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Short Communication

Determination of the malignant hyperthermia (MH) gene status in swine in Croatia

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INTRODUCTION

Malignant hyperthermia (Porcine stress syndrome - PSS) is a genetic disease of swine which usually appears under stress conditions (e.g. bad farming practice, inadequate transportation conditions) *(6, 8, 9)* or during inhalation of anaesthetics. The disease is characterized by muscular cramps, red flushing of the skin, hyperventilation, high fever and subsequnt death of the affected animals. The gene (RYR1) responsible for the disease is situated on the short arm of the sixth chromosome near centromere *(6, 12)*. It encodes the channel protein which regulates the flux of calcium ions in and out of the sarcoplasmic reticulum during muscular contraction *(8, 12, 13)*. Molecular analysis coupled to pedigree analysis revealed that there is a single point mutation on the position 1843 of the RYR1 gene, which is responsible for the abnormal functioning of the protein during muscle contraction *(8, 12, 13)*. The T > C substitution leads to the substitution of Arg 615 with Cys 615. The resultant aberrant protein is responsible for the uncoordinated flow of calcium ions and resultant symptoms of the disease.

Porcine stress syndrome has far-reaching consequences for the meat industry. Osmotic imbalance caused by flooding of the cytoplasm with calcium causes redistribution of water in muscle tissue. The result is pale, soft, exudative meat (PSE meat) which is not accepted on the market. Until several years ago, the susceptibility to the disease has been tested by inhalation of the gas halothane *(3, 4, 9, 11)*. However, this method soon proved to be unreliable because only suceptible individuals (recessive homozygotes - n/n) were detected while some individuals (heterozygotes - N/n), which passed the test, transferred the mutated gene to their offspring.

Recently, a new test for the porcine stress syndrome has been devised *(5, 6, 10)*. The test is based on selective amplification of the mutated part of the RYR1gene using polymerase chain reaction (PCR), and subsequent testing for the restriction fragment length polymorphisim (RFLP) of the amplified fragment *(8, 10)*. The test proved to be very accurate and fast, allowing rapid testing of large number of individuals in short time, and detecting all three malignant hyperthermia genotypes (N/N, N/n and n/n).

The situation with malignant hyperthermia testing in Croatia is unclear. There was never a systematic halothane testing of the breeding stock and subsequnt exclusion of susceptible individuals from futher breeding projects. Consequently, the extent of the mutated RYR1 spread in the pig populations on the farms is unknown.

In this paper, we present the results of the preliminary molecular testing of the pigs from some farms in Croatia for the prevalence of normal/mutated RYR1 gene on one farm in Croatia.

MATERIALS AND METHODS

Blood samples: Between 0,1 - 1 ml of blood was obtained by ear vein puncture. The blood was collected in sterile reactions tubes containing 150 μl 1M EDTA pH 8.0. The samples were shipped in ice to the laboratory and immediately stored at -20C.

Forty eight blood samples were treated at a time. DNA isolation and all subsequent procedures were done as described previously *(10)*. Briefly, the samples were thawed at room temperature for approximately 30 minutes. 100 μl of blood was lysed in 500 μl TE buffer pH 7.4 (10 mM Tris -HCl pH 7.4, 1 mM EDTA pH 8.0) and cell debris was pelleted by centrifugation for two minutes at 13500 rpm using Eppendorf centifuge (5415 C). Supernatants were discarded and pellets were washed 2 - 3 times with 500 μl TE or until the pellets were free of red colour. The pellets were digested with proteinase K in 100 μl of the mixture containing 10X PCR Buffer II Perkin Elmer (100 mM Tris-HCl pH 8.3, 500 mM KCl), 25 mM $MgCl_2$, concentrated Tween - 20 and proteinase K (20 mg / ml) at 56°C for 90 minutes. After digestion, proteinase K was inactivated at 96°C for 10 minutes. Samples were then cooled at room temperature and for a few seconds, centrifuged at 13500 rpm to remove particulate matter. We used 5 μl of each sample for PCR amplification.

DNA surrounding the mutation site was amplified using 30 cycles of a two-step PCR in Perkin Elmer

Thermal Cycler. In the first step, the DNA was denatured at 94°C for 13 seconds; in the second step the template was annealed and extended at 67°C for 80 seconds. Mastermix reagents in 25 μl of reaction media consisted of 10X PCR Buffer II (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 200 μM of each of dATP, dGTP, dCTP and dTTP; 1 mg/ml of the forward (5' - TCCAGTTTGCCACAGGTCCTACCA - 3') and reverse (5' - TTCACCGGAGTGGAGTCTCTGAGT - 3') primer, 50 mU / μl Taq DNA polymerase and 5 μl of DNA isolate.

After PCR, the reaction tubes were maintained at 4°C and restriction enzyme was added in a 10 μl solution containing 3.5 μl NEBuffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DDT, pH 7.9), 0.35 μl bovine serum albumin (10 mg/ml), 0,5 μl of the restriction endonuclease Bsi HKA I and 5,65 μl water. Samples were incubated on 60°C for 2 hours. After restriction enzyme digestion, the samples were loaded and run on the 3% agarose gel containing ethidium bromide and subsequently visualized under UV light.

RESULTS AND DISCUSSION

In our study, 285 swine were tested. The results are summarized in Table 1 and 2. In up to 5% of the samples, due to low amplification and bad staining in the gel, it was impossible to assess the RYR1 status.

The prevalence of heterozygotes (N/n) (11.23%) in population is lower than that in populations of tested swine in Canada, United States and England *(5, 10)*. However, the percentage of susceptible individuals (n/n) (1.05%) is similar to the percentage of susceptible individuals in Canada, United States and England. This results has to be confirmed on much larger sample, since very low number of tested individuals preclude us from inferring the RYR1 status in the breeding population of swine in Croatia.

The frequency of mutated RYR1 gene depends on selection strategy as well as on the previous halothane testing of the herds. Since halothane testing has never been done systematically in Croatia, we presumed that the number of susceptible individuals should have been much higher. However, due to inadequate farming practice, susceptible swine probably die within the first several months of their life. Recently, it was shown that RYR1 gene has profound effect on production traits *(1, 2)* in swine. The application the DNA - based test together with meat quality tests offers the ooportunity to select for N/n pigs with superior meat quality and fattening traits.

TABLE 2

Prevalence of the malignant hyperthermia mutation in swine of different sex and breeding status.

Sex & Breeding Status	No. of Swine Tested	Heterozygotes (%)	n/n homozygotes (%)
Boars	133	13.50	1.5
Gilts	117	7.69	0.85
Sows	35	14.3	0

TABLE 1

Prevalence of the malignant hyperthermia mutation in swine of various breeds.

Breed	No. of swine tested	Heterozygotes (%)	n/n homozygotes (%)
Big Yorkshire	32	6.25	0
Swedish Landrace	105	10.48	1.90
Hampshire	29	20.69	0
Dutch Landrace	53	15.09	1.89
♀ Big Yorkshire × ♂ Swedish Landrace	8	0	0
♀ Swedish Landrace × ♂ Big Yorkshire	52	7.69	0
♀ Dutch Landrace × ♂ Big Yorkshire	6	16.66	0
All Breeds	285	11.23	1.05

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ABSTRACT

Determination of the malignant hyperthermia (MH) gene status in swine in Croatia

The malignant hyperthermia (MH) gene status in pigs on some farms in Croatia was investigated. Molecular test using polymerase chain reaction was used and 285 swine of four breeds and some crossbred pigs were tested. The obtained results showed the existence of approximately 11% of heterozygotes and about 1% of susceptible homozygotes in the breeding population of tested swine. These results are more favorable than the results obtained in England, USA and Canada. The assesed MH status opens the possibilities of selective breeding with the aim of producing animals with better fattening traits and superior meat quality.

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