Equine Polysaccharide Storage Myopathy

A Necropsy Study of 60 Danish Horses

Summary
Equine polysaccharide storage myopathy (EPSM) is a muscle disorder in horses characterized by abnormal polysaccharide accumulation in muscle fibers and with clinical signs of rhabdomyolysis. EPSM has been studied intensively in the last decade and a prevalence of 8 % has been found in overtly healthy horses in the UK and around 20% in horses with neuromuscular disorders. The prevalence of EPSM has not been investigated in Denmark. The objective of the study was to determine 1) if EPSM exists in Denmark 2) the prevalence among horses forwarded for autopsy at the Department for Veterinary Disease Biology at Copenhagen University and 3) if any breed disposition could be identified. Muscle samples were fixated in formalin and examined for pathognomonic changes of EPSM i.e. periodic acid-Schiff-positive, amylase-resistant abnormal polysaccharide inclusions in type 2 muscle fibers. One out of 60 examined horses (1.7 %, 95 % confidence interval: 0.1 %–10.1 %) was identified with EPSM. In conclusion, EPSM does exist in the Danish horse population, with a prevalence of approximately 2 % among the examined horses.

Sammendrag
Equine polysaccharid storage myopati (EPSM) er en muskellidelse hos heste, karakteriseret ved aflejring af abnormalt polysakkarid i myocytterne og tegn på rhabdomyolyse. EPSM har været genstand for intensiv forskning de seneste 20 år, og en prævalens på 8 % er blevet fundet hos raske heste i Storbritannien og ca. 20 % hos heste med neuromuskulære lidelser. Prævalensen af EPSM hos danske heste har ikke tidligere været undersøgt. Formålet med denne undersøgelse var at undersøge: 1) hvorvidt EPSM forekommer i den danske hestepopulation, 2) prævalensen af EPSM hos heste, indsendt til obduktion på Institut for VeterinærSygdomsbiologi ved Københavns Universitet samt 3) undersøge forekomsten af en mulig racedisposition for EPSM. Muskelpøver fikseredes i formalin og undersøges for pathognomoniske forandringer, forenelige med EPSM, dvs. periodic acid-Schiff-positive, amylase-resistente abnormale polysakkarid inklusioner i type 2 muskelfibre. En ud af 60 undersøgte heste (1,7 %, 95 % confidence interval: 0,1 %–10,1 %) diagnosticeredes på denne baggrund med EPSM. Det konkluderes, at EPSM findes i den danske hestepopulation med en prævalens på ca. 2 % af de undersøgte heste. På grund af det lille antal muskelpøver med EPSM kunne der ikke undersøges, hvilke racer der er prædisponerede for at udvikle lidelsen.
Introduction

Dr. S. J. Valberg was the first person to identify Equine Polysaccharide Storage Myopathy (EPSM or PSSM) as a cause of equine exertional rhabdomyolysis in 1992 (1). Since then the disease has been diagnosed in at least 35 different breeds in USA and UK (2) and EPSM is considered to be a common cause of neuromuscular disease in Quarter Horse-related breeds, draft breeds and Warmblood breeds (2). As Warmblood breeds, and to some degree draft breeds, are common in Denmark, it would be important to document the possible existence of EPSM, because of its potential as a differential diagnosis for horses showing signs of rhabdomyolysis, gait abnormalities, muscle atrophy, back pain and poor performance (2, 3, 4) but also because of the potential genetic/breeding aspects.

Background

Etiology

EPSM is today categorized as either Type 1 or Type 2. Type 1 is caused by a gain-of-function mutation in the skeletal muscle glycogen synthase 1 (GYS1) gene resulting in a glycogenosis with excessive accumulation of glycogen. This mutation is inherited in an autosomal dominant manner (5). Horses having EPSM for other, yet unknown, reasons are categorized as having type 2 EPSM. In Quarter Horse-related breeds a modifying gene, the ryanodine receptor 1 (RYR1) gene mutation, has been discovered. Horses with both the GYS1 and the RYR1 mutation often have a more severe, occasionally fatal, clinical phenotype (6).

Clinical Signs

The clinical signs associated with both types of EPSM are all related to skeletal muscle dysfunction, with the most affected muscles being the rump, thigh and back muscles, including the gluteal, semimembranosus, semitendinosus and longissimus dorsi muscles (7). The most prominent clinical sign of EPSM in horses is exertional rhabdomyolysis manifested by muscle stiffness, shifting hind limb lameness, elevated respiratory rate, sweating, firm painful hindquarter muscles and reluctance to move (1, 8).

The average age of onset of clinical signs of EPSM differs among different breeds of horses (Quarter Horses approximately 5 years (9), Warmblood breeds approximately 7 years (10), draft breeds approximately 8 years (11)).

Diagnosis

Type 1 EPSM is diagnosed by a genetic test for the GYS1-mutation (12), whereas Type 2 is diagnosed by evaluation of muscle biopsies. The gold standard for diagnosis of EPSM in some breeds, where the GYS1 mutation is a frequent cause of EPSM, appears to be GYS1 genotyping followed by evaluation of muscle biopsy in those horses tested negative for the mutation (13, 14). Figure 1 shows the Neuromuscular Diagnostic laboratory at the University of Minnesota’s current recommended diagnostic process for EPSM (12).

Histology on muscle biopsies, typically from the semitendinosus or semimembranosus muscle in the horse (1, 15) will show the characteristic features in PAS stained histological sections including increased staining for glycogen and other polysaccharides, especially in the cytoplasm and in the subsarcolemma (Figure 2 and 3). In the amylase treated sections, stained with PAS, amylase-resistant PAS-positive...
abnormal polysaccharide inclusions can be seen in type 2 skeletal muscle fibers, which is pathognomonic for EPSM. Fiber size variation and internal nuclei with prominent nucleoli and muscle cell necrosis is often seen (Figure 4) (1, 11, 15). The accumulation of abnormal polysaccharide in skeletal muscle cells appears to develop over a few years and the accumulation may not be evident in muscle biopsies from affected horses less than 2 years of age (16).

Presence of PAS-positive amylase-resistant inclusions in muscle fibers is definitive for the diagnosis of EPSM (referred to as grade 2 EPSM). Some authors, however, have also included increased staining for amylase-sensitive glycogen in PAS stains, or the presence of PAS-positive sarcoplasmic masses (with or without amylase-resistant polysaccharide) to be diagnostic for the disease (referred to as grade 1 EPSM) (2, 17, 18, 19). The inclusion of amylase-sensitive glycogen as a diagnostic criterion for EPSM greatly increases the sensitivity and decreases the specificity; therefore it results in a higher prevalence of the disease in the population studied (2, 11, 20). The inclusion of this diagnostic criterion may, however, reduce the risk of false-negative results among younger horses, in which the amylase-resistant polysaccharide has not yet been developed (2).

Persistent elevations of serum creatine kinase (CK) and aspartate transaminase (AST), and a minimum of a threefold elevation in CK activity 4 hours after an exercise test (consisting of a maximum of 15 minutes of lunging at a walk and trot), are often seen among EPSM affected Quarter Horses. The median CK and AST activity

### Table 1. Overview of EPSM prevalence found in other countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Breeds</th>
<th>History</th>
<th>Prevalence EPSM (Grade 1)</th>
<th>Prevalence EPSM (Grade 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valentine &amp; Cooper 2005</td>
<td>USA</td>
<td>7 draft horses, 11 Morgan, 34 Arabian Horses, 8 Ponies, 16 Appalosa, 6 Tenessee Walking Horses, 68 Quarter Horses, 27 Paint, 14 Warmbloods, 22 thoroughbreds, 12 other breeds.</td>
<td>Horses examined at necropsy at Oregon State University. Horses &gt; 1 year old.</td>
<td>44.9%</td>
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<tr>
<td>Mc Gowan et al. 2009</td>
<td>UK</td>
<td>Abattoir study: 51 heavy breeds, 21 cob-types, 16 ponies, 6 light breeds. Neuromuscular disorders: 16 thoroughbreds, 6 Quarter Horses, 13 heavy breeds, 8 cob-types, 3 other light breeds.</td>
<td>Apparently healthy horses slaughtered for human consumption.</td>
<td>Overall: 40.1% (grade 1 &amp; 2) Draft horses: 56.9% Warmbloods: 51.5% Quarter Horses: 47.8%</td>
</tr>
<tr>
<td>Mc Cue et al. 2006</td>
<td>USA</td>
<td>66 draft breeds, 51 Warmbloods and 360 Quarter Horses.</td>
<td>Horses presented with a neuromuscular disorder.</td>
<td>Overall: 40.1% (grade 1 &amp; 2) Draft horses: 56.9% Warmbloods: 51.5% Quarter Horses: 47.8%</td>
</tr>
<tr>
<td>Firshman et al. 2005</td>
<td>USA</td>
<td>103 Belgian draft horses.</td>
<td>Farms were selected to participate in the study because EPSM had previously been diagnosed in at least 1 horse from each farm. Horses &gt; 1 year old.</td>
<td></td>
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<tr>
<td>Valentine et al. 2001</td>
<td>USA</td>
<td>37 draft horses.</td>
<td>Horses undergoing necropsy examination, 4/38 were examined specifically for evidence of EPSM. Horses &gt; 1 year old.</td>
<td>66%</td>
</tr>
<tr>
<td>Mc Cue &amp; Valberg 2007</td>
<td>USA</td>
<td>164 Quarter Horses.</td>
<td>Overtly healthy. Horses &gt; 2 years old.</td>
<td>6-12%</td>
</tr>
</tbody>
</table>
in this breed has been measured to be 2809 and 1792 U/L respectively. In contrast, serum CK and AST are often at normal levels among draft breeds and Warmbloods with EPSM (21).

Prevalence
The prevalence of EPSM is greatly dependent on whether the diagnosis of EPSM is based on increased staining for periodic acid-Schiff (PAS)-positive amylase-sensitive glycogen with or without amylase-resistant glycogen (grade 1) or finding of amylase-resistant inclusions in muscle fibers (grade 2). When grade 1 criterion has been used, the prevalence of EPSM has been reported as high as 44.9 %, the same study found the prevalence to be 22.7 % when the grade 2 criterion was used (19).

The prevalence of EPSM is high in draft breeds, varying between 36 % and 45 % (grade 2), and Quarter Horse-related breeds, varying between 6 % and 26 % (grade 2) and lower in light horse breeds such as thoroughbreds (2, 11, 18, 19, 25). A gender predilection for EPSM has not been identified (9,10,11).

In horses with a history of a neuromuscular problem biopsies revealed a prevalence of 40.1% (grade 1) and 21.7% (grade 2) at the Neuromuscular Diagnostic Laboratory, University of Minnesota. A prevalence of 22% (grade 2) has been found in United Kingdom (22). An overview of EPSM prevalence found in other countries is seen in table 1.

The prevalence of the GYS1 mutation in horses with EPSM depends on whether the EPSM diagnosis is based on grade 1 or grade 2 criteria, as the GYS1 mutation was present in 70.1 % of grade 2 and 16.1 % of grade 1 EPSM horses. When based on grade 1 criteria, the prevalence is much lower among all types of breeds or 1.7 % in draft breeds, 22.3 % in Quarter Horse-related breeds, 2.3 % in Warmblood breeds and 5.0% in other light horse breeds. The low prevalence of the mutation among horses with EPSM grade 1 may, to some level, be attributed to a higher number of false-positive diagnoses of EPSM within this group (23). The mutation is common in draft breeds (87.3 %)

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**Figure 3.** Transverse section of an equine skeletal muscle stained with PAS demonstrating glycogen and other polysaccharides in and between muscle fibers. In this muscle sample from an EPSM positive horse there is an increased PAS staining seen as deep purple areas within the cytoplasm (long arrow) and subsarcolemmal aggregates (short arrow).

**Figure 4.** Transverse section of the same muscle sample as in fig. 3. The sample was treated with amylase and stained with PAS. Abnormal PAS-positive, amylase-resistant polysaccharide can be seen within muscle fibers. Some fibers are completely filled up by abnormal polysaccharide (short arrows). Macrophages between muscle fibers containing PAS positive material are indicative of a prior myocyte breakdown (open arrow). Fiber size variation and muscle fiber necrosis (long arrow) can also be noted.

**Textbox 1.** How to obtain and ship specimens for EPSM diagnostics at the Neuromuscular Diagnostic Laboratory at the University of Minnesota.

**How to**

- Obtain a muscle sample

  Obtain a sample from semimembranosus muscle. The best site is midway between the tuber ischii and the origin of the Achilles tendon at about the level of the vulvar lips. Make a vertical incision through the skin, subcutaneous tissue and muscle fascia. Obtain a 5cm long, 1.5cm high and 1.5cm wide biopsy sample. Close any dead space between subcutaneous layers and skin with intradermal sutures or staples.

- Ship a muscle sample

  The fresh muscle sample should be wrapped in saline-moistened gauze, placed in a hard, watertight container, and shipped overnight on ice packs. The sample can also be formalin fixed. If a sample larger than TruCuts are submitted, they should sit in the air for five minutes before being placed in formalin.

- Obtain a sample for genetic testing

  Genetic testing can be performed on either whole blood, on mane or on tail hair samples.
  - Whole Blood: Draw 3-7 mL into a purple top EDTA tube labeled with the horse and owner’s ID.
  - Hair: Pull twenty mane or tail hairs with roots intact. Place the hairs in a completely sealed envelope or plastic bag and label the bag with horse and owner’s ID.

For more detailed information and slideshows on how to obtain samples for EPSM diagnostics, please visit www.cvm.umn.edu/umec/biopsy_instructions/home.html

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and Quarter Horse-related breeds (71.9%) and less common in Warmblood breeds (17.9%) and other light horse breeds (24.0%), when diagnosis of EPSM is based on the grade 2 diagnostic criterion.

**Treatment**
Long-term therapy for any of the varied clinical manifestations of EPSM involves dietary modifications and regular exercise. Dietary management should be aimed at providing adequate, but not excessive, calories by decreasing the glucose load and providing fat as an alternative source of energy. The best response in clinically-affected EPSM horses could be expected from a diet with 4% of digestible energy in starch and 13% of digestible energy in fat (24). Forage and pastures should provide the foundation for the diet of EPSM horses. Providing regular daily exercise and maximum turnout is also important management practice for horses with EPSM (9, 10, 16).

**Prognosis**
Equine polysaccharide storage myopathy cannot be cured; horses with the disease will always have an underlying predisposition for muscle soreness; however the clinical signs can be markedly reduced. If the recommendation regarding low-starch, high-fat diet and daily exercise/turnout is followed, an improvement in clinical signs of 71% and 75% has been archived for Warmbloods and Quarter Horses respectively (9, 10).

**Materials and Methods**
Sixty horses referred for necropsy at the Department of Veterinary Disease Biology at the University of Copenhagen over a 1-year period were included in the study. Age of the horses ranged from 2 to 28 years. Twenty-six of the horses were Warmbloods, 12 Icelandic horses, 8 ponies, 4 draft horses, 3 Quarter-Horse related breeds, 3 mixed breeds, 2 Standardbreds and 2 Arabian horses. The main pathological diagnoses of the horses could be related to the following subclasses: 34 cases related to the gastrointestinal tract, 6 orthopedic cases, 4 metabolic cases, 4 cases of neoplasms, 2 cases related to muscles, 2 cases related to the reproductive, 2 respiratory cases, 2 neurologic cases, 1 cardiovascular and 1 traumatic case and 2 horses not fully necropsied.

The samples obtained were longitudinal rectangular strips of semimembranosus muscle, approximately 70-80 mm long, 10-15 mm high and 10-15 mm wide. The samples were obtained 0 to 4 days (mean ± SD, mean 1.9 ± 1.0) post mortem. The samples were fixed in 10% formalin for 1 to 42 days (mean 3.9 ± 6.9, median 2) and cut in transverse and longitudinal sections. The transverse and longitudinal sections were stained with hematoxylin and eosin (HE). Transverse muscle sections were also stained with PAS-amylase. Each set of three stained muscle specimens (HE transverse, HE longitudinal, PAS-amylase transverse) was evaluated blind to the horse’s identity and clinical signs for histological features typically associated with EPSM, including presence of subsarcolemmal vacuoles, granular cytoplasmic glycogen, subsarcolemmal glycogen and abnormal amylase-resistant crystalline polysaccharide. A diagnosis of EPSM was made if abnormal amylase-resistant polysaccharide inclusions were observed in at least two muscle fibers/biopsy sample (grade 2 diagnostic criterion) (11, 19).

**Results**
Equine polysaccharide storage myopathy was diagnosed in 1 out of 60 horses (1.7%, 95% CI: 0.1%–10.1%). This horse was a 10-year old riding pony, gelding, referred due to colic to the Large Animal hospital, University of Copenhagen. The pony had been euthanized on the owner’s request. The pony had no documented history of muscle related problems. In the HE stained semimembranosus muscle specimen, a marked fiber size variation, muscle cell necrosis and centrally located nuclei could be noted. Subsarcolemmal vacuoles and granular cytoplasmic glycogen could also be seen. In the PAS-amylase stains, abnormal amylase-resistant crystalline polysaccharide was observed in approximately 20–30% of muscle fibers (figure 4).

**Discussion**
The reported prevalence of 1.7% is not consistent with the prevalence observed in other studies, but it is important to note that in the present study only the grade 2 diagnostic criterion was used. This study included horses with a broad variety of diseases, as opposite to only horses with a clinical history of neuromuscular problems and the breed composition of our study population, was not comparable with that investigated by others. The necropsy study performed by Valentine (18) found the prevalence of EPSM (grade 1) to be as high as 44.9% and EPSM (grade 2) to be 22.7%. Their study included 225 horses, of which 49.3% were Quarter Horse-related breeds, a breed known to have a high incidence of EPSM (2, 19, 25). They also included seven draft-related horses, where five of them were Percheron-related, a breed previously recognized to have a higher incidence of EPSM than any other draft breeds studied (18, 19).

A British abattoir study reports a prevalence of EPSM of 7%, but in this study more than half of the horses were draft-related horses, and among these 4/7 of the EPSM-horses were found (22). In the present study only 5% of the horses investigated were draft-related horses and only 5% were Quarter Horse-related breeds. A large percent of Warmbloods were included in this study (44%), none with a known history of neuromuscular disorder. To the authors’ knowledge the prevalence of EPSM in Warmbloods has only been investigated in two studies of horses with neuromuscular disorders. The prevalence in these two studies was found to be 19.1% and 32.6% (grade 2) respectively (2, 10). This relatively high prevalence is probably attributed to the fact that only horses with neuromuscular problems were included, but may also be due to the incorporation of draught horse bloodlines early in development of a variety of Warmblood breeds. It is important to note that all horses investigated in the
present study were diseased horses submitted from a university hospital and might not be representative for the general horse population in Denmark. In conclusion EPSM is a disorder that does exist among Danish horses. Until the prevalence has been investigated among horses with a history of neuromuscular problems, it is hard to predict the clinical relevance of our findings. Based on studies in other countries (2, 3, 10, 22, 26) and the present finding we recommend EPSM to be included as a possible differential diagnosis when dealing with an unexplainable neuromuscular problem or poor performance of Danish horses.

References