Estimated prevalence of polysaccharide storage myopathy among overtly healthy Quarter Horses in the United States

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Objective—To estimate the prevalence of polysaccharide storage myopathy (PSSM) among Quarter Horses in the United States and evaluate possible relationships between muscle glycogen concentration, turnout time, and exercise level.

Design—Cross-sectional study.

Animals—164 overtly healthy Quarter Horses > 2 years old from 5 states.

Procedures—Horses with a history of exertional rhabdomyolysis or any other muscular disease were excluded. Muscle biopsy specimens were examined histologically for evidence of PSSM and were submitted for determination of muscle glycogen concentration. A diagnosis of PSSM was made if amylase-resistant inclusions that stained with periodic acid–Schiff stain were detected.

Results—Prevalences of PSSM on the 2 farms with a history of PSSM were 20% (1/5) and 40.7% (11/27); mean prevalence for the other 4 farms was 6.1% (8/132). Sex was not significantly associated with a diagnosis of PSSM, and age was not significantly different between horses with and without PSSM. Total histologic score, serum creatine kinase activity, and muscle glycogen concentration were significantly higher in horses with PSSM than in horses without.

Conclusions and Clinical Relevance—Results suggested that the prevalence of PSSM among overtly healthy Quarter Horses in the United States is likely to be between 6% and 12%. (J Am Vet Med Assoc 2007;231:746–750)

Polysaccharide storage myopathy was first described as a cause of chronic exertional rhabdomyolysis in Quarter Horses and related breeds when inclusions that stained positively with PAS stain were identified in muscle biopsy specimens.1 Subsequent reports1-3 have suggested that horses with PSSM may display any of a spectrum of clinical signs ranging from overt rhabdomyolysis to subclinical increases in serum CK activity after exercise. A genetic basis for PSSM in Quarter Horses has been suggested from the results of a limited breeding trial3 and consanguinity in the pedigrees of affected horses.4

Polysaccharide storage myopathy has been diagnosed in > 50% of 753 Quarter Horses and horses of related breeds for which biopsy specimens have been submitted to the Neuromuscular Diagnostic Laboratory at the University of Minnesota.3 The true prevalence of PSSM in Quarter Horses, however, cannot be extrapolated from results for this group of horses, all of which had clinical signs of a neuromuscular disease. Previous authors5 have suggested that the prevalence of PSSM among horses of light horse breeds may be as high as 33%. However, alternative diagnostic criteria, such as increased amounts of amylase-sensitive glycogen and histologic evidence of cytoplasmic masses or spots, were used in that study. Although these criteria are highly sensitive when used to diagnose PSSM, they are associated with low specificity.5 Additionally, the study population was not composed solely of healthy individuals, but included horses examined at postmortem. Thus, the estimated prevalence of 33% may be an overestimate of the true prevalence of PSSM in the general horse population, and more information is needed on the prevalence of this condition.

In horses with PSSM, muscle glycogen concentration is between 1.5 and 4 times the concentration in healthy horses.1,8 Excessive glycogen storage does not appear to be caused by deficiencies in the enzymes involved in glycogenolysis or glycolysis.9 Rather, horses with PSSM have heightened sensitivity to insulin and enhanced glucose uptake,10,12 with glycogen accumulation likely being a result of increased glycogen synthesis. Despite the apparent availability of excess glycogen for energy metabolism, horses with PSSM have an energy deficit during submaximal aerobic exercise, as evidenced by accumulation of inosine monophosphate.

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>PAS</td>
<td>Periodic acid–Schiff</td>
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<tr>
<td>PSSM</td>
<td>Polysaccharide storage myopathy</td>
</tr>
<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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in the muscles. However, the relationship between muscle glycogen concentration and clinical signs of exertional rhabdomyolysis is unclear. Feeding horses with PSSM a diet containing a low starch but high fat content appears to ameliorate episodes of rhabdomyolysis, particularly when combined with regular exercise and turnout. To date, however, it has not been clear whether this improvement is linked to lower muscle glycogen concentrations, greater availability of fat for metabolism, or training-induced alterations in skeletal muscle metabolism.\textsuperscript{14,15}

The purposes of the study reported here were to estimate the prevalence of PSSM among overtly healthy Quarter Horses in the United States and to examine possible relationships between muscle glycogen concentration, turnout time, and exercise level.

\textbf{Materials and Methods}

\textbf{Horses}—Horses at 6 farms located in 5 states (Kansas, Oklahoma, Texas, North Dakota, and Wyoming) were included in the study. Four of the farms were selected to participate on the basis of previous professional collaborations. Three were university based (farms A, B, and C), and 1 was a large private farm (farm D); all 4 had large Quarter Horse populations. Three were breeding operations, and 1 was a performance operation. The remaining 2 farms were selected to participate because they were known to have previously produced horses with PSSM. One of these farms (farm E) was selected because it had a large number of horses available to biopsy; PSSM had previously been diagnosed in a single horse on this farm, but the horse was no longer on the premises. The other farm (farm F) was selected on the basis of proximity to one of the university-based herds that had already been enrolled in the study; PSSM had been diagnosed in a single horse on this farm. Feeding and turnout practices were recorded for each farm included in the study.

For each of the farms included in the study, muscle biopsy specimens were obtained from all horses \( > 2 \) years old that were made available by the farm owners, except that horses with a history of exertional rhabdomyolysis or any other muscular disease were excluded. The initial goal was to obtain biopsy specimens from a minimum of 100 horses. Age, sex, primary use, and pedigree information were recorded for each horse from which a biopsy specimen was obtained.

\textbf{Muscle biopsy procedure}—Gluteal muscle biopsy specimens were obtained from all horses by use of a modified, 6-mm Bergstrom biopsy needle. A standardized site 17 cm along a line from the dorsal aspect of the tuber coxae to the head of the tail and 8 cm deep was used for all horses. Approximately 300 mg of muscle was obtained from each horse. Approximately 100 mg of each muscle specimen was rolled in talcum powder to prevent freeze artifacts and immersed in liquid nitrogen for later histochemical analysis. Approximately 200 mg of each specimen was fixed in neutral-buffered 10% formalin, and the remainder of each sample was frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for later biochemical analysis.

\textbf{Histochemical analysis}—Frozen samples were mounted in cross section, and 10-\( \mu \text{m}\)-thick sections were cut with a cryostat. Sections were stained with H&E and PAS stains. Formalin-fixed samples were embedded in paraffin, and 4-\( \mu \text{m}\)-thick sections were cut. Sections were incubated with a solution of amylase (35 mg of amylase in 10 mL of water) for 15 minutes at\( 37^\circ\text{C}\) and then stained with PAS stain.

All sections were examined by a single investigator (SVJ), and a diagnosis of PSSM was made if amylase-resistant, PAS-positive inclusions were seen. In addition, sections were examined for 8 histologic features (intensity of PAS staining, subsarcolemmal glycogen, granular cytoplasmic glycogen, subsarcomemmal vacuoles, rimmed vacuoles, necrosis, centrally located nuclei, and atrophy). A score from 0 (absent) to 3 was assigned to each feature, and a total histologic score ranging from 0 to 24 was calculated for each horse. Finally, the presence or absence of sarcoplasmic masses or sarcocysts was recorded.

\textbf{Biochemical analysis}—Frozen specimens were weighed (approx 10 mg) and boiled in 1M HCl for 2 hours, and glycogen concentration was determined fluorometrically as glucose residues.\textsuperscript{16}

\textbf{Serum CK activity}—At the time of muscle biopsy, a blood sample was obtained from each horse by means of jugular venipuncture. Samples were allowed to clot, and serum was removed and frozen at \(-80^\circ\text{C}\) until analyzed. Serum CK activity was measured in a random sample of 60 horses.

\textbf{Pedigree analysis}—Four-generation pedigrees for each horse were inspected to determine the number of horses on each farm that were related within 4 generations.

\textbf{Statistical analysis}—Prevalence of PSSM was calculated, with detection of amylase-resistant, PAS-positive inclusions used as the gold standard for diagnosis of PSSM, along with sensitivity and specificity of total histologic score and sensitivity and specificity of muscle glycogen concentration. Confidence intervals for prevalence, sensitivity, and specificity were determined by use of the efficient score method.

The Pearson \( \chi^2 \) test was used to determine whether sex (male vs female), farm history (no farm history of horses with PSSM vs farm history of horses with PSSM), or horse use (breeding vs performance) was significantly associated with diagnosis of PSSM (yes vs no). Continuous data were tested by use of the Kolmogorov-Smirnov test to determine whether they were normally distributed. Normally distributed data (ie, serum CK activity and muscle glycogen concentration) were compared between horses with and without PSSM by means of an unpaired, \( t \) test. Data that were not normally distributed (ie, total histologic score, scores for individual histologic features, and age) were compared between horses with and without PSSM by use of the Mann-Whitney \( U \) test.

Values for normally distributed data were expressed as mean \( \pm \) SD; values for data that were not normally distributed were expressed as median and range. All analyses were performed with standard software.\textsuperscript{8} Values of \( P < 0.05 \) were considered significant.

\textbf{Results}

A total of 164 horses were included in the study, including 117 breeding animals and 47 performance
horses (Table 1). This included 4 stallions, 130 mares, and 30 geldings. Overall, PSSM was diagnosed in 20 of the 164 (12.2%) horses (95% CI, 7.8% to 18.5%).

A farm history of having previously produced horses with PSSM was significantly ($P < 0.001$) associated with diagnosis of PSSM, with prevalence of PSSM higher on the 2 farms with a history of having previously produced horses with PSSM than on the 4 farms without any such history (Table 1). Polysaccharide storage myopathy was diagnosed in 16 of the 117 (13.7%) performance animals; horse use (breeding vs performance) was not significantly ($P = 0.39$) associated with diagnosis of PSSM (yes vs no). Similarly, sex (sexually intact male vs gelding vs female) was not significantly ($P = 0.75$) associated with diagnosis of PSSM.

Age of horses with PSSM (median, 7 years; range, 1 to 19 years) was not significantly different ($P = 0.77$) from age of horses without PSSM (median, 7 years; range, 1 to 26 years). Total histologic score for horses with PSSM (median, 8.5; range, 5 to 13) was significantly ($P < 0.001$) higher than total histologic score for horses without PSSM (median, 4; range, 0 to 10). When scores for individual histologic features were examined, scores for intensity of PAS staining, granular cytoplasmic glycogen, rimmed vacuoles, necrosis, centrally located nuclei, and atrophy were significantly higher in horses with PSSM than in horses without (Table 2). Sarcoplasmic masses were seen in biopsy specimens from 5 of the 20 (25%) horses with PSSM and in 46 of the 144 (32%) horses without PSSM. Sarcocysts were an incidental finding in 10 of 164 (6%) of muscle biopsies.

Mean muscle glycogen concentration was significantly ($P < 0.001$) higher in horses with PSSM (182 ± 35 mmol/kg) than in horses without PSSM (111 ± 25 mmol/kg; Figure 1). Mean muscle glycogen concentration in horses without PSSM that were fed grain (113 ± 25 mmol/kg) was not significantly ($P = 0.45$) different from mean concentration in horses without PSSM that were not fed grain (109 ± 25 mmol/kg). Similarly, mean muscle glycogen concentration in horses with PSSM that were fed grain (187 ± 34 mmol/kg) was not significantly ($P = 0.40$) different from mean concentration without PSSM. Sarcocysts were an incidental finding in 10 of 164 (6%) of muscle biopsies.

![Figure 1—Scatterplots of muscle glycogen concentration in horses with (PAS positive; n = 20) and without (PAS negative; 144) PSSM; solid lines represent the mean glycogen concentration.](image)

<table>
<thead>
<tr>
<th>Farm</th>
<th>Selection criteria</th>
<th>Daily turnout time (h)</th>
<th>Grain fed</th>
<th>Total No. of horses</th>
<th>No. of horses tested</th>
<th>No. (%) of horses with PSSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>All broodmares</td>
<td>24</td>
<td>No</td>
<td>38</td>
<td>12</td>
<td>0 (0)</td>
</tr>
<tr>
<td>B</td>
<td>All broodmares</td>
<td>24</td>
<td>No</td>
<td>43</td>
<td>33</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>C</td>
<td>All horses</td>
<td>24</td>
<td>Yes</td>
<td>28</td>
<td>28</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>D</td>
<td>All horses caught</td>
<td>12–14</td>
<td>Yes</td>
<td>225</td>
<td>58</td>
<td>41 (18.5)</td>
</tr>
<tr>
<td>E</td>
<td>All breeding horses</td>
<td>24</td>
<td>No</td>
<td>35</td>
<td>27</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td>F</td>
<td>All horses caught</td>
<td>24</td>
<td>No</td>
<td>7</td>
<td>5</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>376</td>
<td>164</td>
<td>20 (12.2)</td>
</tr>
</tbody>
</table>

*Farms with a history of having a horse with PSSM. NA = Not applicable.

<table>
<thead>
<tr>
<th>Histologic feature</th>
<th>Median score (range)</th>
<th>Percentage of horses with feature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Horses without PSSM</td>
<td>Horses with PSSM</td>
</tr>
<tr>
<td>Atrophy*</td>
<td>0 (0–1)</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>Necrosis*</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>Centrally located nuclei*</td>
<td>0 (0–3)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Subsarcolemmal vacuoles*</td>
<td>0 (0–2)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Granular cytoplasmic glycogen</td>
<td>1 (0–2)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Subsarcolemmal glycogen</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>Rimmed vacuoles</td>
<td>0 (0–0)</td>
<td>0 (0–3)</td>
</tr>
<tr>
<td>Intensity of PAS staining*</td>
<td>2 (1–4)</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>Total*</td>
<td>4 (0–10)</td>
<td>9 (6–17)</td>
</tr>
</tbody>
</table>

Individual features were scored on a scale from 0 (absent) to 3. *Values were significantly ($P < 0.05$) higher for horses with PSSM than for horses without. NA = Not applicable.
in horses with PSSM that were not fed grain (180 ± 35 mmol/kg). Sensitivities of using muscle glycogen concentration to diagnose PSSM were 71.4% (95% CI, 48% to 88%), 72.2% (95% CI, 46% to 89%), and 75% (95% CI, 47% to 92%), respectively, at cutoffs of 153, 165, and 175 mmol/kg. Corresponding specificities were 96.4% (95% CI, 91% to 99%), 95.1% (95% CI, 90% to 98%), and 94.5% (95% CI, 89% to 97%), respectively.

Serum CK activity was measured in 10 horses with PSSM and 50 horses without. Mean serum CK activity in horses with PSSM (mean ± SD, 443 ± 87 U/L) was significantly (P = 0.02) higher than mean value for horses without PSSM (298 ± 109 U/L).

Examination of 4-generation pedigrees revealed that 4 of the 13 horses on farm A that were included in the study were related to each other within 4 generations, as were 27 of the 33 horses on farm B, 19 of the 28 horses on farm C, all 58 horses on farm D, all 27 horses on farm E, and all 5 horses on farm F.

**Discussion**

Accurately estimating the prevalence of PSSM among Quarter Horses in the United States would require testing a random sample of the entire population. The necessity of obtaining muscle biopsy specimens to diagnose PSSM and the expense of traveling to various farms made it difficult to completely randomize horse and farm selection in the present study. Results of the present study suggested that the estimated prevalence of PSSM was affected by farm selection. For example, observed prevalences of PSSM on the 2 farms that had a prior history of PSSM were 20% (1/5) and 41% (11/27), even though horses in which PSSM had previously been diagnosed were excluded. In contrast, on the 4 farms with no known history of PSSM, the prevalence was only 6%. Nevertheless, given the size of the population in the present study and the fact that farms were located throughout the United States, we believe that our findings can be generalized and that the prevalence of PSSM among overtly healthy Quarter Horses in the United States is likely to be between 6% and 12%. Thus, the prevalence of PSSM may be somewhat higher than the prevalence of hyperkalemic periodic paralysis, which is reported to be 4% for the general Quarter Horse population.1

Differences in prevalence of PSSM among farms in the present study did not appear to be due to management conditions. Limited turnout and diets with a high grain content have previously been associated with expression of clinical signs of PSSM.3 However, horses on farms with a high prevalence of PSSM in the present study were not receiving grain and had 24-hour turnout. Thus, the high prevalence on some farms may have been associated with inheritance of PSSM. Limited breeding trials have shown that PSSM is inherited in Quarter Horses,5 and all farms in the present study had horses with close familial relationships. We hypothesized that the higher prevalence of PSSM on farms E and F was the result of passing the genetic mutation for PSSM from 1 generation to the next. Further, we suggest that lower numbers of PSSM-affected broodstock were present on farms A, B, C, and D, resulting in infrequent transmission of the genetic defect to offspring and a lower prevalence of the condition, despite a high degree of relatedness among individuals.

One of the surprising findings in the present study was the high prevalence of PSSM in horses that lacked overt clinical signs of muscle disease. The lack of clinical signs may have been a result of ideal management conditions for horses with PSSM and the lack of forced exercise in the broodmares. Recommended management practices for horses with PSSM include limiting intake of soluble carbohydrates such as grain; providing fat as an alternative energy substrate, if necessary; and maximizing turnout time or providing daily exercise.6,7 These practices have been successful in decreasing muscle stiffness in all horses with PSSM and in eliminating episodes of rhabdomyolysis in approximately 75% of affected horses.8,9 Most horses in the present study had little or no access to grain and had ample time to exercise while foraging on large pastures. Furthermore, the performance horses that were fed grain had daily turnout and a rigorous exercise regimen 5 or 6 days a week. Thus, clinical signs of PSSM may have been unapparent because horses were managed under these conditions.

A high prevalence of subclinical PSSM among otherwise apparently healthy horses is of particular concern, as the inclusion of such horses in the breeding population may increase the prevalence of PSSM in the population. To minimize inadvertent breeding of PSSM-affected individuals, a sensitive diagnostic test is needed. In general, a test for the particular genetic mutation causing PSSM would provide the highest sensitivity and specificity. In the absence of such a test, strict criteria for interpretation of muscle biopsy specimens are needed to obtain a sensitive test that avoids misdiagnosis of healthy individuals or individuals with other neuromuscular diseases.

As was the case in a previous study7 of horses with clinical signs of PSSM, horses in the present study with subclinical PSSM had higher total histologic scores than did horses without PSSM. Thus, use of the histologic score in conjunction with evidence of amylase-resistant, PAS-positive inclusions may improve the accuracy of a diagnosis of PSSM. It is important to note that many of the features included in the histologic score are not specific for PSSM, and the possibility of other neuromuscular diseases that share these features should be taken in to consideration. We also evaluated whether the diagnosis of PSSM could be improved by assessing intensity of PAS staining and measuring muscle glycogen concentrations. Greater intensity of PAS staining, detection of sarcoplasmic masses, and detection of granular glycogen had high sensitivity for PSSM, but low specificity. Thus, it is not surprising that in a previous study6 in which these features were considered diagnostic for PSSM, the prevalence of PSSM was estimated to be twice the prevalence estimated in the present study.

Muscle glycogen concentration ranged from 124 to 234 mmol/kg among horses with PSSM in the present study, whereas muscle glycogen concentrations in healthy trained horses reportedly ranges from 125 to 163 mmol/kg.18 Use of a muscle glycogen concentra-
tion of 165 mmol/kg was associated with a sensitivity of 72.2% and specificity of 95.1%. However, muscle glycogen concentration alone did not appear to be an ideal diagnostic criterion. Thus, 1 possible approach to obtaining the most sensitive and specific diagnostic test for PSSM would be to consider all horses with amylase-resistant, PAS-positive inclusions, along with all horses with muscle glycogen concentration > 165 mmol/kg, as being positive for PSSM.

In conclusion, results of the present study suggested that the prevalence of PSSM among Quarter Horses in the United States is between 6% and 12%. The high prevalence in certain breeding herds was likely the result of unintentional breeding of affected individuals. In the absence of a genetic test, examination of muscle biopsy specimens is the best diagnostic technique available to clinicians. The sensitivity and specificity of this technique could potentially be optimized by considering both the presence of amylase-resistant, PAS-positive inclusions and muscle glycogen concentration. The existence of a large number of breeding individuals. In the absence of a genetic test, examination of muscle biopsy specimens is the best diagnostic technique available to clinicians. The sensitivity and specificity of this technique could potentially be optimized by considering both the presence of amylase-resistant, PAS-positive inclusions and muscle glycogen concentration. The existence of a large number of breeding horses with subclinical PSSM could inadvertently be sustaining or increasing the prevalence of this disease in the Quarter Horse population.

References