

*Full Length Research Paper*

## Allele frequency of hyperkalemic periodic paralysis (HYPP) in quarter horses from Mexico

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**Hyperkalemic periodic paralysis (HYPP) is an autosomal co-dominant genetic disease of Quarter-mile horses which originated by a point mutation of the gene coding the sodium channel protein in the plasmatic membrane of muscular cells. The mutation affects both dominant homozygous and heterozygous animals with myotonia, unpredictable muscular paralysis, weakness and collapse. In some cases, death can occur due to paralysis of the hearth or respiratory muscles. Detection of affected animals can be achieved by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test. Based on the fact that the mutation originated in the stallion "Impressive", whose genetic material is known to have been used in Mexico, it is possible that HYPP have been disseminated among Mexican Quarter horses. Blood samples were obtained by random sampling from 51 Quarter horses and subjected to PCR-RFLP analysis. The results obtained showed 43 recessive homozygous (N/N, normal, 84.3%), seven heterozygous (N/H, affected, 13.7%) and one dominant homozygous (H/H, affected, 2%). Allelic frequencies found were  $N = 0.157$  and  $n = 0.843$ . The total of 15.7% affected animals can be considered a relatively high frequency of the disease; therefore, molecular diagnosis of HYPP is recommended to prevent a further spread of the mutation among Mexican Quarter horses.**

**Key words:** HYPP, quarter horse, PCR-RFLP, Mexico.

### INTRODUCTION

Hyperkalemic periodic paralysis (HYPP) is a genetic disease with an autosomal co-dominant way of inheritance which is present in quarter-mile horses and their crosses (Naylor et al., 1999). The disease has also been reported in horses of the Appaloosa and Pinto breeds (Church 1995; Rudolph et al., 1992a). It causes muscle tremors, weakness, and paralysis of respiratory

muscles, collapse and even death in some cases as a result of stress or during general anesthesia (Pang et al., 2011). Based on the fact that HYPP is a musculoskeletal disease, collapse occurs without loss of consciousness (Lyle and Keen, 2010). These symptoms appear in affected animals (dominant homozygous and heterozygous) between two and three years old.

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**Table 1.** Number of animals analyzed by location.

State sampled	County of animals	Number of animals sampled
Nuevo León	Allende	13
	Santiago	13
Coahuila	Arteaga	8
	Saltillo	10
Tamaulipas	Villagrán	7
<b>Total</b>		<b>51</b>

Symptoms are more severe in the dominant homozygous than in the heterozygous animals, and can be managed successfully given a rapid diagnosis and treatment (Naylor et al., 1999; Pang et al., 2011). Signs of the disease are manifested by a sudden increase in the blood serum potassium concentration, above 8 to 9 mEq/L, indicating an alteration of the normal potassium entrance and exit from muscle cells (Naylor 1994; Zeilmann et al., 1993). The disease is caused by a point mutation originating from the substitution of a cytosine for a guanine and the concomitant replacement of a leucine for a phenylalanine in the gene coding the sodium channel protein (transmembranal S region, subunit alpha) of muscle cells (Cannon et al., 1995; Rojas et al., 1991; Rudolph et al., 1992b; Valbers, 2010).

HYPP was originated with the stallion "impressive", which had a wide diffusion as a breeder in the U.S.A (Bowling et al., 1996; Naylor et al., 1994; Rudolph et al., 1992a). Reported frequencies of HYPP include studies in the U.S.A., in which a frequency of 4.4% of animals (frequency of 0.044) with the mutation was reported for Quarter Horses, corresponding to a allelic frequency of 0.02 (Bowling et al., 1996), as well as frequencies ranging from 0.008 in Quarter Horses to 0.299 in halter American paint horses (Tryon et al., 2009). The mutation has not been found nor described in other breeds or subtypes of horses and no further information of its frequency in horses exist. It is suspected that its lineage has extended into Mexico, since animals belonging to the "Impressive" genetic line do exist in this country. However, in order to determine the frequency of HYPP, random testing (not including only a single blood line) is needed (Bowling et al., 1996). Presently, no such studies exist in México.

The objective of the present work was to establish the genotype and allelic frequencies of HYPP by PCR-RFLP in an open population of Quarter horses and its crosses located at northern Mexico. These results will have an impact on further spreading the disease, since they could lead to the implementation of a screening program to identify animals with the mutation, assuring the genetic quality of the animals.

## MATERIALS AND METHODS

Blood samples were taken from 51 Quarter-mile horses chosen at random from three states and five counties located in north-east Mexico (Table 1). In this procedure, horses were sampled with no knowledge of their pedigree; therefore, the sampled horses did not belonged exclusively to animals with the "Impressive" blood line, but to the general Mexican Quarter-mile horse population. Molecular diagnosis of HYPP allows the determination of genotypes for the disease with a 99% precision (Bowling et al., 1996; Duyn and Hering, 1995). It consists of the amplification by polymerase chain reaction (PCR) of a 92 base pair (bp) segment of the gene coding the cellular sodium channel followed by its digestion with the restriction enzyme *Taq* I (restriction fragment length polymorphism, RFLP). *Taq* I cuts the PCR product when the mutation is absent, originating into two fragments (64 and 28 bp). The mutation destroys the *Taq* I restriction site and therefore no digestion occurs when it is present (Rudolph et al., 1992b).

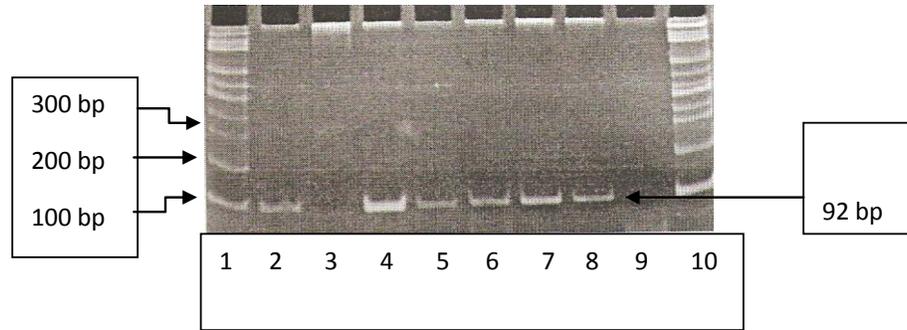
The Puregene kit (genomic DNA Purification kit, Gentra Systems) was used for DNA extraction. PCR primers used were: IVS<sub>2</sub>F4: 5'-GGGGAGTGTGCTCAAGATG-3'; IVS<sub>3</sub>R: 5'-AATGGACAGGATGACAACCAC-3'. These primers amplify a 92 base pair (bp) DNA fragment containing the mutation. PCR conditions were as follows: initial denaturalization at 94°C for 5 min; 35 cycles of denaturalization at 94°C for 1 min, hybridization at 60°C for 1 min, extension at 72°C for 1 min; a final extension at 72°C for 10 min. To each sample of 5 µL genomic DNA diluted to 20 ng/µL was added a reaction mix composed of 10 µL of *Taq* and Go PCR mix (Gentra Systems) containing *Taq* DNA polymerase (2.5 U/sample), PCR buffer solution (1×), dNTP's (200 µM) and MgCl (1.5 mM), plus 0.5 µL each PCR primer (20 pM) and 34 µL molecular grade water for a final volume of 50 µL. Amplifications were carried out in a hot-lid thermocycler, always including a negative control (without DNA). An aliquot of PCR products obtained were subjected to digestion with restriction enzyme *Taq* I for 1 h at 65°C (Rudolph et al., 1992b).

Both the PCR and digestion products were visualized by 30% polyacrylamide gel electrophoresis, staining the DNA with ethidium bromide for detection with a photo documenter according to standard procedures. In order to determine genotypes, fragment size was determined by comparison with molecular ladders. Genotypes: dominant homozygous, H/H, affected: one 92 base pair (bp) band; heterozygous, N/H, affected: one 92, one 64 and one 28 bp band; recessive homozygous, N/N, unaffected: one 64 and one 28 bp band. Genotype frequencies were determined by direct count of animals according to the molecular diagnostic, dividing the number of animal with each genotype between the total numbers of analyzed animals. Allelic frequencies were calculated by direct gene counting, following the standard formulae showed below:

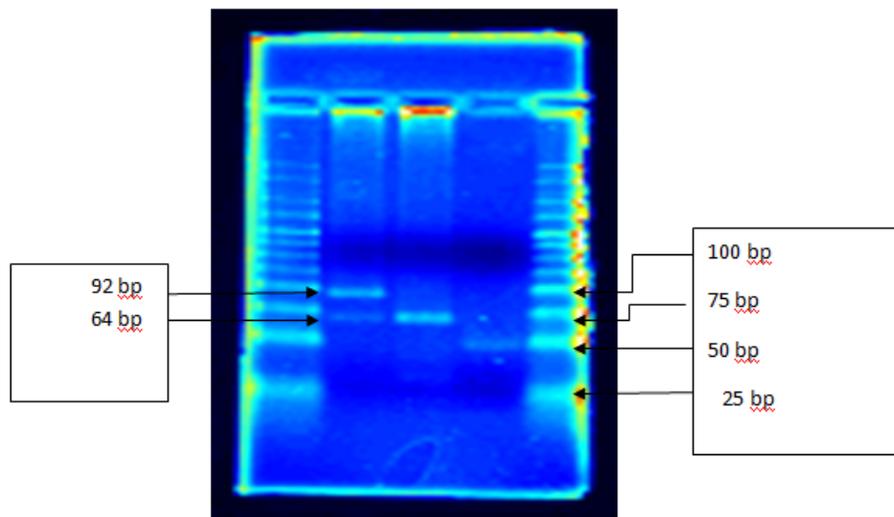
N allele: frequency of  $\frac{N}{N}$  + frequency of N/N; H allele: 1 - N allele.

## RESULTS

The undigested PCR product (92 bp) was obtained from all 51 samples (Figure 1). RFLP analysis showed that the expected 28 bp band could not be detected; however, it was possible to determine the genotype of the animals based on the presence of the other expected bands, since the N/N genotype had only one 64 bp band, the N/H had a 92 and a 64 bp bands and the H/H had only one 92 bp band (Figure 2). Based on this, one animal was dominant homozygous (frequency of 0.02), 7 were heterozygous (0.14) and 43 animals were recessive



**Figure 1.** Visualization of PCR amplification of the HYPP gene. The expected PCR product of 92 base pairs (bp) is shown (lanes 2 and 4 - 8). Lane 3 shows no amplification in this particular experiment and lane 9 is the negative control. Lanes 1 and 10: molecular size marker (100 bp ladder).



**Figure 2.** Visualization of PCR-RFLP analysis. Lane 2 shows the presence of the 92 and 64 base pair (bp) size bands (expected in genotype Nn). Lane 3 contains only the 64 bp size band (expected in genotype nn). The expected 28 bp band is missing in both lanes 2 and 3; however, both the heterozygous affected (Nn) and the normal (nn) genotypes can be distinguished from the homozygous affected (NN) genotype (only a 92 bp band, result not shown). The close to 50 base pair size band in lane 4 is a primer dimer and contained a negative control. Lanes 1 and 5: molecular weight marker (25 b.p. ladder).

homozygous (0.84). In total, 8 animals (0.157) presented the mutation. Allelic frequencies were H = 0.157 and N = 0.843 (Table 2). The 28 bp band was not detected due to its small size, which makes it difficult to visualize in gels.

## DISCUSSION

The frequency of animals with the mutation in the studied population (0.157) can be considered moderately high when compared to previously informed frequencies ranging from 0.008 to 0.299 in the U.S.A. from Quarter Mile horses sampled at random (Bowling et al., 1996; Tyron et

al., 1996). In the present paper, evidence exists of the introduction of HYPP into the Netherlands (Sloet van Oldruitenborgh-Oosterbaan, 1999) and Australia (Church, 1995); furthermore, current cases of HYPP in quarter horses, confirmed by DNA analysis, do exist in countries such as Canada (Pang et al., 2011). These results are in agreement with those presented in this paper and confirm the need of strengthening the procedures for the verification of the status for HYPP by laboratory analysis. Some evidence at the Western part of México exists on the presence and frequency of animals with HYPP. At a local Congress held in 1995, a frequency of animals with HYPP of 40% (0.4) was found among animals that shared

**Table 2.** Number and frequency of animals by genotype.

Genotype (%)	Number of animals	Genotype frequency
Dominant homozygous (NN, affected)	1	1.96 (1/51)
Heterozygous (Nn, affected)	7	13.70 (7/51)
Recessive homozygous (nn, normal)	43	84.30 (43/51)

the “Impressive” blood line (Ayala-Valdovinos et al., 2006, unpublished results).

As mentioned earlier, random sampling is needed in order to obtain the allelic frequency. The frequency of animals found in the present study (0.157) is considerably lower than the mentioned Western México, which can be explained for the kind of animals sampled. However, our results give additional evidence of relatively high allelic frequency for HYPP in quarter horses in Mexico. In our study, close to 16% of the animals sampled were positive for HYPP. We considered that the results obtained in the present study are only applicable to the Mexican north-east Quarter-mile horse population, and in order to determine the whole frequency of horse HYPP in Mexico, further studies should be performed at other areas of the country, as well as in other horse populations such as in the Pinto and Appaloosa breeds.

This result indicates that although the pedigree record must include a certification of HYPP-free of Quarter horses imported from the U.S.A. to Mexico, the mutation still exists in the genetic pool of this breed and its crosses. However, since no such laboratory test is required for animals born inside Mexico, a genetic test based on PCR-RFLP analysis could contribute to the elimination of the disease from the quarter horse population in this country, which is in agreement with the conclusions obtained by other authors indicating that the use of DNA analysis for selective breeding is important for the reduction and eradication of HYPP, and that only normal (N/N) horses should be bred (Nollet and Deprez, 2005).

On the other hand, the American Quarter Horse Association have ruled that foals born in 2007 or later that tested homozygous for HYPP (H/H) would not be eligible for registration (Bettley et al., 2012). This type of measures could be implemented in Mexico in order to obtain the same goal of reducing or eradicating HYPP.

### Conflict of interests

The author(s) have not declared any conflict of interests.

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