MALIGNANT HYPERTHERMIA GENE STATUS OF PIGS IN SOUTH AFRICA

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Abstract – Porcine Stress Syndrome (PSS) is a genetic disorder caused by a recessive mutation in the halothane (HAL) gene and results in sudden death of pigs when placed under stress during transport and pre-slaughtering conditions. Animals that are affected by the mutation in the HAL gene tend to develop pale, soft and exudative (PSE) meat, that result in an economic loss. A commercial DNA based test is available for testing for the recessive gene and applied by the pig industry. In this study the frequency of the Mh gene was firstly determined at the nucleus level and boars used in AI stations on samples collected over a period of two years. It was found that there has been an increase in the number of recessive alleles, Nn and nn (alleles of genotypes) between 2000 and 2003 that now necessitates further study to ensure optimum carcass quality.

Key Words – porcine, stress, PSE meat, ryanodine receptor

I. INTRODUCTION

Malignant hyperthermia (Mh) is a pharmacogenetic disease that affects calcium regulation in muscle and results in sudden death and (or) in PSE meat [16]. Mh in pigs has been associated with a recessive mutation in the gene coding for porcine calcium release channel, also called the ryanodine receptor gene (*ryr-1* locus) or halothane gene (*Hal*) [10], which is located on chromosome 6 [13]. In pigs, it has been shown that the primary defect resides in a single point mutation (Arg614Cys) in the porcine RYR1 protein. The ryanodine receptor regulates Ca++ transport across the cell membrane in muscle cells [1]. The test for Mh was discovered and patented in the early 1990's by the University of Toronto with an accuracy approaching 100%. The DNA test provides the pork industry with a powerful tool to detect the HAL gene in live pigs and to eradicate it from the industry. Currently, in South Africa an estimated 50% - 60% of all slaughtered offspring are sired through artificial insemination (AI). The distribution of the MH gene through AI, (especially the heterozygous alleles), will not only be spread at a rapid rate through commercial herds, but substantial financial losses will occur further down the supply chain. In South Africa, during the period 1992 – 1999, the frequency of the NN homozygous alleles (non carriers) increased from 0.62 to 0.77, the Nn heterozygous alleles (carriers) decreased from 0.29 to 0.19 and the frequency of the nn homozygous recessive alleles (inherently associated with in transit deaths and poor meat quality) decreased from 0.08 to 0.03. During the period 2000 - 2003 a total of 1194 pigs were tested for the MH-gene. During this time the frequency of the NN homozygous alleles decreased from 0.80 to 0.65. The Nn heterozygous alleles increased from 0.18 to 0.28 and the frequency of the nn homozygous recessive alleles increased from 0.02 in 2000 to 0.07. These figures have stressed the importance to test a wider sample of the pig population in South Africa that includes commercial and indigenous breeds. The aim of this study was to study the frequency of the Mh gene at the nucleus level and boars used for AI from samples collected over a period of 2 years.

II. MATERIALS AND METHODS

A total of 14 seed stock herds and AI stations provided samples to the ARC for testing for phase 1 of the project. (Tables 1 and 2). A total of 439 hair samples were included representing the SA Landrace, Large White, Duroc, Pietrain, Chester White and Kolbroek. For phase 2 of the project, a total of 1500 samples were collected at abattoirs around the country (Table 4). The laboratory assay consisted of the DNA extraction from hair

roots, followed by polymerase chain reaction (PCR), gel electrophoresis, UV visualization and analysis. DNA was extracted from the hair roots using a modified Proteinase K digestion method [14]. The premix PCR solution consisted of the Hal gene specific primers (20 µM), 100 µM each of dATP, dCTP, dGTP, and dTTP, Taq polymerase 0.3mM of MgCl2 buffer and deionized water. The Hal gene-specific primers were 5'- GTTCCCTGTGTGTGTGCAATGGTG-3' (forward; MHF) and 5'-ATCTCTAGAGCCAGGGAGCAAGTTCTCAG TAAT-3' (reverse; MH-R). The PCR program used included a denaturing step at 95 °C for 1 min, followed by annealing of the primers at 58 °C for 2 min, with an extension step at 72 °C for 2 min. Forty cycles of this 3-step procedure was performed in a thermal cycler. The samples were run on an acrylamide gel, stained with ethidium bromide and visualization under ultra violet light. Controls with known genotypes as well as no template controls were included in each run. There are approximately 86 abattoirs distributed throughout the country. A total of 19 pork producers have been identified, but only 15 producers were included for phase 2. A hundred animals per producer were sampled. Sampling was done at abattoirs by the field technicians from the Agricultural Research Council – Animal Production Institute.

Table 1 Geographic distribution, number of boars and breed

Province	Number of samples	Breed
Gauteng	62	Kolbroek Large White SA Landrace
Limpopo	25	Duroc Large White SA Landrace
KwaZulu Natal	107	Pietrain Duroc Large White SA Landrace
Western Cape	67	Large White SA Landrace Duroc
Northern Cape	23	Large White Duroc Chester
North West Province	24	SA Landrace Large White
Total	308	

Table 2. Number of breeding companies, number of boars and synthetic lines included

Breeding Company	Lines 1	2	3	4	5	Number of samples
1	12	25	5	8		50
2	13	17	25	4	2	61
3	15	2	1	2		20
Total						131

III. RESULTS AND DISCUSSION

Results of phase 1 are presented in Table 3. For phase 2 of the project, 96.4% of the pigs tested did not carry the mutation. Fifty one (3.4%) of the pigs were carriers (Nn) and three animals (0.2%) were homozygous (nn), having inherited a copy of the mutation from both parents. There was a marked difference in the incidence of carriers of the mutation observed in samples from different producers, ranging from 0% to 12.7%. This, however, may reflect different approaches with regard to breeding policy, as the three affected animals that inherited the mutation did not originate from producers where the incidence of carriers was high (>10%). The adverse effects of pork obtained from non carriers of the MH mutation (NN individuals) as a result of transport stress are well documented [11; 7; 18].

Table 3. Breeds, number of animals tested, carrier and recessive boars of Phase 1

Breed	No. of Boars	MH Test Results					
		NN	**%**	**Nn)**	**%**	**nn**	**%**
SA Landrace	90	85	94	5	6	-	
Large White	158	157	99	1	1	-	
Duroc	42	42	100	-		-	
Pietrain	4		-	3	75°	1	25°
Chester	3	3	100°	-		-	
Kolbroek	11	11	100°	-		-	
Synthetic lines	131	123	94	8	6	-	
Total	439	421		17		1	

°Percentage based on low sample size

Table 4. Overall results of Phase 2

Genotype	Number of animals
NN (Normal)	1446
Nn	51

nn	3
Total	1500

Results obtained from Phase 1 of this project indicate the MH gene status in the male stud and prominent stock AI stations. From the animals tested, it is evident that the presence of the MH gene is low in South African seed stock herds. The use of this information can contribute to future management and informed breeding programmes to effectively reduce or eliminate the MH gene. It can be recommended that, when purchasing new breeding material (animals, semen and embryos), producers should request that all purchases be certified MH- gene free. It was also noted that transport over a substantial distance to abattoirs is a reality for many of the slaughter pigs in South Africa. The absence of the MH mutation does not imply resistance to adverse changes in pork meat. However, the presence of the mutation in pigs is certain to result in inferior meat [9; 17] and therefore the removal of the MH mutation from breeding stock should eliminate a major contributing factor to PSE meat in South Africa. By the turn of the century it was generally concluded world-wide that it is imperative to remove the MH mutation from the pig population as the market discriminates against pork of inferior quality [19]. The economic implications of this result have great benefits for the South African pork industry.

IV. CONCLUSION

The low frequency of the MH gene amongst the breeding stock of the South African pig population is a first step in eliminating a major contributing factor to PSE meat in South Africa. The economic implications of this result have great benefits for the South African pork industry.

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REFERENCES

1. Bjurström, S., Carlsten, J. and Jönsson, L. The reduction of skeletal muscle lesions after experimental stress in stress-susceptible pigs protected with datrolene. Zentralbl Veterinarmed A, 42 (10): 659-667, Dec. 1995.

2. De Smet, S.M., Pauwels, H., De Bie, S., Demeyer, D.I., Callewier, J. and Eeckhout, W. Effect of halothane genotype breed, feed withdrawal and lairage on pork quality of Belgian slaughter pigs. J Anim Sci, 74 (8): 1854-1863, Aug. 1996.

3. Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V.K., Weiler, J.E. et al. (1991)

4. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253: 448-451.

5. Geers, R. A modulating effect of beta-endorphin on pigs coping strategies during transport.

6. Physical Behaviour, 57: 1057-1060, June 1995.

7. Geers, R., Bleus, E., Van Schie, T. Villé, H., Gerard, H., Janssens, S., Nackaerts, G. Decuypere,

8. E. and Jourquin, J. Transport of pigs with respect to the halothans gene: stress assessment. Journal Animal Science, 72: 2552-2558, October 1994.

9. Goodwin, R.N. Genetic parameters of pork quality traits. PhD thesis, Iowa State University, Ames, 1994.

10. Fujii J, Otsu K, Zorzato F, et al.: 1991, Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448-451.

11. Hambrecht, E., Eissen, J.J., de Klein, W.J.H., Ducro, B.J., Smits, C.H.M., Verstegen, M.W.A. and den Hartog, L.A. Rapid chilling cannot prevent inferior pork quality caused by high preslaughter stress. Journal Animal Science, 82: 551-556, February 2004.

12. Hambrecht, E., Eissen, J.J., Newman, D.J., Smits, C.H.M. den Hartog, L.A. and Verstegen, M.W.A. Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions. Journal Animal Science, 83: 440-448, February 2005.

13. Harbitz I, Chowdhary B, Thomsen PD, et al.: 1990, Assignment of the porcine calcium release channel gene, a candidate for the malignant hyperthermia locus, to the 6p11-q21 segment of chromosome 6. Genomics 8:243.

14. Higuchi, R. & Bradley, D., 1998. DNA typing from single hairs. Nature 332, 543-546.

15. Kauffman, R.G., van Laack, R.L., Russell, R.L., Pospiech, E., Cornelius, C.A., Suckow, C.E. and Greaser, M.L. Can pale, soft, exudative pork be prevented by postmortem sodium bicarbonate injection? Journal Animal Science, 76: 3010-3015, December 1998.

16. Mitchel, *G.*, and J.J.A. Heffron. 1982. Porcine stress syndromes. Adv. Food Res. 28:167.

17. Monin, G., Larzul, C., le Roy, P., Culioli, J., Mourot, J., Rousset-Akrim, S., Talmant, A., Touraille, C. and Sellier, P. Effects of the halothane genotype and slaughter weight on texture of pork. Journal Animal Science, 77: 408-415, 1999.

18. Nyberg, L., Lundström, K., Edfors-Lilja, I. and Rundgren, M. Effects of transport stress on concentrations of cortisol, corticosteroid-binding globulin and glucocorticoid receptors in pigs with different halothane genotypes. Journal Animal Science, 66: 1201-1211, May 1998.

19. Wendt, M., Bickhardt, K., Herzog, A., Fischer, A., Martens, H. and Richter, T. Porcine stress syndrome and pse meat: clinical symptoms, pathogenesis, etiology and animal rights aspects. Berl Munch Tierarztl Wochenschr, 113: 173-190, May 2000.