

An Equine Necroscopic Study to Determine the Sensitivity and Specificity of a Dual Antibody Test

Franklin L. Pellegrini, DVM and Scott D. Carter, PhD

Overview

The ability to detect the source of fecal blood in the equine digestive tract would provide an important diagnostic for the overall health of the animal. There are several proximate causes of gastric bleeding, including but not limited to ulcers (both gastric and colonic), parasitism, infection, surgery and disorders such as protein-losing enteropathy (PLE). These problems, and the subsequent loss of blood, can adversely affect digestive health, causing pain and discomfort. Untreated, they can lead to anemia, colic and even death. Although frank blood loss may not be associated with all degrees of severity of these conditions, the detection of blood is nevertheless an excellent proxy for pathologies involving any compromise of the mucosal lining of the GI tract (Pellegrini, 2005).

Gastric lesions due to ulcers and parasites can often be visualized with a three-meter endoscope, but visualization of the rest of the equine GI tract is restricted to surgical or necroscopic inspection only. It is thus very difficult to isolate and properly treat non-specific bleeding. A test that could distinguish the source of a hemorrhage would be highly beneficial for veterinarians attempting a differential diagnosis.

Although some researchers have posited that equine blood may be completely digested by bacteria in the colon, previous research by the authors has shown that gastric bleeding can reliably be identified in fecal matter (Carter, Pellegrini, 2007). Although the blood is occult, it can be detected with guaiac stains and also by highly specific and sensitive antibody assays. In particular, a recently developed field test can quickly and accurately detect equine hemoglobin (Hg) in feces from both gastric and colonic lesions.

Enzymes, including pepsin and trypsin in the stomach and duodenum, are known to completely digest certain components of plasma, including albumin (Ab). Thus any equine Ab detected in fecal matter must have originated caudal to the common bile duct. Possible sources include colonic ulceration, PLE, and right-dorsal colitis (RDC) due to non-steroidal anti-inflammatory drug (NSAID) usage.

This suggests a novel technique to help identify the location of a bleeding lesion. Whereas Hg detection indicates bleeding anywhere along the GI tract, the presence of fecal Ab specifically indicates hind-gut bleeding. One caveat is that less serious injuries may produce Ab in the absence of Hg.

This study was designed to test the ability of antibody assays (calibrated to only detect Hg and Ab levels that are above background) to correctly predict observed levels and locations of GI tract ulceration.

Methods

In this blind study, 86 horses in a Canadian abattoir were submitted to a thorough examination of both their stomachs and colons. Immediately after each horse was euthanized, fecal balls were recovered from the anus using a sterile glove. The samples were tagged and kept with the cadaver. This material was tested using two antibody assays in the form of "rapid test" kits: one for hemoglobin and one for albumin. This test took about 20 minutes, and the results were then numbered and logged.

The digestive tract was then removed and the stomach and colon were tied off for separate examination. The stomach and colon were split open and examined for ulcers. Gastric ulceration was categorized by reference to the *Dorland's Illustrated Medical Dictionary*, and included no reference to the etiology of any injury. Dorland's defines gastric ulcer grades from 0 to 4 as:

0. Normal, unulcerated tissue. The epithelium is intact and there is no thickening or abnormal coloring.
1. The mucus lining is intact, but there are areas of thickened, discolored tissue.
2. Small, single or multiple ulcers present.
3. Large, single or multiple ulcers.
4. Extensive, deep ulceration.

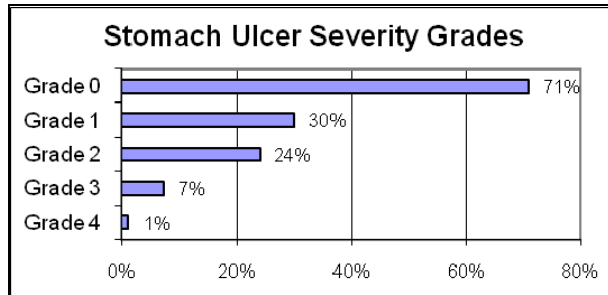
A scale similar to the gastric ranking was used for rating colonic ulcers:

0. No visible damage
1. Lightly dispersed underlying tissue damage covering less than 5% of any quadrant or small, non-bleeding focal damage not penetrating the mucosa.
2. Dispersed underlying tissue damage covering at least 20% of any quadrant or focal damage with bleeding. Note that grades 0 and 1 are defined as non-bleeding ulcers.
3. Dispersed, dark underlying tissue damage covering at least 50% of any quadrant or full thickness focal damage and bleeding.
4. Dispersed, very dark underlying tissue damage covering 90% of any quadrant or large, full thickness (to musculature) focal damage and bleeding.

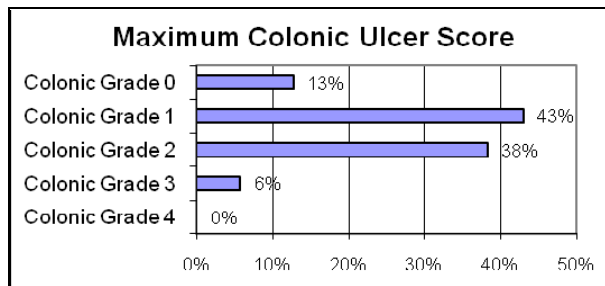
These gross anatomical observations were then numbered and logged.

Results

Using the gastric scale, the observed severity of stomach ulcers in the euthanized horses was rated:



For these same horses, the following incidence of colonic ulceration was observed:



The results of the antibody tests were compared to these anatomical observations to check their predictive values.

For **hemoglobin**, the background-corrected antibody test correlated to the overall level of observed GI ulceration, where the positive gastric and colonic cutoff was set to grade 2 and above:

Ulcer score	≥2	<2	total
positive	53	2	55
negative	13	18	31
total	66	20	86
	Accuracy:		77%
	Sensitivity:		75%
	Specificity:		87%
	P-value:		.02
	Predictive Val Pos:		96%

Note that the overall hemoglobin sensitivity (true positives divided by the sum of true positives and false negatives) is 75% and the specificity (true negatives divided by the sum of true negatives and false positives) 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 background-corrected antibody test was correlated exclusively to the level of colonic ulceration, where the cutoff was set to grade 1 and above:

Ulcer score	≥1	<1	total
positive	62	3	65
negative	8	13	21
total	70	16	86
	Accuracy:		81%
	Sensitivity:		83%
	Specificity:		73%
	P-value:		3%
	Predictive Val Pos:		95%

Note that the colonic albumin sensitivity is 83% and the specificity is 73%. Here the P-value is 3%.

Discussion

Due to the difficulty and expense of endoscopy, equine veterinarians are often forced to rely on symptomology and treatment response as a diagnosis for GI tract pathology. Occasionally, owing to this lack of diagnostic accuracy, such a regimen may actually worsen the problem. Given that gastric and colonic ulcers are prevalent in horses – especially performance and show horses – and that ulcers can lead to colic and even death, an accurate differential test would be invaluable.

This study demonstrates that a two-part Hg and Ab test can be designed to effectively provide the following differential diagnostics:

- **Positive Hg and positive Ab:** indicates hindgut compromise, but may include gastric bleeding as well.
- **Negative Hg and positive Ab:** indicates hindgut mucosal compromise.
- **Positive Hg and negative Ab:** indicates gastric bleeding with a low probability of colonic mucosal compromise.
- **Negative Hg and negative Ab:** indicates no colonic ulceration and a Gastric Ulcer score of 0 or 1 (no or low-grade injury).

This two-part test should become a welcome addition to the equine veterinarian's toolkit. As well as a powerful diagnostic of localized ulceration, it can provide invaluable post-operative feedback and help in the diagnosis of other GI diseases such as PLE and RDC.

References

- Pellegrini, Franklin L. Results of a Large-Scale Necroscopic Study of Equine Colonic Ulcers. *J Equine Vet Sci* 2005; 25 (3) 113-117.
- Carter, S., Pellegrini, F.L. The Use of Novel Antibody Tools to Detect the Presence of Blood in Equine Feces. September, 2007.