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26 for the *GYS1* mutation. Cases usually respond well to management changes, in
27 particular a diet low in starch and high in fat when it is accompanied by regular
28 exercise.

29

30 **Introduction**

31 Exertional rhabdomyolysis is a syndrome of muscle damage that is usually
32 precipitated by exercise. Once considered a single disease entity, it is now
33 understood to represent a common clinical presentation of several very distinct
34 disease processes (Valberg et al. 1999).

35

36 First described by Valberg et al. in 1992 in Quarter horse and Appaloosa related
37 breeds with exertional rhabdomyolysis, PSSM has subsequently been identified in
38 a number of different breeds found in Europe and the United States (Valentine et
39 al. 2000; McCue et al. 2006).

40

41 Polysaccharide storage myopathy is characterized by the accumulation of
42 excessive glycogen and diastase resistant amylopectin polysaccharide inclusions
43 within skeletal muscle fibres (Valberg et al. 1992) (Figure 1). Unlike normal
44 glycogen stored in muscle fibres these polysaccharide inclusions are resistant to
45 digestion with diastase and therefore are not broken down in the normal manner.
46 The discovery of a mutation in the glycogen synthase 1 (*GYS1*) gene in some but
47 not all horses with the disease (McCue et al. 2008) suggested that PSSM is in fact
48 a group of diseases of different aetiologies, and that the pathogenesis is more
49 complex than initially thought. As not all horses with PSSM possess the *GYS1*
50 mutation (McCue et al. 2008); this led to the disease being re-classified as type 1
51 PSSM (PSSM1) referring to individuals that possess the gene mutation, and type 2
52 PSSM (PSSM2) for individuals that have the characteristic histopathology in their
53 skeletal muscle but do not possess the mutant allele. Many of the earlier studies of
54 horses with PSSM performed by Dr Valberg and co-workers are now understood

55 to have involved horses with the *GYS1* mutation and therefore refer to PSSM1
56 (Valberg-personal communication). It remains possible that the polysaccharide
57 inclusions in horses with PSSM2 may be a common end-point of several different
58 pathological processes. As more is understood about this subset of horses without
59 the *GYS1* mutation several abnormalities of glycogen metabolism may be
60 identified and this group subdivided further, as with human glycogen storage
61 diseases. This paper reviews our current understanding of type 1 PSSM (PSSM1;
62 horses with the *GYS1* mutation) for which a definitive test is currently available.

63

64 **Skeletal muscle pathology**

65 Histopathology of muscle from horses with PSSM1 reveals excessive glycogen
66 alongside abnormal non-lysosomal bound polyglucosan bodies containing less
67 highly branched glycogen with protein aggregates (Valentine et al. 2001;
68 Annandale et al. 2004; McCue et al. 2009). The presence of subsarcolemmal
69 vacuoles, predominantly in type 2 muscle fibres (those with a propensity for
70 glycolytic metabolism) are also a common finding (Valberg et al. 1992) (Figure 1).
71 No disruption of the important membrane associated protein dystrophin has been
72 identified in affected horses (Naylor et al. 2012). Non-specific chronic myopathic
73 changes such as internalised nuclei and variation in muscle fibre size are
74 consistent with previous muscle damage and regeneration. Affected horses also
75 have a shift in muscle fibre type from type 2x to type 2a fast twitch fibres (Naylor
76 et al. 2012). As the disease affects type 2 muscle fibres, muscles that contain a high
77 proportion of these fibres such as the semimembranosus or gluteal muscle are
78 usually selected for biopsy. The severity of histopathological abnormalities will
79 likely reflect the fibre type proportion of a particular muscle. It is intriguing that

80 similar muscle pathology has recently been described in a large number of marine
81 mammals, although unsurprisingly the clinical histories for these species are
82 unknown (Sierra et al. 2012).

83

84 How the characteristic polysaccharide inclusions relate to the clinical signs in
85 patients with PSSM is unclear. There is evidence to support a metabolic defect
86 leading to a reduction of energy availability within affected muscle fibres
87 (Annandale et al. 2005). This may explain the observation of clinical signs in
88 young foals with the disease in the absence of extensive change on muscle biopsy
89 (Byrne et al. 2000; De La Corte et al. 2002), Therefore the polysaccharide
90 inclusions may be a co-incidental marker of the disease rather than causative. In
91 addition, it has been proposed that the physical presence of the polysaccharide
92 inclusions and subsarcolemmal vacuoles may disrupt the arrangement and
93 function of the myofibrillar proteins and their attachments, a theory supported by
94 the correlation between the severity of histopathology and muscle enzyme
95 activity (Naylor et al. 2012). It is difficult to reconcile the clinical improvement
96 observed in response to management changes if physical interference was solely
97 responsible for the clinical signs observed, as the polysaccharide inclusions do not
98 change with such husbandry modifications.

99

100 **Aetiology of PSSM**

101 In contrast to Thoroughbred horses with Recurrent Exertional Rhabdomyolysis
102 (RER) no abnormality of muscle contracture was detected in *in-vitro* studies of
103 muscle from horses with PSSM, suggesting a different pathogenesis of the two
104 diseases (Lentz et al. 1999). The absence of any derangement in the ability of

105 affected horses to utilize glycogen or produce lactate during exercise led to the
106 suggestion that PSSM results from abnormal glycogen storage rather than a defect
107 affecting glycogen utilization (Valberg et al. 1999). This is also supported by the
108 finding that the greatest increases in CK activity were observed in PSSM horses
109 performing submaximal rather than maximal exercise (Valberg et al. 1999).
110 Studies evaluating the role of insulin sensitivity in this abnormal glycogen storage
111 disease have yielded conflicting results in different breeds of horses (Annandale
112 et al. 2004; Firshman et al. 2008). Quarter horses with PSSM were shown to have
113 increased insulin sensitivity relative to control horses (De La Corte et al. 1999;
114 Annandale et al. 2004) whilst no difference was found between Belgian draught
115 horses and controls (Firshman et al. 2008). These differences may reflect inherent
116 breed differences in other genes regulating insulin sensitivity or differences in
117 muscle fibre type proportions (Firshman et al. 2008). Suggesting that mechanisms
118 other than heightened insulin sensitivity may be resulting in the accumulation of
119 abnormal polysaccharide within skeletal muscle of some horses with PSSM1.

120

121 There are eleven skeletal muscle glycogenoses recognized in humans, many of
122 which produce histopathological features similar to PSSM. These diseases are
123 associated with autosomal recessive defects in specific enzymes of glycogen
124 metabolism, lysosomal abnormalities or defects of AMP-dependent protein kinase
125 (DiMauro and Lamperti 2001). The clinical phenotypes observed in some of these
126 diseases are similar to those seen in horses with PSSM1. Initial work into PSSM in
127 the horse logically focused on evaluating the activity of these key enzymes in
128 affected horses, however no abnormalities were identified (Valberg et al. 1998).
129 Similarly no difference in the content of the major insulin sensitive glucose

130 transporter GLUT4 in muscle from affected horses was found (Annandale et al.
131 2004), although more recently many more GLUT receptors have been identified in
132 the skeletal muscle of horses, such as GLUT8 and GLUT12 (Lacombe 2014), and
133 their role in PSSM is yet to be evaluated.

134

135 Enhanced glycogen synthesis was suggested when horses with PSSM were shown
136 to re-synthesise glycogen more rapidly following exercise depletion than normal
137 horses (De La Corte et al. 1999b). As normal glycogen synthesis is under the
138 control of two enzymes; glycogen synthase and glycogen branching enzyme, an
139 alteration in the activity of one of these enzymes would likely affect the relative
140 branching of the glycogen molecule formed leading to an altered 3-dimensional
141 structure (Annandale et al. 2004) that may impart a resistance to digestion with
142 diastase.

143

144 **Type 1 PSSM**

145 In 2008, an autosomal dominant, gain of function, mis-sense mutation (R309H) in
146 the glycogen synthase 1 gene (*GYS1*) was identified in association with many but
147 not all cases of PSSM (McCue et al. 2008). Glycogen synthase is an enzyme
148 responsible for the production of glycogen, by joining glucose monomers via alpha
149 1,4 linkages, under the influence of insulin and glucose-6 phosphate. This point
150 mutation leads to a single amino acid substitution, from arginine to histidine that
151 results in increased glycogen synthase activity (McCue et al. 2008).

152

153 To date the *GYS1* mutation has been identified in a large number of breeds across
154 Europe and North America (McCue et al. 2008b; McCue et al. 2009; Stanley et al.

155 2009b; McCue et al. 2010; Jöhlig et al. 2011; Schwarz et al. 2011; Herszberg, et al.
156 2009; Baird et al. 2010) (table 1). A particularly high prevalence has been
157 identified in Quarter horses, Percheron and Belgian draft horses (McCue et al.
158 2008b), whilst to the authors knowledge it remains to be identified in a pure bred
159 Thoroughbred horse. Given that genotyping affected horses has only been
160 commercially available for the last five years, it is highly likely that as more cases
161 are genotyped we can expect to find the mutation in a greater range of breeds. The
162 particularly high prevalence in hardy draught breeds led some authors to suggest
163 that the disease phenotype may have imparted an evolutionary advantage in these
164 breeds, which is partly supported by a recent hereditary study (McCoy et al. 2014).

165

166 **Clinical presentation**

167 Whilst there is a distinct correlation between the *GYS1* genotype and the severity
168 of histopathology (Naylor et al. 2012) there remains considerable variation
169 between the clinical signs associated with PSSM1, from exertional rhabdomyolysis
170 to vague signs of poor performance, suggesting that other genetic and
171 environmental factors may act to modify the disease phenotype (Valberg et al.
172 2011). McCue and co-workers have shown that the ryanodine receptor (*RYR1*)
173 mutation associated with malignant hyperthermia leads to a more severe
174 phenotype in Quarter horses with PSSM1 (McCue et al. 2009b), whilst
175 environmental factors such as diet and exercise are known to attenuate clinical
176 signs (Firshman et al. 2003; Ribeiro et al. 2004; Borgia et al. 2010). It is plausible
177 that many of the previously suggested acquired causes of rhabdomyolysis, such as
178 hormonal imbalances or anti-oxidant deficiencies may exert an effect by
179 modifying the phenotype of an already genetically susceptible individual.

180

181 Whilst many horses with PSSM1 are asymptomatic (Johlig et al. 2011; Naylor et al.
182 2012), exertional rhabdomyolysis is the most commonly and perhaps easily
183 recognized clinical syndrome. Exertional rhabdomyolysis was reported in over
184 90% of affected cases in one study of horses where biopsies were obtained to
185 investigate poor performance (McCue et al. 2009). In one family of Warmblood
186 horses 59% of those with the *GYS1* mutation also had a history of exertional
187 rhabdomyolysis (Johlig et al. 2011), and those with the mutation were 7.1 times
188 more likely to show signs of exertional rhabdomyolysis than those without.
189 However more subtle clinical signs may easily be over-looked and these include
190 poor performance, muscle fasciculations, muscle atrophy, gait abnormalities,
191 generalized or pelvic limb stiffness, undiagnosed lameness, paresis or back pain
192 (Quiroz-Rothe et al. 2002; McCue et al. 2009). Interestingly PSSM affected horses
193 are often described as having a calm demeanor (Valberg et al. 2011).

194

195 **Shivers**

196 Early reports suggested a possible link between PSSM and the incidence of
197 Shivers, a neuromuscular condition characterized by a reluctance to lift the pelvic
198 limbs, and to back-up, often associated with fasciculations of the musculature of
199 the pelvic limb and tail (Firshman et al. 2005). This was supported by the high
200 prevalence of weakness in horses with PSSM and a report of two Belgian horses
201 with weakness and Shivers that were diagnosed with severe PSSM on
202 histopathology of skeletal muscle at post-mortem examination (Valentine et al.
203 1999). However no association between the two conditions was identified in two
204 larger studies of 103 Belgian draught horses (Firshman et al. 2005) or 132

205 Warmblood horses (Hunt et al. 2008). It appears that both PSSM and Shivers are
206 neuromuscular disorders that commonly occur in similar breeds of horses,
207 occasionally concurrently, and that there is no causative relationship.

208

209 **Cardiac disturbances**

210 Human glycogenoses are often associated with specific cardiac phenotypes that
211 contribute to exercise intolerance. In particular enhanced atrio-ventricular
212 conduction leading to arrhythmogenesis and cardiac failure are seen (Arad et al.
213 2005; Soliman, et al. 2008). Given that polysaccharide inclusions have been
214 reported in the myocardium of affected horses at post-mortem examination
215 (Valentine et al. 1997; Valentine et al. 2001; Larcher et al. 2008) and that sudden
216 death has been described in horses with PSSM, further investigation of the cardiac
217 phenotype of horses with PSSM1 was performed (Naylor et al. 2012b). No
218 significant structural changes or arrhythmias were detected in affected horses
219 when compared with matched controls (Naylor et al. 2012b).

220

221 **Diagnosis**

222 Resting muscle enzyme activity may be used to screen for subclinical muscle
223 damage in possible PSSM1 cases, however increases in basal creatine kinase (CK)
224 and aspartate transferase (AST) activity may not be observed in affected horses,
225 particularly in those that are heterozygous for the *GYS1* mutation (Naylor et al.
226 2012). Measuring skeletal muscle enzyme activity following 20 minutes of
227 submaximal exercise (e.g. trot and canter work) may increase the sensitivity of
228 this assessment, particularly for horses with signs of exercise intolerance (Valberg
229 et al. 1999). A significant difference was observed between horses with PSSM1

230 and controls in CK activity at 4 hours post-exercise but not AST activity 4 or 24
231 hour post-exercise in one study of Belgian draught horses (Naylor et al. 2012),
232 whereas significantly higher post-exercise AST activities were observed in horses
233 with PSSM1 relative to controls in an earlier study of Haflinger horses (Schwarz et
234 al. 2011). These studies suggest that there maybe breed differences in muscle
235 enzyme responses to exercise in horses with PSSM1 or may simply reflect small
236 sample sizes. Furthermore the changes in muscle enzyme activity following
237 exercise may be relatively small (increases of less than 50% above resting levels)
238 compared to those typically seen in other diseases such as RER and importantly
239 there is considerable overlap between the response of unaffected control horses
240 and those heterozygous for the *GYS1* mutation. Therefore raised muscle enzyme
241 activity should increase the index of suspicion of a myopathy and prompt further
242 investigations such as muscle biopsy or *GYS1* genotyping. However, PSSM cannot
243 be excluded in the absence of large changes of muscle enzyme activity following
244 exercise.

245

246 A diagnosis of PSSM has traditionally been made on histopathology of muscle
247 biopsy samples, however this technique is unable to clearly differentiate between
248 PSSM1 and PSSM2. The identification of the *GYS1* mutation has allowed the
249 development of a restriction fragment length polymorphism (RFLP) assay to
250 diagnose type 1 PSSM (McCue et al. 2008). This is performed on DNA extracted
251 from EDTA whole blood samples or hair roots (approximately 30 required- easily
252 collected from the mane or tail). This assay is a less invasive method for testing for
253 PSSM1 than the traditional muscle biopsy, and is particularly useful in breeds
254 known to have a high prevalence of the *GYS1* mutation. The blood test may also be

255 useful in younger individuals where changes on histopathology are fewer, as it is
256 known that the severity of the polysaccharide accumulations increases as an
257 animal ages (De La Corte et al. 2002). Furthermore genotyping affected animals
258 may provide useful prognostic information for making decisions with regards to
259 training and breeding, as it has been shown that the severity of the skeletal muscle
260 pathology correlates with the number of copies of the mutant allele with
261 homozygotes having more severe histopathology than those heterozygous for the
262 *GYS1* mutation (Naylor et al. 2012).

263

264 It is often useful to consider the breed of the animal when deciding which
265 diagnostic test(s) to perform. In breeds with a particularly high prevalence of
266 PSSM1 such as Draught and Quarter horse related breeds it may be preferable to
267 genotype the horse for the *GYS1* mutation initially. A particularly high prevalence
268 is found in continental European breeds, such as the Percheron, whilst the
269 prevalence in UK derived breeds such as the Clydesdale or Shire is much lower
270 (McCue et al. 2010). Conversely in breeds with a lower prevalence of the *GYS1*
271 mutation, such as Cobs and Welsh ponies or indeed those where the mutation has
272 yet to be described such as Thoroughbred horses, that would more likely have
273 PSSM2 or another myopathy, a skeletal muscle biopsy remains the most
274 appropriate diagnostic test currently available. Skeletal muscle biopsy samples
275 should be harvested from the *gluteal* or the *semimembranosis* muscles, and are
276 easily obtained from the standing sedated animal (Ledwith and McGowan 2004).
277 Biopsy samples are best preserved when placed in an empty sterile pot and
278 transported immediately on ice packs to the laboratory (Stanley et al. 2009). It is
279 recommended to liaise with the diagnostic laboratory prior to collecting the

280 biopsy and avoid posting samples at the end of the week, to avoid unnecessary
281 delays in transport.

282

283 **Treatment of PSSM**

284 The aim of managing horses with PSSM is to limit the constant synthesis of
285 glycogen within skeletal muscle by reducing circulating insulin and promoting
286 glycogen metabolism through regular exercise. In addition an alternative energy
287 source such as fat can be provided as long as the horse is not overweight. These
288 recommendations are based on research performed in horses with PSSM1. To date
289 there are no controlled studies in horses with PSSM2 although it is assumed that
290 similar recommendations apply. Regular daily exercise in addition to pasture
291 turnout is advised, in conjunction with a diet low in starch and sugar (<10%
292 digestible energy (DE) as non-structural carbohydrates (NSC)) and relatively high
293 in fat (13-20% DE) (Ribeiro et al. 2004; Borgia et al. 2010). Horses should continue
294 to receive 1-2% of their bodyweight as forage, ideally with a low (<12%) NSC
295 content (Borgia et al. 2011) and in some cases, depending on workload and energy
296 requirements, further caloric supplementation may not be required. Grazing may
297 need to be restricted at certain times of the year when the NSC content of grass is
298 particularly high. There are specifically formulated commercial diets available
299 (such as Dodson and Horrell ERS Pellets or Saracen ReLeve), although adequate
300 fat will only be provided if fed in quantities recommended by the manufacturers.
301 Alternatively a low starch diet may be supplemented with vegetable oil, up to a
302 maximum of 1ml/kg to provide sufficient calories. In some cases diets with a
303 slightly lower fat content may be more palatable yet still be sufficient to control

304 the condition. A high lipid diet increases the requirement for anti-oxidants,
305 therefore a feed balancer containing vitamin E may be beneficial.

306

307 The prognosis is favorable in cases where dietary and exercise recommendations
308 are followed, and these horses are significantly more likely to have an
309 improvement in the severity and frequency of clinical signs relative to those cases
310 where only one (exercise or dietary) recommendation is followed (Firshman et al.
311 2003). A clinical improvement may be observed within 6 weeks although
312 complete adaptation to these diets likely takes several months (Ribeiro et al.
313 2004).

314

315 **Type 2 PSSM**

316 Horses with PSSM2 are not easily distinguishable from those with PSSM1 on the
317 basis of clinical signs and histopathology, although subtle differences in the
318 morphological appearance of polysaccharide inclusions have been suggested
319 (McCue et al. 2009). Fine granular, often diastase negative, inclusions are
320 frequently located close to the sarcolemma in PSSM2 as oppose to the coarse
321 granular diastase positive granules frequently observed in PSSM1 (McCue et al.
322 2008; McCue et al. 2009). Type 2 PSSM may be a result of one sole enzymatic
323 defect, or perhaps more likely may reflect a group of glycogen storage diseases,
324 with a similar histopathological end-point. Further work is needed to further
325 elucidate the pathophysiologic process(es) involved in these horses.

326

327 **Conclusion**

328 Polysaccharide storage myopathy is a disease seen in a variety of breeds
329 throughout the UK and Europe as well as North America. The particularly high
330 prevalence of PSSM1 in some Draught breeds likely reflects a prior evolutionary
331 advantage. The recent identification of the *GYS1* mutation could allow for
332 eradication of the disease from these breeds by the implementation of
333 coordinated breeding programmes. This remains controversial, however, in those
334 breeds with a low incidence of clinical signs. The clinical presentation can vary as
335 can muscle enzyme activity in affected horses, therefore muscle biopsy and
336 genotyping for the *GYS1* mutation is required to establish a definitive diagnosis.
337 Whilst no specific treatment is currently available affected horses usually respond
338 well to management changes, in particular a low starch high fat diet in conjunction
339 with regular exercise. With appropriate management the prognosis is favorable.

340

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501

502 Table 1: Breeds in which the *GYS1* mutation has been identified in to date.

Breed
Quarter Horse
Paint
Appaloosa
Warmblood
Haflinger
Morgan
Mustang
Rocky Mountain horse
Belgian draught
Percheron
Shire
Suffolk Punch
Hanoverian
Rhineland
Cob Normand
Connemara x Welsh pony
Connemara x Thoroughbred
Cob
Argentinian polo pony
Polo pony
South German Coldblood
Saxon-Thuringian Coldblood

Exmoor pony

Continental European draught breeds

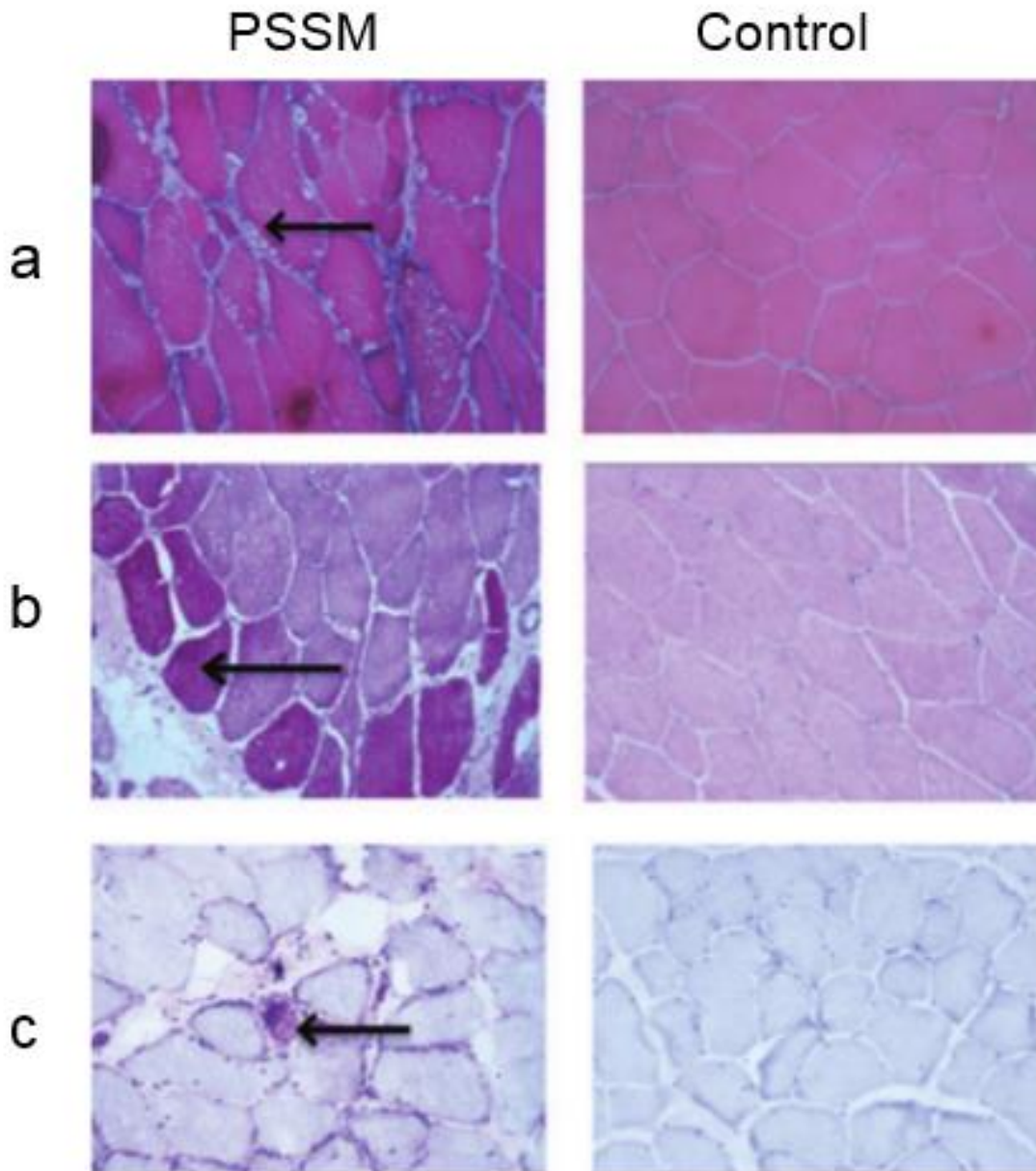
e.g. Ardenner, Belgian trekpaard

Crossbreeds

503

530

531 Figure 1: Characteristic skeletal muscle histopathology in type 1 PSSM compared
532 with muscle from a matched control stained with a) haematoxylin and eosin
533 showing sub-sarcolemmal vacuolation (arrow) and marked variation in fibre
534 size, b) periodic acid Schiff (PAS) showing increased glycogen accumulation
535 (arrow) and c) periodic acid schiff following predigestion with diastase revealing
536 abnormal diastase-resistant polysaccharide (arrow). ×20 magnification.
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