

# Satellite Article

## Muscle biopsy: a routine diagnostic procedure

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### Common equine neuromuscular disorders

#### **Equine rhabdomyolysis syndrome (ERS) and polysaccharide storage myopathy (EPSM)**

Equine rhabdomyolysis syndrome is a condition described in most breeds of horses; synonyms include **Monday-morning disease, set-fast, tying-up and azoturia**.

The **diagnosis** is usually made on clinical signs and increased serum activities of creatine kinase (CK) and aspartate aminotransferase (AST). **Signs** include repeated bouts of muscle pain that range from stiffness to inability to move, sweating and palpably hard muscles. It occurs in 5 to 7% of Thoroughbreds in race training (MacLeay *et al.* 1999; McGowan *et al.* 2002a) and has been shown to occur as frequently in polo horses (McGowan *et al.* 2002b) and competition event horses (C. McGowan, unpublished data).

**Many cases of ERS are attributed to one of 2 underlying causes, both thought to have a genetic basis:**

- **A defect in intracellular skeletal muscle calcium regulation** (similar to malignant hyperthermia) in Thoroughbred horses (Lentz *et al.* 1999).
- **EPSM** in Quarter Horses, Warmbloods, draught breeds and their crosses (Valberg *et al.* 1992, 1997; Valentine *et al.* 2000, 2001a).

While EPSM is usually manifest as ERS (Valberg *et al.* 1997), it has been reported to be associated with post anaesthetic myopathy, generalised weakness (Valentine *et al.* 1997; Valentine 1999) and with gait abnormalities of the hindlimbs including shivering (Valentine 1999; Valentine *et al.* 1999). While it was initially reported in the USA, it has recently been reported in Europe (Quiroz-Rothe *et al.* 2002) and the UK (McGowan *et al.* 2003). Its incidence has not been fully investigated, but may occur in as many as 50% of draught and related breeds in the USA (Valentine *et al.* 2001a).

While the exact pathogenesis is unknown, EPSM is associated with abnormal glucose uptake into muscle of affected horses (De La Corte *et al.* 1999). It is characterised (**Fig 1**) histologically by accumulations of glycogen and amylase resistant complex polysaccharide within the *type 2* myofibres (Valberg *et al.* 1992; Valentine *et al.* 1998). Myofibre size variation and hypertrophy, internal nuclei and interstitial fat accumulation may also be observed (Valentine *et al.* 1997, 1998).

#### **Equine motor neuron disease (EMND)**

Equine motor neuron disease was first described in horses by Cummings *et al.* (1990) in the USA and has subsequently been recognised worldwide (Divers *et al.* 2001). It is characterised by loss of lower motor neurones in the brainstem and spinal cord leading to a neuromuscular wasting disease. **It is usually found** in mature horses with Quarter Horses, Appaloosas and Standardbreds over-represented and lack of grazing is associated with an increased risk (Divers *et al.* 2001).

The **clinical** signs vary with the stage of disease and range from poor performance, fatigue and muscle wasting to trembling, muscular fasciculations, abnormal stance, gait and raised tail head. In 30% of cases, ophthalmic examination reveals a diffuse pattern of abnormal yellow-brown pigment in the tapetal area with linear deposition in the tapetal- nontapetal junction (Divers *et al.* 2001).

**Laboratory abnormalities** are lowered plasma Vitamin E. Other findings are inconsistent but include increased serum activities of creatine kinase (CK) and aspartate aminotransferase (AST); lower peak of blood glucose on an oral glucose absorption test; and increased serum ferritin concentration. **Diagnosis** is based on clinical signs, laboratory findings and biopsy of the ventral branch of the spinal accessory nerve (Jackson *et al.* 1996) or a muscle biopsy (Valentine *et al.* 1998). The muscle biopsy is usually preferred as it does not require general anaesthesia and is technically less demanding. As *type 1* myofibres are predominantly affected, the recommended biopsy site is the *sacrocaudalis dorsalis medialis* or tail head muscle. The histopathological findings are marked variation in fibre size with angular atrophy of some, hypertrophy of others and interstitial infiltration of fibrous tissue (**Fig 2**).

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Other neuromuscular disorders in horses are not routinely diagnosed using a muscle biopsy. Although horses with shivering may have no lesions on muscle biopsy (C. McGowan, unpublished data), shivering caused by EPSM should be ruled out. Muscle inflammatory disorders in horses have been described infrequently in the literature (Barrott *et al.* 2004).

## Diagnosis of muscle disorders (why perform a muscle biopsy?)

Following clinical examination and clinical pathology (muscle enzyme activities and urinary electrolyte fractional excretion), veterinarians are often left without further diagnostic protocols to provide additional information to clients and formulate a rational therapeutic plan and prognosis. Further investigation of ERS by the authors includes an exercise test and a muscle biopsy. Following collection of a pretest blood sample for measurement of serum activities of CK and AST, the horse is lunged at a trot, or worked on the track at similar exercise intensity, for 15–20 mins and CK is collected 4 h post exercise. Many horses with recurrent ERS will show at a rise of CK by 1000 u/l or greater, indicating ongoing recurrence.

A muscle biopsy is useful for all forms of muscle disorders. It may allow differentiation of the cell infiltrate in a myositis, assessment of degree of atrophy and replacement of muscle with fibrous tissue in EMND, and both confirming ongoing myopathic changes and revealing the extent of involvement of muscle in cases of ERS. A muscle biopsy is useful in differentiating EPSM as a specific cause for ERS. Knowing the specific cause for ERS can help in determining heritability and prognosis. Also, while both ERS and EPSM are managed principally by dietary therapy (Valentine *et al.* 2001b), ERS in light-bred horses, if shown not to be caused by EPSM, may be amenable to medical therapy. Equine rhabdomyolysis syndrome in these horses has been suggested to be a calcium channel disorder based on *in vitro* contracture testing, and drugs that affect muscle calcium regulation, e.g. phenytoin (Beech *et al.* 1988), may therefore be used.

## Considerations in taking a muscle biopsy

**After an acute episode of ERS**, it takes 1–3 weeks for the muscle to have recovered fully, including the return of nuclei

to the periphery of the fibre (McGavin and Valentine 2001). If you are attempting to differentiate a recurrent from a sporadic case of ERS, it is advisable to wait this period of time. This waiting period is not necessary if the biopsy is to confirm muscle damage in ERS, to rule out EPSM as a cause of ERS, or if investigating other suspected neuromuscular disorders, such as any case with localised or generalised muscle atrophy or weakness.

For ERS or EPSM, it is best to choose the *semimembranosus* or *semitendinosus* muscle groups for a surgical biopsy or the middle gluteal for a **needle biopsy**. For suspected EMND, the tail head muscle (*sacrocaudalis dorsalis medialis*) biopsy is recommended. For **shivering**, *semimembranosus* or *semitendinosus* muscle biopsy is recommended to look for EPSM or other neuromuscular disorder as the underlying cause. For localised muscle inflammation or atrophy, the muscle group affected is biopsied surgically in most cases (**Table 1**).

## Techniques

Each technique is generally most safely performed on an adequately sedated and restrained horse. **The use of equine stocks is advised.**

### Needle biopsy

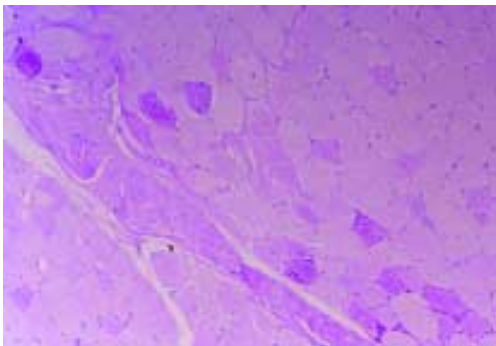
Biopsies are usually taken from the middle gluteal muscle (**Fig 3**), approximately 15 cm (in a 500 kg horse) caudodorsal to the *tuber coxae*, using a stainless steel biopsy needle (Snow and Guy 1976). The biopsy site should be clipped and scrubbed with antiseptic appropriate for surgical preparation. The area is desensitised by injection of 1 ml 2% mepivacaine (Intra-Epicaine)<sup>1</sup> subcut. into the site using a 25 gauge needle.

**Care should be taken not to inject into the muscle**, as this would disrupt the normal histological architecture. A stab incision through skin and fascia is made using a sterile No. 10 scalpel blade. The biopsy needle<sup>2</sup> consists of 3 parts: a 6 mm diameter outer needle portion, with a pointed tip and a 'window' opening near the tip; an inner, sharp-edged cutting cylinder, fitting tightly within the needle; and a stilette to enable removal of the specimen from the needle (**Fig 4**). Following removal of the stilette, the biopsy needle should be

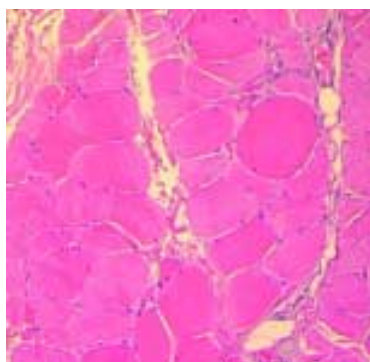
**TABLE 1: Major clinical indications for performing a muscle biopsy, differential diagnoses and preferred technique**

Major clinical sign	Main neuromuscular differential diagnoses	Preferred muscle biopsy technique
Recurrent episodes of rhabdomyolysis	ERS, EPSM, nutritional myodegeneration	Surgical (semimemb/tend) OR Needle (middle gluteal)
Persistent elevations of muscle enzymes and poor performance	ERS, EPSM, nutritional myodegeneration	Surgical (semimemb/tend) OR Needle (middle gluteal)
Generalised muscle wasting	EMND, myositis	Surgical – tail head
Muscle weakness/fasciculations	EMND, EPSM	Surgical – tail head (and semimemb/tend optional)
Shivering	EPSM, idiopathic	Surgical (semimemb/tend) OR Needle (middle gluteal)
Localised muscle atrophy	Myositis, neurogenic atrophy	Surgical or needle – affected muscle

EMND = Equine motor neuron disease, ERS= Equine rhabdomyolysis syndrome, EPSM = Polysaccharide storage myopathy, semimemb/tend = *semimembranosus/semimembranosus* muscles, tail head = *sacrocaudalis dorsalis medialis* muscle.



**Fig 1:** Semimembranosus muscle biopsy of a horse affected with polysaccharide storage myopathy (magnification x100). The sample was stained using Periodic Acid Schiff following incubation with diastase for 10 mins. Note the accumulations of diastase-resistant polysaccharide.



**Fig 2:** Sacrocaudalis dorsalis medialis muscle biopsy of a horse affected with equine motor neuron disease (H&E, magnification x100). Note the marked fibre size variation, with angular atrophy of some and hypertrophy of others.



**Fig 3:** Site for needle biopsy of the gluteal muscle is clipped.

inserted at right angles to the skin surface to a depth of 8 cm and 4–8 quick cuts made (**Fig 5**).

Increased yield of muscle can be achieved by ensuring the window is facing away from the operator and, once inserted fully, angling the needle dorsally in order to engage more muscle into the window. **Alternatively**, application of negative pressure (suction) to the cutting cylinder by use of a 50 ml syringe can have a similar effect. The stilette is used to extract a cylindrical piece of muscle from the needle (**Fig 6**).



**Fig 4:** The biopsy needle consisting of (from lower field to upper) a 6 mm diameter outer needle portion, with a pointed tip and a 'window' opening near the tip; an inner, sharp-edged cutting cylinder, fitting tightly within the needle; and a stilette to enable removal of the specimen from the needle.



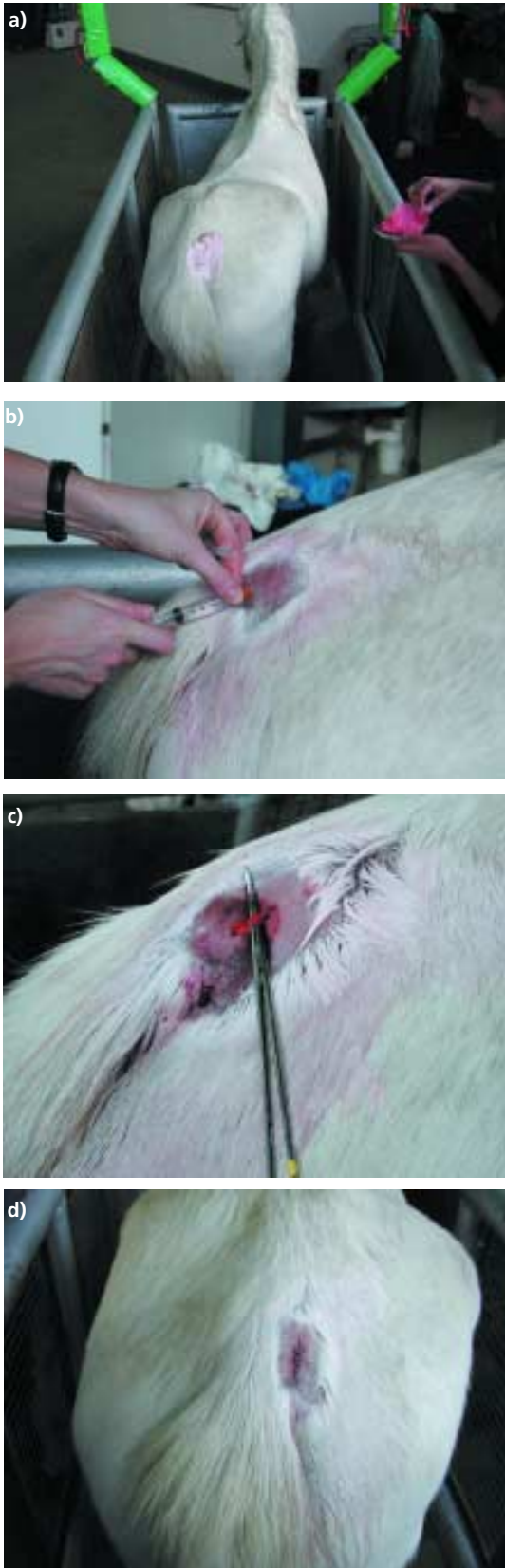
**Fig 5:** Collection of a needle biopsy of the gluteal muscle.



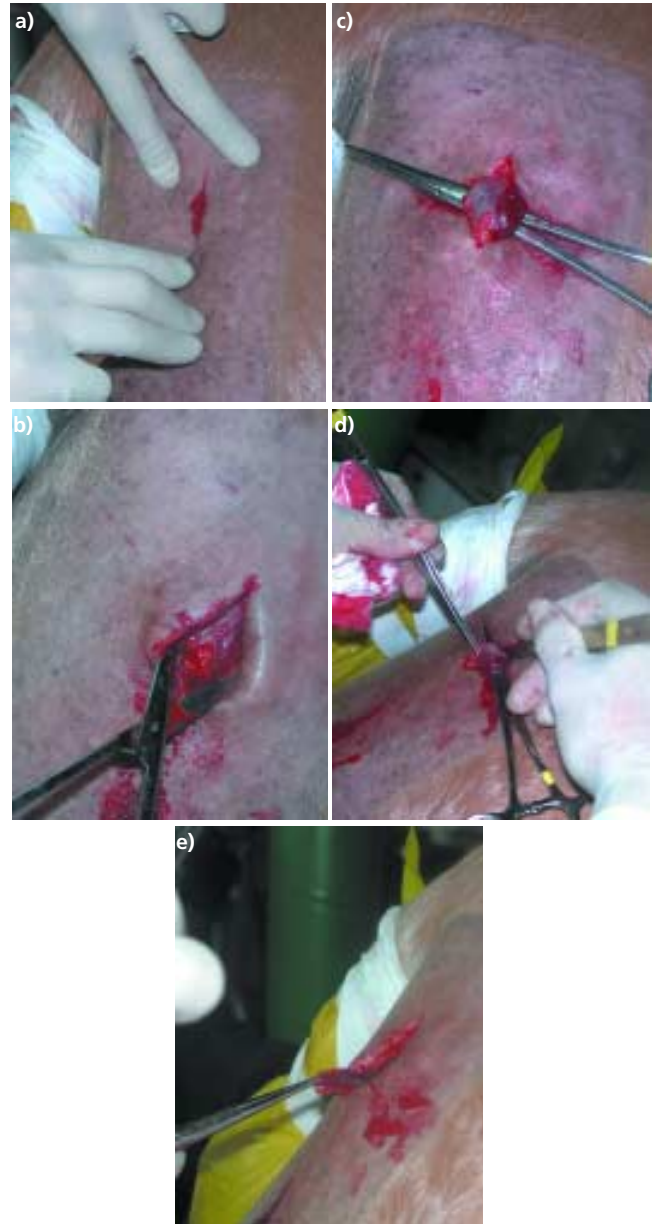
**Fig 6:** The stilette is used to extract the muscle from the cutting cylinder of the needle.



**Fig 7:** Site for collection of a surgical biopsy of the semimembranosus/semitendinosus muscle is clipped and surgically prepared.



**Fig 8: Demonstrating a sample being taken from the sacrocaudalis dorsalis medialis muscle. a) Site for tail head biopsy; b) local anaesthetic infiltration; c) isolation of muscle; d) skin staples.**



**Fig 9: Demonstrating a surgical biopsy of the semimembranosus/semitendinosus muscle. a) Initial incision for surgical biopsy; b) blunt dissection down to muscle; c) isolation of portion of muscle; d) sharp incision of undermined muscle; e) sample held ready for excision.**

Aligned fibres are teased out and fixed in 10% buffered formalin or frozen with the technique described below.

The **advantages of this method** are that it is minimally invasive, quick to perform and requires no time off work. The needles are reusable, rarely need sharpening and are able to be sterilised with other stainless steel instruments. The **disadvantages** are that it requires some experience to take appropriate samples and the needles are expensive to purchase. Also, as the sample size is small, some experience and care is required to align the fibres correctly to allow true transverse and longitudinal sections to be made.

## Surgical biopsy

The technique for the *semimembranosus* muscles involves clipping and surgically preparing a 20 x 30 cm area adjacent to the *tuber ischii*, proximally or distally, 5 cm lateral to the tail (Fig 7). The tail head muscle is sampled in a similar fashion to the *semimembranosus/semitendinosus*, although the muscle is smaller (Figs 8a–d).

Mepivacaine is used for a subcutaneous 'L' block, which avoids infiltration of local anaesthetic into the area to be sampled. A 10 cm vertical incision is made, followed by blunt dissection down onto the muscle. Using curved haemostats, a 0.5–1.0 cm diameter by 2 cm long section of muscle, aligned in the same plane as the skin incision, is bluntly dissected free and elevated using the haemostats. A scalpel is used to cut one end of the muscle then, gently holding the free end, the fixed end is cut (Figs 9a–e). Contraction of the sample following incision is expected, such that the resultant sample is approximately 1 cm in length. The muscle sample should then be placed on a piece of cardboard or tongue depressor to maintain alignment of the fibres and fixed in 10% buffered formalin or frozen. The skin is then closed with staples or sutures.

The **advantages of this method** are that it is technically straightforward and produces a large sample size, which may increase the chance of finding inclusions characteristic of EPSM in myofibres. The **disadvantage** is that it requires a short period of convalescence as the suture line heals.

## Adverse sequelae

The needle biopsy is minimally invasive and adverse sequelae are rare. In **the authors' experience**, the only sequela that has occurred in hundreds of biopsies is some mild haemorrhage that responded to digital pressure.

Wound dehiscence and superficial infection has been reported following surgical biopsy of the *semimembranosus/semitendinosus* muscles, but not following surgical biopsy of the tail head muscle (Valentine *et al.* 1998). This complication appears to be rare and healing by second intention is recommended if dehiscence occurs.

## Fixation and laboratory analysis of samples

Formalin fixation is usually achieved with 10% buffered solution but care should be taken to ensure the sample is no larger than 0.5–1.0 cm diameter, or the centre portion will not be fixed adequately. The disadvantage of this technique is that there may be some shrinkage artefact or cracking on the cut sections.

**Freezing** reduces these artefacts and is necessary for fibre typing; however, in the authors' opinion, is not necessary for routine clinical work. Methods of freezing employed to reduce freezing artefacts include rolling the sample in talcum powder prior to immersion in liquid nitrogen, cooling slowly on the top of liquid nitrogen in an aluminium 'boat', or freezing in isopentane cooled in liquid nitrogen. Very few commercial laboratories routinely process frozen muscle samples, and

those that do often recommend sending a **fresh sample**, doing the freezing at the laboratory.

Not all laboratories routinely perform the special stains required to assess muscle biopsies or are comfortable interpreting the resulting slides. Few laboratories perform analysis of frozen specimens. **It is advisable to contact your laboratory** prior to sample collection to check whether they are happy to receive the sample and how they would like it fixed and transported. **The diagnosis of EPSM is dependent** on observation of abnormal aggregates of glycogen and inclusions of complex polysaccharide that are amylase- or diastase-resistant. These are demonstrated by staining with periodic acid-Schiff (PAS). PAS solution, once made, is labile so it is important to ensure that laboratory technicians are using freshly prepared stain to avoid poor stain quality. If there is any ambiguity regarding interpretation, it is advisable to recut and stain the slides.

## Conclusions

**In conclusion, muscle biopsy provides a simple, minimally invasive method of providing further information about a neuromuscular disease case. The incorporation of muscle biopsies into the diagnostic protocol of practising veterinarians will further our understanding of muscle disorders and denervating conditions in horses.**

## Manufacturers' addresses

<sup>1</sup>Arnolds Veterinary Products Ltd., Shrewsbury, Shropshire, UK.

<sup>2</sup>Kruise UK Ltd, Leeds, West Yorkshire, UK.

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