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COMPARISON OF BLOOD MARKER AND DNA ANALYSES FOR DETECTION OF THE HALOTHANE GENOTYPE OF PIGS

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INTRODUCTION

The nature of the genetic mutation giving rise to malignant hyperthermia (MH) and the Porcine Stress Syndrome (PSS) has been elucidated in the laboratory of Dr. David MacLennen of the University of Toronto (Fujii *et al.*, 1991). This discovery allows a diagnostic test which appears to identify the three genotypes (NN, Nn and nn) with respect to the MH or halothane gene. Although the blood marker approach has been found to be 90-95% accurate for Swedish Landrace and Yorkshire pigs (Gahne and Juneja, 1985), DNA analysis:
1) eliminates the requirement for halothane testing;
2) is technically much simpler than the blood marker approach (Gahne and Juneja, 1985) in that blood or other tissues from one pig, rather than blood from families of pigs, will allow the determination of the genotype; and
3) is applicable in a population in which the frequency of the MH mutation is very low.

The discovery of the genetic mutation leading to MH in pigs and extension of the discovery to an analytical method for detecting its presence has to date included 182 pigs distributed among six breeds (Yorkshire, Landrace, Duroc, Hampshire, Poland China, Pietrain) (Fujii *et al.*, 1991) and 338 British Landrace pigs from the ABRO research herd (Otsu *et al.*, 1991). Although these researchers suggest that this technique will distinguish the three MH genotypes in 100% of pigs, this has only been verified within rather discrete swine populations.

The present study was designed to determine the reliability of DNA analysis for identifying the MH mutation in comparison to the blood marker method within a swine population of wider genetic base than has been used previously.

MATERIALS AND METHODS

Design

During a period of approximately two years of blood marker testing within Yorkshire and Landrace pig herds in Alberta, Canada, pigs from 83 different litters displayed positive halothane tests. As many litter mates of the halothane-positive pigs as possible were halothane tested and blood was collected from all halothane tested pigs (n=540) and from their parents (n=124) for determination of MH genotype by both blood marker and DNA analyses.

Halothane test

Pigs were halothane-tested at eight weeks of age by administering4.5% halothane in oxygen for a maximum of four minutes. Those exhibiting muscular rigidity will be designated as halothane-positive. The test was terminated when a positive reaction was noted.

Blood marker analysis

Blood samples were collected from the vena cava or the jugular vein into EDTA-treated vacutainer blood collection

tubes. Blood plasma and erythrocytes were then prepared according to Gahne and Juneja (1985) and stored frozen. The erythrocyte markers, PHI (phosphohexose isomerase) and PGD (phosphogluconate dehydrogenase), for the halothane gene locus were identified by the one dimensional electrophoresis method of Gahne and Juneja (1985). The plasma markers, PO2 (postalbumin 2), for the halothane gene locus, and PI1 and PI2 (alpha-protease inhibitors), PO1 (postalbumin), TF (transferrin), HPX (haemopexin), all of which can be used to check correctness of parentage were identified by the two dimensional electrophoresis technique of Juneja and Gahne (1987). Marker haplotyping was conducted as described by Gahne and Juneja (1985).

DNA analysis

Erythrocyte fractions had been stored at -20°C for one to four years after blood marker analyses prior to DNA analysis. Crude DNA was extracted by washing the erythrocyte fractions with Tris/EDTA buffer and subsequent treatment with proteinase K (in 0.5% Tween 20 for two hours at 56°C) as reported by Innis *et al.* (1990). Samples were then stored at -20°C until DNA analysis by the approach of Otsu *et al.* (1992). For PCR (Polymerase Chain Reaction) amplification of the DNA fragment containing the mutation site, a modification of the procedures recommended by Perkin Elmer/Cetus was used. The primers have been described by Otsu *et al.* (1992). $MgCl_2$ concentration was 1.5mM. The inclusion of 1μl Reagent C (HRI Research Inc., Concord, California, U.S.A.) per 50μl PCR reaction mixture reduced the interference due to hemoglobin. Amplification was repeated for 35 cycles, each cycle comprised of 94°C for one minte followed by 65°C for one minute in a Pharmacia LKB Gen ATAQ thermal cycler. The amplified fragment was then treated with the restriction enzyme, BSI HKA (New England Nuclear, Boston, Mass., U.S.A.), for one hour at 56°C. The resulting DNA fragments were separated by electrophoresis on 8% polyacrylamide gels and visualized by staining with ethidium bromide. The electrophoresis patterns have been described by Otsu *et al.* (1992).

RESULTS AND DISCUSSION

Of the 83 litters having at least one halothane-positive pig, 80 were from heterozygous (Nn) parents and did not show any errors in parentage by the blood marker method of Juneja and Gahne (1987). Within these 80 litters 50 pigs could not be successfully genotyped by the blood marker method. The numbers of pigs, litters and sires included in the method comparison is shown in Table 1 for each breed type. The majority of the pigs were of the Yorkshire breed. In total 459 offspring and 119 different parents were included. These originated from 12 different herds. Eleven sires originated from the Canadian Swine Artificial Insemination Centres. Pedigrees extended to all parts of Canada and into Europe. Thus a wide diversity of genetic backgrounds was included.

The frequencies of halothane genotypes determined by blood marker and DNA analyses are presented in Table 2. DNA analyses were in agreement with blood marker analyses for 93.0, 95.6 and 95.8% of pigs for NN, Nn and nn genotypes, respectively, or 95.2% of pigs with all genotypes combined.

The frequencies of offspring within the genotypes, NN, Nn and nn, were expected to approximate a 1:2:1 ratio. For several reasons this was not the case. Blood marker genotypes were always obtainable for halothane-positive pigs, but in several instances could not be determined successfully in halothane-negative litter mates due to insufficient numbers of pigs available for testing in a litter or due to incompatibility of marker haplotypes. Only pigs for which halothane-marker haplotypes could be determined are included.

The greatest single source of disagreement between the two analytical methods occurred because of errors in halothane test results. The blood marker method appears to have mis-genotyped 3.3% of pigs due to inaccuracies in the halothane test. Table 3 shows the correspondence of the DNA and blood marker analyses with the halothane test results. The blood marker method indicated that 6.6% of nn pigs were halothane-negative and by its very nature it is incapable of detecting false positive halothane tests. By DNA analysis 7.9% of nn pigs gave false negative halothane tests while 3.7% of NN and Nn pigs gave false positive halothane tests.

In 1.5% of pigs discrepancies between the two methods were most logically explained by genetic recombination between the halothane gene and the blood marker genes.

In only 0.4% of analyses (two pigs), discrepancies could not be explained by obvious deficiencies in the blood

marker method. These anomalies may have been due parentage error or to technical error. The method of DNA analysis for the MH mutation has thus been confirmed as a robust method for the detection of pigs carrying the gene which in its homozygous state results in a positive halothane test and the Porcine Stress Syndrome.

CONCLUSION

Comparison of the blood marker method and the DNA-based method for detection of halothane genotype in 459 Landrace/Yorkshire pigs of very diverse genetic background indicated that the methods were in agreement for 95% of pigs. For all but two pigs (0.4%) the disagreements could be explained in terms of known weaknesses in the blood marker method. This confirms that DNA analysis is accurate and is the method of choice for the determination of halothane genotypes of pigs.

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Table 1. Number of pigs, litters and different sires within each breed type.

	Breed			
	Yorkshire (Y)	Landrace (L)	YxL	All
Pigs	333	60	66	459
Litters	54	12	14	80
Sires	21	9	9	39

Table 2. Frequencies of halothane genotypes by blood marker and DNA analyses.

Genotype from blood markers	Genotype from DNA analysis			All
	NN	Nn	nn	
NN	80 (93.0)[a]	6 (7.0)	0 (0.0)	86 (100)
Nn	5 (2.5)	196 (95.6)	4 (2.0)	205 (100)
nn	2 (1.2)	5 (3.0)	161 (95.8)	168 (100)
All	87	207	165	459

[a] Numbers in brackets represent frequencies within a row as percentages.

Table 3. Frequency (%) of phenotypes from the halothane test for the three genotypes from DNA and blood marker analyses.

Genotype	Halothane test	Blood Markers	DNA analysis
NN	negative	100.0%	97.7%
Nn	negative	100.0	98.6
nn	negative	6.6	7.9
NN	positive	0.0	2.3
Nn	positive	0.0	1.4
nn	postive	93.4	92.1