DIAGNOSIS

Examining the Evidence

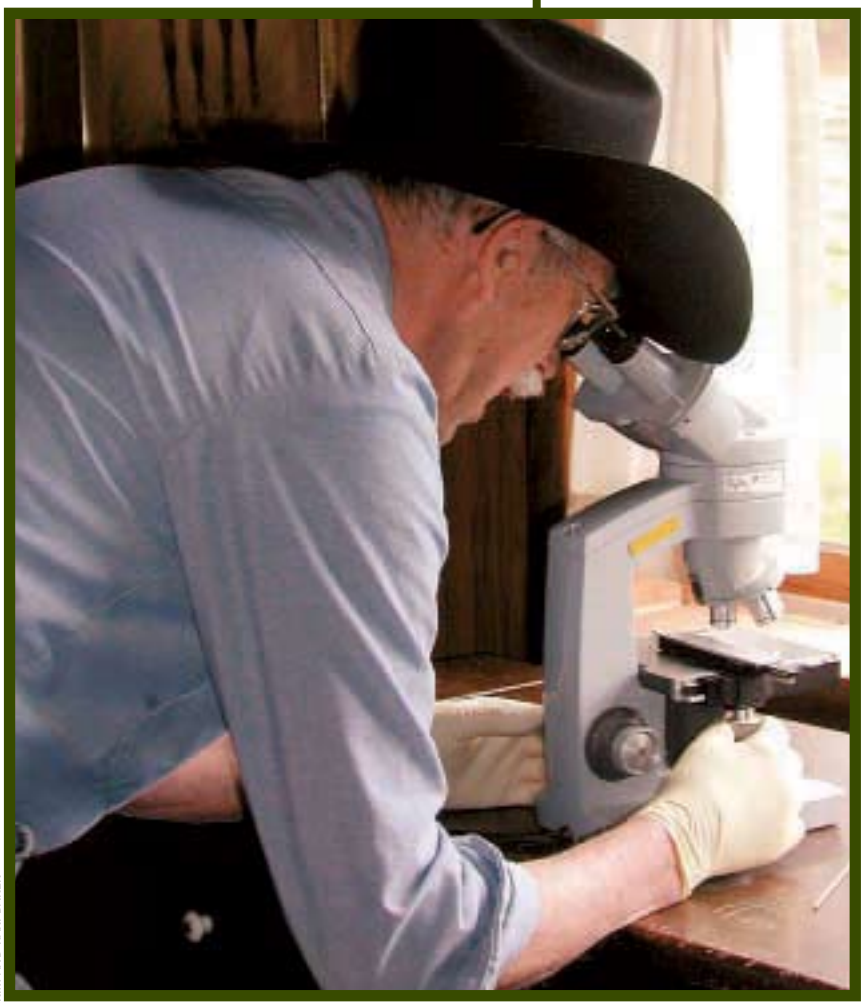
PARASITE PRIMER—PART 6

BY KAREN BRIGGS, WITH
CRAIG REINEMEYER, DVM, PHD;
DENNIS FRENCH, DVM, MS, DIPL. ABVP;
AND RAY KAPLAN, DVM, PHD

How do you really know if your worm control program is working? If your horses are looking good, are they doing as well as they could be? If they are not doing as well as you would like despite frequent deworming, is the problem due to worms or to something else? How can you really tell?

The truth is that few horse owners know for sure if they are properly controlling worms in their horses. Most people equate treating a horse with dewormer on a regular basis with providing adequate control. Unfortunately, this is almost always not the case.

Why? Largely because high levels of drug resistance, present throughout North America, render treatments on many farms relatively ineffective. To further complicate matters, the biology of the parasites we need to control has changed over the years—so the commonly used every-eight-week treatment program many of us subscribe to often fails to adequately control worms even when drug resistance is not a major problem.



KIM AND KARI BAKER

New recommendations for parasite control include examining the feces of horses to determine worm egg counts. Money spent on proper diagnosis will lead to less money spent on dewormers for horses that don't need them (or need them less often), less drug resistance, and thus better control of worms.

Another important issue is the way we view our treatment programs. Most horse owners treat all horses with the same regimen, which has been taught in the past. The reality, however, is that worm demographics might not be the same from horse to horse. Studies have demonstrated that about 20-30% of your horses harbor about 70-80% of all the worms (see page 25). Some horses carry extremely high worm burdens, while other horses—for reasons we still don't fully understand—have strong immunity and are infected with fewer worms.

Knowing this, does it really make sense to deworm all of our horses the same way? The answer is a definitive no. And new recommendations for worm control in horses (which will be detailed in a later article) are based on treating some horses more and others less. A "selective" deworming program will result in fewer treatments given (leading to less drug resistance), but if used properly will still result in better overall parasite control.

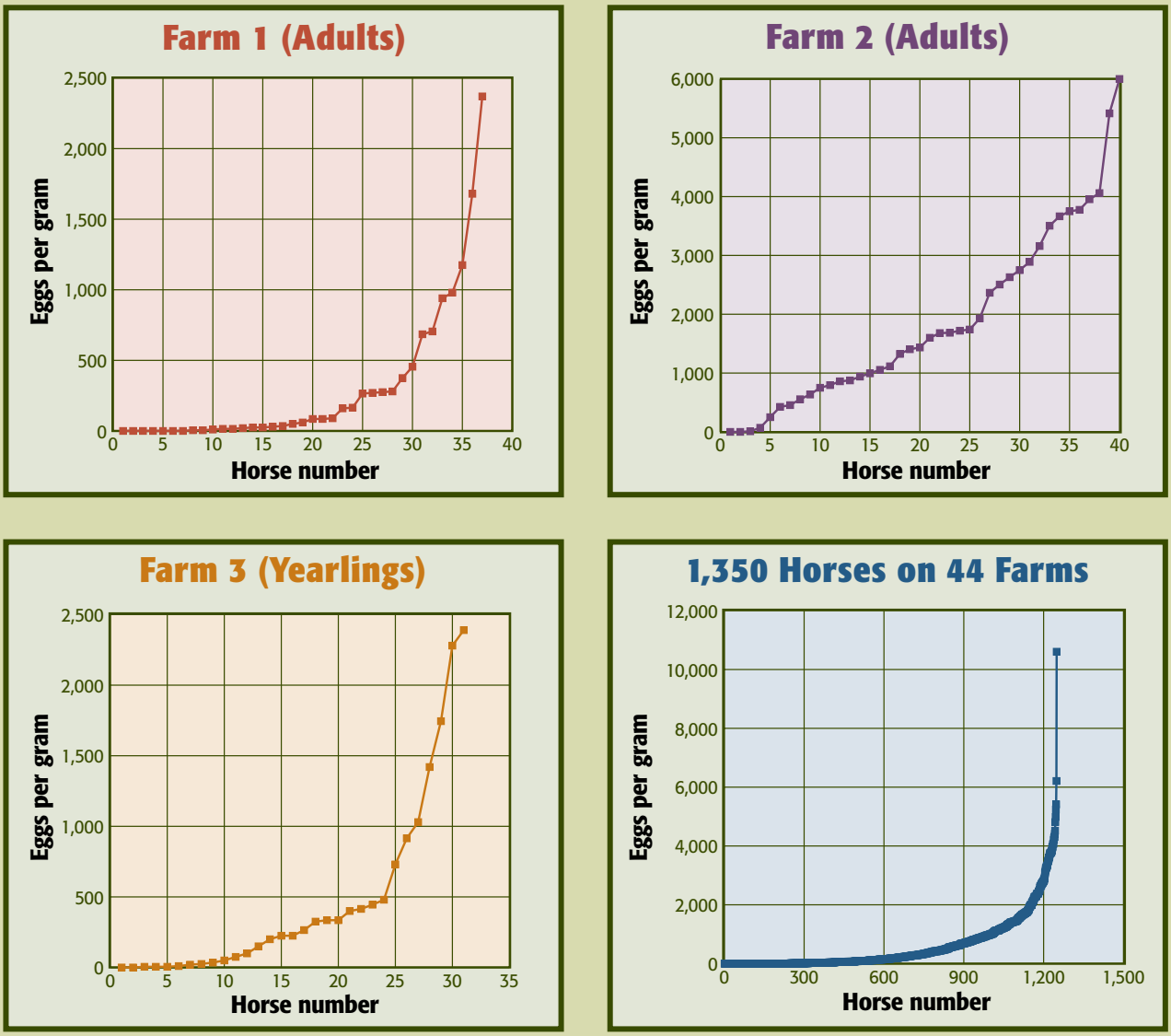
How do we know which horses to treat and not to treat? The answer is simple—we must examine the feces of horses to determine worm egg counts. Money spent on proper diagnosis will lead to less money spent on dewormers, less drug resistance, and better control of worms. Doesn't it make sense to control worms using a medically based approach that considers the needs of individual patients, rather than a recipe-based approach that treats all horses the same?

Eggs by the Dozens

Fecal examinations are done much less frequently than they ought to be, and when they are performed, most times they are only qualitative in nature, meaning that they merely demonstrate the presence or absence of parasite eggs. This can be helpful on occasion, but most often does not yield useful information. For example, small strongyle eggs account for at least 95% (and up to more than 99%) of all worm eggs passed in the manure of adult horses. Essentially, then, we can assume that all horses which have the opportunity to graze are infected with small strongyles. The presence of their eggs in a fecal sample doesn't tell the whole parasite story. Other worm eggs are less commonly seen, and when present, are usually present in low numbers.

Instead of just determining if eggs are there or not, fecal examinations should be quantitative, meaning that an actual number of eggs per gram of manure is determined. Quantitative techniques require that a standard amount of feces be tested, that the quantity of flotation solution is measured, and that a standardized volume of the mixture is examined under the microscope for counting the worm eggs

DISTRIBUTION OF FECAL EGG COUNTS IN HORSES



GRAPHS COURTESY DR. RAY KAPLAN

These graphs show the distribution of fecal egg counts (FEC) in horses on three farms in Georgia (Farms 1-3) and all horses on 44 farms in Florida, Georgia, Kentucky, and Louisiana. Farms 1 and 2 only had adult horses, farm 3 had only yearling horses, and the combined graph represents horses of all ages. Each colored square represents the FEC of a single horse, which is read on the Y axis. Note that in each case the distribution of FEC in the stable is the virtually the same. The shape of these graphs shows the aggregated nature of parasite infections, where a small percent of the animals harbor most of the parasites. In yearlings, because many have not yet reached their immune potential, the shape of the graph is a little less steep.

This aggregated pattern of parasite distribution among animals is always seen. The only thing that changes is the magnitude of the parasite level. From these graphs it is obvious that some horses need much more attention to worm control than do others. Therefore, only by performing FEC can worm control programs be optimized to achieve the desired level of control.

—Ray Kaplan, DVM, PhD

therein. Fortunately for the long-suffering soul squinting through the microscope, a quantitative test doesn't need to enumerate the whole spectrum of parasite clues; it's sufficient to quantify just the strongyle and ascarid eggs, which are the primary targets of worm control programs. Numbers of eggs of other worms, including tapeworms, are less informative, and typically are just noted as being present or absent.

Determining the number of small strongyle eggs being passed in the manure is the only reliable means of estimating the number of adult small strongyles infecting a horse. In young horses (less than 15 months of age), eggs of ascarids (*Parascaris equorum*) can also be present in large numbers.

Results of fecal egg counts (FEC) can be evaluated to:

- Identify the animals in need of the most intensive control measures;
- Assist in stable-wide parasite management decisions;
- Determine if the drugs you're using are effective; and to
- Monitor the success of your worm control program on an ongoing basis.

Checking FEC on a regular basis is the single most important thing you can do to improve your parasite control program. Future articles in this series will address how to interpret and use the results of an FEC to keep your anti-parasitic efforts at their peak, but first, let's discuss how a FEC is performed. This can be done in a lab or in your home with the right equipment.

MCMASTER METHOD

PHOTOS COURTESY DR. RAY KAPLAN

The preferred quantitative method for performing FEC is the McMaster method. A McMaster FEC is a fairly easy procedure to perform, and it is something that individual horse owners can do provided they receive adequate training and obtain the necessary equipment and supplies. Top photo: Materials required; bottom photo: McMaster's egg counting slide.

D.I.Y. Egg Counting

The preferred quantitative method for performing FEC is the McMaster method. A McMaster FEC is a fairly easy procedure to perform, and it is something that individual horse owners can do provided they receive adequate training and obtain the necessary equipment and supplies. Alternatively, veterinarians and local diagnostic labs often will perform quantitative FEC if the service is requested. If this service is not offered, ask your veterinarian why not, and encourage the vet's lab to provide this valuable test. The procedure for performing McMaster FEC is explained in detail shortly and an outline of the procedure is given in "Modified McMaster Egg Counting" on page 27.

Fecal worm egg examination methods are based on the principle of differential density. In other words, parasite eggs sink in water, but they will float in various chemical solutions that are more dense than water (technically, they have a higher specific gravity) because the eggs are lighter than the fluid used as a flotation solution. Ideally, you want the manure to sink, and the eggs to float.

The most common solution used for fecal flotation is saturated sodium nitrate, which is inexpensive, stable, and non-toxic. You probably can arrange to buy some from your veterinarian or a pharmacy. Zinc sulfate is also available commercially and can be used for the same purpose. Theoretically, one could use a concentrated solution of many other common compounds such as sugar, table salt, or Epsom salts. However, saturated sodium nitrate is the preferred solution and can be purchased pre-made or easily prepared using the recipe provided in "Saturated Sodium Nitrate For Flotation and McMaster's Techniques" on page 27. One quart of flotation solution is sufficient for about 30 McMaster examinations.

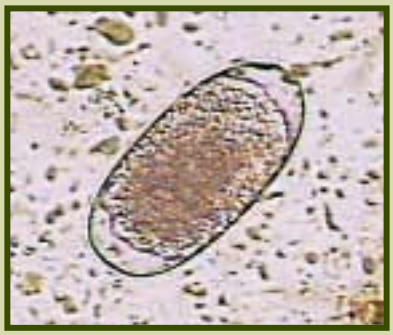
The first step is to collect freshly passed manure balls that are uncontaminated by soil or bedding. (Scavenging manure dropped on concrete or rubber mats, as your horse stands in the cross-ties, is one way to achieve this.) A Ziplock bag can be inverted over the hand and used to pick up one or two fecal balls. Seal the bag and label it with the name of the horse and the date of collection. Fresh samples work best, but accurate results can be obtained up to seven days after collection if the sample is kept refrigerated during the interim. (Do warn your friends and family if you're going to store manure in the fridge, lest they think you've finally lost it!) If samples are not refrigerated, the eggs will hatch within 12 to 24 hours. Once hatched, they cannot be counted.

Just before preparing the sample, the feces should be mixed thoroughly by kneading the bag or by stirring the contents with a wooden tongue depressor or other flat utensil.

The next step is to obtain *measured* amounts of manure and flotation solution. The solution can be measured easily with a syringe or a graduated cylinder, but

WHAT TO LOOK FOR

Ascarid eggs are round and about 90-100 microns long. They should be counted separately from the strongyle eggs.



Strongyle eggs are oval-shaped and about 90 microns long.

IMAGES COURTESY DR. RAY KAPLAN

Tapeworm eggs are D-shaped, and are not counted in the fecal egg count procedure (which focuses primarily on strongyles and ascarids).

unless one has a postal scale or laboratory balance, it could be difficult to weigh the desired quantity (four grams) of feces accurately. Fortunately, both measurements can be made simultaneously using items that veterinary practices discard on a daily basis. Empty syringe casings from 35- or 60-milliliter syringes work very well for this purpose. Ask your veterinarian to save a few for you. Small translucent plastic cups also work well.

Using a large syringe, add 26 mL of tap water to the empty casing or cup, then use a permanent marker to make a ring around the casing at the top of the water line. Then, using the syringe again, add another 4 mL of tap water and make a second line at the 30 mL mark.

Now you're ready to perform a quantitative egg count.

1. Add flotation solution (saturated sodium nitrate or zinc sulfate) to the first line (26 mL) on the calibrated cup or syringe casing.
2. Next, add small quantities of feces until the fluid level rises to the second line. (The intent is to add four grams of feces, and since one mL of water weighs 1.0 grams, the fluid displacement is a reasonable approximation of weight.)
3. Pour the mixture into a disposable paper cup or a container that can be washed between samples and mix thoroughly with a tongue depressor or flat utensil.
4. An optional—but highly recommended—step is to filter the fecal solution through two layers of cheesecloth or a tea strainer. This step removes the larger particles and makes the sample much cleaner and easier to read under the microscope.

   The next step requires a calibrated apparatus known as a McMaster's slide (see page 26). These can be purchased commercially (see "McMaster Grid Sources" on page 28 for sources), are extremely durable, and they will last a lifetime. A McMaster slide has two chambers, with a calibrated grid superimposed above each chamber. The volume under each grid is 0.15 mL, so if one counts eggs in both chambers, the volume examined is 0.3 mL, or 1% of the total fecal mixture (30 mL).
5. Mix the solution well (remember the eggs are in the flotation fluid and will start to float as soon as the mixing stops).
6. Immediately draw up about one mL of the fecal mixture into a disposable plastic pipette or a small syringe (empty one-mL or three-mL syringes work well).
7. Inject the liquid in the pipette or syringe into both chambers until the area under each grid is filled with liquid. If you get any large air bubbles, you will need to

suck the solution back out and refill the chamber.

8. Wait one or two minutes, then examine under a mechanical stage microscope (which allows the slide to move around) at 100X total magnification (10X ocular lens and 10X objective lens). If the lines in the grid are in focus, you are in the appropriate focal plane to see any parasite eggs that will float to the top of the liquid interface. It is critical that the slide be examined in the correct focal plane. If you are not looking at the top surface of the liquid where the floating eggs are found, you will not see the eggs. You can be sure you are doing this correctly if you see little black circles—these are microscopic air bubbles and are almost always present. These bubbles will float and be in the same place as the eggs. Start at an outside corner of the grid and stay between the lines so you can count the entire chamber without missing individual eggs or counting them twice. At 100X the field of view through the microscope will take up an entire column width of the grid. You will see just the edges of the vertical grid lines on the sides of the field of view. Go down one column and over and up on the next and so forth until you have counted all the eggs in the six columns of the grid. Then repeat on the second chamber grid.
9. When finished, rinse the slide and syringe casing thoroughly with warm water and you're ready to check the next horse. Do not let the slides soak in soapy water for extended periods—this causes them to become cloudy. Instead, rinse them right away with warm water and, if needed, only soak in soapy water for 10 minutes or less before rinsing again.
10. The number of eggs counted should be multiplied by 100. (Because you've examined 1% of the sample, each egg observed within the grid actually represents 100 eggs in the entire mixture.) But because you started with four grams of feces, you must divide this number by four to report the results correctly in eggs per gram (EPG). You'd get the same numerical results if you just multiply the number of eggs counted by 25.

What this means in practical terms is that for every egg you see on the McMaster slide (within the grid area only), there are 25 eggs in one gram of feces. Therefore, if feces have fewer than 25 EPG, the result will be negative (no eggs seen). This does not mean that the horse is not infected, only that infection is at a very low level. As

FECAL EGG COUNT PROCEDURE

Modified McMaster Egg Counting

Materials:

McMaster's egg counting slide*, saturated sodium nitrate**, graduated beaker, tongue depressor, balance, cheesecloth, disposable plastic pipette, paper towels, compound microscope.

Notes:

- A fresh fecal sample should be collected and it should be kept refrigerated until tested.
- A kit containing all needed supplies is available from Chalex Corp.

Procedure:

1. Weigh out 4 g of feces in a small beaker or paper cup.

2. Add 26 mL of sodium nitrate flotation solution (to bring the volume up to 30 mL) to feces. Mix well.

- *Note: If you do not have a scale, you can add feces to the 26 mL of solution and when the volume reaches 30 mL, you have added 4 g.*

3. Strain through one or two layers of cheesecloth (or tea strainer), mix well.

4. Immediately withdraw about 1 mL of the suspension with a pipette or syringe and fill both counting chambers of the McMaster slide. Work quickly, stirring as you draw up fluid. Let the slide stand for one to two minutes to allow eggs to float to top. If visible air bubbles are present, remove the fluid and refill.

- *Steps three and four should be done at the same time without letting the sample sit between steps, since eggs are in flotation fluid and will immediately begin to rise to the top of the fluid. You want to be sure to get a representative sample of the mixed solution.*
- *Once chambers are filled, step three can be started for the next sample.*
- *Once filled, the chambers can set for 60 minutes before counting without causing problems. Longer than this and drying/crystal formation can begin.*

5. Count all eggs inside of grid areas (only count the eggs which have more than half of their area inside the grid) at 100x total magnification (10x ocular lens and 10x objective lens). Focus on the top layer, which contains the very small air bubbles (small black circles). Count both chambers. Count only strongyle eggs (oval-shaped, about 90 microns long; see page 26). Ascarid eggs (round, about 90-100 microns long) can also be counted, but should be counted separately from the strongyle eggs. Do not count strongyloides (oval, about 50 microns long), tapeworm eggs (D-shaped), or coccidia (various sizes)—only notations are made as to the presence of these other parasites.

6. Total egg count (both chambers) x 25 = eggs per gram (EPG).

- *Each chamber has a depth of 1.5 mm and holds a volume of 0.15 mL. Two chambers hold 0.3 mL of fecal mixture, which is 1/100th of the total volume of 30 mL. Therefore, the number of eggs counted must be multiplied by 100. However, since you began with 4 g of feces, to yield eggs per gram, you must instead multiply by 100 and divide by 4 (or multiply by 25).*

**** McMaster Grid Sources***

Chalex Corporation, 5004 228th Ave. S.E., Issaquah, WA 98029-9224; 425/391-1169; fax 425/391-6669; chalexcorp@att.net; www.vetslides.com.
Focal Point, PO Box 12832, Onderstepoort, 0110, South Africa; +27 12 329-1210; www.mcmaster.co.za; e-mail eddy@icon.co.za.

*****Saturated Sodium Nitrate For Flotation and McMaster's Techniques***

- Sodium nitrate (laboratory grade), 400 grams
- Hot water, 1,000 mL
- Heat with stirring until boiling, then let cool at room temp. Store at room temperature—do not refrigerate as additional solute will precipitate.
- Specific gravity should be 1.20 to 1.25. This can be checked with a hydrometer. If specific gravity is too high, additional water can be added.

Note: *Fecal flotation solutions are also commercially available and can provide more accurate results.—Karen Briggs, with Craig Reinemeyer, DVM, PhD; Denny French, DVM; and Ray Kaplan, DVM, PhD*

we mentioned before, if a horse is eating grass, then he is—without exception—infested with small strongyle worms!

You'll probably have to spend a little time with your veterinarian's lab technician to learn to identify strongyle and ascarid eggs confidently, but this is not a difficult task (see images on page 25).

Those Elusive Tapeworms

Although fecal examination is excellent for uncovering the presence of ascarids and strongyles, it is a poor method for detecting tapeworm eggs. The reason for this is that tapeworm eggs are not released from the worms the same way they are from roundworms. Instead of continuously shedding eggs as do roundworms, tapeworms intermittently release segments of the worm that contain the eggs. The eggs are then released into the manure as the segment disintegrates. This results in both uneven shedding of eggs and uneven distribution of eggs within feces. Therefore, it is quite common to *not* find eggs in a fecal examination of a horse which is actually infested with tapeworms—this tends to make it difficult for a veterinarian to prove that a given horse is harboring these parasites.

However, if many horses sharing a pasture are tested, the chance of finding eggs increases. And if one infested horse turns up, chances are that his companions are infested, too.

Because tapeworm eggs are so difficult to find in feces, a method such as the McMaster, which only examines a small percent of the total sample, is not the method that should be used. Instead, a large amount of feces (at least five grams) should be examined using a procedure that concentrates the eggs by using a centrifuge. Theoretically, this procedure can detect one egg in five grams of feces, in contrast to the McMaster method that can only detect 25 or more eggs in one gram of feces. (Needless to say, you'll probably want to rely on your veterinarian for this test, unless you're in the business of collecting and shipping semen and have a centrifuge at your disposal.)

In 1995, researchers in the United Kingdom developed a test that could detect antibodies to the tapeworm *Anoplocephala perfoliata* in the blood of horses. A positive result indicated exposure to tapeworms, but not necessarily a current infection with adult worms. This test was used in a survey in the United States, as described in an earlier article in this series (see www.TheHorse.com/emag.aspx?id=4917). The U.K. test ultimately was refined so the results could be correlated to worm numbers. Unfortunately, this test is not readily available for clinical purposes at the present time.

Next issue, join us for a discussion of your horse's environment and how it contributes to his parasite load, as well as what you can do to reduce the impact!

ANNE EBERHARDT

TESTING YOUR HORSE

How Often Should You Test?

How often should fecal egg counts (FEC) be performed? The answer is not straightforward and will vary from farm to farm. When FEC monitoring is first introduced to a stable, many more samples will need to be tested than later on when your program is established.

To start, all drugs used on a farm should be tested to determine their efficacy. This can be done with one drug at a time, about two weeks after administration. More details on how to do this will be included in our future article on anthelmintic resistance in the September 2004 issue.

Once you know which drugs are doing the best job of keeping egg counts low, FEC should be performed on all horses before the next scheduled deworming with that drug. This will tell you how well your worm control program is working. The reappearance of eggs in the manure will generally take about four weeks if you've used pyrantel or one of the benzimidazoles, six to eight weeks if you've used ivermectin, and 12-16 weeks with moxidectin. These numbers will also depend on the horse—youngsters and those carrying very heavy parasite loads will generally have eggs reappear more quickly.

You can also use FEC before a scheduled deworming to determine which horses need treatment. Some horses in your herd might have low FEC (less than 100 eggs per gram, EPG) and probably don't need to be dewormed. Other horses will have high (greater than 500 EPG) or very high counts (greater than 1,000 EPG), which suggests the interval being used for treatments is too long for these horses. Doing a fecal count first might cut down on the number of tubes of dewormer your farm goes through and help fight the battle against drug resistance.

Performing FEC at frequent intervals in this manner during the first year or two (at every scheduled deworming) will show you some important things that will enable you to improve your worm control, including:

- Which drugs are effective on your farm;
- Which horses tend to always have low FEC (require less frequent deworming);
- Which horses tend to always have high FEC (require more frequent deworming);
- The interval between treatments that is required for the anthelmintics you're using (this may vary from drug to drug); and
- Are fecal egg counts getting lower over time, indicating that worms are being controlled better than when the program was started?

Once you've established an effective routine on your farm, the need for frequent FEC will be reduced because you'll have identified patterns in your herd. Thereafter, you can probably get by with only periodic monitoring.—*Karen Briggs, with Craig Reinemeyer, DVM, PhD; Denny French, DVM; and Ray Kaplan, DVM, PhD*