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FREQUENCY OF PORCINE STRESS SYNDROME MUTATION IN COMMERCIAL POPULATIONS IN MEXICO

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Introduction

Porcine stress syndrome (PSS) is a hereditary predisposition manifested in affected individuals as sudden death induced by stressful situations. This syndrome may be triggered while handling or transporting susceptible animals, during mating, delivery, or exposure to extreme environmental temperatures. After sacrifice, PSS-susceptible pigs develop a muscle degeneration known as pale, soft, exudative muscle (PSE). Furthermore, PSS and PSE susceptible pigs show extreme susceptibility to certain drugs such as succinylcholine or volatile anesthetics such as halothane. Exposure to this compound causes a syndrome known as malignant hyperthermia (MH) (Mitchell and Heffron, 1982).

It is currently known that the same genetic defect is responsible for PSS, PSE and MH in pigs, which is a mutation involving a substitution of cytosine (C) for thymine (T) at the nucleotide 1843 in the ryanodine receptor gene (RYR), which codifies for a calcium release channel at the sarcoplasmic reticulum. This mutation has been identified in practically all commercial pig breeds (Fujii et al., 1991; Otsu, Khanna, Archibald, MacLennan, 1991; Harbitz,, Kristensen., Bosnes, Kran & Davies, 1992).

The PSS caused annual losses estimated at 230 to 320 million dollars per year to the American pork industry. It had been estimated that approximately 30% of South African and German Landrace pigs were susceptible to MH (Mitchell et al., 1982). Similarly, gene frequency for MH was 0.42, 0.34 and 0.22 in Swedish Landrace, Yorkshire and Duroc breeds respectively (Gahne and Juneja, 1985).

The epidemiology of porcine stress syndrome is currently unknown in Mexico. Furthermore, the economic impact of this condition on the national pork industry and the need to systematically control the disease, have not been assessed.

Objectives

The objective of the present study was to determine the frequency of the RYR mutant allele in Mexican commercial porcine populations.

Methodology

2.1. Samples

A total of 464 blood samples were drawn from hybrid commercial pigs at the moment they were sacrificed at municipal slaughterhouses of Ecatepec in the State of Mexico (n=158), Pachuca (n=271) and Tizayuca (n=35) in the State of Hidalgo. Samples were selected randomly during several visits to each slaughterhouse over a 3 months period.

2.2. Mutation characterization

DNA was extracted from the blood samples using the sodium chloride exclusion method (Miller, Dykes, Polesky, 1988). A DNA segment containing the porcine ryanodine gene was amplified by the polymerase chain reaction (PCR). Amplified products were immobilized in a nylon membrane and hybridized with radioactively labeled oligonucleotides specific for the normal and mutant alleles (Fujii et al., 1991).

The amplification reactions were done in a final volume of 50 µl containing 50 mM KCl; 10 mM Tris-HCl, pH 8.3 10; 1µg/ml gelatin; 1.5 mM $MgCl_2$; 200 µM of each triphosphate deoxynucleotide (dATP, dCTP, dGTP, dTTP); 100 µg genomic porcine DNA; 50 pmols of each primer: RYR1 (5'-CTCCAGTTTGCCCACAGGTCCTACC-3) and RYR2 (5'- CATTCACCGGAGTGGAGTCTCTGAG´-3') (Fujii et al., 1991) and 2.5 units Taq-DNA polymerase (Biogenica, México). Amplification conditions were as follows: an initial denaturating cycle at 94^{0}^C for 3 min; 30 subsequent cycles consisting of denaturation at 94^{0}^C, 30 s; annealing at 62^{0}^C, 30 s; and extension at 72^{0}^C, 30 s; and a final extension cycle at 72^{0}^C for 3 min. The amplification products were assessed on 3% agarose gels stained with ethidium bromide and visualized under UV light according to conventional methods (Sambrook, Fritsch, Maniatis, 1989). Afterwards, 30 µl of 0.6M NaOH was added to 20 µl of the amplification product. After 10 minutes incubation, 50 µl of cold 2M ammonium acetate was added, and 40 µl of this mixture was applied on two nylon membrane replicas (Magna, MSI, INC. Westboro, MA. USA) and UV crosslinked. After that the membranes were hybridized either with the mutant specific oligonucleotide 15T (5'- GGCCGTGTGCTCCAA), or with the normal allele specific oligonucleotide 17C (5'-TGGCCGTGCGCTCCAAC) at 55^{0}^C. Both oligonucleotides were labeled with polynucleotide kinase and gamma-P^{32}^ATP following previously described protocols (Sambrook et al., 1989). Hybridized membranes were washed twice with 6X SSC, 0.1% SDS at 55^{0}^C (for the 15C probe) or at 65^{0}^C (for the 17C probe), and later exposed to X-ray film (Kodak X-Omat) for 1-2 days at –70^{0}^C. Each membrane contained the amplified products of previously typified animals (CC, CT and TT) in duplicate as internal hybridization controls. Genotyping was performed by comparing the hybridization signals of each replica (Figure 1).

2.3. Genetic and statistical analysis

Allelic frequencies were estimated directly by allele counting. In addition, observed heterozygosity (Ho) and expected heterozygosity (Hs) were used to estimate the fixation

index Fis = 1- Ho/Hs, (Nei, 1987), which indicates the excess or deficiency of homozygotes in a population. In order to test whether this value differs from zero, we used the chi-square test with one degree of freedom $\chi#$^{2}^= nFis^2; where n equals the number of individuals in a population.

Results & Discussion

Although several methods have been used to predict the quality of pork (Cheah, Cheah, Lahucky, Mojto, Kovac, 1993), molecular biology techniques are very promising. The identification of the RYR mutant and normal alleles by allele-specific oligonucleotide hybridization used here was appropriate to assess large number of samples, and was a reliable and sensitive method, in spite of the disadvantages implicit in using radioactive isotopes. We also tested the use of restriction enzymes to detect the mutation (O'Brien, Shen, Cory, Zhang, 1993**),** however this assay was unsuccessful due to the recurrent low activity of the commercial restriction enzyme available.

The prevalence of the mutation responsible for the porcine stress syndrome, namely the total number of animals carrying at least one mutant allele (mutant homozygotes plus heterozygotes), was 40.3% in commercial Mexican pigs (Table 1). Studying over 10,000 pigs, O'Brien et al. (1993) reported that the prevalence of the mutant allele was 16%. The prevalence of the mutation in Mexican commercial populations was more than 2.5 fold higher. In the same study, O'Brien et al. (1993) found a high prevalence of the mutant allele in commercial breeds, presumably breeding lines: 97% in Pietran, 35% in Landrace, 19% in Large White, 19% Yorkshire, 15% Duroc, and 14% in Hampshire breeds.

In Mexico most of the commercial pig populations are obtained by crossing hybrid dams (Hampshire-Yorkshire or Landrace–Yorkshire) with purebred sires, mainly Duroc or Hampshire. Although, also are used hybrid sires with crosses of Hampshire, Yorkshire or Duroc breeds (CONAPOR, 1998). Because, it is known the association of the mutant T allele to greater muscularity and lean carcasses (Leach, Ellis, Sutton, McKeith, Wilson, 1996), this mutation has been introduced in breeding pig lines in order to have heterozygotes in the final commercial pig. However, this breeding tactics imply the rational use of typed animals. The high prevalence of the T allele in our data indicates that the T allele is being intensely introduced into the Mexican porcine populations. This probably is the result of using breeding animals with high prevalence of the T allele. Unfortunately, there is no current available data of the frequency of RYR genotypes in Mexican breeding pig populations and it seems the pig industry is not performing surveillance and control of this gene.

Table 2 shows the allelic frequencies for the RYR gene, the observed heterozygosity (Ho), the expected heterozygosity (Hs) and the fixation index (Fis) estimated in the slaughterhouse porcine population. The gene frequency for the T allele was 0.214. Remarkably, the population was not in genetic equilibrium ($\chi#$^{2}^= = 6.62, $P<0.05$). This disequilibrium was mainly due to an excess of heterozygous genotypes in this population, as measured by negative Fis values (-0.119), and was apparently due to a reduced number of the frequency of expected mutant homozygotes (2.6% vs 4.6%) (see Table 1).

Our results suggest that there is a strong selection pressure against the T allele in commercial animals reaching the slaughterhouses. This would explain why the actual genotype frequency of TT (2.4%) was lower than expected (4.6%) In fact, the contribution of TT individuals to the chi-square value was almost 4 units of the total estimated chi-square value of 6.6. This means that almost half of these animals die due to porcine stress syndrome. Because our samples came from different farms and locations, we were expecting to find the existence of isolated reproductive populations (Wahlund effect; Nei, 1987) with an homozygosis excess. However, we rather found a homozygous deficiency. This means that the entire pig populations is behaving as a mendelian population, and the selection process is affecting negatively the TT genotypes.

Considering that around 13.5 million pigs per year are sacrificed in Mexico (Gallardo-Nieto, Villamar, Barrera, Ruiz, 2004), and if the 95% confidence interval for the T allele frequency is between 0.1617 and 0.2663; then, it would be expected that annually around 351,514 and 953,399 pigs would be homozygotes TT. Moreover, and if we regard an homozygote mortality estimated of 43.58%, then we may expect that between 153,005 and 414,992 pigs could die in some point prior to the sacrifice due to the porcine stress syndrome. Of course, the later the loss within the productive process, the greater the economic harm. However, considering that the investment cost for each pig that reaches the slaughterhouse is about $100.00 dollars, the economic losses caused by porcine stress syndrome can be estimated at 15.3 to 41.5 million dollars per year. Obviously, these figures may be even higher if we consider the losses caused by the PSE.

The samples that we studied were obtained from municipal slaughterhouses who process pigs from at least 17 different Mexican states. Although this is a preliminary study, it offers the first estimation of the prevalence of the RYR T 1843 C mutation in commercial porcine populations of Mexico. It will be important further studies in larger samples and in breeding populations.

Conclusions

Considering the high incidence found in Mexican commercial pig populations, and its association with PSS and PSE, the decision to maintain or eliminate the RYR mutant allele is a fundamental issue to be raised in the Mexican porcine industry. It seems that in most countries the current recommendation is the systematic elimination of the T allele from pig populations. If Mexican porcine industry follows the same trend, then it will have to be establish genetic services to identify carrier animals, as well genetic controls for the imported and locally commercialized breeding animals. This eventually will develop better surveillance and quality control systems of the porcine germoplasm, leading to few economic losses for the local porcine industry.

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Tables

Table 1. Observed and expected absolute and relative genotypic frequencies of the ryanodine receptor alleles in Mexican commercial pigs.

	CC		CT	CT	TT	TT	Total
	N	%	N	%	N	%	
Observed	277	59.7	175	37.7	12	2.6	464
Expected	286.75	61.8	155.90	33.6	18.5	4.6	464

N= Number of individuals registered with each genotype.
%= percentage of individuals identified for each genotype.

Table 2. Allelic frequencies for the ryanodine receptor gene, observed heterozygosity (Ho), expected heterozygosity (Hs) and fixation index (Fis) estimated in the porcine population.

C	T	s.e.	Ho	Hs	Fis	χ^2
0.786	0.214	0.0267	0.377	0.337	-0.119	6.62*

C= normal allele. T= mutant allele. s.e.= Standard error. χ^2= Chi-square values. *=P<0.05. ç