Fecal fluency: A review of fecal tests and how to interpret the results

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Abstract

Gastrointestinal parasites are the greatest threat to the health and productivity of small ruminants and camelids in the United States, and in many other parts of the world. The steadily escalating problem of multi-drug resistant parasites has resulted in an increase in treatment failures, often with lethal consequences. Effective control programs for *Haemonchus contortus* and other important parasites require knowledge of which parasites are present on farms, the magnitude of the infection among animals, and which anthelmintics are still effective. This presentation will focus on fecal analyses used to detect important nematodes that affect goats, sheep, llamas and alpacas, and methods used to determine anthelmintic resistance.

Key words: small ruminants, camelids, parasites, *Haemonchus*, anthelmintics

Résumé

Les parasites gastro-intestinaux représentent la plus grande menace à la santé et à la productivité des petits ruminants et des camélidés aux États-Unis et dans plusieurs autres parties du monde. Le problème croissant de multirésistance aux médicaments chez les parasites a augmenté la prévalence d'échec thérapeutique causant souvent la mort. Les programmes efficaces de contrôle du vers *Haemonchus contortus* et de d'autres parasites importants nécessitent de connaître le type de parasites présents dans les fermes, le degré d'infection parmi les animaux et quels anthelminthiques sont encore efficaces. Cet article se penche sur les analyses fécales qui sont utilisées pour détecter les nématodes importants qui affectent les chèvres, les moutons, les lamas et les alpagas et sur les méthodes utilisées pour déterminer la résistance anthelminthique.

Introduction

Camelids and small ruminants are hosts to a vast array of trichostrongyle nematodes including *Haemonchus contortus*, *Teladorsagia circumcincta (formerly Ostertagia circumcincta), Trichostrongylus colubriformis*, *Cooperia* spp, *Nematodirus* spp, *Oesophagostomum* spp, and *Bunostomum* spp.[9] *Haemonchus contortus* is the most dangerous pathogen of the group as blood feeding by fourth-stage larvae and adult worms in the abomasum (camelid third compartment) can lead to fatal anemia in the host. In addition, the progressive decline in the efficacy of anthelmintics in multi-drug resistant *H. contortus* populations has compromised the ability of producers and veterinarians to manage worms with anthelmintics.[2,5,9] Diagnostic testing is essential to determine what type of worms are in the hosts, and to gauge infection intensity. Further, surveillance programs that monitor the efficacy of anthelmintics against the resident trichostrongyles on farms are necessary aspects of good livestock management. Anthelmintic resistance increases over time in worm populations in response to spontaneous mutations and selection pressure imposed by anthelmintic treatments. Detection of declining efficacy before it reaches clinically significant levels allows producers to make anthelmintic choices that save money and time that would be otherwise lost on ineffective treatments, and to preserve animal viability.

Fecal Sample Collection and Handling

Approximately 8-12 fecal pellets should be collected from the rectum using lubricated, gloved fingers. Owners often prefer to collect freshly passed feces off the ground. Animals can be put onto a "clean" floor until they defecate to reduce contamination of the fecal sample. Nematode larvae will hatch from eggs in the presence of oxygen and warmth in a few hours to days, so feces should be stored in a zip-lock bag from which air has been evacuated. A study conducted in South Africa showed that vacuum-packed fecal samples stored at room temperature retain sufficient diagnostic quality for fecal egg count determination for up to 3 weeks.[8] The Reynolds Handi-vac System is inexpensive (less than $25.00) and is readily available in most grocery stores. Refrigeration will also delay egg hatching. If a fecal culture is needed, then the fecal sample needs to be transported to the testing lab and processed within 3 days of collection. Chilling the eggs for more than 48 hours and/or prolonged air exclusion can result in failure of the larvae to hatch in the laboratory.

The Fecal Egg Count

Fecal egg counts (FEC) are preferred to simple fecal smears or non-quantitative floatation techniques when assessing trichostrongyle infections. The FEC provides an estimate of the magnitude of the worm burden, particularly for highly fecund worms such as *Haemonchus contortus*. This relationship is evident when the majority of the worms are patent adults at the time of testing. Fecal egg counts are useful, but less reliable estimates of adult worm burdens in lower egg shedders like *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. Many trichostrongyle nematode eggs look alike under the microscope, with the exceptions of *Nematodirus* spp and *Marshallagia* spp; these eggs are much larger and contain dark clusters of cells.[9] The smaller trichostrongyle eggs are counted separately from *Nematodirus* spp and *Marshallagia* spp eggs, as well as other parasitic ova or oocysts. Most diagnostic laboratories report the fecal egg count for "strongyles" or "trichostrongyles" (terms used interchangeably) but do not make any distinction as to worm genus. Trichostrongyle eggs need to be cultured, hatched to larval stages, and examined microscopically in order to distinguish them to the genus level.

Peanut Agglutination Test

Recently, a fluorescein-labeled peanut agglutination (aka "lectin staining") test was developed to rapidly distinguish *Haemonchus contortus* eggs from those of less pathogenic trichostrongyles.[4] Peanut agglutinin adheres specifically to the outer wall of *Haemonchus contortus* eggs. Researchers conjugated peanut agglutinin to fluorescein isothiocyanate so that the agglutinin-bound *Haemonchus* eggs could be easily visualized under a microscope equipped with ultraviolet illumination. Good correlations were found between results of fecal culture and the peanut agglutination test.[4] This test is available at Oregon State University Diagnostic Laboratory and at the University of Georgia College of Veterinary Medicine. Contact Bob Storey (head research scientist in Dr. Ray Kaplan's Laboratory, UGA Department of Infectious Disease; 706 542-0742) regarding sample submission; cost is $45 per sample.

Interpretation of the Fecal Egg Count

Many asymptomatic small ruminants and camelids have fecal egg counts of several hundred eggs per gram, which in the vast majority of cases is consistent with a mild infection.[9] Since mild worm burdens are well tolerated, and considered beneficial in that they stimulate host immunity, asymptomatic healthy hosts with low fecal egg counts do not need anthelmintic treatment. Fecal egg counts greater than 3,000 eggs per gram can be associated with morbidity in camelids and small ruminants, but resilient small ruminants can be asymptomatic with FECs as high as 5,000 eggs per gram. Heavy *Haemonchus* burdens can result in FECs of 20,000-100,000 eggs per gram.[a]

Interestingly, worm burdens are not uniformly distributed among herd/flock mates. Typically, 20-30% of the animals in the herd shed 80 percent of eggs.[2] Fecal egg count, FAMACHA score, and hematocrit reflect the ability of the host to retard establishment of worm infections and to cope with parasitic challenge, and these traits are heritable in small ruminants.[7] As a result, these tests can be used to select small ruminants for worm resistance, particularly when the *Haemonchus contortus* challenge is moderate-to-high.[7]

Fecal Egg Count Determination

The traditional McMaster's technique (see Appendix 1) is commonly used to calculate fecal egg counts in small ruminants and camelids.[9] This technique requires less equipment and time than centrifugation methods, and it has a sufficiently sensitive end point (25 or 50 eggs per gram, depending on technique) to make relevant clinical evaluations. A known weight of feces is thoroughly mixed in a known volume of floatation solution, and then strained into a second container. Sodium nitrate solution (specific gravity 1.18) is the preferred floatation solution; it can be prepared or purchased as Fecasol solution. The fecal suspension is pipetted into the chambered McMaster slide, and allowed to stand for 5 minutes so eggs have time to rise in the solution. The trichostrongyle eggs within the grids are counted under a microscope at 10 X 10 magnification, and then multiplied by the appropriate factor to convert the count to eggs per gram (epg).

Recently, a 3-chambered McMaster's slide was developed that improved the sensitivity of the McMaster's technique to 8.3 epg.[3] The 2- and 3-chambered McMaster's slides are available from Chalex Corporation (Issaquah, WA).

Centrifugation methods such as the Stoll technique (single centrifugation step) and Wisconsin technique (2 centrifugation steps; see Appendix 2) increase the FEC sensitivity to 5 epg. Centrifugation techniques are used commonly in camelid practice and bovine practice. A study that compared the sensitivity of a double centrifugation technique with a traditional McMaster's technique demonstrated that more large coccidian oocysts, *Trichuris* spp eggs, and *Nematodirus* spp eggs were detected in camelid fecal samples with the centrifugation technique.[1] Howell et al subsequently compared the sensitivity of the new 3-chambered McMaster's technique with the Stoll and Wisconsin tests in equine, camelid, and bovine fecal samples.[3] The 3-chambered McMaster's

technique yielded equal or improved fecal egg detection sensitivity in both camelid and equine samples compared to the centrifugation techniques. The centrifugation technique was most advantageous when bovine samples were analyzed; the darkening of the sodium nitrate in the presence of some factor in bovine feces made the eggs difficult to visualize on the McMaster's slide.[3] The 3-chambered McMaster's technique warrants further evaluation in camelids and small ruminants, because the low cost of materials, the ease of the technique, and excellent sensitivity make it an attractive alternative to more cumbersome and time-consuming centrifugation methods.

Detection of Anthelmintic Efficacy Reduction

Anthelmintic resistance can be detected using an *in vivo* test such as the fecal egg count reduction test (FECRT), and by using an *in vitro* larval developmental assay.[2,5] Testing for anthelmintic resistance should be performed every couple of years.

The FECRT is considered to be the "gold standard" method for detecting parasitic anthelmintic resistance in many livestock species. Despite this distinction, the PECRT is far from perfect, as biologic variability and methodology can negatively impact the results. In essence, the FECRT compares the mean post-treatment fecal egg count with either the mean pre-treatment fecal egg count, or with the mean FEC of an untreated control group.

Fecal egg count reduction tests can be used to test several different anthelmintics at the same time, if there are sufficient numbers of animals with sufficient worm burdens available for testing. The animals should not have received anthelmintic treatment for at least 4 weeks prior to testing. An additional caveat is that animals with profound morbidity (severe anemia, peripheral edema, wasted body condition) should not be left in a control group. The best strategy is to treat severely parasitized animals immediately with a combination of anthelmintics from different classes, and to exclude them from the FECRT.

The FECRT gives the most reliable results when at least 10 to 15 animals are assigned to each anthelmintic test group, and to an untreated control group. The untreated control group provides the best comparison data, as factors that influence fecal egg counts (weather, time of year, diet) will be taken into account. If less than 10 animals are available for each group, then extra effort is required to balance the level of infection among the groups. Pre-treatment fecal egg counts can be performed, ordered from highest to lowest, and the animals assigned to treatment groups in a manner that reasonably balances the fecal egg counts among them. Depending on fecal egg count methodology used, the cutoff for pre-treatment egg count can be determined. For instance, animals with FECs below 150 epg groups should not be used if the level of the detection of the FEC technique is only 50 epg. More sensitive tests are needed in order to use animals with lower FECs. An alternate approach to performing pre-treatment fecal egg counts is to balance the level of infection among the groups using FAMACHA scores. The animals can be scored as they come through the chute, weighed, and treated according to assignment. This approach (using FAMACHA) is more cost-effective, and it saves time and labor because treatments are given during the initial visit. It is important to weigh animals and to treat them orally with the appropriate dose of anthelmintic according to their assigned group. Post-treatment fecal egg counts are collected 10 to 14 days after dosing from both the treated animals and controls. The mean fecal egg count of the untreated control group is compared with the post-treatment fecal egg count means of each drug-treated group using the formula:

$$\%\ FECR = \frac{control\ mean\ FEC - post\ tx\ mean\ FEC}{control\ mean\ FEC} \times 100$$

If pre-treatment (pre-tx) FEC means are being compared to the post-treatment (post-tx) FEC mean, then the formula is changed to:

$$\%\ FECR = \frac{pre\ tx\ mean\ FEC - post\ tx\ mean\ FEC}{pre\ tx\ mean\ FEC} \times 100$$

An automated program can be used to calculate the percent fecal egg count reduction. The RESO FECRT Program is available online at: sydney.edu.au/vetscience/sheepwormcontrol/software/FECR4.xls

An efficacious treatment will result in a FECRT of 95 to 100%. Clinical benefit is still perceived when resistance level is low (≤ 90%), but once efficacy slips to 50% or lower, treatment failure is obvious.

Larval Developmental Assay (DrenchRite®)

The larval developmental assay is available at the UGA Department of Infectious Disease through Professor Ray Kaplan's Laboratory. The DrenchRite® assay (Horizon Technology, 1996) detects resistance to benzimidazoles, levamisole, and the avermectin/milbemycin class from a single pooled fecal sample that contains sufficient numbers of viable parasite eggs (> 350 epg). Sensitivity or resistance to moxidectin can be determined based on the ivermectin dose response.[6] Eggs are isolated from the feces, and placed into wells in a specialized microtiter plate containing growth media and varying concentrations of anthelmintics. The efficacy of the anthelmintic is calculated based on the concentration

of the drug required to inhibit larval development in the wells. Results are reported in approximately 14 days. The resistance status of the anthelmintics is classified as "susceptible", "suspected (low level) resistant", and "resistant". The test requires specialized equipment and many hours of skilled labor, which is reflected in the cost ($450 per sample). Inquiries about the larval developmental assay should be directed to Sue Howell at jscb@uga.edu.

Endnote

[a] Bob Storey, personal communication

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Appendix 1. Modified McMaster's Technique

Materials: fecal floatation solution, graduated beaker, tongue depressors, weigh scale, cheesecloth, pipettes, McMaster's counting slide*, paper towels, compounded microscope and fecal samples.

Procedure:

1. Weigh out 2g (for sheep/goats) or 4g (for cattle and horses) of feces in small beaker (Nalgene 50 ml beakers work well).
2. Add 28 ml (2g) or 26 ml (4g) of sodium nitrate flotation solution to feces, and mix well.
   - For sheep and goats -- unless feces are very soft, add only a few ml. of solution at first and let feces soak for 10-15 min to soften them, then break up with tongue depressor and add remainder of solution
3. Strain through 1 or 2 layers of cheesecloth (or tea strainer), mix well.
4. Immediately pipette a sample of the suspension and fill both sides of counting chamber. Work quickly, stirring with pipette as you draw up fluid. If it takes more than a few seconds to load the first chamber, then mix fecal solution again and refill pipette before loading the second chamber. Let stand for several minutes to allow eggs to float to top. If visible air bubbles are present, remove the fluid and refill.
   - Steps 3 and 4 should be done at the same time without letting sample sit between steps - eggs are in flotation fluid and will immediately start floating. You want to add a homogeneous sample of fecal float solution to chamber.
   - Once chambers are filled, step 3 can be started for the next sample
   - Once filled, the chambers can set for about 60 min before counting without causing problems - longer than this and drying/crystal formation may begin
5. Count all eggs inside of grid areas (greater than 1/2 of egg inside grid) using low power (10x) objective. Focus on the top layer, which contains the very small air bubbles (small black circles). Count only trichostrongyle eggs (oval shaped, ~ 80-90 microns long) in both chambers. (Notations are made as to the presence of other types of eggs and oocysts).
6. Total egg count (both chambers) x 50 (2 gm) or 25 (4gm) = EPG (eggs per gram).

*Chalex Corporation 5004 228th Ave. S.E., Issaquah, WA 98029-9224(425) 391-1169; (425) 391-6669 (FAX); contact: chalexcorp@att.net; web site: www.vetslides.com

Appendix 2. Modified Wisconsin Technique

- Place 5 grams of feces with a tongue depressor in a plastic cup.
- Add H_20 sufficient to bring volume to 75 ml.
- Breakup and mix the feces well with a tongue depressor to get a homogenous suspension of feces and water.
- Pour through a single layer of cheesecloth to remove large fecal debris and squeeze out excess water as much as possible.
- Immediately after straining the fecal solution, mix again and quickly pipette 3 ml of the fecal slurry mixture into a 15ml plastic conical tube with a pipette with the end cut off at a 45 degree angle (to enlarge opening).
- Fill the tube up to the 15 ml mark with water.
- Centrifuge at 1500 rpm for 10 minutes.
- Draw off the supernatant with a pipette –careful not to disturb the top layer of sediment.
- Add approximately 5-10 ml of Sheather's sugar solution to the tube and mix well by using an applicator stick.
- Fill the test tube to the top with Sheather's sugar solution. (Slightly overfill so that there is a positive meniscus, but do not cause tube to overflow. If filled to the correct level the coverslip adheres, but there is enough space so a small air bubble is present.)
- Top with a coverslip.
- Centrifuge at 1500 rpm for 10 minutes.
- Remove the coverslip and place on a glass slide.
- Place another coverslip on the test tube (you may need to add a couple of drops of Sheather's sugar solution to get a slight meniscus). Let stand for 10 minutes.
- Remove the second coverslip and place it next to first coverslip on slideCount and differentiate all the eggs under the entire area of both coverslips.
- Multiply the total count by 5 (sensitivity = 5 epg).

Recipe for Sheather's sugar solution

Sugar (table sugar)	500 grams
Distilled water	320 ml

Add sugar to hot distilled water and stir until dissolved. Allow to cool, then pour solution into a container that is suitable for easy dispensing (a laboratory wash bottle works well). Refrigerate.