Take Home Message—Interpretation of clinical pathology values can be difficult if consideration of the vagaries of testing, the reason for sampling, and organ or tissue specificity are not considered. Initial testing with comprehensive panels interpreted with consideration of clinical exam findings will enable more targeted laboratory testing to monitor progress of condition.

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I. INTRODUCTION

This presentation is not intended to be a comprehensive review of the entire spectrum of clinical pathological investigation of the horse. Rather, it seeks to highlight considerations in the interpretation of common and uncommon clinical pathology results, and review common and interesting clinical scenarios in equine practice.

II. CONSIDERATIONS WHEN INTERPRETING CLINICAL PATHOLOGY VALUES

Why Did You Take the Blood in the First Place?

Client pressure to exhaustively test the horse and a veterinary desire to not miss anything creates an environment where over-testing is possible. Maximum information for the financial expenditure is the goal. Consider the likelihood that the test in question will yield useful information. The predictive value of the test is increased in situations where the likelihood of abnormality or disease is higher.

Chances of an Abnormality are High with Multiple Analytes

The reference interval is set by convention to encompass the central 95% of the reference values obtained from a healthy population of comparable individuals to that for which the test is designed to provide meaningful information. Statistical methods are used for the selection of reference limits, being based on the number and distribution of the reference values. The 2.5th and 97.5th percentiles serve as the lower and upper reference limits, that is, the bottom 2.5% and upper 2.5% are set outside the normal range. This means a total of 5% of values will occur outside the reference interval for a ‘normal’ population of tested animals even though the animal may be judged ‘healthy’.

The chance of getting a value within the reference range for any single analyte is therefore 0.95. Routine biochemical panels measure up to 20 separate clinical variables. Using this as an example, the chance of getting all 20 variables within the normal range in a healthy horse is \((0.95)^{20}\), or 0.36. The probability of getting at least one value outside the normal range in these circumstances is therefore equal to \(1-(0.95)^{20}\), or 0.64 i.e. 64% of the time at least one (or more) result will lie outside the reference range in a healthy horse. When 10 analytes are measured, this chance is equal to 40%.

Duration and Method of Venipuncture

The hemogram is altered by venipuncture for longer than 30 seconds due to splenic contraction resulting from the actions of the sympathetic-adrenal and hypothalamic-pituitary activity. Regarding method, the use of vacuum tubes was associated with cell damage in one study, and higher-gauge needles are considered preferable by some practitioners. Significant differences in hematological parameters between venipuncture and intravenous catheter blood draws has not been shown.

Order of Sample Drawing

The order of blood tube filling can be important. EDTA tube prior to a serum or heparin tube for biochemical testing can allow contamination with the potassium EDTA anticoagulant on the needle of the collection syringe, yielding a spurious high potassium concentration.

Proper Tube Mixing

Shaking can lead to hemolysis. Invert all tubes with additives to mix the additive evenly with the blood (8-10 times) to prevent clotting.

Correct Specimen Volume

Ensure the proper amount of blood is added to the tube for the amount of additive. This is very important when assessing clotting times (citrate) and using small volume tubes.

Sample Handling
Lysis of erythrocytes due to poor sample handling (heat, direct sunlight or delayed analysis) can spuriously elevate K levels, therefore sample protection and timely assay post collection is necessary. Glucose can be artificially low due to cellular consumption, therefore blood collection in a fluoride-oxalate tube (to cease cellular metabolism) or cold storage (on ice, refrigerator) to minimize consumption is advised. A whole blood specimen should be centrifuged and the serum or plasma removed from the red blood cells within two hours after the venipuncture, and once separated will be stable at room temperature for eight hours and up to 48 hours at 2-4°C.\(^4\)

**Breed**

Light horse breeds or ‘hot-blooded’ have higher RBC numbers, hemoglobin (Hb), hematocrit (Hct) and blood volume compared with draft horses or ‘cold-blooded’ breeds. Thoroughbreds have smaller mean cell volume (MCV) values than draft horses. Breeds ancestrally closer have minor differences in Hb, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). A seemingly low hematocrit can therefore be found in healthy draft horses and pony breeds. American miniature horses have lower RBC numbers, hemoglobin, and hematocrit but higher MCV, MCH and MCHC than other breeds.\(^5\) Donkeys are similar to ponies, but have a much higher MCV.\(^6\) With respect to leukocytes, hot-blooded horses have higher numbers when compared with cold-blooded horses.\(^7\) Neutrophil:lymphocyte ratios also differ, with Thoroughbreds and Arabians having a ratio of 1.0 compared with cold-blooded horses and miniature horses with ratios of 1.7 and 0.67, respectively.\(^8\)

**Post-Testing Factors**

Reporting of results – do they seem appropriate for the reference range selected or could transcriptional errors have occurred? The values obtained should be in the appropriate unit for the analyte measured, and examination of the full report for completeness should not find blank areas corresponding to requested testing. Values that are reported at levels incompatible with life or that do not seem reasonable given patient presentation may prompt consideration of a repeated laboratory assay or sample submission.

### III. CLINICAL PATHOLOGY VALUES AND WHAT THEY MEAN

**Hematology**

**Erythrocytes**

Parameters including size, shape and color are widely used to detect abnormalities. Diagnostic significance can be attached to other findings. An increased erythrocyte count can be relative (dehydration) or absolute (polycythemia).

Decreased erythrocyte counts can have many causes.\(^6\) Erythrocyte-associated parasites, such as *Babesia* spp., may be seen; however, absence does not rule out infection, as numbers are low and appearance in peripheral blood cyclic. Agglutination can be confused with rouleaux formation (normal finding in equine blood); however, agglutination produces clumping. Agglutination suggests an immune-mediated anemia, which may be idiopathic (viral or parasitic infection), drug-related, or secondary to heparin administration. Oxidative damage is indicated by the presence of Heinz Bodies or eccentricocytes (onion, Red Maple leaf intoxication). Schistocytes indicate vascular disease, including disseminated intravascular coagulation (DIC), hepatic disease, thrombosis, or ongoing vasculitis (local or generalized).

Blood loss anemia is most recognized in association with a precipitating event (internal trauma, laceration, foaling) and along with hemolytic diseases (intravascular, extravascular) will not be covered. Anemia due to decreased erythrocyte production is more difficult to assess due to an often-insidious onset.\(^6\) Iron deficiency is usually a foal problem due to a marginal iron supply from mare’s milk but in adults may result from chronic blood loss. It is initially normocytic and normochromic, but with time iron deficiency progresses to microcytic and hypochromic anemia. Iron deficiency may also occur with chronic parasitism, gastric ulceration or coagulation problems. Evaluation of a bone marrow aspirate for iron and finding a predominance of rubricytes and metarubricytes is confirmatory of iron deficiency. The anemia of chronic disease/inflammation is the most common form of anemia in the horse. This form of anemia is usually mild and suggestive of underlying disease. With renal disease a definitive marrow response to sometimes decreased erythropoietin is suspected, or a reduced erythrocyte lifespan may be responsible. Myelophthisic diseases are rare but when present they displace or destroy the normal precursor cells of the bone marrow. This results in anemia and, even more commonly, a pancytopenia result. Neoplastic infiltrates are most common, and atypical cells may not be released into the peripheral circulation, requiring bone marrow biopsy for classification.

Toxic damage to the precursor cells may result in aplastic or hypoplastic anemia, as can be seen with some immune-mediated processes.\(^6\) While uncommon and usually idiopathic, rare cases have been reported with administration of phenylbutazone and other medications (antimicrobials such as penicillin). The combination of anemia or pancytopenia in peripheral blood with a bone marrow biopsy demonstrating severe hypoplasia or aplasia and replacement of marrow by fat is confirmatory of toxic damage.

**Leukocytes**

Interpretation of leukocytosis depends on the cells represented.\(^6\) Leukocytosis with a mature neutrophilia is the most common finding and is associated with inflammation or iatrogenic secondary to corticosteroid administration. The stress leukogram manifests as slight to moderate leukocytosis with neutrophilia and eosinopenia. Monocytosis may or may not be present. With the stress leukogram, band cells and other immature leukocytes are absent. Band cells are consistent with the acute inflammatory response, with a regenerative left...
shift defined by mature neutrophils in greater numbers than band cells and a degenerative left shift showing the number of band cells (and possibly more immature myeloid cells) exceeding mature neutrophils (consistent with severe inflammation). Lymphocytosis results from antigenic stimulation, as part of a generalized leukocytosis, or with lymphoid neoplasia. Monocytosis is uncommon but can be part of a stress leukogram or where necrosis or chronic inflammation occurs. Other uncommon findings are eosinophilia and basophilia, in which circumstances one should consider parasitic infestation, allergic reactions, hypersensitivity reactions or antigenic stimulation, mast cell conditions or myeloproliferative disease involving these cell lines.

Leukopenia may involve any or all of the cell lines, with a mild decrease in an otherwise healthy horse most likely of no significance. Leukocyte migration as a result of an inflammatory or infectious stimulus (e.g., Salmonellosis) and decreased production are of clinical importance. Leukopenia may involve any or all of the cell lines, with a mild decrease in an otherwise healthy horse most likely of no significance. Leukocyte migration as a result of an inflammatory or infectious stimulus (e.g., Salmonellosis) and decreased production are of clinical importance. Destruction of bone marrow during myelophthisic disease usually causes pancytopenia. Myeloid neoplasia may or may not affect peripheral leukocyte count; however, abnormal myeloid cells usually appear in circulation. Bone marrow aspiration and organ biopsy (lymph node, spleen, liver) confirms myeloid neoplasia.

Lymphoid leukemia has atypical or immature lymphoid cells in peripheral blood in approximately 50% of horses usually in small numbers. Lymphocytosis with large numbers of peripheral neoplastic lymphocytes is associated with bone marrow infiltration. Acute lymphoid leukemia results in peripheral lymphocytosis with atypical and immature cells. Chronic lymphocytic leukemia results in large numbers of small mature and well-differentiated lymphocytes entering peripheral blood. Plasma cell tumor (multiple myeloma) is rare in horses, with neoplastic proliferation of plasma cells found in bone marrow, spleen, and lymph nodes and concurrent monoclonal gammopathy.

**Platelets**

Thrombocytopenia may result from destruction (immune-mediated), consumption during coagulation (DIC), whole blood loss, sequestration (spleenic) and decreased production. Thrombocytopenia is associated rebounding post thrombocytopenia, chronic inflammation, neoplasia (secondary or during a myeloproliferative disorder), and with general marrow stimulation. Platelet function is also important to consider, and this can be tested by mucosal template bleeding time assessment. Nonsteroidal anti-inflammatory drugs may result in decreased platelet function. Decreased platelet function also has been reported with uremia, liver disease, anemia, neoplasia and a variety of immune-mediated and hereditary conditions. The first step in the investigation of a suspected platelet induced coagulopathy (prolonged patient bleeding in the absence of abnormal laboratory bleeding times) is to obtain a manual platelet count from blood collected into a sodium citrate tube. If platelet count is within range, a platelet function disorder should be suspected.

**IV. ELECTROLYTE ABNORMALITIES**

Sodium: Hypokalemia results from the loss of sodium (Na) containing fluids: reflux, diarrhea, diuretic usage, renal disease, sweat, exudative wounds or burns over large areas. Accumulation of Na-rich fluids in body cavities (ie, the third space effect), high-volume pleural effusion, ascites, and hepatic insufficiency can also be responsible. Hyperkalemia is also seen in chronic renal disease or psychogenic polydipsia with decreased renal function or an absence of access to salt, and with hypoadrenocorticism resulting from prolonged exogenous steroid administration. Hyperlipidemia, hyperproteinemia and hyperglycemia may also cause hypokalemia. Hyperkalemia results from loss of water relative to the electrolyte content. This may occur with the use of diuretics, renal disease, diabetes insipidus, or inadequate water intake (deprivation, exhaustion, reduced thirst mechanism). More common reasons are diarrhea and high volume nasogastric reflux.

Potassium: Hypokalemia results from gastrointestinal (GI) or renal loss, diuretic usage, renal disease, or total body depletion as a result of decreased intake and increased loss. Alkalosis from rapid bicarbonate administration may shift potassium (K) from the extracellular to intracellular compartment. Hyperkalemia elevation may be artifactual, resulting from hemolysis or a delay in separation of serum from blood cells or the clot. Clinically significant hyperkalemia occurs in metabolic acidosis, hyperkalemic periodic paralysis (HYPP), renal disease, ruptured bladder, urethral obstruction, hypoadrenocorticism, and tissue necrosis.

Chloride: Hypochloremia usually occurs in conjunction with and proportional to hyperkalemia, being the result of overhydration. A decrease in chloride independent of decrease in Na occurs with metabolic alkalosis (exhaustion syndrome with loss of Cl in sweat) or as compensation for respiratory acidosis. Hyperchloremia paralleling hyperkalemia is usually the result of dehydration, but can also be seen with metabolic acidosis or compensation for respiratory alkalosis.

Phosphorus: Hypophosphatemia is typical of chronic renal disease or reduced GI absorption. Hyperphosphatemia occurs with decreased glomerular filtration rate, acute renal disease, or hypoparathyroidism. An artifactual increase occurs with a delay in separation of serum from the cells or clot.

Calcium: Hypocalcaemia occurs with hypomagnesaemia, acute or chronic renal failure, colitis, anorexia, rhabdomyolysis, oxalate toxicity, rapid oxytetracycline administration, furosemide usage, excessive bicarbonate administration, cantharadin (blister beetle) toxicity, lactational tetany, and exhaustion syndrome in endurance horses. While decreases in calcium may occur with decreased albumin, ionized calcium can be normal. Hypercalcemia occurs as a paraneoplastic syndrome in lymphoma or gastric squamous cell carcinoma, but also other malignancies. It may occur in chronic renal failure; however, hypercalcemia is sometimes seen. Hypercalcemia with hypophosphatemia occurs in primary hyperparathyroidism, and in secondary
hyperparathyroidism in conjunction with hyperphosphatemia (high grain ration with grass hay).9,10

Magnesium: Decreased serum Mg should be considered a differential diagnosis during tachycardia post colic surgery or during colitis. As parathyroid hormone (PTH) is Mg-dependent, hypocalcemia will be worsened and may be refractory due to hypomagnesemia.9

V. SERUM PROTEINS

The combined level and relative amounts of the individual blood protein components is routinely measured and often relied upon to interpret disease. Elevated total protein (TP) with elevated albumin and normal or elevated globulin level is consistent with dehydration. Increased TP with normal or decreased albumin but elevated globulins suggests dehydration, decreased production or selective loss of albumin in association with dehydration (chronic liver disease, glomerulonephritis), inflammation (albumin production is decreased) or immune stimulation. Decreased TP with both decreased albumin and decreased globulins indicates a protein-losing enteropathy, whole blood loss with subsequent dilution, and rarely a protein-losing nephropathy. Decreased globulins (leading to possibly decreased TP while albumin remains normal) are consistent with immunosuppression or the incomplete passive transfer of antibodies to the neonate.

Serum protein electrophoresis, while a specialized test, is useful where globulin evaluation is present.11 Monoclonal gammopathy suggests lymphoproliferative disease, classically multiple myeloma but other lymphoid malignancies may be responsible. Alpha globulin and beta globulin elevations are often due to either reactive or inflammatory processes. Elevations in beta globulins are characteristic of chronic hepatic disease or chronic parasitism. Polyclonal gammopathy is consistent with chronic immune stimulation due to bacterial or viral infection (chronic abscessation, bacterial hepatitis or chronic endotoxin hepatic insult). Hypogammaglobulinemia suggests immune dysfunction such as selective or combined immunodeficiency, immune suppression, or partial/complete failure of passive transfer to the neonate.

VI. EVALUATION OF SPECIFIC ORGAN SYSTEMS AND CONDITIONS

Kidney

Elevations in urea and creatinine reflect decreased glomerular filtration rate.10 This may be associated with pre-renal, renal, or post-renal conditions. Pre-renal azotemia is readily corrected with appropriate fluid therapy, and may be suggested by history of fluid deprivation or excessive losses, clinical signs of dehydration and concurrent elevations in urine specific gravity. In addition to serum creatinine and urea, consideration of albumin and electrolyte levels (notably Na) may be suggestive of renal compromise. Evaluation of urine specific gravity and urinalysis with ancillary tests to rule out concurrent disease that may affect the kidney provides the basis for determination of whether or not renal disease is present. Elevation of creatinine and urea with a concurrent decrease in urine specific gravity is associated with renal disease, with 65-70% of the nephrons dysfunctional before this occurs. Additional tests include urinary protein, urinary creatinine clearance ratios (urinary fractional excretion of electrolytes), urinary GGT: urinary creatinine ratios, and the presence of tubular casts on urinalysis.10 Care should be exercised in interpreting results for the horse on intravenous fluids as increased urine production can alter urinary enzyme concentration and wash out products of tubular degeneration. Hypostenuria (active dilution of the glomerular filtrate) necessitates the presence of appropriate renal function. A renal biopsy is needed to provide definitive diagnosis of the pathological process involving the kidney, its severity of the disease, and likely prognosis. Post renal conditions (ureteral or urethral obstruction, urinary tract disruption leading to uroperitoneum or subcutaneous urine accumulation) display reduced or absent urine production with a disproportionate increase in urea compared with creatinine.

Polyuria and Polydipsia (PUPD)

With extensively managed horses, or even those with periodic turnout, it is difficult to ascertain water intake, frequency of urination and urine volume. Diet, workload, environment and housing stabilizing conditions all affect intake and output. However, collection of a single urine sample may be informative, with finding of a specific gravity below 1.020 suggesting at least a partial decrease in concentrating ability. Urine specific gravity < 1.008 suggests active dilution of urine due to increased water intake (renal function is intact).10

Renal disease is suggested in PUPD by increases in urea, creatinine, and isothenuria (urine specific gravity 1.008-1.015). The finding of granular casts during urinalysis (rapid evaluation post collection) is indicative of tubular degeneration, with excessive protein associated with this and glomerular disease. Urinary creatinine clearances ratios for electrolytes also assess renal tubular function. Where renal function appears normal, psychogenic or primary polydipsia must be considered (important to exclude other causes of PUPD especially pituitary pars intermedia dysfunction and diabetes insipidus).

Liver

An absence of hepatic enzyme elevation does not eliminate the possibility of chronic liver disease with minimal ongoing hepatocellular damage. Decreased protein levels, especially decreased albumin, may be the only indicator of failure and in rare chronic cases no biochemical abnormalities exist.

Elevations in γ-glutamyltransferase (GGT) occur due to biliary damage or cholestasis and although present in other ductular epithelium systemic elevations are generally considered solely due to biliary disease; however, pancreatitis may possibly
cause elevations. Active hepatocellular damage is indicated by elevations in sorbitol dehydrogenase (SDH).\textsuperscript{12}

Increased direct (conjugated) bilirubin is considered a reliable sign of hepatic disease, with levels in excess of 30% of total bilirubin levels suggesting cholestasis. Elevations in indirect (unconjugated) bilirubin occur with hemolysis, intestinal obstruction, circulatory compromise, and administration of corticosteroids. Neonate levels are consistently higher than adults.

Bile acid assay is useful when there is either clinical or biochemical evidence of hepatic disease. Elevations can occur with prolonged fasting; however, elevations are considered highly sensitive but non-specific for the presence of hepatic compromise and elevate within 24-48 hours of onset. Low values are considered a highly reliable indication of hepatic disease absence. Persistent or large elevations are associated with a poor prognosis (e.g. pyrrolizidine alkaloid intoxication).

**Gastrointestinal Tract**

The glucose absorption test may suggest malabsorption of carbohydrates if peak absorption is depressed or delayed, but is non-specific and does not reflect malabsorption of other categories of nutrients. Intestinal alkaline phosphatase (AP) elevations may reflect irritation of the gastrointestinal tract, but normal values do not rule out focal abnormalities or low-level inflammatory or infiltrative processes.

**Muscle**

Evaluation of suspected myopathies includes aspartate aminotransferase (AST), creatine kinase (CK), serum electrolytes and in some cases urinary creatinine clearance ratios (not consistently helpful; however, this may detect electrolyte imbalances or abnormalities associated with exertional rhabdomyolysis).\textsuperscript{13} Differentiation of urinary myoglobin from hemoglobin with pigmenturia is useful (myoglobin spills over into urine before discoloration of serum in contrast to hemoglobin).

A submaximal exercise test, while useful in some individuals, does not rule out the possibility of periodic rhabdomyolysis if results are normal. Collect baseline values (CK and AST activities), then 2-4 hours post-exercise CK should be less than double the resting value with little or no change in AST noted. In normal horses, at 24 hours post-exercise CK returns to baseline levels, AST increases by no more than 50%, and no clinical signs of stiffness or muscle pain are evident.

**REFERENCES**


