DIAGNOSTICATION OF HYPERKALEMIC PERIODIC PARALYSIS IN HORSES

DIAGNOSTICAREA PARALIZIEI HIPERKALEMICE PERIODICE LA CAI

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Hyperkalemic periodic paralysis (HYPP) is a muscle disease which has been first reported in 1985 in the USA, in a group of 4 horses with episodic weakness associated with intermittent serum hyperkalemia. The condition is caused by a mutation in the gene of the alpha-subunit responsible of sodium and potassium regulation pump in muscle inherited as a dominant autosomal trait. This mutation generates the production of an abnormal protein which alters the structure and function of the sodium channel. Because the mutation destroys the recognition site for the restriction endonuclease TaqI, the development of a PCR-RFLP method for HYPP diagnostication was possible. Our objective was to identify the normal homozygous, heterozygous carriers and affected homozygous horses for HYPP trait, using this method. Results suggest that the genetic test will be useful in identifying horses heterozygous for the HYPP trait and foals with HYPP.

Key words: horse, HYPP, PCR-RFLP, diagnostication.

Introduction

Hyperkalemic periodic paralysis (HYPP) is a muscle disease which has been reported in certain lines of registered Quarter Horses, Appaloosas and Paints in 1985 in USA (Cox JH, 1985). Affected horses often display sporadic attacks of muscle tremors (shaking or trembling), weakness and/or collapse. Attacks can also be accompanied by loud breathing noises resulting from paralysis of the muscles of the upper airway. Occasionally, sudden death can occur following a severe paralytic attack, presumably from heart failure or respiratory muscle paralysis (Cox JH and col. 1990). This modification may be seen in stallions, mares or geldings, can cross breeds and, therefore, is not limited to stock-type horses.

The disease is caused by a single point mutation (C→G) in the alpha-subunit of the muscle sodium channel gene. This mutation induces the substitution of the Phenylalanine with Leucine in the transmembrane domain IVS2 of the alpha-subunit of the muscle sodium channel (Rudolph JA and col., 1992). The genetic mutation is responsible for the production of an abnormal protein which alters the structure and function of the sodium channel. Normally sodium regulates the voltage current of the muscle cell, thus allowing for contraction or relaxation. In
HYPP horses sodium is allowed to leak into the muscle fibres, which causes them to fire indiscriminately. As a consequence of these involuntary muscle contractions potassium can leak out of the muscle cells to the blood and in these cases an elevated potassium level can be measured.

Materials and Methods

A group of 50 horses from Jegălia stud was analysed. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

**PCR Amplification and Sequencing**

We used one set of unlabelled primers which amplify a fragment from the alpha-subunit of the muscle sodium channel gene containing or not the single point mutation. PCRs were optimized by varying the annealing temperature (50–60°C) on a gradient thermocycler IQCycler (BioRad).

The amplified fragments were sequenced by ABI Prism 310 Genetic Analyzer, using the ABI Prism ® BigDye Terminator Cycle Sequencing Ready Reaction Kit after purification with the Wizard System Kit (Promega). The sequences were processed using DNA Sequencing Analysis 5.1 Software (AppliedBiosytems) and the nucleotide sequences were aligned with the Clustal X program and refined manually.

**PCR Amplification and HYPP diagnostication**

For HYPP diagnostication we used the same set of primers with forward primer labelled with 6-FAM dye.

PCR was done using a GeneAmp 9700 PCR System (AppliedBiosystems). The reactions were carried out in 25-μl final volume containing PCR Buffer, MgCl₂, 200 μM of each dNTP 0.5 μM of each primer (F-6-FAM-ACGCGCCGTGTTGCTCAAGAT; R-TGTTACCTGTCTACTGTTGGTG), 0.5 units of AmpliTaq Gold DNA Polymerase, diluted DNA and nuclease-free water. PCR amplifications were performed in 0.2 ml tubes using 30 cycles with denaturation at 95°C (30 s), annealing at 57°C (30 s) and extension at 72°C (60 s). The first denaturation step was of 10 min at 95°C and the last extension was of 15 min at 72°C.

PCR products were digested with restriction endonuclease TaqI at 65°C for 2 hours and the restriction fragments were loaded together with the GeneScan-500 ROX Internal Size Standard (AppliedBiosystems) into ABI PRISM 310 DNA Genetic Analyzer (AppliedBiosystems).

The results were analysed using the GeneScan 3.1.2. Software (AppliedBiosystems) which assigns a base pair size for each signal. GeneScan data can then be exported directly to Genotyper 2.5.2. Software (AppliedBiosystems) for automated genotyping.
Results and Discussions

Our goal was to develop an easy and efficient method which can be used to correctly identify the normal and the carriers horses for HYPP trait and to sequencing a fragment from the gene that contain the mutation.

The profile of the region from the PCR product that may contain the point mutation and Clustal X fragment alignment of a region from alpha-subunit of the muscle sodium channel gene and our PCR product are shown in Figure 1 and 2.

![Image](image1.png)

**Figure 1:** The sequence of the region from the PCR product that may contain the single point mutation inside the recognition site for **Taq I**.

![Image](image2.png)

**Figure 2:** Clustal X fragment alignment of a fragment from alpha-subunit of the muscle sodium channel gene and our PCR product.

For HYPP diagnostication we used PCR-RFLP method. The set of primers was designed to amplify only a 100 bp fragment from the alpha-subunit of the muscle sodium channel gene containing or not the single point mutation (C→G). This mutation modifies the recognition site for **TaqI** restriction endonuclease.
(T\textsuperscript{1}CGA → TGGA). The PCR conditions were selected in such a way that the two primers could amplify the DNA from normal, carrier and HYPP affected horses.

In our experiment successful amplification and digestion with \textit{TaqI} yielded one or two allele peaks with an expected size of 65 and (or) 100bp. The number of allele peaks depends on whether the individual tested is a heterozygote (carrier) or homozygote (normal or HYPP affected). For a normal horse we must obtain one peak at 65 bp since the enzyme cuts the PCR product in two fragments: one of 65 bp and one of 35 bp. Taking in account that only one primer is labelled with 6-FAM dye the laser scanning detects only the labelled fragment. For homozygous affected horse only one peak of 100bp is obtained because the mutation modified the restriction site. Two peaks of 65 and 100 bp are obtained for the heterozygous carriers because one allele is normal and the other one contains the single point mutation.

In our study all the analysed horses were normal. We did not find any carrier or HYPP affected horse.

In Figure 3 are shown the PCR-RFLP profile for homozygous normal horses.

**Figure 3:** Genotype analysis of PCR-RFLP profile for normal homozygous horse.

**Conclusions**

The major focus of this study has been to identify the normal homozygous, heterozygous carriers and affected homozygous horses for HYPP trait in Romanian horse breeds and to implement a useful diagnosis methodology in order to assist veterinarians and breeders in the disease controlling.

Developing genetic tests for this disease will allow breeders to identify carriers of HYPP and thus, focusing their breeding programs. To eliminate this trait from certain lines, stallion and mare owners should have their horses tested prior to breeding and select normal horses for their breeding stock.

Consequently, it will be of interest to use the genetic test to investigate the possible occurrence of the affected gene among from other countries horse populations and among different breeds.
Bibliography


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Paralizia hipercalemică periodică (HYPP) este o maladie musculară care a fost pentru prima oară descrisă în 1985 în SUA la un grup de patru cai care prezentau perioade de slăbiciune, asociate cu creşteri ale nivelului de potasiu seric. Ea este cauzată de o mutație într-o genă moștenită autozomal dominant care reglează concentrația de sodiu și potasiu deoarece codifică subunitatea alfa a canalului de sodiu. Această mutație determină formarea unei proteine anormale care alterează structura și funcția canalului de sodiu. Deoarece această mutație determină modificarea situsului de reacționare a enzimei TaqI, a fost posibil punerea la punct a unui test bazat pe tehnica PCR-RFLP pentru diagnosticarea HYPP. Obiectivul nostru a fost de a identifica homozigotoi normali, purtătorii heterozigotți și homozigotoii afectați de HYPP în cazul exemplarelor analizate, utilizând această metodă. Rezultatele obținute ne asigură că metoda de testare dezvoltată de noi va fi utilă atât în diagnosticarea caiilor purtători ai genei recesive, cât și a celor homozigotți recesivi.

**Cuvinte cheie:** Cal, HYPP, PCR-RFLP, diagnosticare.