisole). No nation-wide studies have been conducted for anthelmintic resistance in cattle nematodes the United States, but certainly in the southern United States, macrocyclic lactone resistance appears to be highly prevalent in *Cooperia* spp. and *Haemonchus* spp. and emerging in *O. ostertagi*.[8] No recent studies have been published on anthelmintic resistance in sheep and goat farms, but Howell and colleagues[7]in the southern United States and Crook and colleagues[4]in the mid-Atlantic region indicated that resistance in *H. contortus* was widespread to the benzimidazoles and ivermectin, with resistance in levamisole and moxidectin less prevalent. Concern was raised at the time of the study that

almost 50% of the farms studied in the southern United States had resistance to all three classes of anthelmintics.

Who and when should we test for parasites?

Young, growing beef cattle are particularly susceptible to gastrointestinal nematode infections as they are still developing an immunity to the parasites.[12] This includes calves during the latter part of nursing, stocker cattle six to eight weeks after turnout, and replacement heifers. Beef brood cows in the winter that are nursing or heavily pregnant are also at risk of succumbing to parasitic disease. For example, cows in the southern United States which are on poor-quality pasturesduring severe weather, are subjected to a high infective-larval challenge, and are additionally challenged by liver fluke infections may develop clinical signs of parasitic gastroenteritis. As a general rule, males are more susceptible to nematode infections than females. Beef bulls may therefore also require additional monitoring of fecal egg counts.

In sheep and goats, the FAMACHA© system is useful for identifying individuals that are clinically ill from hemonchosis and this eliminates the frequent need to use fecal egg counts to identify animals in need of treatment.[9] Fecal egg counts are very useful in small ruminants, however, to confirm parasitic disease in an individual or a herd or flock, especially when *H. contortus* is not the predominant parasite. They are useful for monitoring the parasite infections in the herd or flock over a period of time (such as the summer). Fecal egg counts should be done when new stock are introduced to the farm, prior to the new stock being mingled with existing herd or flock. Introduced animals should be kept in quarantine, their fecal egg counts checked before deworming and their egg counts rechecked 10 to 14 days after treatment to make sure the treatment was effective.

In cattle, sheep, and goats, fecal egg counts are, of course, an integral part of conducting a FECRT.

Limitations of fecal egg counts

Fecal egg counts are a measurement of the concentration of eggs in the feces. As such, factors that affect the consistency of the feces (such as excess fluid in diarrheic feces) will affect the egg count (by diluting the feces) and this needs to be borne in mind when the feces are less formed or drier than expected. Although they may be the only option available to us for assessing parasite burden, fecal egg counts are a poor measurement of that worm burden. Parasites are known to be overdispersed in a host population—20% to 30% of the individuals in a herd or flock will harbor 70% to 80% of the parasites. There is thus a need to evaluate a sufficient number of animals in the group. Strongylid (trichostongyle/strongyle) eggs cannot easily and reliably be identified to species level, with the exception of *Nematodirus* eggs. We therefore need to perform coprocultures and identify the third-stage larvae thus cultured or we need to use molecular techniques to determine the species composition of the eggs.

Fecal sample collection

Rectal fecal samples should be collected if at all possible. In the case of cattle, samples collected from dung pats produced freshly overnight may be acceptable in certain circumstances but collecting samples in this way has not been validated for use in FECRTs. When collecting samples, fill the container as full as possible to exclude air, and seal the container. Transport the samples to the laboratory on ice, but not in direct contact with ice packs. Refrigerate samples that cannot be shipped or processed in the laboratory immediately, but never freeze fecal samples. How much feces is required? About a tablespoon of feces per animal may be

sufficient; more is needed when coprocultures need to be prepared. If a fecal egg count on a pooled sample is desired (for reasons of cost or time), collect the samples individually and ask the laboratory to pool the samples. This permits the laboratory staff to weigh off the same amount of feces from each sample before pooling the feces.

Quantitative methods of fecal egg count

For the diagnosis of gastrointestinal nematode parasitism to be meaningful, we almost always require that a quantitative fecal examination technique be used. Never use a simple passive flotation method for ruminants and expect to make meaningful recommendations based on your findings. Aside from the fact that centrifugal fecal flotation methods (discussed below) consistently yield more eggs than passive flotations,[5] simple passive flotation methods are not quantitative.

The McMaster method[6]and the Mini-FLOTAC[3] are two reliable quantitative methods that can be performed in the veterinary practice. For a description on how to perform the McMaster method and centrifugal fecal flotation described below, please refer to an appropriate text, such as the *Veterinary Clinical Parasitology*.[17]Detailed instructions accompany the Mini-FLOTAC kits. The McMaster method is useful when moderate and high infections are expected. This is because the multiplication factor used when multiplying the number of eggs counted to derive a value for the eggs per gram of feces is high, for example 50. Generally, the McMaster method best lends itself to use in sheep and goats where fecal egg counts lower than 50 epg would not be considered clinically significant. With the Mini-FLOTAC method, the multiplication factor is low and the method lends itself to use in cattle where egg counts are generally lower than in sheep and goats. The multiplication factor for processing ruminant samples by the Mini-FLOTAC method is 5, which means that counts as low as 5 epg may be enumerated. An additional advantage of the

Mini-FLOTAC is that a scale is not required to weigh the sample. A conical collector is built into the apparatus and is designed to accommodate a known weight of feces. The fecal suspension is passed through a sieve which is self-contained within the apparatus which means that there is less mess than with the McMaster method. On the other hand, it may require more time to process and examine a sample using the Mini-FLOTAC system than with the McMaster method. Both methods require the use of a standard compound microscope.

Centrifugal fecal flotations may be used in a quantitative manner, though the flotations are best suited for screening for protozoan parasites (e.g., *Cryptosporidium*) and parasite eggs present in very low concentrations (e.g., *Trichuris*).

Recently, automated methods of performing fecal egg counts have been commercialized. One such system has been developed by Parasight System Inc. for fecal egg counts in sheep and goats.[15]

Diagnostics for *Fasciola hepatica* and lungworms

Liver fluke eggs may be detected using a sedimentation method. Commonly, the FLUKEFINDER® [a] diagnostic system is used to sieve the feces before the filtrate is subjected to a series of washing and sedimentation steps. The final sediment is stained with methylene blue and examined under the microscope for fluke eggs. If the feces are weighed prior to processing, a liver fluke egg count may be obtained. The Mini-FLOTAC method may also be used to enumerate *F. hepatica* eggs if a zinc sulfate solution is used as the flotation solution.

While not available for routine diagnostics in the United States, a copro-antigen ELISA and ELISAs for ovine and bovine sera or bovine milk are available in other countries, and they may or may not become available in the United Statesin the future.

If lungworms are suspected, submit feces to the laboratory and request recoveryof any larvae present using the Baermann technique.

Parasite identification

The limitation with regard to identification of strongylid eggs in feces has been discussed. Certain laboratories therefore offer identification of *H.contortus* eggs in feces using a fluorescein-labeled peanut agglutinin method.[13] This method has also been incorporated into an automated method of fecal egg counts for sheep and goats.[2] A limited number of laboratories offer coproculture for recovery and identification of the infective third-stage larvae. The culture process mimics the natural process of egg hatching and larval development to the third stage. Specialized training is required to identify the larvae.

Recently developed molecular techniques offer perhaps greater hope of more widespread ability to provide parasite identification in fecal samples than the classical parasitology techniques. Techniques include multiplex PCR (qPCR)[14] which is used to identify and quantify eggs of the main gastrointestinal nematodes of ruminants. This may be a test that is offered at commercial laboratories in the near future. Nemabiomemetabarcoding[1] is a next-generation sequencing approach which is currently used almost exclusively as a research tool but may be made available at more laboratories in the future. Of particular promise for future diagnostic use is the adaptation of Oxford Nanopore sequencing technology for nematode identification.[b]

FECRT

New guidelines of the World Association for the Advancement of Veterinary Parasitology have been published that have clarified the way in which FECRTs should be conducted to test for anthelmintic resistance.[10] The basic guidelines require that pre- and post-treatment fecal egg

counts be conducted. The interval between treatment and collection of post-treatment samples varies according to the product used (Table 1). A control group is not required, nor is a specified minimum fecal egg count per animal. Rather, a specified minimum total number of eggs (not epg) is required pre-treatment. The method used to perform the egg count may be chosen based on the mean fecal egg count of the group being tested and the number of animals being tested. The McMaster and Mini-FLOTAC methods are both acceptable, but centrifugal fecal flotation methods are not recommended. Both so-called 'research' and 'clinical' protocols are described. As the name implies, the research protocol should be used in research studies while the clinical protocol is more suited for general on-farm diagnostic use. As anexample, using the clinical protocol, if the total number of eggs counted is 320, a minimum of 8 animals is required. The reader is referred to the publication for further details regarding minimum numbers of eggs to be counted and animals to be included. A website has been developed which allows the fecal egg count data to be entered. The website will provide values for an upper and lower 90% confidence interval or other appropriate statistical test results by which the worm population on the farm may be classified. Based on the calculated confidence interval, the worm population is classified as susceptible, resistant, or inconclusive.

Insert Table 1 near here.

Endnotes

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247 **Table 1.**Recommended intervals between treatment and collection of post-treatment fecal

248 samples when conducting a fecal egg count reduction test (from Kaplan and colleagues[10]).

Host	**Type of anthelmintic**	**Interval**
Sheep and goats	Non-persistent drugs	10 to 14 days
Cattle	Non-persistent drugs	10 to 14 days
Cattle	Macrocyclic lactone drugs	14 to 17 days
Cattle	Moxidectin	17 to 21 days
Cattle	Specially formulated long-acting macrocyclic lactone products	21 to 28 days

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