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Inheritance of Resistance to Neonatal *E. coli* Diarrhoea in the Pig: Examination of the Genetic System

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Summary. Evidence is presented that a dominant allele, *S*, is expressed as a receptor for K88 on the brush-border surface of the pig intestinal cell. The homozygous recessive *(ss)* lacks this receptor. The receptor enables K88 - positive coliforms to adhere to the gut of the piglet which they must do if they are to cause neonatal diarrhoea. The homozygous recessive is thus a disease resistant animal.

A possible reason for the persistence of the dominant (susceptible) gene is given.

Key words: Disease - Susceptibility - Polymorphism - Resistance - *E. coli*

K88 is a protein on the surface of some coliforms which enables the bacterium to combine with a receptor on the surface of the intestinal epithelium of the pig. This adhesion of bacteria to the cells lining the gut is a necessary though not a sufficient condition for pathogenicity. K88 is therefore a virulence factor for *E. coli* in the neonatal pig (Arbuckle 1970; Sojka 1971; Jones and Rutter 1972; Smith and Linggood 1972).

The receptor for the K88 antigen is not present in all pigs. We have devised a simple test in which the brush borders are isolated from the mucosal cells of the small intestine and are suspended with K88-positive bacteria. Adhesion of bacteria to these brush borders occurs in preparations from those animals which have the receptor (positive pigs); no adherence is seen in preparations from animals lacking the receptor (negative pigs) (Sellwood, Gibbons, Jones and Rutter 1975). It is a necessary consequence of the first paragraph that the negative pigs should be resistant to infection by K88-positive *E. coli* and this has been shown experimentally to be so (Rutter, Burrows, Sellwood and Gibbons 1975).

To study the genetics of this phenomenon it is necessary to decide how to approach the problem, the difficulty being that the phenotype cannot be observed until the animal is dead. The normal way in which genetic data are presented can only, in this case, be done retrospectively. We feel it more useful to present the experiments with the reasoning that we actually employed, rather than force the data into a different mould, even though such distortion would result in a more conventional approach. We are able to take advantage of the fact that the pig is a polytocous animal and it is possible to divide litters into three types: 1. non-segregating susceptible (all litter susceptible) 2. non-segregating resistant (all litter resistant) and 3. segregating (litter contains piglets of both phenotypes). While some of our information is derived from the phenotypes of randomly selected animals as they are sent for slaughter, the most useful data is obtained from experiments in which we slaughter and phenotype an entire litter.

The picture which emerges is that of a very simple genetic system with very few discordant observations to date. We also try to deal with the problem of the continuing existence of a gene conferring susceptibility to a disease. In human population genetics this is a long-standing problem to which good answers exist in a few cases only (Motulsky 1962).

A preliminary survey has also been made of the association of this characteristic with other genetic factors in the pig to assess linkage and the results to date are reported.

Materials and Methods

The Compton pig herd consists of about 150 breeding sows, 'Large White' × 'Landrace' crosses. The foundation was hysterotomy derived in 1966 and has been

closed until recently; it is maintained as a minimal disease herd. Some exotic genes have been lately introduced by AI. Animals have been phenotyped using a technique previously described (Sellwood et al. 1975).

Transferrin typing was done by the electrophoretic technique of Kristjansson (1963). Lymphocyte antigens were detected using specific antisera as described by White et al. (1973). Aqueous extracts of meconium (Rapoport and Buchanan, 1950) were titrated in serial twofold dilutions for inhibition of agglutination of human *A* and *O* red cells by human anti-*A* and eel anti-*H* sera, (Jonsson 1944).

Results and Discussion

It was established at an early stage that this phenotypic difference was independent of the age of the pig and was fully established at birth. It was, by our test, an all-or-none characteristic. A genetic explanation was clearly likely and we observed that we could pick out three boars all of whose progeny were susceptible. We tested 58 piglets sired by these three animals in 17 different matings; clearly this suggested that the susceptible phenotype was the expression of a dominant gene. All three possible kinds of litter could be observed among the progeny of other boars, including non-segregating resistant litters; but boars which sired this type of litter did not do so consistently, i.e. with other sows segregating or non-segregating susceptible litters were observed. An outline of the position early in the investigation after the first 20 litters had been examined is given in Table 1. On this basis we made the simplest assumption, i.e. that the underlying genetic system was a single Mendelian locus with two alleles, *S* (positive, susceptible) and *s* (negative, resistant), *S* being dominant over *s*. All matings which gave a non-segregating resistant litter of more than six piglets were considered to be derived from homozygous recessive parents. There is of course always a possibility that all offspring had received the same (resistant) chromosome from a heterozygous parent; the larger the litter the less likely this becomes. The chance of error is 1 in 2^n where n is the number of animals in the litter, consequently we accept the statistical probability of the incorrect assignment of genotype once in 128 times. The homozygous recessive animals thus provisionally identified were mated to unknown animals and the resulting litter was phenotyped. The unknown parent may now be assigned a provisional genotype (and phenotype):

Table 1. Susceptible and resistant phenotypes in individual litters of piglets sired by different boars

Boar		No. of whole litters examined	Phenotype Susceptible	Resistant
1	S	0	0	0
	NS	2	22	0
2	S	0	0	0
	NS	1	12	0
3	S	5	23	24
	NS	6	0	51
4	S	2	13	7
	NS	0	0	0
5	S	1	5	7
	NS	3	0	26

S = Segregating litters
NS = Non-segregating litters

Table 2. Phenotyping of whole litters of which the phenotype of at least one parent is known

Type of Mating	No. of Matings	Litters		Phenotype +	-
- × -	9	S*	0	0	0
		NS*	9	0	84
+ × -	10	S	8	36	32
		NS	2	23	0
- × ?	26	NS	5	50	0
		S	16	71	71
		NS	5	0	44
+ × ?	5	NS	4	46	0
		S	1	6	2

S* = Segregating litter
NS* = Non-segregating litter
+ = Susceptible phenotype
- = Resistant phenotype

if the litter segregates, it is a heterozygote; if it does not segregate and all piglets are susceptible, it is a homozygous dominant; or it is a homozygous recessive, if all piglets are resistant.

The breeding stock were not normally slaughtered unless they had ceased to be useful for breeding, but to date 26 of our genotyped animals have been slaughtered and the assigned phenotype was in every case correct. In addition animals which were the offspring of two homozygous recessive parents could be assigned the *ss* genotype and therefore the resistant phenotype. From Table 2 it may be seen that in all cases where the phenotypes are known to be resistant the litters are all resistant; additionally we have animals arising from the same type of mating where the homozygous recessive genotype of the parents can only be

inferred. Fifty-three of these animals (a random selection) have been examined when slaughtered at pork weight and all but three were found to be resistant. We believe that these three animals (two of which were in one batch) have been mislabelled either on farm or in the laboratory.

Further tests for this simple genetic system are available, we can compare the observed and expected ratios in the phenotypes of offspring from known matings. These are given in Table 2. In the 24 matings of phenotypically resistant *(ss)* animals where the other parent may be assigned genotype *Ss* because the litter segregates, we have 210 piglets in the ratio 107:103. Finally two putative heterozygotes were mated and produced a litter of 11 surviving piglets. The litter was reared and mated to homozygous recessives. The resultant litters were slaughtered so that the original $Ss \times Ss$ litter could be genotyped. We found 5 *SS*, 5 *Ss* and 1 *ss*. One each of the three different genotypes was slaughtered to confirm the phenotype, and confirmation was obtained.

Our primary objective has been to identify genotypes, for which purpose we need the homozygous recessives, consequently it has been essential to breed up numbers of these animals. We have not been able to carry out a traditional experiment in which heterozygotes derived from two homozygotes are backcrossed or interbred in order to observe the segregation of phenotypes in the F_2 generation. The validity of the genetic system proposed must rest on the type of evidence adduced in this paper.

No correlation could be found between the *H* or *A* human blood group receptor in the mucin of the piglet (Chadwick et al. 1949) and either disease susceptibility (in two experimentally infected litters) or the susceptible phenotype (3 litters, 34 animals). None of the lymphocyte SL-A antigens examined was associated with the genes *s* or *S*, nor in a $Ss \times Ss$ mating giving 15 offspring did any of the antigen systems segregate with either gene. Again genes at the transferrin locus are randomly associated with the two genes *S* and *s* in 83 piglets in 8 litters investigated, but it was of interest that in one mating $Ss\ Tf^{A}\ Tf^{B} \times ss\ Tf^{B}\ Tf^{B}$ gene Tf^{A} and *S* segregated together (see Table 3). The odds against this occurring fortuitously are 1:1024 and we conclude that there is linkage between the *Tf* and *S* loci. In another mating, included in Table 3, there is apparently a crossover; the linkage therefore is not close. In none of the other matings examined would it have been possible to detect linkage or crossing over with certainty.

Table 3. Segregation of Tf^{A} and *S* in two unrelated matings $Ss\ Tf^{A}Tf^{B} \times ss\ Tf^{B}Tf^{B}$ yielding 14 and 9 piglets respectively

Transferrin phenotype	Susceptible (+)/Resistant (-) phenotype	
	+	-
13 AB	13	0
10 BB	1	9

Inherited resistance to disease in animals is well-known, see for example Allison (1954), Kostromotinov et al. (1972); Payne (1973); McDevitt and Bodmer (1974) and Przytulski and Porzeczkowska (1976). In most instances the underlying basis is either not known or is concerned with the inheritance of the immune-response (Ir) genes (Hildemann 1973; Svejgaard et al. 1975). However, an instance has recently come to light in which resistance to a pathogen *(Plasmodium vivax)* is determined by the absence of a cell-surface receptor in the homozygous recessive (Miller et al. 1975), a system closely analogous to that reported here. In passing we may point out that the presence or absence of specific pathogen receptors on the cell surface probably underlies inter-species host-pathogen specificity as well as the intra-species difference noted here.

In herds from which we have examined samples, both genes have been present with *S* predominating. In an environment containing virulent K88-positive *E.coli* the gene *S* is clearly at a disadvantage with respect to *s* and it is necessary to seek a reason for the persistence of *S*. There are many explanations based on a compensatory selection against the homozygous recessive not all of which can be ruled out. There is however no discernible evidence for such a compensatory selection. We were unable to find any statistically significant difference between resistant and susceptible animals in a sample of 112 pork weight animals with respect to the economically important parameters growth rate, food conversion efficiency, or carcase quality; and there is no reason to suspect artificial selection by the breeder. The explanation we would tentatively advance is based on a consideration of the alternative way in which piglets

are, under natural conditions, protected from neonatal colibacilliosis, namely by antibody in the colostrum.

As shown by Rutter and Jones (1973) high concentrations of immune anti-K88 globulin the the colostrum of the dam confer on the litter substantial (~ 85 %) protection against artificial infection with virulent K88-positive organisms. In the course of an epidemic, as the dams become immune, the selective advantage of the *ss* genotype will become much reduced; its significant advantage will be restricted to the initial stages after the introduction of virulent K88-positive *E.coli* into an immunologically naive herd. Living pathogens in the gut can stimulate antibody in colostrum and milk without there being any evidence of systemic invasion by the pathogen (Bohl and Saif 1975; Kohler 1974; Goldblum et al. 1975) and there is some recent evidence that stimulation of systemic immune IgG may be undesirable (Brandtzaeg and Tolo 1977). Bourne (1977) has suggested that gut associated lymphoid tissue may migrate, as precursor cells, to the mammary gland. The key factor however, lies in the responsiveness of the two phenotypes to immunological stimulation by bacteria in the small intestine. There is much interest in the immunological response of the gut to intestinal contents - not only to bacterial flora but also to antigens in the food (Watson 1969; Ferguson 1972). Generally this response is poor unless the antigen is ingested as a live non-indigenous bacterium - heavy and persistent dosage is required to elicit any reasonable antibody response to killed organisms (Marwick et al. 1968; Kinne-Saffran et al. 1968). Live *E.coli* give a good response, as measured by protection conferred (Kohler 1974) and exotic organisms give a better response than to autochthonous bacteria (Dubos et al. 1965; Berg and Savage 1972). We may reasonably postulate that, to the homozygous recessive sow, the K88-positive bacterium is not to be differentiated from other commensal coliforms, whereas to susceptible sows it is a potential if not an actual pathogen. As such it is much more likely to stimulate antibodies in the susceptible animal. Some preliminary experimental evidence is available to support this hypothesis. Of 12 sows fed a culture of K88-positive non-pathogenic organisms pre-partum, both homozygous dominants and three out of four heterozygotes produced precipitins in their colostrum. One of six homozygous recessives did also. A full report of this experiment will be given elsewhere.

Once a good degree of natural immunity has been established in a herd, the offspring of both homozygous dominants and heterozygotes will be quite well protected against the disease. The offspring of homozygous recessives will be protected only if they are themselves homozygous recessives. The heterozygous offspring of homozygous recessives therefore, are now at a considerable disadvantage compared with the rest of the young pig population; they are genetically susceptible and minimally protected by antibodies in the colostrum or milk. Thus we have selection against a particular class of heterozygotes, a circumstance which will in general tend to eliminate the less common gene.

It might be imagined that selection for the gene *s* in the homozygote would be balanced by an equal selection against the same gene in a fraction of the heterozygotes. This balance is unstable however. This may not be obvious but a brief mathematical analysis (given as an appendix) makes it clear. It is the inverse case where the heterozygote is favoured over both homozygotes which gives rise to a stable balanced polymorphism, e.g. sickle cell anaemia (Allison 1954).

The sequence of events would appear to be as follows: a chance event causes a rise in the number of K88-positive coliforms in the environment of a herd. Susceptible animals become infected and there is high selection in favour of the resistant gene. The susceptible breeding sows now commence to supply immune bodies in their colostrum but this happens to a much lesser extent, if at all, in the resistant sows. The selective advantage of the recessive homozygote is reduced and simultaneously, from the second source, selective pressure is brought to bear against some of the heterozygotes. The gene frequency of *s* now drops, but disease incidence diminishes, pathogenic *E.coli* tend to disappear and selective pressures on the gene frequency in both directions relax. The frequency of *s* will now fluctuate at a low level until herd immunity is lost and another disease episode presents itself.

The instability of the situation means that in the presence of the pathogen selection against *s* in a highly immune herd may, indeed theoretically should, reduce its frequency to zero (Felsenstein 1976). On

the other hand, should the frequency of *s* rise above the equilibrium figure in the absence of substantial increase in the selection against the heterozygote, or in particular should it rise above the critical figure of 0.5, the frequency of *s* should then increase rapidly to 1. The pig population of this country does not consist of a series of isolated breeding populations, but nevertheless one would expect to find herds with a nil or low *s* frequency and herds entirely homozygous for *s* with few intermediate herds. In the Compton herd the gene frequency of *s* was initially 0.6 and is tending to rise, but a certain amount of artificial selection is taking place. In three other herds examined the frequency was low. The effect of an immunisation programme would be to diminish selection pressure both in favour of and against the resistant gene. At present no quantitative prediction can be made as to which it would affect most, but it should be noted that it is possible that it could increase the chance of the elimination of the potentially valuable recessive gene.

Appendix

If x = gene frequency of *s*
K_1 = selection coefficient for genotype *ss* (>1)
K_2 = selection coefficient for genotype *Ss* born to mothers of genotype *ss* (<1)

Genotype frequencies are

$$ss = x^2$$

Ss out of *ss* ♀ $= x^2(1-x)$

all other *Ss* $= (2x - x^2)(1-x)$

$$SS = (1-x)^2 .$$

Hypothetical genotype frequencies in F_1 subjected simultaneously to selection pressures K_1 and K_2

$$\overbrace{K_1x^2}^{ss} \quad \overbrace{K_2x^2(1-x)+(2x-x^2)(1-x)}^{Ss} \quad \overbrace{(1-x)^2}^{SS}.$$

Frequency of gene *s* therefore is

$$\frac{2K_1x^2+K_2x^2(1-x)+(2x-x^2)(1-x)}{2[K_1x^2+K_2x^2(1-x)+(2x-x^2)(1-x)+(1-x)^2]}$$

so that for the change in x produced by selection, Δx, we have

$$\Delta x = \frac{x^2(1-x)[2K_1+K_2-3+2(1-K_2)x]}{2[K_1x^2+K_2x^2(1-x)+(2x-x^2)(1-x)+(1-x)^2]} .$$

Since the bottom line will always be positive, it follows that there will be an equilibrium when $x = (2K_1+K_2-3)/2(K_2-1)$. But it follows easily that, if x is greater than the equilibrium value, Δx will be positive if $K_2 < 1$ (which is assumed in this model) and the equilibrium will be unstable.

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