PREVALENCE OF THE PORCINE STRESS SYNDROME (PSS) MUTATION IN BREEDING STOCK OF SWINE IN CROATIA

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INTRODUCTION

Porcine stress syndrome (PSS, malignant hyperthermia) is an economically important genetic disease of pigs characterized by muscular hypermetabolism and skeletal muscle contractions. Molecular basis of the disease is a hypersensitive triggering mechanism of the Ca^{++} - release channel of sarcoplasmic reticulum (2). The PSS defect renders muscle hypersensitive to stimulation by various stressors. The gene for porcine Ca^{++} channel (*RYR1*) was sequenced revealing that the single point C - T mutation in the second exon of the gene converts ^{615}Arg to ^{615}Cys. The incidence of PSS in swine varies from breed to breed and from country to country. It is likely that 12% homozygous swine die of PSS and up to 50% of the carcasses of homozygous animals are devaluated due to pale, soft, exudative (PSE) meat. Knowing the reason for the PSS, it is likely that the efforts taken to get rid of swine stock with this apparently deleterious gene could easily carried out. However, this mutated gene may have both beneficial and deleterious effects. The beneficial effects of the mutated gene in heterozygous pigs is associated with muscle hypertrophy which appears to add 1 - 3% to lean dressed carcass weight. In selection of breeding stock for such traits as large ham conformation, large loin eye area and excessive leanness, the DNA - based test for the mutation associated with the PSS should be used. The aim of this study was to determine the prevalence of the PSS mutation of various breeds of swine in Croatia.

MATERIALS & METHODS

Blood samples (1ml) from pigs at two farms with over 5,000 sows of various breeds were collected. Genomic DNA was isolated from 0.5 ml blood sample which was added to 50 ml of TE buffer pH 8.0 and spun for 10 sec at 1300 g. Then the pellet was washed 3 times with TE and resuspended in 100 μl of Perkin Elmer Cetus gelatin - free buffer supplemented with 0.5 % Tween 20 and 10 μg / ml Proteinase K.The mixture was incubated at 60°C for 30 minutes and reaction terminated by immersion in boiling water for 10 min. This mixture was used to isolate 659 bp fragment by PCR amplification. The reaction was done in Perkin Elmer Cetus PCR buffer containing 1 mM $MgCl_2$. Genomic DNA (200 - 400 ng) and 100 ng of each of primers were added to the mixture and program carried at 94° of 1 min, 53° for 2 min, and 72° for 3 min. The forward primer was 5' - TCCAGTTTGCCACAGGTCCATACCA - 3' and the reverse primer was 5' - ATTCACCGGAGTGGAGTCTCTGAG - 3'. The amplified sequence was cut with HgiAI to detect the presence of C / T mutation and restriction fragments were resolved on 3 % agarose gel.

RESULTS

From a total of 873 pigs of different breeds the lowest percentage of heterozygous carrier of the PSS mutation was funded in Hampshire swine while being the highest in Hypor (commercial synthetic line) (Table 1). Of the common breeds Landrace (L) swine were affected much higher than Yorkshire (Y) and the crossbreed between LxY, respectively. Occurrence of homozygotes for PSS was rare, observed in only 2 of 195 Landrace, 2 of 204 Yorkshire, and 4 of 361 their F1 hybrid, respectively.

Table 1. Prevalence of the porcine stress syndrome (PSS) mutation in breeding stock from various breeds of swine in Croatia

Breed	No. of swine tested	No. and (%) of Heterozygous swine	No. and (%) of PSS swine	GF*
Hypor (Hy)	21	8 (38.1)	1 (4.8)	0.238
Landrace (La)	195	57 (29.2)	2 (1.0)	0.156
Yorkshire (Yo)	204	37 (18.1)	2 (1.0)	0.100
Hampshire (Ha)	19	1 (5.3)	1 (5.2)	0.079
(La×Hy)F1	75	24 (32.0)	1 (1.3)	0.173
(La×Yo)F1	361	54 (15.0)	4 (1.1)	0.086

* PSS gene frequency = [(No. of PSS homozygotes × 2) + No. of heterozygotes] / No. of tested swine

DISCUSSION

This study confirmed application of a method for large-scale, rapid, accurate, DNA-based laboratory diagnosis of the mutation associated with susceptibility to PSS (3). Prevalence of the PSS mutation varied markedly with breed. Of the common breeds of swine, Landrace (Gr 0.156) by affection to PSS followed Hypor-the synthetic line of swine (Gf 0.238). With the development of the PSS test described here, the mutation of RYR1 gene can be used in selection of swine for increased muscularity and leanness. However, it is likely that each breed should be tested for live performance and carcass characteristic, comparing them among normal, heterozygous, and homozygous for PSS mutation to get the economic impact of the beneficial effects.

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