Short communication

Frequency of the malignant hyperthermia gene in the South African pig industry

P. Soma[1#], E. van Marle-Köster[2] & L. Frylinck[1]
[1] Agricultural Research Council, Private Bag X2, Irene, 0062, South Africa
[2] Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, 0001, 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 this mutation tend to develop pale, soft and exudative (PSE) meat, which results in an economic loss. In South Africa, the frequency of the number of carriers (Nn) and recessive (nn) pigs has increased by 21% to 30% from 2000 to 2003. This study aims to determine the prevalence of the malignant hyperthermia (MH) gene in breeding boars at nucleus or seed-stock level, and the prevalence at commercial abattoirs across the South African pig industry. Results indicate a low number of carriers (Nn = 17) and recessive (nn = 1) pigs at seed-stock level. For commercial abattoirs, 96.4% of the pigs tested did not carry the mutation. The low incidence of the MH mutation from breeding stock should eliminate a contributory factor to PSE meat in South Africa. Transport over long distances to abattoirs may ultimately have an effect on pork obtained even from non-carriers of the MH mutation.

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[#] Corresponding author: Pranisha@arc.agric.za

Malignant hyperthermia (MH) is a genetic disease that affects calcium regulation in muscle, and results in sudden death and/or pale, soft and exudative (PSE) meat (Tarrant *et al.*, 1986). In pigs, MH has been associated with a recessive mutation in the gene coding for the porcine calcium release channel, also called the ryanodine receptor gene (*ryr-1* locus) or halothane gene (*Hal*) (Fujii *et al.*, 1991), which is located on chromosome 6 (Harbitz *et al.*, 1990). The primary defect resides in a single point mutation (Arg614Cys) in the porcine RYR1 protein. The ryanodine receptor regulates Ca^{2+} transport across the cell membrane in muscle cells (Bjurström *et al*., 1995). In the past, a halothane challenge test was done, in which pigs were subjected to inhalation of halothane gas in order to identify carriers of the mutation (Basic *et al*., 1997). In the event of a reaction to the halothane test, both parents of the animal were suspected of being carriers of the mutation. Pigs were then classified as halothane positive or halothane negative. The DNA test for MH was discovered and patented in the early 1990s by the University of Toronto with accuracy approaching 100%. Genomic testing allowed for the identification of the MH genotypes, MH heterozygous (Nn), MH homozygous (nn) and non-carriers (NN) of the MH mutation. The test provides the pork industry with a powerful tool to detect the HAL gene in live pigs and eradicate it from the industry. The elimination of the MH mutation from breeding stock has a major benefit towards producing PSE-free meat (Goodwin, 1994; Monin *et al*., 1999; Wendt *et al*., 2000; Lahucky, 2002).

Currently in South Africa, an estimated 50% - 60% of all slaughtered offspring are sired through artificial insemination (AI) (South African Pork Producers Organization (SAPPO), 2014, personal communication). The distribution of the MH gene through AI (especially the heterozygous alleles) may have an effect in commercial herds, and cause substantial financial losses further down the supply chain if not controlled. Data from the Agricultural Research Council database collected between 1992 and 1997 (Nel *et al.*, 1993) indicated that the prevalence of NN homozygous (non carriers) was low in the early 1990s, with more than 77% of the population being non carriers (NN). However, from 2000 to 2003, a total of 1194 pigs,

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both sows and boars, were tested for the MH gene. A decrease was found in the frequency of the NN homozygous genotypes and an increase in the Nn heterozygous genotypes (21% in 2000 to 30% in 2003) (ARC, unpublished report). These figures stressed the importance of testing a wider sample of the pig population that includes all breeds that contribute to the commercial market. A project jointly funded by the Red Meat Research and Development Trust (RMRDT) and the Agricultural Research Council Animal Production Institute (ARC-API) was initiated in 2005 to determine the status of the MH gene in the South African pig industry. The aim of this study was to establish the frequency of the MH gene at nucleus level and commercial level from samples collected over two years between 2005 and 2007, using DNA technology.

A total of 439 hair samples from boar were received from 11 seed-stock herds and three AI stations for the study (Phase I). The samples represented the major pig breeds used in the South African industry, namely SA Landrace, Large White, Duroc, Pietrain and Chester White. The Kolbroek, an indigenous pig breed, was added as an outgroup (Table 1).

Table 1 Geographic distribution, number of boars, and breeds studied

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	24	SA Landrace, Large White
AI Company 1	50	Represents 4 composite lines
AI Company 2	61	Represents 5 composite lines
AI Company 3	20	Represents 4 composite lines
Total	439	

AI: artificial insemination.

For the investigation at commercial level (Phase II), 1500 hair samples of randomly selected commercial/slaughter pigs were collected from 15 major pork producers distributed throughout South Africa. Terminal sires for commercial pigs are Large White, Landrace or Duroc. Some AI stations use the Pietrain or Duroc breeds as terminal sires (Voordewind, SA Studbook, personal communication). One hundred animals per producer were sampled. All hair samples were numbered and stored in paper envelopes pending extraction. The sex of the pigs was not reported for these hair samples.

Table 2 Test for homogeneity of independent breeds showing *P*-values

Breed	*P*-value
Chester	1.0000
Composite	0.0271
Duroc	0.3798
Kolbroek	1.0000
Large White	0.0193
Pietrain	<0.0001
South African Landrace	0.7532

The laboratory assay consisted of DNA extraction from hair roots, followed by polymerase chain reaction (PCR), gel electrophoresis, UV visualization and analysis. DNA was extracted with a modified Proteinase K digestion method (Higuchi *et al.*, 1998). The premix PCR solution consisted of Hal-gene-specific primers (20 µM), 100 µM each dATP, dCTP, dGTP and dTTP, Taq polymerase 0.3 mM $MgCl_2$ buffer and deionized water. The HAL gene-specific primers were: 5'-GTTCCCTGTGTGTGTGCAATGGTG-3' (forward; MHF) and 5'-ATCTCTAGAGCCAGGGAGCAAGTTCTCAGTAAT-3' (reverse; MH-R) (Accession No. M91452; Fujii *et al*., 1991). The PCR programme included a denaturing step at 95 ºC for 1 minute, followed by annealing of the primers at 58 ºC for 2 minutes, with an extension step at 72 ºC for 2 minutes. Forty cycles of this three-step procedure were performed in a thermal cycler. The samples were run on an acrylamide gel, stained with ethidium bromide and visualized under ultra-violet light. Controls with known genotypes, as well as no template controls, were included in each run. Genotypic data were stored in an Excel database and analysed with the test for the homogeneity of independent samples (Strasheim *et al*., 1999).

The percentage of genotypes with the MH gene observed in the seed-stock boars and from AI stations is presented in Table 3. The frequency of carrier animals (Nn) was low for all breeds, with no carrier animals in the Chester and Kolbroek breeds.

Table 3 Percentage of boars for the three malignant hyperthermia genotypes for boars representing stud breeders and artificial insemination stations (Phase I)

BREEDS	No. 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°	-		-	
Composite	131	123	94	8	6	-	
TOTAL	**439**	**421**		**17**		**1**	

[#] Percentage based on low sample size.

Results from the pigs slaughtered at the various abattoirs indicated that 96.4% of the pigs tested did not carry the mutation. Fifty one (3.4%) of the pigs were carriers (Nn), and three (0.2%) were homozygous (nn), having inherited a copy of the mutation from both parents. Statistical analyses using the test for homogeneity of independent samples (Strasheim *et al*., 1999) indicated no significant differences in the prevalence of the MH gene in the Duroc and South African Landrace breeds. The Composite, Pietrain and Large White breeds showed differences among the MH gene in these breeds, compared with the MH gene over all the breeds (Table 2).

Results from this study indicated that the MH gene status in the boars from seed-stock farmers and AI stations is low in South Africa. Some of the pig breeds under review showed that there are breed effects, which in some cases are related to the presence or absence of the MH gene. Breeds such as the Pietrain, with outstanding carcass characteristics, tend to have a higher incidence of carriers (Monin *et al*., 1981). Most breeders are aware of the adverse effects of the MH gene, and aim to avoid importation of carrier animals (Global Meat, online). DNA testing is an essential tool for controlling MH genes in the herd.

There was a marked difference in the incidence of carriers of the mutation in samples from different producers, ranging from 0% to 12.7%. This may reflect different approaches to breeding, as the three animals that inherited the mutation did not originate from producers where the incidence of carriers was high (>10%). 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, and poor meat quality obtained from non carriers of the MH mutation (NN individuals) as a result of transport and other stress is well documented (Hambrecht *et al*., 2004; 2005; Geers *et al*., 1994; Nyberg *et al*., 1998).

From this study, there is low prevalence of the MH gene in the seed-stock sector and breeders have access to DNA testing for monitoring the status of the MH gene in South African herds. However, contributing factors, such as handling and transport, which cause PSE meat, require further attention.

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