Diagnostic Approach to Muscle Disorders

Stephanie J. Valberg, DVM, PhD, Diplomate ACVIM

To identify a specific muscle disorder, a standardized approach of evaluating horses with potential muscle disease should include accurate history, careful physical examination, and selection of diagnostic tests such as serum biochemistry, imaging techniques, exercise testing, electromyography, muscle biopsy, and genetic testing. Author’s address: Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN 55108; e-mail: valbe001@umn.edu. © 2006 AAEP.

1. Introduction

The fundamental approach to diagnosing muscle diseases in horses is based on a thorough history, careful physical examination, complete blood count, and serum biochemistry profile. After this evaluation, a diagnosis can be made if muscular signs are part of a primary or secondary disease process. Primary muscle disorders usually fall into one of five broad clinical categories: (1) focal muscle strain, (2) rhabdomyolysis, (3) weakness and exercise intolerance without rhabdomyolysis, (4) abnormal muscle contraction/conduction, and (5) muscle atrophy (Table 1). Additional diagnostic tests can then be selected to localize the muscle groups affected by the disorder and to identify the disease process that is causing the presenting clinical signs.

Localizing the extent and severity of muscle strain or rhabdomyolysis may be assisted by thermography, ultrasonography, scintigraphy, or electromyography, and its specific cause may be determined by historical information or in some cases, by muscle biopsy. Exercise testing, electromyography, muscle biopsy, and molecular genetic testing may identify causes of exercise intolerance, weakness, and abnormal muscle contractility. Determining if muscle atrophy is myogenic or neurogenic in origin can be assessed by electromyography and muscle biopsy as well as other tests such as cerebrospinal fluid (CSF) taps, Western blot for equine protozoal myeloencephalitis (EPM), and serum vitamin E concentrations. Detailed information regarding specific diagnostic testing is provided in the accompanying sections.

2. History and Physical Examination

A detailed history is often required when assessing muscle disorders in horses, because many disorders are intermittent in nature and triggered by certain environmental stimuli. A careful description of the horse’s muscle tone, muscle mass, gait, degree of pain, exercise intolerance, and weakness when showing clinical signs as well as the duration and frequency of signs becomes important. Further characterization of possible eliciting factors requires a detailed account of the horse’s exercise schedule, diet, vaccination history, signs of concurrent disease, and current medications.

Inspection of the horse at a distance while it is standing with forelimbs and hindlimbs exactly square is imperative in assessing muscle mass, symmetry, and signs of atrophy. Subsequently, palpa-
tion of the entire muscle mass of the horse provides valuable information regarding muscle tone, heat, pain, swelling, subtle muscle atrophy, and fasciculations. Firm and deep palpation of the lumbar, gluteal, semimembranosus, and semitendinosus muscles may reveal pain, cramps, or fibrosis. The triceps, pectoral, gluteal, and semitendinosus muscles can be tapped with a fist or percussion hammer and observed for prolonged contracture suggestive of myotonia. Running a blunt instrument such as a hemostat, needle cap, or pen over the lumbar and gluteal muscles provides information regarding back pain. Extension (swayback) followed by flexion (hogback) of the back is expected in healthy animals. Guarding against movement may reflect abnormalities in the pelvic or thoracolumbar muscles or pain associated with the thoracolumbar spine or sacroiliac joints.

A lameness evaluation, including flexion tests, is often indicated as part of evaluation of the muscular system. Muscle pain may be secondary to changes in movement caused by lower limb lameness. The horse should be observed at a walk or trot for any gait abnormalities and in some cases, lunged for 15 min or ridden until clinical signs are elicited. If the horse shows any signs of neurologic disease, a detailed neurologic examination should also be performed.

3. Biochemical Profiles

Skeletal muscle necrosis may be identified by determining the activity in serum of creatine kinase (CK), aspartate transaminase (AST), and lactate dehydrogenase (LDH). Carbonic anhydrase III and serum myoglobin have also been suggested as markers of equine muscle necrosis. The permeability of the muscle-cell membrane, rate of enzyme production, alternate tissue sources of the enzyme, and rate of enzyme excretion/degradation may also influence serum-enzyme activities.

Serum CK

Isoforms of CK are found in skeletal muscle (MM), cardiac muscle (MB), and nervous tissue (BB). CK, a relatively low molecular-weight protein (80,000 Da), is liberated within hours of muscle damage or increased cell-membrane permeability into the extracellular fluid, and it usually peaks at 4–6 h after muscle injury. A three- to five-fold increase in serum CK from normal values is believed to represent necrosis of ~20 g of muscle tissue. Rhabdomyolysis results in a proportionately greater increase in the MM isof orm than the MB isof orm, but some studies disagree about the tissue specificity of serum CK isof orms in the horse. Limited elevations in CK (<1000 U/l) may accompany training or transport. Extremely fatiguing exercise (e.g., endurance rides or the cross-country phase of a three-day event) may result in CK activities being increased to >1000 U/l, but they are usually <5000 U/l. Under these circumstances, serum CK activities rapidly return to baseline (i.e., <350 IU/l in 24–48 h). Recumbent animals may have slightly elevated CK activities of <3000 U/l. In contrast, more substantial elevations (from several thousand to hundreds of thousands of U/l) in the activity of this enzyme may occur with rhabdomyolysis.

Serum AST

Serum AST, previously known as serum glutamicoxaloacetic transaminase (SGOT), is a larger molecular-weight protein that has high activity in skeletal and cardiac muscle as well as the liver, red blood cells, and other tissues. Elevations in AST are not specific for myonecrosis, and increases could be the result of hemolysis or muscle, liver, or other organ damage. AST activity rises more slowly in response to myonecrosis than does CK, often peaking between 12 and 24 h after the insult. In addition, AST is cleared slowly by the reticuloendothelial system and may persist for 2–3 wk after rhabdomyolysis.

Comparing serial activities of CK and AST provides information concerning the progression of myonecrosis. Combined elevations in CK and AST reflect relatively recent or active myonecrosis; persistently elevated serum CK indicates that myonecrosis is likely to be continuing. Elevated AST activity accompanied by decreasing or normal CK activity indicates that myonecrosis has ceased. The degree of elevation of CK and AST does not necessarily reflect the severity of clinical signs.

Serum LDH

LDH is a tetramer made up of combinations of the M and H subunits with five isoenzyme forms found in various organs within the body. Electrophoretic separation suggests that the M4 (LDH5) and M3H (LDH4) isof orms are found predominantly in skeletal muscle. Elevations in LDH may be detected in horses with rhabdomyolysis, myocardial necrosis, and/or hepatic necrosis. Therefore, concurrent measurement of serum CK is necessary to establish if rhabdomyolysis is present.

Myoglobin

Elevation in plasma/serum myoglobin concentrations indicates acute muscle damage. Myoglobin is a low molecular-weight protein (17.6 kDa) that leaks into plasma immediately after muscle damage and is rapidly cleared in the urine by the kidney; ~200 g of muscle or more must be damaged before it is detectable in the urine in human patients. Normal serum concentrations in resting horses have been determined by nephelometry (range = 0–9 μg/l), and measured concentrations from horses with rhabdomyolysis range from 10,000 to 800,000 μg/l.

Vitamin E and Selenium Concentrations

Whole-blood selenium concentrations or glutathione peroxidase measured in EDTA or heparin tubes are of value in assessing muscle disorder in animals.
housed in areas deficient in selenium. Vitamin E concentration can be measured in serum samples; however, variability in serum levels can be quite large, and pooling of several samples is often recommended to accurately assess a deficiency.

4. Urinalysis

Urinalysis can occur between and within individual horses. It is particularly important in horses with myoglobinuria, elevations in creatinine, or suspected electrolyte imbalances. Urine specific gravity, protein content, white blood cell count, red blood cell count, and evaluation of cast formation should be performed to assess the potential for concurrent renal disease. A positive hemastix test (orthotoluidine) in the absence of hemolysis or red blood cells in urine is highly suggestive of myoglobinuria. Further differentiation of myoglobin from hemoglobin is sometimes warranted, and where available, electrophoresis, nephelometry, or spectroscopy may be used.

Renal Fractional Excretion of Electrolytes

Determination of electrolyte, mineral, and creatinine concentrations in urine and blood may be useful to identify electrolyte balance in horses with muscle cramping or exertional rhabdomyolysis. Ideally, urine should be collected as a freely voided sample at a standardized time of day before exercise and feeding. Ion-specific electrodes commonly used for analyses in small animals are useful in determining creatinine and chloride concentrations in horses. They are of limited value for testing sodium for analyses in small animals are useful in determining creatinine and chloride concentrations in horses. They are of limited value for testing sodium, because the high urinary-potassium content of equine urine often interferes with analysis for sodium. Use of flame photometry or emission spectrophotometry can provide more accurate measurements of Na, K, Ca, Mg, and P. Where Ca, P, and Mg are to be analyzed, samples should not be centrifuged before analysis but acidified to dissolve urine crystals. Values below the reported ranges are suggestive of conservation and possibly inadequate dietary intake that may require supplementation; however, wide variations can occur between and within individual horses.

Renal fractional excretions (FE) can be calculated using the following formula (x = measured electrolyte and Cr creatinine):

\[
FE\% (X) = \left( \frac{[Cr]_{\text{plasma}} \times [X]_{\text{urine}}}{[X]_{\text{plasma}} \times [Cr]_{\text{urine}}} \right) \times 100
\]

Normal values for FE of electrolytes are dependant on a horse’s diet. Normal values in acidified urine for horses consuming grass hay and a sweet-feed mix with available salt are FE\text{Na} = 0.04–0.08%, FE\text{K} = 35–80%, FE\text{Cl} = 0.4–1.2%, FE\text{Ca} = 5.3–14.5%, FE\text{P} = 0.05–4.1%, and FE\text{Mg} = 14.2–21.4%.11

5. Exercise-Response Test

Diagnosing chronic exertional rhabdomyolysis may be problematic in horses that do not have acute clinical signs and have normal serum AST and CK at rest. In such cases, an exercise challenge can be helpful in detecting subclinical exertional rhabdomyolysis. In addition, quantifying the extent of rhabdomyolysis during mild exercise is helpful in deciding how rapidly to put a horse back into training. Blood samples should be taken before exercise and ~4–6 h after exercise to evaluate peak changes in CK. Serum CK activity measured immediately post-exercise will not reflect the amount of damage occurring during the exercise test. Small fluctuations in serum CK activity may occur with exercise because of enhanced muscle-membrane permeability, particularly if exercise is prolonged or strenuous and the horse is untrained. A submaximal exercise test is often valuable for detecting rhabdomyolysis, because it provides more consistent evidence of subclinical rhabdomyolysis than maximal exercise tests. Fifteen minutes of trotting is often sufficient to produce subclinical muscle damage in horses prone to exertional myopathies. If signs of stiffness develop before this time, exercise should be concluded. A normal response would be a <three- to four-fold increase from basal CK.

6. Imaging

Thermography

Thermography may be useful for identification of superficial abnormal temperature changes caused by muscle damage, but it has limited value in deeper injuries; there are many potentially confusing issues such as recent removal of a rug or tack or grooming. Careful comparisons of the left and right sides should be made. Muscle inflammation is seen as a hot spot in the skin directly overlying the affected muscle. The most common sites of muscle strain identified thermographically include the longissimus dorsi, the origin or body of the middle gluteal, the insertion of the gluteals on the greater and third trochanters of the femur, biceps femoris, semitendinosus, semimembranosus, and adductor.18

Nuclear Scintigraphy

Nuclear scintigraphy is particularly useful for identification of areas of deep muscle damage that had not been suspected based on clinical examination. Scintigraphy has been used most commonly in horses with a history of poor performance, with or without stiffness after exercise, to confirm a diagnosis of equine rhabdomyolysis. Technetium 99m methylene diphosphonate (MDP) is taken up in damaged muscle in the horse and is best seen in the bone-phase images (i.e., 3 h after injection). The mechanism of MDP binding is unknown, but the release of large amounts of calcium from damaged muscle or the exposure of calcium binding sites on protein macromolecules in the damaged muscle may
be responsible. The use of scintigraphy for the diagnosis of other muscle injuries has not been documented in the horse, but it can be helpful in some cases involving either proximal forelimb or hindlimb muscles. Uptake of the radiopharmaceutical tends to be much more focal and much less intense than in cases of equine rhabdomyolysis.

Ultrasonography
If there is physical disruption of the muscle, diagnostic ultrasonography is potentially very useful for identification of muscle trauma and fibrosis. Careful comparisons must be made between similar sites in contralateral limbs in both transverse and longitudinal images, because the typical striated echogenic pattern varies according to the muscle group. The appearance of muscle is also sensitive to the way the horse is standing and the tension on the muscle, so it is important that the horse is standing squarely and bearing weight evenly. Muscle fascia appears as well-defined relatively echo-dense bands. Care must be taken in identifying large vessels and artifacts created by them.

In an acute injury, muscle-fiber disruption is seen as relatively hypoechoic areas within muscle with loss of the normal muscle striation. The jagged edge of the margin of the torn muscle may be increased in echogenicity. Tears in the muscle fascia may be identified. The defect in muscle may be filled by a loculated haematoma that is slowly replaced by hypoechoic granulation tissue. Muscle repair shows a progressive increase in echogenicity. Relatively hyperechoic regions may develop because of fibrous scarring. Hyperechoic regions causing shadowing artefacts reflect mineralization.

7. Electromyography
A specific diagnosis of the cause of muscle atrophy, muscle fasciculations, or myotonic dimpling after tapping the muscle can be aided by performing electromyography (EMG). EMG of normal skeletal muscle shows a brief burst of electrical activity when the needle is inserted in muscle and then quiescence; this is not the case if motor units are recruited (motor-unit action potentials) or the needle is very close to a motor-end plate (miniature end-plate potentials). Normal muscle shows little spontaneous electrical activity unless the muscle contracts or the horse moves. Horses with abnormalities in the electrical-conduction system of muscle or denervation of motor units show abnormal spontaneous electrical activity in the form of fibrillation potentials, positive sharp waves, myotonic discharges, or complex repetitive discharges. Specific patterns of waveforms may indicate specific disorders such as denervation atrophy, hyperkalemic periodic paralysis (HYPP), or myotonia.

8. Muscle Biopsy
There have been many recent advances in obtaining diagnostic information regarding specific muscle disease by using the muscle-biopsy technique. Specialized laboratories are often most helpful in processing frozen muscle biopsies and interpreting muscle pathology to provide a specific diagnosis and treatment.

To obtain the best diagnostic information, it is important to select an appropriate muscle to biopsy. With exertional rhabdomyolysis, samples of the semimembranosus muscle and gluteus medius muscle are often examined because of their consistent involvement in exertional rhabdomyolysis and the ease of collection with open- and needle-biopsy techniques, respectively. An open-surgical biopsy of the semimembranosus muscle is usually obtained in veterinary practice because of the ease of orienting a longitudinal biopsy and ease of treating complications such as dehiscence. A site ~8 cm distal to the tuber ischii provides ample muscle tissue without leaving a readily evident scar should dehiscence occur (Figs. 1 and 2). The biopsy is performed using sedation and local anesthesia directed into the SC, but not muscle, tissue. After a vertical incision in the skin and muscle fascia, two parallel incisions 2 cm apart and 4–5 cm long are made in
the muscle. The muscle is grasped in one dorsal corner using forceps to avoid crushing other portions of the biopsy (Fig. 1). A cross-secting incision is made dorsally; the muscle sample is then excised in a ventral direction to a depth of 1–2 cm, and the sample is excised ventrally. Preparation of the sample for shipment is described below. Good closure of fascia and SC tissue is the key to prevent dehiscence. Staples or non-absorbable skin sutures are used to close the skin. Horses need to be stall rested for 2–4 days after this biopsy procedure, and sutures should be removed in 10–14 days.

Another technique that decreases recuperation time uses a specialized 6-mm outer diameter percutaneous biopsy needle. A 1-cm skin incision is made through a bleb of local anesthetic, and the needle is inserted into the gluteus muscle to a depth of 6 cm. If necessary, repeated needle insertion is performed to obtain enough muscle to form at least a 1.5-cm² sample. Samples obtained using the needle biopsies do not tolerate shipment on ice packs to an outside laboratory as well as samples obtained by surgical biopsy.

A few muscle disorders are amenable to evaluation using Trucut biopsy samples. This is best applied to diffuse disorders like immune-mediated myopathies that affect muscles such as epaxial or gluteal muscles that are difficult to sample using open surgical techniques. Several Trucut biopsies placed in formalin are required. Trucut samples are not of value in assessing samples from horses with exertional rhabdomyolysis or equine motor-neuron disease, because they do not provide an adequate sample size to make a diagnosis.

Sample Preparation
Before obtaining a muscle biopsy, the laboratory where samples will be evaluated should be contacted to determine their preferred method of fixation. Many laboratories specializing in neuromuscular diseases prefer to obtain samples that are fresh within 24 h of sampling, because frozen sections provide better preservation and visualization of muscle properties. On arrival in the laboratory, these fresh samples are prepared for freezing in isopentane suspended in liquid nitrogen. Preparation of samples for shipment includes wrapping the muscle in gauze moistened with saline (damp but not soaking wet) and placing it in a small plastic container (Fig. 3). Samples are shipped overnight on ice packs in a Styrofoam container. If placing samples in formalin, either stretch the sample by pinning it on a tongue depressor or allow the sample to sit in open air for 2–5 min before fixing; this minimizes contraction bands within fibers. Laboratories that use formalin fixation avoid the more intensive preparation that is required for frozen samples; however, formalin fixation has the disadvantage of creating a number of artifacts including cracking, sedimentation, and leaching of glycogen that make it less than ideal.
IN-DEPTH: MUSCLE DISORDERS

9. Genetic Testing

A DNA test\(^3\) is available to identify horses carrying the mutation for HYPP found in the extended pedigree of “Impressive” descendants. Mane or tail hairs with intact roots are submitted for analysis to determine if horses are normal, heterozygous, or homozygous for HYPP. Other genetic mutations in other bloodlines that might cause HYPP are not detected by this test. A DNA test\(^3\) is also available to identify if horses are carriers of glycogen-branching enzyme deficiency (GBED) or if foals or aborted feti are homozygous for this disorder. Testing is performed on hair samples with intact roots or liver samples.

Based on the information obtained by this type of thorough evaluation, a diagnosis can usually be obtained. A classification system may be helpful in narrowing down muscle diseases in horses, such as GBED, immune-mediated myopathies, exertional rhabdomyolysis, polysaccharide storage myopathy, and shivers.

References and Footnote

13. Beech J, Lindborg S, Braund KG. Potassium concentrations in muscle, plasma and erythrocytes and urinary fractional excretion in normal horses and those with chronic intermit-

### Table 1. Classification of Muscle Disorders

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1. Non-exercise associated rhabdomyolysis | i. Inflammatory myopathies  
Clotridial myositis  
Influenza myositis  
Sarcocystis myositis  
Immune mediated myopathy  
ii. Nutritional myopathy  
Vitamin E and selenium deficiency  
iii. Toxic myopathy  
Ionophore toxicity  
Pasture myopathies  
Rayless golden rod/white snake root  
Cassia occidentalis  
Atypical myoglobinuria |
| 2. Exertional rhabdomyolysis | i. Focal muscle strain  
ii. Sporadic tying-up (historically first episode, normal AST)  
iii. Chronic tying-up  
Dietary imbalances, vitamins, minerals, electrolytes  
Polysaccharide storage myopathy  
Recurrent exertional rhabdomyolysis  
Idiopathic chronic exertional rhabdomyolysis |
| 3. Exertional myopathy with normal CK | i. Mitochondrial myopathy  
ii. Myogenic atrophy  
Severe rhabdomyolysis  
Diuse  
Cushing’s disease  
Immune-mediated myositis (rapid atrophy)  
ii. Neurogenic atrophy  
Equine protozoal myelitis  
Local nerve trauma  
Equine motor neuron disease |
| 5. Muscle fasciculations | i. Pain, fear  
ii. Electrolyte abnormalities  
iii. Hyperkalemic periodic paralysis  
iv. Otobius Megnini ear tick infestation  
v. Myotonic dystrophy  
vi. Stiff horse syndrome  
vii. Shivers |
| 6. Muscle atrophy | i. Focal muscle strain  
ii. Sporadic tying-up (historically first episode, normal AST)  
iii. Chronic tying-up  
Dietary imbalances, vitamins, minerals, electrolytes  
Polysaccharide storage myopathy  
Recurrent exertional rhabdomyolysis  
Idiopathic chronic exertional rhabdomyolysis |
| 3. Exertional myopathy with normal CK | i. Mitochondrial myopathy  
ii. Myogenic atrophy  
Severe rhabdomyolysis  
Diuse  
Cushing’s disease  
Immune-mediated myositis (rapid atrophy)  
ii. Neurogenic atrophy  
Equine protozoal myelitis  
Local nerve trauma  
Equine motor neuron disease |
| 5. Muscle fasciculations | i. Pain, fear  
ii. Electrolyte abnormalities  
iii. Hyperkalemic periodic paralysis  
iv. Otobius Megnini ear tick infestation  
v. Myotonic dystrophy  
vi. Stiff horse syndrome  
vii. Shivers |

Staining

A number of basic pathologic responses can be detected in hematoxylin and eosin (H&E), periodic aid Schiff’s (PAS), and amylase PAS stains of muscle tissue. Further histochemical stains of frozen sections include acid and alkaline phosphatases for necrosis and degeneration, myosin adenosine triphosphatase stains for fiber typing, mitochondrial stains, lipid stains, and immunohistochemical stains. Although not routinely performed because of expense, glutaraldehyde fixation of small muscle samples can be of value for electron microscopy.


*Jørgen Kruuse A/S, Marslev Byvej 35, Marslev 5290, Denmark.*