Rapid Diagnosis of Equine Infectious Respiratory Disease

By: Roger Maes, DVM, PhD; Steve Bolin, DVM, PhD; Annabel Wise, DVM, PhD

Equine infectious respiratory disease is common worldwide, is often contagious, and is caused by several viruses and bacteria. Fever, cough, nasal discharge, and lethargy are signs of respiratory disease. Infectious respiratory disease in horses is seen in all months of the year, but it is most commonly encountered in the months of spring. The disease is often seen in facilities, or after events, that congregate horses. Viruses such as equine influenza virus (EIV), equine herpesvirus-1 (EHV-1), equine herpesvirus-4 (EHV-4) and equine arteritis virus (EAV) all play a role, as do bacterial agents including Rhodococcus equi and Streptococcus equi subspecies equi and zooepidemicus.

EIV has been reported to be the most common viral agent involved in equine infectious respiratory disease. The classical symptoms include coughing, serous to mucopurulent nasal discharge, and rectal temperatures of 103F-105F. Similar to influenza in humans, the spread of equine influenza viruses in a population of susceptible horses is very rapid.

Herpesviruses EHV-1 and EHV-4 are primary pathogens of horses that are capable of producing respiratory disease, reproductive disease, and neurologic disease in horses. Respiratory disease caused by these viruses is manifested by a range of conditions that vary from subclinical infection to widespread outbreaks of severe respiratory disease. Severity of respiratory disease by EHV-1 and EHV-4 is dependent upon both viral and host factors. EHV-4 usually is associated with respiratory infections in weanlings up to two-year-old horses. Occasionally, EHV-4 is linked with abortion in older horses. In contrast, EHV-1 induced respiratory symptoms are often followed by abortions in susceptible pregnant mares, and with certain strains of EHV-1, respiratory disease by neurological disease.

In an excellent recent overview of EAV, Holyoak et al.2 stated that: "Natural outbreaks of clinical disease are characterized by one or more of the following: abortion in pregnant mares; fulminant infection in neonates associated with severe interstitial pneumonia or enteritis; systemic illness in adult horses; and persistent infection in stallions." The respiratory disease component is likely to be more pronounced in young foals. Infections acquired by mares from persistently infected stallions tend to be subclinical.

Streptococcus equi subsp. equi causes submandibular and retropharyngeal lymph node infections, lymph node swelling and sometimes abscessation. The lymph node swelling leads to the pharyngeal constriction, which is commonly referred to as strangles. Streptococcus equi subsp. zooepidemicus causes foal pneumonia and lower airway disease, but is also associated with endometritis and abortion in mares. Rhodococcus equi frequently induces pneumonia and enteritis in foals. Initial

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Interpreting Serum Trace Mineral Concentrations from Sick Animals

By: Tom Herdt, DVM, DACVN

Recently, a veterinarian submitted for trace mineral determination (Primary Trace Nutrients Panel) a serum sample from an acutely ill calf. Results from this assay are indicated as “sick call” values in Table 1. For this calf, concentrations of iron and zinc were in the deficient range, while the serum copper concentration was more than adequate. An immediate assumption was that iron and zinc deficiency may have been the cause of, or a contributing factor to, the calf’s illness. Before making dietary adjustments, however, we recommended that samples be tested from additional, healthy appearing calves in the herd. The average serum trace mineral concentrations from five normal appearing calves are also shown in Table 1. The values were all within the reference range. What happened? Why was there such a difference in the serum mineral concentrations between the sick calf and its normal herd mates, all of which were receiving the same diet?

This case demonstrates the care that must be taken in interpreting serum trace mineral concentrations from single animals, especially those that are clinically ill. First we need to recognize that the sensitivity of serum mineral concentrations to dietary deficiency varies with the mineral. Serum and whole-blood selenium concentrations are sensitive indicators of dietary insufficiency; serum copper and iron are of intermediate sensitivity, and dietary deficiency of zinc and manganese generally must be prolonged before the deficiency is reflected in reduced serum concentration. However, in addition to dietary effects, there are important metabolic factors that may also affect serum trace mineral concentrations.

Inflammation is an important factor influencing serum trace mineral concentrations, and was the probable reason for the results seen in the case of these calves. Inflammation generally will cause a dramatic drop in serum iron, a drop in serum zinc, and an increase in serum copper concentrations. In this case, the sick calf had severe, acute peritonitis caused by a perforated abomasal ulcer, which was diagnosed on necropsy. This resulted in a massive inflammatory lesion.

During generalized inflammatory processes, cytokines direct hepatic metabolism and result in the sequestration of zinc and iron in the liver. At the same time, there is a reduction in the rate of transferrin synthesis and secretion, and an increase in the rate of ceruloplasmin synthesis and secretion. These proteins, which are synthesized in the liver, are the serum carrier proteins for iron and copper, respectively. Thus, changes in their serum concentrations affect the concentrations of minerals they carry. The net result of a change in these proteins is a drop in serum iron and an increase in serum copper during inflammation. From a functional standpoint, these processes probably benefit the body by providing additional hepatic zinc to support the hepatic synthesis of inflammatory proteins, reduce iron availability for bacterial growth and replication, and increase the availability of ceruloplasmin to enhance antioxidant defenses.1

This case illustrates the intricacies of serum trace mineral metabolism and the importance of using caution in making nutritional decisions from animals with clinical illness. Nutritional decisions should always be based on the analysis of samples from multiple animals that are either clinically normal, or represent the general condition of a group of animals.

### Table 1: Serum nutritional trace mineral concentrations from a sick calf compared to the average (± SE) serum mineral values from five normal calves in the same herd.

<table>
<thead>
<tr>
<th>Serum Concentrations of</th>
<th>Range of Adequate Values</th>
<th>Deficiency Value</th>
<th>Sick Calf</th>
<th>Average of Five Normal Herd Mates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (ug/mL)</td>
<td>0.6 to 0.8</td>
<td>&lt;0.4</td>
<td>1.0</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Iron (ug/dL)</td>
<td>110 to 170</td>
<td>&lt;60</td>
<td>42</td>
<td>133 ± 15</td>
</tr>
<tr>
<td>Zinc (ug/mL)</td>
<td>0.9 to 1.7</td>
<td>&lt;0.6</td>
<td>0.4</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

There are a number of measurements that are useful in the assessment of calcium disorders, including serum ionized calcium (iCa), parathyroid hormone (PTH), parathyroid hormone-related protein (PTHRP), and 25-hydroxyvitamin D.

**Calcium Measurement**

Total calcium is composed of three fractions: ionized, complexed, and protein-bound. Changes in any one fraction will impact the tCa measurement. Despite the fact that only the iCa fraction is physiologically active, the calcium status of animals has typically been based on evaluation of serum tCa concentration, assuming that it is directly proportional to iCa. This assumption may lead to erroneous interpretation of laboratory data in many clinical conditions. In over 1600 canine samples, the diagnostic disagreement between serum iCa and tCa was 27%, and in dogs with chronic renal failure, this disagreement was 36%. In over 400 cats, the diagnostic disagreement between iCa and tCa was 40%.

It has been reported that canine serum tCa concentrations should be “adjusted” relative to the serum total protein or albumin concentration to improve diagnostic interpretation. These formulas, however, were developed without verification by iCa measurements. In over 1600 canine cases, the use of an adjustment formula to predict iCa status showed a higher diagnostic discordance (37%) than did measurement of serum tCa alone (27%). In 490 dogs with chronic renal failure, diagnostically significant concordance between adjusted tCa and iCa measurement increased to 53%, indicating poor performance of the adjustment formulas in the prediction of iCa status. Adjustment formulas performed poorly as they were based solely on the protein-bound fraction of calcium, ignoring complexed calcium, which can vary greatly among patients.

Ionized calcium is the biologically active form of calcium, and for accurate assessment of calcium status, iCa must be measured directly. Fasting serum or heparinized plasma samples should be submitted for analysis. Oxalate, citrate, and EDTA anticoagulants should not be used, because calcium is bound to these chemicals and becomes unavailable for analysis.

**Parathyroid Hormone**

Serum PTH concentration should be evaluated with simultaneous measurement of serum iCa concentration. In primary hyperparathyroidism (HPTH), both iCa and PTH are elevated. Early in the course of the disease, elevation of iCa may be mild to moderate, with a high normal concentration of PTH. If parathyroid glands are normal, hypercalcemia (parathyroid-independent) should be associated with low PTH concentration, and hypocalcemia should be associated with elevated concentrations of PTH. Animals with renal failure and secondary hyperparathyroidism have increased serum PTH with normal or decreased iCa concentration.

**Parathyroid Hormone-Related Protein**

PTHRP is not strictly a calcium-regulating hormone, but measurement is useful in some cases. PTHrP is a peptide originally isolated from human and animal tumors associated with humoral hypercalcemia of malignancy. PTHrP should be measured in all cases when a malignancy is suspected. However, a negative PTHrP result does not rule out the possibility of malignancy, as tumors may secrete other factors that can result in hypercalcemia. PTHrP is very susceptible to degradation and should be measured in EDTA plasma. Serum is not recommended for measurement of PTHrP. In a recent study, paired plasma and serum samples were analyzed for PTHrP in 35 dogs with malignancy. There was a 50% false negative rate when using serum to measure PTHrP, most likely due to proteolysis of PTHrP in serum.

**Vitamin D Metabolites**

The 25-hydroxyvitamin D concentration is a good indicator of vitamin D ingestion and can be used to diagnose hypovitaminosis D; either serum or plasma (EDTA or heparin) can be used for measurement. Metabolites resulting from the ingestion of cholecalciferol present in rodenticides will be measured with the 25-hydroxyvitamin D assay; however, calcipotriene, the vitamin D analog found in antipsoriasis creams, is not measured with the assay for 25-hydroxyvitamin D.

### TABLE 1: Anticipated changes associated with calcium disorders

<table>
<thead>
<tr>
<th>Condition</th>
<th>iCa</th>
<th>iCa</th>
<th>Pi</th>
<th>PTH</th>
<th>PTHrP</th>
<th>25-OH-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary HPTH, Renal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N, ↑</td>
<td>↓</td>
</tr>
<tr>
<td>Secondary HPTH, Nutritional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓, low N</td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓, N</td>
</tr>
<tr>
<td>Vitamin D Toxicity (cholecaciferol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓, low N</td>
</tr>
<tr>
<td>Vitamin D Toxicity (calcipotriene)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Idiopathic Hypercalcemia (cat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Hypoadrenocorticin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Dehydration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

### Additional Reading


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