H Blood Types in Pigs as Predictors of Stress Susceptibility

Abstract. *At least two genotypes in the H system of blood groups in pigs are responsible for blood types associated with the porcine stress syndrome (PSS), and at least three genotypes are responsible for blood types associated with freedom from PSS. Two blood types, each of which apparently may result from more than one genotype, are associated with PSS in some pigs and not in others.*

The porcine stress syndrome (PSS) is characterized by sudden death in pigs subjected to stress, which may result from physical exertion. It occurs most frequently in heavily muscled pigs at the time of marketing (when they weigh 100 kg or more), although it may occur earlier or later (*1*). Death is typically preceded by rigidity of muscles, increased heart rate, open-mouthed respiration, elevated body temperature, and blotchy cyanosis of the skin. Rigor mortis occurs promptly after death. Susceptibility to PSS can be diagnosed with considerable accuracy by exposing pigs to halothane anesthesia (*2*). Susceptible pigs develop muscular rigidity within 3 minutes when exposed through a face mask to 6 percent halothane for 1 minute and 2 percent halothane for the following 2 minutes with oxygen as the only carrier of gas. Most pigs for which PSS susceptibility is diagnosed in this way recover, although not all do.

We here present evidence that PSS is associated with blood type in the H system of red cell antigens in pigs, so that H type can be used to predict PSS susceptibility. The H system has previously been shown to be important in swine productivity and reproduction (*3*). The H system is a complex system of blood groups controlled by at least seven alleles, with at least five different blood factors (*4*).

Pigs from 26 litters from a herd selected for susceptibility to PSS were tested for PSS by the development of rigidity under halothane (by L.L.C.); independent tests for red cell antigens were also made (by B.A.R.). The pigs were from 7 to 10 weeks of age when tested and most were of the Yorkshire breed. Some litters were part Duroc. Blood factors A, O, Ba, Bb, Ca, Da, Ea, Eb, Ed, Ee, Ef, Eg, Fa, Ga, Gb, Ha, Hc, Ja, Ka, Kb, La, Lb, Lf, and Lg were identified, but data for only A, O, Ha, and Hc are reported here. The others did not appear to be involved in PSS.

Table 1 shows the results of tests of 144 pigs; in some litters not all pigs were tested. The tests for A and O are divided into two categories, those giving hemolysis with antibody to A (anti-A) or antibody to O (anti-O) and those giving no hemolysis with either. The A system in pigs is like ABO of humans in that A is dominant to O, so pigs are either A or O (there is no B type), or they may be negative for both A and O because of their H-system genotype: In American Durocs and Yorkshires, certain pigs homozygous for blood factor Ha have red cells that lack A and O (*5*) as do certain miniature pigs in Czechoslovakia (*6*). However, in a semi-inbred line of Landrace in Czechoslovakia where only two alleles, H^{cd} and H^{-}, were segregating, pigs homozygous for $H^{cd}H^{cd}$ lacked A and O (*6*).

The A reagent used for the tests summarized in Table 1 was prepared from a normal serum of a cow and the O reagent was prepared from the normal serum of a goat; all of the H-system reagents were prepared from isoimmune antiserums produced by immunizing one pig with red blood cells of another (*4–6*). For the tests for blood factor Ha, two Ha antiserums were used, both of which were dosage reagents in that they caused more hemolysis sooner with red blood cells from homozygous pigs with two doses of blood factor Ha and were less reactive with red blood cells from heterozygotes with only one dose. This dosage is shown in Table 1 as presence or absence of hemolysis after ½ hour of incubation. Equal volumes (1/30 ml each) of a 3 percent suspension of red blood cells in saline, diluted antiserum (reagent), and absorbed rabbit complement were incubated at room temperature (23° to 26°C) in covered microtiter plates (*5*). For hemolytic tests for Hc, three different Hc reagents were used.

All 17 pigs of H type a/a (*7*) that were negative for A and O were susceptible to PSS, whereas the 38 a/a pigs positive for A or O were PSS-free (Table 1). It appears that among our pigs, there are two different alleles that produce Ha-positive types. Homozygotes for one allele are A-negative or O-negative and are susceptible to PSS, whereas pigs with a second H allele, which gives an Ha-positive, A- or O-positive, type when either homozygous or heterozygous, are PSS-free.

Of 16 pigs of H type -/-, 15 were classified as PSS-susceptible, and the other one, although classified as PSS-free, was noted as having a slight reaction to halothane. This pig may be of a different genotype for -/-, but it is probably somewhat PSS-susceptible. The halothane test seems to be more than 90 percent accurate in diagnosing PSS (*2*); virtually all pigs positive for the test have been susceptible to PSS, but approximately 10 percent of pigs diagnosed as susceptible by other criteria do not go completely rigid within 3 minutes under halothane anesthesia.

Of 49 pigs classified as H type a/- (Table 1), the 14 susceptible to PSS are presumably heterozygous for H^{-} and the allele that gives an Ha-positive, A-negative or O-negative, type when homozygous, whereas the 35 pigs free of PSS are presumably heterozygous for the second allele giving Ha-positive, A-positive or O-positive, types when homozygous or heterozygous.

Of the remaining types, the 12 pigs with H type a/c were PSS-free, as were 11 of 12 of type c/-. The single c/-, PSS-susceptible pig was probably heterozygous for a different allele for Hc, which results in a susceptible type in combination with other alleles for PSS or when homozygous. It has been reported that some Hc homozygotes are negative for A and O (*6*), and these may be PSS-susceptible types.

Of the 47 susceptible pigs in this herd, PSS could have been predicted on the basis of blood type in 32: 17 a/a, A-negative or O-negative, and 15 -/-. The one -/- pig classified as PSS-free should also probably be in the susceptible category. Of the 97 pigs classified as PSS-free, 50 had distinctive blood types: 38 a/a, A- or O-positive, and 12 a/c. It is likely that some a/c pigs are susceptible (heterozygotes for two alleles associated with PSS), but none were

Table 1. Reactions of pig red blood cells with antiserums to blood factors Ha, Hc, A, and O, and susceptibility of pigs to PSS (porcine stress syndrome) as determined by development of rigidity under halothane anesthesia.

H type	A or O type	PSS-susceptible (No.)	PSS-free (No.)	Hemolysis			
				½ hour incubation with anti-Ha	5-hour incubation with:		
					Anti-Ha	Anti-Hc	Anti-A or anti-O
a/a	–	17	0	+	+	0	0
a/a	+	0	38	+	+	0	+
a/-	+	14	35	0	+	0	+
a/c	+	0	12	0	+	+	+
c/-	+	1	11	0	0	+	+
-/-	+	15	1	0	0	0	+

observed in this herd. The a/- pigs were the most frequent type for which PSS susceptibility could not be predicted. However, in seven of the eight litters in which PSS-susceptible and PSS-free a/- pigs were both present, the red blood cells from the PSS-free pigs reacted slightly more quickly and strongly with one of the Ha reagents than did those from the susceptible type. The difference in degree of hemolysis was slight, and the other Ha reagent did not show any difference. It is possible that additional Ha reagents would show a greater difference between genetically different a/- types, and it may be possible to produce reagents which react with red blood cells from one of the types and not the other. Similarly, a reagent might be prepared in such a way as to distinguish the c/- PSS-susceptible type from the c/- PSS-free type; the red blood cells from the PSS-free types reacted more quickly and completely with the Hc reagents than did those from the susceptible pig. If this difference were to prove consistent and other reagents were so prepared as to clearly distinguish between all H-system genotypes, it might be possible to accurately predict PSS susceptibility by blood typing for H-system factors.

In addition to effects of the H system on productivity and reproduction, the relation between H system and PSS is important because PSS is frequently associated with pale, soft, exudative pork (*8*). It has been suggested that the basic cause of PSS and of undesirable pork is a difference in permeability of muscle fibers. Research on H blood types has not included studies of H factors on muscle membranes (*4–6*).

PSS is also a model in pigs for a similar syndrome in humans, the malignant or fulminant hyperthermia syndrome (*9*), and it is also a possible model for the sudden infant death syndrome.

Although there have been no previous reports of association between red blood cell antigens and stress susceptibility in any species, the association between antigens in the M system of blood groups in sheep and high versus low levels of red blood cell potassium (*10*) is an example of another profound physiological effect associated with the red cell antigenic type, which also affects productivity and reproductive performance (*11*).

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7. In this report, a/a refers to H types having two doses of blood factor Ha; such pigs may be homozygous for one allele that produces an Ha-positive type, or they may be heterozygous for two different alleles, each of which produces an Ha-positive type. There are at least two alleles, H^a and H^{ab}, that produce Ha-positive types in Miniature pigs and crossbred pigs in Czechoslovakia (*4*). The correspondence between these alleles and alleles in American pigs has not been determined.
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14 October 1975; revised 2 December 1975

Dimethyl Disulfide: An Attractant Pheromone in Hamster Vaginal Secretion

Abstract. *Dimethyl disulfide, isolated from estrous hamster vaginal secretion and identified by gas chromatography–mass spectrometry, is an attractant for male hamsters.*

That olfactory pheromones are involved in the reproductive behavior of many animals now seems to be established, but the identification of the active chemical compound or compounds for mammalian species has proceeded more slowly than for invertebrate species (*1*). In the laboratory or under seminatural conditions, many mammals do not rely exclusively on olfaction for the initiation or maintenance of normal sex behavior. However, the male golden hamster is particularly dependent on its vomeronasal and olfactory systems since their deafferentation regularly interferes with normal responses to an estrous female (*2*). The estrous female hamster produces copious amounts of vaginal secretion in synchrony with behavioral receptivity (*3*). The odor of this material has a number of different effects on male behavior, such as decreasing aggression (*4*) and flank gland marking (*5*) and increasing the number of inappropriate mounts attempted with surrogates (*6*). Independent of its ability to increase mounting, this odor elicits approach and intense investigatory behavior by both sexually experienced and inexperienced male hamsters (*7*). Because of the uniformity of the behavior pattern of approaching, sniffing, and digging, we decided to use this pattern as the basis of an assay to identify the attractant pheromone in hamster vaginal secretion.

The vaginal secretions were collected on filter paper during the evening of day 1 of the females' estrous cycles and stored at −20°C (*8*). The male behavioral assays were conducted in plastic cages (42 by 21 by 20 cm) with solid plastic covers. Two odor ports, each a cluster of five small holes (6 mm inside diameter), were drilled through the cage bottom 30 cm apart. These ports were covered by a fine wire mesh. Glass jars (30 ml) containing odorants could be firmly fixed beneath each port by a perforated screw cap attachment, which allowed test or control odors to diffuse from the jars, through the ports and the 2-cm layer of Sanicell bedding, and into the cage. A typical behavioral assay began with a preliminary 3-minute period in which both jars contained a suitable control odor, to which no test animal ever responded. Then followed a test period during which one of the jars, selected randomly, contained the odor to be evaluated. A positive response was recorded if the male approached the area over one of the odor ports and began to sniff and dig through the bedding in an attempt to gain access to the jar. The latency and duration (both sniffing and digging time) were recorded. One minute after the beginning of a response the test jar was removed and further response time at the test port was accumulated for an additional minute. In the absence of any positive response the test was terminated after 7 minutes.

Prior to being used in the behavioral assays, each male was given a 7-minute sex behavior test with a receptive female. Approximately 95 percent of the males were observed to mount and intromit on this single test and were retained in the colony. The response of these animals to the odor of vaginal secretion (from three females) was then measured in a plastic test cage. This substance attracted all of the animals. Occasional animals that spontaneously dug at the ports in the absence of odor stimuli were removed from the colony. Thus all males used to evaluate chemical samples were known to respond sexually to receptive females, were strongly attracted