New perspectives in equine digestive health

Introducing the SUCCEED® Equine Fecal Blood Test™

A new antibody test that helps practitioners diagnose GI health conditions in their equine patients.
Practicing equine veterinarians are generally familiar with ulcers, colic and other digestive health issues, and how these ailments can afflict their equine patients. What may be less understood is just how widespread these problems are.

Several studies have shown that approximately 90% of race horses and 60% of show horses, have active gastric ulcers. Even leisure horses are affected, with almost 40% exhibiting gastric ulceration. What is more, a recent peer-reviewed study has shown 63% incidence of colonic ulceration in performance horses. Ulcers can have a serious deleterious effect on the performance of these animals.

Unfortunately, these sad statistics are mostly due to our own mismanagement. In particular, the way horses are fed disregards how their digestive systems have evolved. Horses in the wild graze continuously and the intermittent, high-carbohydrate feed that is provided in a barn is unnatural and potentially harmful. Many of the behavior and performance issues that are observed with stabled horses are caused by this unnatural feeding regimen, not to any innate limitations of the horse or its training. Ulcers may be responsible for much of the subclinical anemia, listlessness, weight loss and general poor health noted by veterinarians for years. In fact, the number one complaint about horses discovered to have ulcers is that their performance is declining. With these animals, environment has a much larger impact on their ability than any genetic factor.
As bad as this is, the gastric region that is home to EGUS represents less than 10% of the entire volume of the equine GI tract. The bulk of the work of digestion takes place in the hindgut. Gastric ulcers in horses are fairly well understood because they can be inspected by endoscopy, but colonic ulcers are much harder to observe. Colonoscopies are impractical due to the difficulty of evacuating the equine colon without endangering the health of the horse. Lacking evidence to the contrary, it has erroneously been thought that equine colonic ulcers are rare.

The horse's hindgut includes the large intestine, comprised of the cecum and the colon. Like humans, horses cannot digest fiber on their own. But in the hindgut of the horse, billions of bacteria and other micro-organisms ferment the structural carbohydrates that constitute fiber and convert them to VFAs which the horse can digest. These VFAs represent the primary source of energy for the horse.

As noted, however, horses today have less fiber in their diets. Instead, they are fed concentrates such as processed grain, pellets, or sweet feed. Without chaff (chopped hay) to encourage chewing and saliva, the rapid ingestion of concentrates allows these to reach the hindgut undigested. Microorganisms in the cecum and colon convert a portion of these high-energy feeds and starches into lactic acid, which can lead to hind gut acidosis. This acidic environment can shift the balance of normal microorganisms and pathogenic bacteria, leading to an inability to properly ferment forage into VFAs ("poor doers") and further can lead to colonic ulceration.
How common are colonic ulcers? In 2004, Freedom Health conducted a large-scale necropsy of 180 horses. The study revealed that 87% had gastric ulcers and 63% had colonic ulcers, resulting in a cumulative ulceration rate of 97%. The results are summarized in the following graph:

Prior to the dissection of these horses, a fecal sample was collected. This manure was tested with a guaiac stain and correlated to the gross examination of intestinal tissue. Overall, the guaiac test proved to be highly specific and significant for the existence of an ulcer, but the existence of false negatives lowered the overall accuracy of the test to 65%, roughly comparable to human outcomes with such a test.

Gastric works by binding hemoglobin and turning blue in the presence of hydrogen peroxide. The false negatives are likely due to the fact that the guaiac reaction is not very sensitive, requiring 10mg of hemoglobin per gram of stool to produce a positive result. Since many ulcers bleed only lightly or sporadically, these may be missed by the guaiac fecal blood test (gFBT).

This study clearly demonstrated that the problems with the equine digestive tract extend past the stomach. It also showed that traditional fecal blood testing kits may not be sensitive enough to detect ulceration originating in either the gastric or the colonic area. However, this initial study provided important evidence that blood can be detected in manure, and led to a fresh examination of the diagnostic possibilities.

Because the currently available diagnostic technologies are limited, many veterinarians rely on symptomology. They treat a possible ailment and, if the horse improves, they may assume the original diagnosis was appropriate. This approach, however, is notoriously unreliable, costly and can easily aggravate the original (improperly diagnosed) problem.

As a result of these limitations, Freedom Health LLC undertook an analysis of two potential marker proteins found in blood that might help to distinguish foregut from hindgut lesions. In an experiment conducted with researchers from Island Whirl Equine Colic Research Laboratory in Florida, equine blood was introduced through a gastric cannula to two experimental horses and fecal samples were then taken periodically for the next 18 hours.

This study looked at albumin, a blood protein that is known to be degraded by enzymes such as pepsin and trypsin in the stomach and duodenum. Because it is digested in this way, albumin detected in fecal matter is indicative of a hindgut lesion at a point caudal to the common bile duct in the small intestine. The study also looked at hemoglobin, which the previous research had shown can survive gastric enzymatic degradation as well as colonic bacterial digestion. Thus, albumin can serve as a proxy for hindgut lesions, while the stability of hemoglobin allows its use as an indicator of either foregut or hindgut lesions. Taken together, detection of these two proteins provides a novel technique for distinguishing these two disjoint areas of ulceration.

These two protein markers were analyzed using an Enzyme-Linked ImmunoSorbent Assay (ELISA). When the results were plotted, it was possible to see how the levels of hemoglobin peaked and then slowly fell over the 18-hour period, while albumin levels remained consistently low due to the gastric source of the serum. This provided strong support for the utility of use these two markers in a differential diagnosis.

Figure 1: OD450 results from an averaged ELISA time-course assay on two horses. Note the slow decay of detected hemoglobin and the consistently low levels of albumin.
A highly targeted test kit

Based on these promising preliminary results, Freedom Health designed a point-of-care test kit using purified antibodies targeted to equine albumin and equine hemoglobin to produce a highly specific and sensitive reaction.

The test includes two antibody wells. A couple of drops of diluted fecal material are placed in each well and after a few minutes, the presence of albumin and hemoglobin are determined and a diagnosis can be made in the barn. The following grid shows the possible test results and their likely meanings:

<table>
<thead>
<tr>
<th>Results</th>
<th>Negative albumin</th>
<th>Positive albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative hemoglobin</td>
<td>No detectable bleeding</td>
<td>Hind gut bleeding</td>
</tr>
<tr>
<td>Positive hemoglobin</td>
<td>Foregut bleeding</td>
<td>Hind gut bleeding Possible foregut bleeding</td>
</tr>
</tbody>
</table>

This test allows the practitioner to avoid a crude and potentially harmful analysis by symptom and helps to explicitly localize the source of GI lesions. A proper course of treatment should only be initiated upon an accurate appraisal, and this test seems to provide a high level of confidence.

Testing the test

To validate and calibrate the test kit, Freedom Health performed another necropsy study in August 2007, this time at a Canadian abattoir. In this study, fecal material was recovered from 86 euthanized horses and analyzed with the test kit.

The digestive tract of each cadaver was then removed and the stomach and colon were examined for ulcers. Gastric ulcers were categorized by reference to the *Dorland’s Illustrated Medical Dictionary*, using grades from 0 to 4, with the following results:

A scale similar to the gastric ranking was used for rating colonic ulcers:

The results of the antibody tests were compared to these anatomical observations to check their predictive values. For hemoglobin, the test correlated well to the overall level of observed GI ulceration when the positive gastric and colonic cutoff was set to grade 2 and above.

Diagnostic sensitivity is defined as true positives divided by the total actual positives (true positives + false negatives) and indicates how likely a positive result reflects a true positive. Specificity indicates the likelihood that a negative test result indicates a true negative.

Note that the overall hemoglobin sensitivity for our test is 75% and the specificity is 87%. The positive predictive value, which indicates the probability that a positive test predicts a genuine underlying problem, is 96%. The P-value here is 2%, indicating a high degree of significance for this assay at this cutoff value.

For albumin, the antibody test was correlated exclusively to the level of colonic ulceration, where the cutoff was set to grade 1 and above. Note that the colonic albumin sensitivity is 83% and the specificity is 73%. Here the P-value is 3%.

### Analysis of hemoglobin as an indicator of Gastric or Colonic Ulceration

<table>
<thead>
<tr>
<th>Ulcer score</th>
<th>≥2</th>
<th>&lt;2</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>63</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>negative</td>
<td>13</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>total</td>
<td>76</td>
<td>20</td>
<td>86</td>
</tr>
</tbody>
</table>

**Accuracy:** 77%
**Sensitivity:** 75%
**Specificity:** 87%
**P-value:** 2%
**Predictive Val Pos:** 96%

### Analysis of albumin as an indicator of Colonic Ulceration

<table>
<thead>
<tr>
<th>Ulcer score</th>
<th>≥1</th>
<th>&lt;1</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>62</td>
<td>3</td>
<td>65</td>
</tr>
<tr>
<td>negative</td>
<td>8</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>total</td>
<td>70</td>
<td>16</td>
<td>86</td>
</tr>
</tbody>
</table>

**Accuracy:** 81%
**Sensitivity:** 83%
**Specificity:** 73%
**P-value:** 3%
**Predictive Val Pos:** 95%
Implications for the practitioner

The SUCCEED® Equine Fecal Blood Test™ (FBT) lets you conduct a simple test on a horse at the point of care, without invasive and costly diagnostics or referrals. Veterinarians can explore the possibility of foregut and hindgut lesions and related conditions in their patients without resorting to hit-or-miss symptomology. The two-part diagnostic consists of Test A to detect the presence of albumin and Test H to indicate the presence of hemoglobin. These tests can be performed in the barn in a few minutes with no extra equipment—a fecal sample and approximately 3 oz. of clean tap water along with the contents of a single kit, are all that is required to test one horse. The results are easy to read directly from the window of the rapid-test kit, and appear within 5 minutes.

The result is a strongly differential diagnostic aid that can help to guide the veterinarian’s treatment with greater confidence. Because the SUCCEED FBT can be performed within minutes, it is possible to test a number of horses in a barn or other boarding environment in the course of a typical client visit. Given the ease and affordability of the FBT, practitioners can easily test all of their clients’ horses on a regular schedule. Consistent testing is especially important for performance horses, or wherever the care, feeding and general husbandry are less than ideal for digestive health, including intermittent feeding, high-grain diets, stall confinement, etc.

Testing a client’s horses, especially those assumed to be in good digestive health, can provide an opportunity for the veterinarian to educate their clients about these hidden GI issues. It can help you to provide a proper physiological context for many of the performance or behavioral issues horse owners and trainers face regularly, but which are often attributed to training or the horse’s individual attitude or ability.

The SUCCEED Equine Fecal Blood Test is immediately available from veterinary supply distributors, including MWI, Milburn Equine, Butler Animal Health, Webster Veterinary, Midwest Veterinary Supply and Professional Veterinary Products.

The SUCCEED® Equine Fecal Blood Test™ is available exclusively to veterinarians through veterinary supply distributors, including Butler Animal Health Supply, Milburn Equine, MWI Veterinary Supply, Webster Veterinary, Midwest Veterinary Supply, and Professional Veterinary Products.

Also available from Freedom Health...

SUCCEED® Digestive Conditioning Program®
in oral paste and top-dress granules.