RESEARCH REPORT
ANIMAL WELFARE

Title: The frequency of the HAL-1843 mutation of the RYR gene in dead and non-ambulatory/non-injured pigs on arrival at the packing plant. – **NPB #05-069**

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Abstract:

Four Midwestern packing plants were visited on 53 occasions and tissue samples were collected from 2019 pigs to determine the frequency of the HAL-1843 mutation of the RYR gene in dead (DOA), non-ambulatory/non-injured (NANI), and normal animals. The pigs sampled came from a total of around 130,000 animals from 454 farms that were transported on 861 trailer loads with ~152 pigs/load, with an average weight of ~125 kg/pig. Frequency of animals with the HAL-1843 mutation was low with only 2.7% of pigs being either homozygous recessive (nn; 0.45%) or carriers (Nn; 2.3%) for the mutation and 97.3% of pigs being homozygous for the normal allele (NN). The mutation was present in all three classes of pig with 1.8% of normal, 1.8% of NANI, and 4.7% DOA animals having at least one copy. There was a trend (P = 0.08) for the frequency of carriers to be different between Normal, NANI, and DOA animals (1.64, 1.61, and 3.74%, respectively; SEM 0.93). The 55 pigs with at least one copy of the mutation came from 53 different farms and, therefore, the mutation was relatively widespread, being present, in ~11% of farms sampled. The results of this study suggest that, although the HAL-1843 mutation is still present in commercial pig populations in the U.S., its low frequency means that it is not a major cause of transport losses.

Introduction:

Losses of harvest-weight pigs during transport to the slaughter plant (dead and non-ambulatory animals) are of concern to the US pork industry from both welfare and economic perspectives. Historically, the HAL-1843 mutation of the RYR gene (commonly called the Halothane or Stress Gene) was responsible for a substantial proportion of transport losses. The specific HAL-1843 mutation was identified in 1991 (Fujii et al., 1991) and a DNA-based test for this has been widely available since that time. Subsequently, most breeding stock suppliers selected against this unfavorable mutation and there are claims that it has been eliminated from most commercial swine populations. There is evidence, however, that the mutation is still present in US commercial pigs. Even at a low frequency, this mutation could be an important causal factor in transport losses. Quantifying the frequency of the HAL-1843 mutation of the RYR gene in pigs that are lost during transport in the contemporary commercial pig population of the U.S. is an essential first step to determining the extent of its involvement in the dead-on-arrival and non-ambulatory/non-injured pig issue. The study reported here was carried out to investigate the frequency of the unfavorable HAL-1843 mutation of the RYR gene in pigs lost in transport (dead and non-ambulatory/non-injured) and in a random sample of contemporary normal animals arriving at Midwest packing plants.

Objectives:

To determine the frequency of the HAL-1843 mutation of the RYR gene in dead (DOA), non-ambulatory/non-injured (NANI), and normal animals on arrival at the packing plant.

Procedures:

Slaughter Plants: Four large commercial packing plants (designated as plants A, B, C, and D) operating in the Midwest of the US were chosen for this study on the basis that they were handling a large number of animals that would be representative of the population of commercial pigs slaughtered in the US.

Plant Visits and Samples Collected: The plants were visited on several occasions over a five-month period (from January 2006 to May 2006) to collect tissue samples for DNA analysis (Table 1). Visits were made on 53 occasions, with the number of visits to plants A, B, C, and D being 25, 9, 10, and 9, respectively. Samples from Normal and NANI animals were obtained on 34 visits. However, because of the relatively low incidence of DOAs observed, extra visits were required to complete the sampling of this class of animal. In total, samples from 2019 animals were obtained consisting of 511, 555, 459, and 494 samples from plants A, B, C, and D, respectively (Table 2). These represented samples from 649 Normal animals, 726 NANIs, and 644 DOAs.

Identification of Dead and Non-ambulatory Animals: After trailers were unloaded, packing-plant employees identified dead and non-ambulatory pigs and University of Illinois investigators identified non-ambulatory/non-injured individuals from among the non-ambulatory animals. Non-ambulatory/non-injured animals were defined as non-injured pigs that either could not walk or were having difficulty in walking and could not keep up with the remainder of the group, and were

showing physical symptoms of stress (open-mouthed breathing, skin discoloration, muscle tremors, and/or abnormal vocalization).

Additional Information Collected: Information collected on either the animals that were sampled or the trailer loads that they came from included source of animals, gender, number of pigs per load, average live weight per pig, transport company, trailer design, and the number of dead and non-ambulatory pigs. However, to protect proprietary interests, identification of specific plants and sources of animals are not included in this report nor will they be released at any time in the publication of the results.

Sampling Procedures: Ear tissue samples were obtained using an ear punch. Samples from DOAs were obtained in the holding pens at the plant after the truck had been unloaded. The procedure for sampling the contemporary Normal animals was as follows. When a dead and/or a NANI animal had been identified, a normal animal (identified by the common trailer load tattoo number) was randomly selected from the same trailer load. Subsequently, samples from the previously identified NANI and contemporary Normal animals were obtained on the slaughter line before the head was removed from the carcass. Immediately after collection, the samples were placed in a plastic bag which was sealed, stored on ice and, subsequently, transferred to a freezer (-20ºC) where they were held until preparation for shipping to the laboratory for DNA analysis. The sample storage bag was labeled with animal identification, the class of the animal (DOA, NANI, Normal), the slaughter plant, and the date of sample collection.

HAL-1843 Genotyping: All genotyping was carried out by a commercial laboratory (GenAlysis Laboratory, Inc., Lakeside, OH). A thin slice of tissue from each frozen ear sample was placed in a pre-labeled, 0.65 ml micro centrifuge tube and shipped to the laboratory. The DNA was

extracted according to the laboratory's standard operating procedures and genotyping was carried out according to the procedure described by Fujii et al. (1991).

The genotypes were defined as follows:

Homozygous normal (NN)

Heterozygous carrier (Nn; mono-mutant with one copy of the mutation)

Homozygous recessive (nn; di-mutant with two copies of the mutation)

Quality Assurance: All procedures used for collection, identification, and handling of samples and all recording of associated information were carried out according to standard Good Laboratory Practices. The laboratory used for the DNA genotyping had a long and successful history of carrying out such analyses and employed its own in-house standard operating procedures to ensure the accuracy and validity of the results.

Statistical Analysis: The total number and frequency (percentage) of the three genotypes [i.e., homozygous dominant (NN), heterozygous carrier (Nn), and homozygous recessive (nn)] were determined for each class of animal (Normal, NANI, and DOA), each plant (A, B, C, and D), and each class of animal within each plant by using the PROC FREQ procedure of SAS. The frequency data were not normally distributed, and, therefore, these data for all three genotypes were transformed using a Chi-square rank-based test by using the PROC RANK procedure of SAS. Transformed data were analyzed with the PROC MIXED procedure of SAS with the model including the fixed effects of HAL-1843 genotype and plant.

Results:

The number of trailer loads of pigs and number of farms from which DOA, NANI, and/or Normal animals were sampled are summarized by plant in Table 3. Across the four plants, pigs from a total of 861 trailer loads representing 454 different farms provided samples of either DOA, NANI, and/or Normal animals for this study. Number of pigs per load averaged around 152 animals with an average live weight of 125 kg (276 lb). Approximately half of the trailers from which pigs were sampled were of the pot-belly design (51%) with the remainder being of the straight-deck design (49%). The 2019 pigs that were sampled consisted of 56% of barrows and 44% of gilts that came from a total of around 130 thousand animals (861 trailer loads with ~152 pigs/load) and, therefore, the pigs that were sampled represented 1.6% of the pigs transported.

The frequency of the three genotypes broken down by plant and class of animal are presented in Tables 4 and 5, respectively. Overall, the frequency of animals with the HAL-1843 mutation was relatively low with 2.7% of animals having at least one copy of the mutation (i.e., either Nn or nn) and 97.3% of animals being homozygous for the normal allele (NN). Of the animals with the mutation, 46 (2.3%) of the 2019 pigs tested in this study, were carriers (Nn) of the mutation (Table 4). The frequency of homozygous recessive pigs (nn) was relatively low with only 9 animals or 0.45% of all pigs tested having two copies of the mutation. Of these, all were from different farms, and for the 46 pigs that were carriers (*Nn*) two farms had two pigs represented, with the remaining 42 animals coming from different farms. The sample of pigs used in this study came from a total of 454 farms (Table 3), and, therefore, the mutation was present in animals from around 11% of farms sampled, with 2% of farms having homozygous recessive animals and 9% of farms having carrier animals.

There was a difference ($P < 0.02$) among the plants for the frequency of animals having the mutation with two of the plants having a frequency of animals with at least one copy of the mutation of around 1%, whereas, for the other two plants the frequency was 3.46 and 3.72%, respectively (Table 4).

The mutation was present in all three classes of pig, i.e., normal, NANI, and DOA animals (Table 5). The frequency of dead animals having at least one copy of the mutation (4.7%) was more than double that of normal (1.8%) and NANI (1.8%) pigs (Table 5), however, these differences in frequency were not statistically significant ($P > 0.05$). However, there was a trend ($P = 0.08$) for the frequency of carrier animals to be higher in DOA pigs (3.74%) than in the Normal (1.64%) or NANI (1.61%) animals (Table 5).

Discussion:

In this study, the animals that were sampled came from a total of 454 different farms and, therefore, can be considered to represent a relatively large producer base in the Midwest of the U.S. Samples were obtained from the vast majority of DOA and NANI pigs that were found at the packing plants on the days of the visits. Thus, the samples of DOA and NANI animals used in this study are likely to be representative of the respective populations. However, the 861 trailer loads that were sampled transported around 130 thousand pigs to the plants and, therefore, the Normal animals sampled in this study represent less than 0.5% of all Normal animals delivered to the plant in the loads that were sampled. Therefore, care must be taken when interpreting the genotypic frequencies in Normal pigs as the animals involved are a very small sample and may not be representative of the entire population.

The frequency of pigs with at least one copy of the HAL-1843 mutation at 2.7% of animals tested was relatively low. There are no recently published studies that have estimated the frequency

of this mutation in contemporary U.S. pig populations. Murray and Johnson (1998) published the results of a survey of the frequency of this mutation in pigs arriving at two packing plants in Western Canada. This survey showed that the frequency of the homozygous recessive (nn), carrier (Nn), and normal (NN) genotypes for animals that were dead on arrival or died prior to harvest at the plant were 27.7, 25.2, and 47.1%, respectively. Obviously, the frequency of the HAL-1843 mutation in dead pigs was much higher in the Canadian survey than in the current study. The pigs sampled in the Canadian study were likely to be from different genetic suppliers than represented in the current study. Also, a significant period of time has elapsed since the study of Murray and Johnson (1998) was conducted during which the frequency of the mutation could have changed, particularly if breeding stock suppliers have actively selected against the mutation as they have claimed.

Overall, these results suggest that, although the HAL-1843 mutation is at a relatively low frequency in contemporary pigs in the U.S. (less than 3% of pigs tested in this study), it is still relatively widespread, being present in around 11% of farms that were represented. Historically, most commercial programs that aimed to exploit the potential benefits of the HAL-1843 mutation (i.e., improved feed efficiency and increased carcass yield and lean content) were based on a sireline that was a carrier of the mutation (i.e., Nn) and a negative dam line (i.e., NN). However, these results suggest that on some farms the mutation is present on both the sire and dam side of the pedigree.

There were differences among plants in the frequency of carrier animals, however, these differences were relatively modest. The numbers of animals sampled from each plant (~500) was relatively small and, given the low frequency of the mutation observed, a larger sample size would

be required to detect any difference in the frequency of homozygous recessive animals between the plants.

Among the three classes of animal there was no statistical difference in the frequency of either homozygous recessive or carrier animals. Numerically, there was a higher frequency of animals with the mutation (nn or Nn) in DOA pigs (~4.7%) than in NANI or Normal animals (~1.8%). In all classes, however, the frequency of the mutation was low which suggests that, although this mutation may be a factor in transport losses for individual animals, it is not a major cause of losses. Consequently, efforts to reduce the incidence of transport losses should focus on other causes, both genetic and non-genetic. This is not to say that the industry should discount efforts to eliminate this mutation from pig populations. However, given the relatively low frequency of the mutation observed in this study, the cost compared to the benefit of further testing must be considered.

Lay Interpretation:

The HAL-1843 mutation of the RYR gene (commonly called the "Stress" or "Halothane" gene) is associated with increased stress susceptibility and death loss in pigs. Death loss and non-ambulatory pigs during transport are an important economic issue for the swine industry as well as being a major welfare concern. Over the last 10 to 15 years, most breeding stock suppliers have selected against this mutation and there are claims that it has been eliminated from the most commercial populations. This study was carried out to establish the frequency of the mutation in pigs from contemporary U.S. populations that arrived at packing plants either dead or in a non-ambulatory/non-injured condition, and in a random sample of contemporary normal animals.

The results of the study suggest that the mutation is still relatively widespread, being present in pigs from ~11% of farms sampled. However, the frequency of animals with the mutation was

low (<3% of animals tested) suggesting that it is not a major causal factor in transport losses (either dead or non-ambulatory/non-injured animals).

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Table 1. Number of visits per plant to obtain tissue samples for each class of pig

	Plant				
Class of pig[a]	A	B	C	D	TOTAL
Normal	19	5	6	4	34
NANI	19	5	6	4	34
DOA	25	9	10	9	53

[a]Normal = no visible physical problem; NANI = non-ambulatory/non-injured; DOA = Dead on arrival

Table 2. Number and class of pigs sampled within the four packing plants

	Class of pig[a]			
Plant	Normal	NANI	DOA	Total
A	175	171	165	511
B	160	222	173	555
C	148	156	155	459
D	166	177	151	494
Total	649	726	644	2019

[a]Normal = no visible physical problem; NANI = non-ambulatory/non-injured; DOA = Dead on arrival

Table 3. Number of trailer loads and number of farms from which pigs were sampled within each packing plant.

Plant	A	B	C	D	A – D
No. loads	208	187	293	173	861
No. farms	93	129	134	98	454

Table 4. Genotypic frequencies broken down by plant.

Plant	A	B	C	D	SEM	P Value
Genotype						
Homozygous dominant (NN)						
Number	487	549	439	489	-	-
Percentage	95.29	98.89	85.67	98.99	0.98	0.07
Heterozygous carrier (Nn)						
Number	19	6	16	5	-	-
Percentage	3.72[a]	1.11[b]	3.46[a]	1.03[b]	0.78	0.02
Homozygous recessive (nn)						
Number	5	0	4	0	-	-
Percentage	1.00	0.00	0.87	0.00	0.28	0.16

[a,b]Means with differing superscripts are different ($P < 0.05$).

Table 5. Genotypic frequency broken down by class of pig.

Class of Pig[a]	Normal	NANI	DOA	SEM	P Value
Genotype					
Homozygous dominant (NN)					
Number	637	713	614	-	-
Percentage	98.14	98.10	95.33	1.17	0.10
Heterozygous carrier (Nn)					
Number	11	11	24	-	-
Percentage	1.64	1.61	3.74	0.93	0.08
Homozygous recessive (nn)					
Number	1	2	6	-	-
Percentage	0.17	0.29	0.94	0.34	0.41

[a]Normal = no visible physical problem; NANI = non-ambulatory/non-injured; DOA = Dead on arrival