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Is genetic resistance to Salmonella uniform in pigs?

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Abstract
Previous experimental Salmonella infection studies in pigs in Denmark have shown considerable differences in antibody response where some pigs remained seronegative after infection. To investigate the level and uniformity of Salmonella resistance in crossbred pigs an experiment was conducted to test if antibody response is genetically controlled. In total 611 Salmonella seronegative offspring of Duroc sires and L×Y sows were orally infected with *S. typhimurium* via the feed at 15-20 kg live weight. On day 15 post inoculation, all pigs were blood sampled and sera were tested for Salmonella antibodies. Pigs with low antibody response in this tested sample were re-tested twice (on days 22 and 29 p.i.). Almost 7% of the pigs showed none or very low antibody response. An animal model with repeated measurements of antibody response was used to estimate genetic parameters. A heritability significantly greater than 0 was detected, indicating that immune response to Salmonella resistance in pigs has a genetic component. It was tested whether the genetic component in Salmonella response negative status in pigs could be explained by a single recessive allele. The hypothesis of a single recessive allele causing resistance to Salmonella was rejected. Nonetheless, the existence of genetic variation allows the use of selection to change the level and reach a high uniformity in antibody response to Salmonella in pigs.

Introduction

Salmonella infection studies in Denmark have shown that some pigs remain faecal culture negative and seronegative despite oral inoculation (Nielsen et al., 1995). Resistance to bacterial gut infections thus may have a genetic component. Just as for E.coli-149 F4 infection in pigs a single recessive gene was found to cause resistance to diarrhoea in piglets (Jørgensen et al., 2003).

Salmonella challenge studies in chicken and in lamb indicate that resistance has a genetic component and that selection for reduced carrier status is possible (Beaumont et al., 2006; Moreno et al., 2003). The mechanism proposed for genetic resistance to Salmonella in the gut is that bacteria are unable to adhere to the epithelial cells of the intestine and will thereby not colonize the host. This means that the bacteria will not cause infection in resistant animals, characterised by animals remaining seronegative, i.e. not producing antibodies, and culture-negative upon challenge with Salmonella infection.

Genetic resistance to Salmonella in pigs will have a potential impact on food safety, design of housing, production systems and animal health management.

A challenge experiment was carried out to investigate whether genetic variation in resistance to *Salmonella typhimurium* exists in Danish pig breeds. The aim was to investigate the genetic background to resistance and to test the feasibility of genetic improvement. Two hypotheses were tested: 1) additive genetic variation in Salmonella resistance in pigs exists and 2) Salmonella resistance in pigs may be caused by a single recessive allele.

Materials and methods
In a multiplying herd 72 Landrace*Yorkshire (LY) crossbred sows were inseminated with semen from 72 Duroc (D) boars. The sows originated from 15 sires and 65 dams. The Duroc sires originated from 66 sires and 69 dams. Each boar and each sow produced only one litter. A total of 773 piglets were born of which the 67 largest litters were included in the study. Also, obviously sick animals were discarded.

The multiplying herd had been seronegative for Salmonella for 4 years up to the start of the study as shown by monthly blood sampling as part of the Salmonella control program in Danish breeding herds. The sows included in the study were 3 1st parity sows, 30 2nd parity sows and 34 3rd parity sows. In total 611 piglets were weaned at 7 kg live weight (approx. 28 days) and moved to a Salmonella-free research facility in 6 test groups of about 100 animals per test group. Each test group of animals was housed in a separate stable section with 10 pigs/pen. Upon arrival to the research facility, it was ensured that pigs were negative for Salmonella by examining pooled faecal samples.

All pigs in a test group were inoculated on the same day at a live weight of 15-20 kg. The pigs were orally inoculated via the feed with approximately 10^9c.f.u. *S. typhimurium*. On day 15 post inoculation (p.i.) all pigs were blood sampled and sera were examined for antibodies against Salmonella using the Danish mix-ELISA (Nielsen et al., 1995). Several test groups were blood sampled on the same day, hence sharing the same calibration for OD%-level. The trait recorded was antibody response, measured as optical density (OD%). A low value, OD% <27 was considered seronegative for antibody to Salmonella indicative of negative infection status despite inoculation. On day 22 and 29 p.i. pigs with negative antibody response in the first blood sample were re-tested serologically. A control group of pigs with OD% >26 at first sampling was also re-tested at least once. Seronegative pigs with antibody levels <27 OD% were also examined for Salmonella by standard bacteriological methods using 3 consecutive faecal samples collected at day 20-22 and 27-29 p.i.

Genetic variation analysis
To detect the possible genetic variance an animal repeatability model with random effects was considered:

$$g(OD_{ij}) = s_i + c_i + d_i + b_1 x_i + b_2 (x_i)^2 + L_i + a_i + e_i + \varepsilon_{ij} \qquad (1)$$

where OD_{ij} is the response level of optical density recorded by animal i on measurement j, g is a link function, s_i is a two level sex effect, c_i is a group effect of the six test groups, d_i is the effect of blood sampling day, b_1 and b_2 are regression coefficients of the first and second order value of age at inoculation time x_i. Then random litter effect is denoted $L_i \sim N(0,\sigma_L^2)$, and $a_i \sim N(0,\sigma_a^2\mathbf{A})$ is the genetic component of animal i with genetic variance σ_a^2 and $\mathbf{A}$ is the additive relationship matrix. The environmental variance between animals is described by $e_i \sim N(0,\sigma_e^2)$ and the variance between repeated measurements within the same animal is described by $\varepsilon_{ij} \sim N(0,\sigma_\varepsilon^2)$. The link function g is assumed to be $g(OD_{ij})=\sin^{-1}[(OD_{ij}/182)^{0.5}]$, where 182 is the upper bound of OD value in the experimental data. The variances of the OD value decrease if the measurements are close to the lower or upper boundary, and the link function stabilizes variances across the range of OD values.

The significance of fixed effects were obtained in a mixed model similar to (1) omitting the animal part, a_i. The animal model was estimated by REML using VCE (Neumaier and Groeneveld, 1998) and the mixed model was estimated using 'proc mixed' in SAS .

Single gene test
To investigate whether resistance may be caused by a single recessive allele it was assumed that both parents were heterozygous for the recessive allele to be able to show resistance in the offspring. Due to this assumption, the segregation ratio for Mendelian inheritance was calculated for

families with affected offspring only and with correction for families that were not segregating. The Singles method (Li and Mantel, 1968; Davie, 1979; Christensen, 2002) takes into account families with at least one resistant offspring. Thus, in these families:

$$\hat{p} = \frac{A - A_1}{T - A_1} \qquad \text{and} \qquad \mathrm{Var}(\hat{p}) = \frac{T - A}{(T - A_1)^3}\left[A - A_1 + 2A_2\frac{T - A}{T - A_1}\right]$$

where $\hat{p}$ is the segregation ratio, *T* is the total number of offspring in families with at least one resistant offspring and A is the total number of resistant offspring. A_1 and A_2 are the number of families with respectively 1 and 2 affected offspring.

The single gene test was obtained by:

$$Z^2 = \frac{(\hat{p} - p)^2}{Var(\hat{p})}$$

Z^2 is Chi-squared distributed with 1 degree of freedom.

Results and discussion

Pigs with low antibody response, OD% less than 27, were considered seronegative and thereby resistant to Salmonella. In total 62 pigs had an OD% <27 at the first test (Table 1). A total of 42 pigs (6.9%) remained seronegative in all three blood samples until 30 days p.i. A control group of 25 pigs with higher values at the first test was re-examined at least once.

Table 1: Means and standard deviation of antibody response in pigs inoculated with *Salmonella typhimurium*

Grouping	No. of pigs	% of all pigs	Mean OD% (±SD)		
			15 days p.i.	22 days p.i.	29 days p.i.
1st blood test, all pigs	611	100	77 (40)	-	-
Low response 1st test (<27 OD%)	62	10.1	14 (7.1)	22 (13.4)	21 (14.8)
Low response all tests (<27 OD%)	42	6.9	13 (7.3)	15 (7.8)	14.7 (7.0)
High response 1st test (>26 OD%)(control group)	25	4.1	85 (41.9)	73 (39.4)	86 (30.4)

The 42 pigs with OD-values <27 originated from 33 different litters. Three full-sibs originated from each of 4 litters, 2 full-sibs from each of 6 litters and 1 pig from each of 18 litters. Assuming that antibody response is connected to resistance, 41.8% of the litters with in total 324 pigs thus had one or more resistant pig and 14.9% of the litters had two or more resistant full sibs/litter.

The repeatability mixed model showed significant differences in OD% mean values in the six test groups (p=0.0001) and at the 8 days of blood sampling (p=0.0001) (Table 2). There was no significant sex effect (p=0.84), and also the regression coefficients related to the age at inoculation time were insignificant (Table 2).

From the animal model the heritability for antibody response was estimated to be 0.175 (Table 3). Thus, there is clear evidence of genetic variation in resistance to Salmonella.

Table 2: Number of levels, estimates, and significance of fixed and random effects estimated in a linear mixed model omitting the genetic component of model (1).

	Number of levels	Estimate	P-value of significance
Sex, s_i	2	-	0.84
Test group effect, g_i	6	-	0.0001
Day of blood sampling, d_i	8	-	0.0001
Regression coefficients, b_1	1	0.03821	0.2783
Regression coefficients, b_2	1	-0.00019	0.3426
Litter, σ_L^2	1	0.00516	0.0017
Between animals, σ_e^2	1	0.01723	0.3156
Within animals, σ_ε^2	1	0.02909	0.2085

Table 3: Variances, ratios, and standard errors of ratios of random effects estimating the animal model in (1).

	Variance	Ratio	Standard error
Litter, σ_L^2	0.000	0.000	0.000
Animal, σ_a^2	0.010	0.175	0.050
Between animals, σ_e^2	0.033	0.613	0.051
Within animals, σ_ε^2	0.011	0.212	0.019

Hypothesis testing of a single recessive allele causing resistance by “Singles method“ was performed. It was assumed that $H_0 : \hat{p} = p.$ The total number of offspring in affected families were 324 and number of resistant offspring were 42, number of affected litters with one resistant offspring were 18 and litters with two resistant offspring were 6. The estimated frequency was, $\hat{p} = 0.0784$ with $Var(\hat{p}) = 0.0003$. Hence, with the expected value of p=0.25 the chi-square value was obtained to be Z^2 =85.3. This shows that the estimated $\hat{p}$ was statistically significantly different from the expected value of p=0.25. The hypothesis about a single recessive gene alone causing resistance was rejected.

This lead us to conclude that there is not sufficient evidence to assume that a single recessive allele is causing the genetic variation in antibody response to Salmonella in Danish pigs. The heritability found in our study shows clear evidence of genetic variation in resistance to Salmonella. The analysis demonstrates substantial and significant heritability and indicates that several genes are acting in the process of antibody response but the mechanism remains unknown.

Genetic control of resistance depends on several factors such as the Salmonella strain, inoculation dose and time interval from inoculation to blood testing p.i. This underlines the importance of precision in measurements and the choice of measured traits due to the complexity of genetic resistance. The faecal sampling results in the experiment did not demonstrate a strong relationship between Salmonella-positive feces tests and high OD% values. This may be ascribed to the low sensitivity of the culture method, the limited number of fecal samples collected per pig, or a combination of both. It should be borne in mind that the purpose of analyzing fecal samples was to catch any Salmonella-excreting pig that for some reason was unable to mount an antibody response. Indeed, our study showed that there were some pigs with low OD% values that showed positive results in faeces, probably due to this fact. Finally, additional typing in the laboratory confirmed that the strain of Salmonella was the same throughout the experiment, indicating the absence of Salmonella from other sources.

The results with 7% resistant pigs indicate that the level of resistance is low, however the uniformity is high. The aim will be to increase the level of resistant animals. By increasing resistance the uniformity will decrease until a certain point, before it increases again.

Selection for improved genetic resistance to Salmonella based on fecal samples will be difficult and very expensive. Blood sample based selection provides a much easier and cheaper method with substantial heritability but it would still require challenge test with pathogens. However, effective selection for uniformly high levels of genetic resistance to Salmonella would most likely require the identification of causative genes, completely obviating the need for any challenge test.

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