The Use of Novel Antibody Tools to Detect the Presence of Blood in Equine Feces

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Overview

The equine digestive tract can suffer from a variety of injuries, such as parasitism, bacterial infection and ulcers, which can result in internal blood loss, pain and diminution of performance. The presence of these injuries can compromise overall digestive health and function, and may lead to larger problems such as colic and even death.

Although gastric ulcers can be directly observed with a three-meter endoscope, this procedure requires a high level of training and can be quite costly. Due to the inability to safely evacuate the hind gut of a horse, colonic visualization is not practical, leaving the equine veterinarian with few options. Unfortunately, it is now known (Pellegrini, 2005) that colonic ulcers are common in horses and may, due to their unique etiology, respond differently to treatment. Any technique that would allow a differential diagnosis of GI tract problems would thus be greatly welcomed.

While staining techniques using guaiac acid have been employed, the sensitivity and specificity of such tests is poor and prone to interference (Pellegrini, 2005). Antibody technology, on the other hand, is known to provide extremely sensitive and specific results. Blood-specific antibodies have been used with great success in humans, but such methodologies have yet to be developed for equine use.

The purpose of this experiment was to test the specificity and sensitivity of two newly developed antibody assays specific for components unique to equine blood as a foundation for readily detecting digestive tract bleeding in horses. After verifying sensitivity using ELISA techniques, these antibodies were then used to detect the presence of two specific antigens in fecal blood over an 18-hour period after the introduction of blood into the stomachs of two horses. In addition, 50 horses were tested for the presence of these antigens to see if they would match the rates of ulceration seen in previous necroscopic studies.

Methods

Antibody Production: Two polyclonal antibodies, one specific for equine hemoglobin (Hg) and the other for albumin (Ab) were prepared in a rabbit model. A peptide sequence unique to each protein was chosen and synthesized in the lab, further conjugated to enhance immunogenicity, and then injected into rabbits. At 2 and 3 months, the rabbits were given booster injections of the peptide sequence to further enhance their immune reaction and maximize antibody production. At the conclusion of three months on the protocol, bleeds were taken from each rabbit, serum was separated, and all serum samples were pooled.

Antibodies were then purified using an affinity column containing the original peptide sequence, ensuring that only antibodies to the chosen sequence were in the final antibody preparations:

AB1: Prepared using the 35 AA C-terminus of equine albumin.

AB2: Prepared using both the 22 AA N-terminus and 36 AA C-terminus of equine hemoglobin.

Sample Collection: In experiment 1, equine blood (40mls) was introduced through a gastric cannula to two experimental horses. Representative fecal samples were taken periodically for the next 18 hours, mixed with a 1X TRIS buffered saline and stored frozen. In experiment 2, representative fecal samples were taken from 25 active racing horses and 25 pleasure horses, mixed with 1X TRIS buffered saline and stored frozen.

ELISA: Standard ELISA methodology was used to prepare each sample which were then adhered to standard test wells. For detection, samples were first exposed to either AB1 or AB2 for primary detection, and then further reacted with an anti-rabbit antibody labeled with peroxidase for colormetric optical density (OD_{450}) detection. All samples were read using a standard microwell plate reader.

Results

Experiment 1: Figure 1 (below) represents data collected from an averaged time-course assay on two cannulated horses. Data points have been corrected for background and plotted for 18 hours of fecal collection.

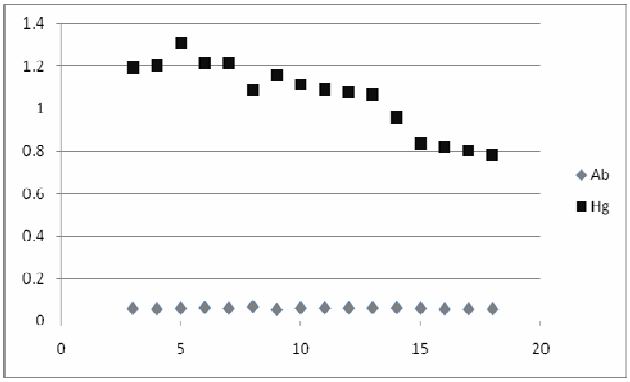

Figure 1: OD_{450} results from an averaged ELISA time-course assay on two horses. Note the slow decay of detected hemoglobin and the consistently low levels of albumin.

Experiment 2: Figure 2 (below) represents one-time point data for race and pleasure horses. As the results indicate, Ab was detected in approximately 30% and Hg was detected in approximately 60% of all horses. Both Ab and Hg were detected in about half of the race horses. Among pleasure horses, Ab was detected in fewer than 10%, while Hg was found in almost 70%.

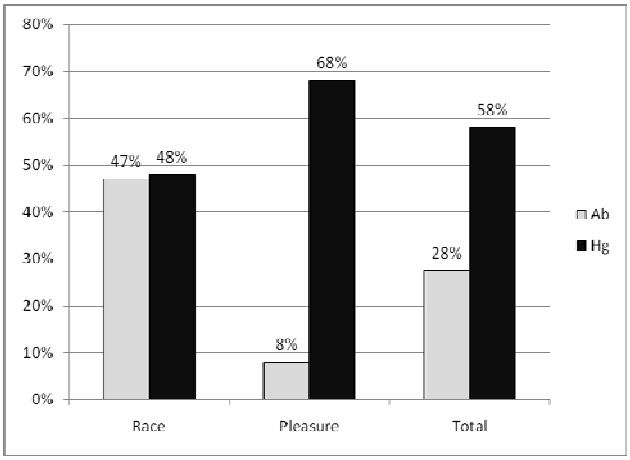

Figure 2: Percentage incidence of albumin and hemoglobin detected for horses grouped by activity level.

As in experiment 1, both proteins were detected in fecal samples, corrected for background and averaged.

Discussion

This paper details the use of novel antibody techniques to detect the presence of equine serum albumin and hemoglobin in fecal matter. These proteins have peptide sequences that are uniquely equine, and thus would only be present in the equine digestive tract from either ingested equine blood or from bleeding occurring at some point in the digestive tract.

Currently, equine veterinarians rely on endoscopy, symptomology and/or treatment response for diagnosis. All of these approaches have severe limitations. Endoscopy only accounts for the gastric region and a three-meter endoscope may not be readily available. Gross observation is highly subjective and is hindered by the fact that external symptoms may be attributable to a variety of causes. Finally, treatment response takes time and may actually add to the problem if misdirected.

The use of antibodies for detection of human fecal occult blood has been well established for the diagnosis of cancer and ulcers (Barrows, 1978), but this technology has not yet been used in equines. The ability to reliably detect occult fecal blood in horses would radically improve the equine practitioner's diagnosis, leading to more timely and accurate treatment options. As well as the detection of ulcers, such a test would enhance the veterinarian's ability to monitor an equine patient following intestinal resection, where blood loss is of particular concern and may not be detected for 24 hours or more running serial packed cell volumes.

Also useful would be the ability to distinguish between blood of cranial origin (bleeding prior to the duodenum) and of caudal origin (bleeding located in the cecum and colon). These two regions of the GI tract have completely different ecologies and demand targeted treatment. To accomplish this differentiation, a test would have to employ indicators particular to each region of interest.

This study looked at albumin, a protein that is degraded by enzymes such as pepsin and trypsin in the stomach and duodenum. It also looked at hemoglobin, a protein that we have shown can survive enzymatic degradation as well as bacterial digestion in the colon (Pellegrini, 2005). Thus, serum Ab serves as an indicator of caudal bleeding only, while the stability of Hg allows its use as an indicator of either caudal or cranial bleeding. Taken together,

detection of these two proteins can provide an important differential diagnosis.

While these results had initially been confirmed in vitro using blood added to manure, experiment 1 establishes the viability of the technique in vivo. From whole blood (containing both Hg and Ab) introduced into the stomachs of two horses via cannula, it was possible to detect levels of Hg peaking and falling over an 18-hour period, while Ab levels remained consistently low.

Experiment 2 was conducted to further establish the validity of this dual antibody approach. 50 horses were selected at random, 25 from an active racing population, and 25 from a leisure horse population, and one- time point fecal samples were taken and evaluated using ELISA techniques identical to experiment 1. As indicated by the results, approximately 60% of all horses in the study have indications of cranial bleeding or caudal bleeding, while approximately 40% of all horses had indications of caudal bleeding only. This shows a good correspondence to previous work looking at incidences of gastric and colonic ulcers during necropsy (Pellegrini, 2005, Bedding, Pellegrini, 2006).

When looked at as a whole, these experiments confirm the validity of both detection of fecal occult blood in equine manure, as well as the potential differentiation of cranial and caudal bleeding in horses. The implications of this research are far reaching and set the stage for development of diagnostic tools that can help the equine practitioner more precisely and quickly diagnose digestive tract health issues in equine patients.

References:

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