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Genetic variation for resistance to clinical and subclinical diseases exists in growing pigs

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Abstract

The objective of this study was to test that genetic variation for resistance to clinical and subclinical diseases exists in growing pigs. A total of 13 551 male growing pigs were assessed for resistance to five categories of clinical and subclinical disease: (i) any clinical or subclinical disease, (ii) lameness, (iii) respiratory diseases, (iv) diarrhoea, and (v) other diseases (i.e. any clinical or subclinical disease with the exception of (ii), (iii), and (iv)). Additive genetic variation for resistance to each disease category was estimated by fitting a Weibull, sire-dam frailty model to time until the pigs were first diagnosed with a disease from that category. Genetic correlations among the resistances to each disease category were approximated as product-moment correlations among predicted breeding values of the sires. Additive genetic variation was detected for resistance to (i) any clinical or subclinical disease (additive genetic variance for log-frailty (± s.e.) = 0·18 ± 0·05, heritability on the logarithmic-time scale = 0·10), (ii) lameness (0·29 ± 0·11, 0·16), (iii) respiratory diseases (0·24 ± 0·16, 0·12), (iv) diarrhoea (0·30 ± 0·27, 0·16), and (v) the other diseases (0·34 ± 0·15, 0·19) and there were generally positive and low-to-moderate correlations among the predicted breeding values (–0·03 to + 0·65). These results demonstrate that additive genetic variation for resistance to clinical and subclinical diseases does exist in growing pigs, and suggests that selective breeding for resistance could be successful.

Keywords: *disease resistance, genetic variation, pigs.*

Introduction

Pig production is often hindered by disease, which can cause mortality, reduced production performance, increased costs and poor animal welfare. Methods currently used to control disease include eradication, sanitation, quarantine, culling, vaccination and medication. A complementary, albeit longer-term, approach to disease control is to selectively breed pigs for resistance. Selective breeding should be possible given that genetic variation for resistance to specific pathogens is present in most, if not all, animal populations (Nicholas, 1987; Müller and Brem, 1991; Straw and Rothschild, 1992) and this is the case for pigs in relation to pathogens that have been investigated (Straw and Rothschild, 1992; Rohrer and Beattie, 1999). During pig production, resistance is assessed, not in relation to specific pathogens, but as the incidence of clinical and subclinical disease (e.g. lameness, respiratory diseases, diarrhoea, reduced food consumption). This has the advantage of enabling large numbers of pigs to be assessed while they are exposed to the pathogens encountered during production. However, clinical and subclinical diseases can be the outcome of infection by many different pathogens, and much of the variation for resistance among pigs is due to environmental factors, such as unpredictable exposure to the pathogens. Despite these drawbacks, in the species where most work has been done in this field, namely adult dairy cattle, resistance to economically important clinical diseases (e.g. mastitis, digestive diseases, feet and leg disorders) still exhibit low

levels of additive genetic variation (h^2 <0·10 for disease incidence assessed as a categorical trait) (e.g. Philipson *et al.*, 1980; Lyons *et al.*, 1991; Mäntysaari *et al*, 1991; Simianer *et al.*, 1991; Uribe *et al.*, 1995; Heringstad *et al.*, 2000). This has lead to the inclusion of resistance to clinical diseases in cattle breeding programmes (e.g. Pedersen *et al.*, 1993; Pedersen and Aamand, 1999). Similarly, in the few studies presented to date on piglets and growing pigs, low levels of additive genetic variation have been documented for resistance to respiratory diseases, diarrhoea, and arthritis (Smith *et al.*, 1962; Lundeheim, 1979 and 1988; Lingaas and Rønningen, 1991). These results suggest that at least low levels of additive genetic variation for resistance to clinical and subclinical diseases exists in pigs, and that selection for resistance to clinical and subclinical disease can be successful.

In this study, additive genetic variation was estimated for resistance to clinical and subclinical diseases in growing pigs. Male growing pigs from the nucleus breeding population of the Danish pig breeding programme (DanAvl) were assessed for resistance to five categories of disease (i.e. any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases) while they were performance tested for production traits (e.g. growth rate, food efficiency, and lean tissue content). Additive genetic variation was estimated for resistance to each disease category, while genetic correlations among the resistances were approximated as product-moment correlations among predicted breeding values. The objective was to test that additive genetic variation for resistance to clinical and subclinical diseases exists in growing pigs.

Material and methods

Performance test

Between 1995 and 1998, 13 551 male growing pigs from the Duroc, Yorkshire, Landrace, and Hampshire breeds were assessed for clinical and subclinical diseases while they were performance tested for production traits at Bøgildgård test station. Bøgildgård is the central test station of DanAvl and is used to performance test approximately 5000 male pigs each year. On average, the performance test for each pig lasted 83 days, beginning when the pigs were 9 weeks old (±4 days) (approx. 30 kg live body weight) and finishing when they attained slaughter weight (90 kg) at approximately 21 weeks of age. However, there were pigs that were removed from the performance test as early as day 7 and as late as day 123. Pigs removed during early stages of the performance test either died, were injured, or were severely ill (i.e. growth was severely affected, and they would not respond to treatment).

Pigs

The pigs performance tested at Bøgildgård were from DanAvl's nucleus breeding population (Figure 1). Each was bred at one of 49 breeding farms and was selected to be performance tested because it had the potential to be genetically superior for the production traits by virtue of its ancestry. At the conclusion of the performance test, those pigs found to be genetically superior were selected to produce semen used to artificially inseminate sows at the breeding farms. The remaining pigs were slaughtered. The artificial insemination of the sows at the breeding farms resulted in the next generation of the nucleus breeding population.

The 49 breeding farms were responsible for maintaining DanAvl's nucleus breeding population. The majority of the farms maintained one or two of the pig breeds, although there were farms that maintained as many as all four breeds.

Pedigree

The pigs performance tested at Bøgildgård were from 1032 sires and out of 7104 dams. The number of pigs from each sire ranged between 1 and 101 (mean 13·1 (s.e. 5·2)) and the number of pigs out of each dam ranged between 1 and 11 (1·9 (s.e. 1·2)). The total number of individuals in the pedigree structure after tracing animals back from the sires and dams of the pigs was 14 458.

Rearing of pigs

Bøgildgård operates by an 'all in-all out' policy. The pigs were performance tested in stall groups, where the pigs in each group started and finished the performance test at the same time. Specifically, the pigs arrived at Bøgildgård from their respective breeding farms as 4-week-old piglets (approx. 8 kg live body weight). Upon arrival, they spent 5 weeks in acclimatization pens (Figure 2). The pigs were then allocated to a stall within a test facility to be performance tested.

The test facility consisted of 16 stalls. Each stall was divided into eight pens, and each pen maintained between 12 and 14 pigs (i.e. approx. 100 pigs per stall). Pigs allocated to the same stall finished the period of acclimatization at the same time. However, pigs allocated to the same pen within each stall were from the same breed and were a mixture of pigs from different acclimatization pens. After each stall group had been performance tested, the pigs were removed

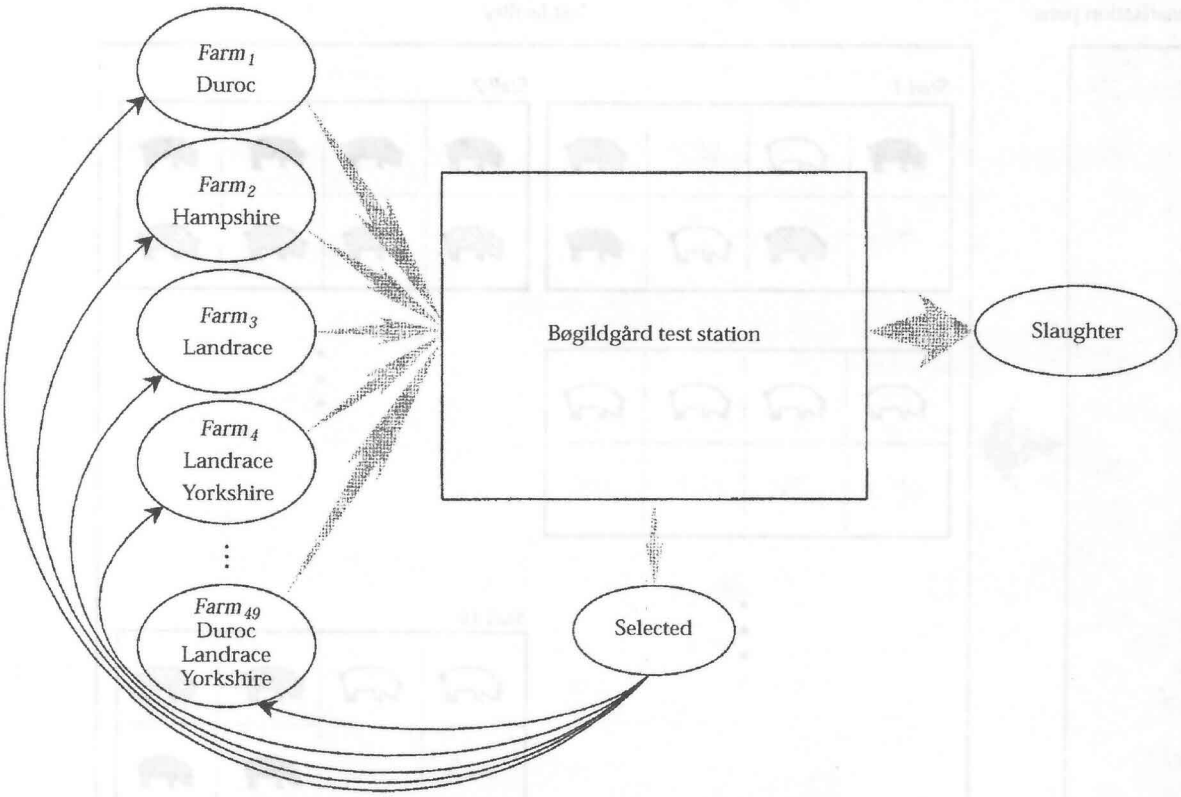

Figure 1 Transfer of pigs within DanAvl's nucleus breeding population. Pigs selected to be performance tested for production traits at Bøgildgård test station were bred at 49 breeding farms. The majority of these breeding farms maintained one or two of the pig breeds (i.e., Duroc, Yorkshire, Landrace or Hampshire), although there were farms that maintained as many as all four breeds. At the conclusion of the performance test, those pigs found to be genetically superior were selected to produce semen used to artificially inseminate sows at the breeding farms. The remaining pigs were slaughtered.

and the stall made ready for the next group to be performance tested.

Diagnosis of clinical and subclinical diseases

During the performance test, a pig was assumed to have been diagnosed with a clinical or subclinical disease when it was treated for the disease. Each time a pig was treated, a record was made of the disease and the date of treatment. The clinical and subclinical diseases diagnosed are presented in Table 1.

There were cases where non-diagnosed pigs were treated to prevent them from being infected with a clinical or subclinical disease (i.e. majority of pigs within a pen group were treated for a disease although only a few individuals in the group were diagnosed with the disease). However, preventive treatments were not recorded in a way that was distinguishable from the treatments of diagnosed pigs. Therefore, to account for the preventive treatments, when more than 75% of the pigs in a pen group were treated for a particular disease on a given day, all of these treatments were assumed to be preventive and removed from the data set. This enabled pigs treated on subsequent days to be identified as the diagnosed pigs.

Table 1 *Clinical and subclinical diseases diagnosed while growing pigs were performance tested for production traits at Bøgildgård test station*

Clinical	Lameness, respiratory diseases, diarrhoea, skin disorders, sneezing, snout deformation, boils, cramp, tail bitten
Subclinical	Reduced food consumption

The clinical and subclinical diseases were grouped into the following categories: (i) any clinical or

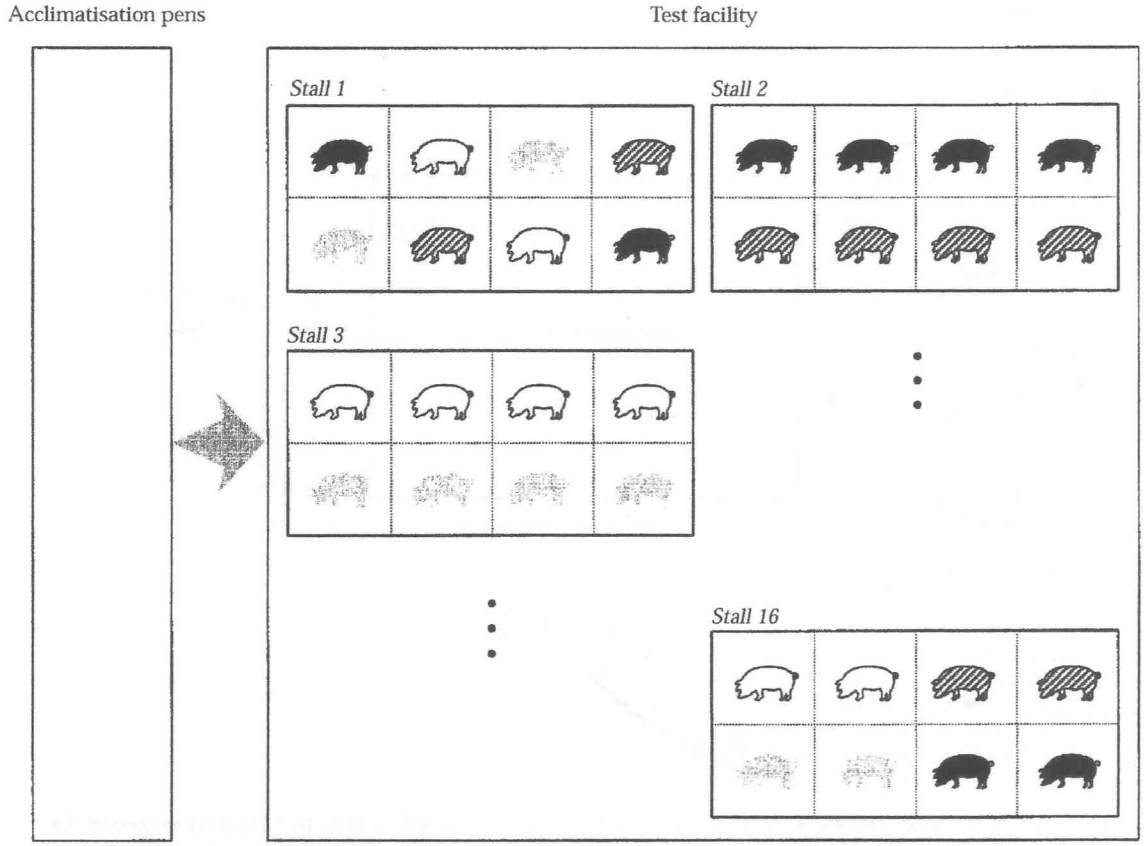

Figure 2 Allocation of pigs at Bøgildgård test station. After arrival at Bøgildgård from their respective breeding farms, the pigs spent 5 weeks in acclimatization pens. The pigs were then allocated to a test facility to be performance tested. The test facility consisted of 16 stalls. Each stall was divided into eight pens, and each pen maintained between 12 and 14 pigs. Pigs allocated to the same pen within each stall were from the same breed, and were either Duroc (represented by), Yorkshire ( ), Landrace ( ) or Hampshire ( ) pigs.

subclinical disease (i.e. includes all diseases), (ii) lameness, (iii) respiratory diseases, (iv) diarrhoea, and (v) other diseases, which included reduced food consumption and all the clinical diseases with the exception of (ii), (iii), and (iv).

The diseases were grouped into these categories, as preliminary analysis demonstrated that lameness, respiratory diseases, diarrhoea, and reduced food consumption were the most prevalent diseases. By contrast, less than 0·6% of the pigs were treated for skin disorders, sneezing, snout deformation, boils, cramp and tail bitten.

Assessment of disease resistance

Resistance of the pigs to each disease category was assessed as time (days) from the start of the performance test until first diagnosis of a disease from that category. By assessing resistance in this way, it was assumed that all pigs would eventually be diagnosed with a disease from each category. Pigs that were not diagnosed with a particular disease category were assumed to have a censored record for that category (i.e. assumed that they would be diagnosed some time after the performance test).

Assessing resistance as time until first diagnosis provides a measure of the degree of resistance (i.e. the longer it takes before a pig is diagnosed, the greater the resistance). In terms of a selective breeding programme, the aim is to produce pigs whose time until diagnosis would be some time after they have been performance tested. For growing pigs, this is after they have been slaughtered.

Statistical analysis

Kaplan-Meier estimate of the survival function. The Kaplan-Meier estimate of the survival function (Kaplan and Meier, 1958) was plotted for any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and the other diseases. The slope of the survival functions estimate the daily rate (i.e. proportion) of pigs first diagnosed over the course of the performance test.

Model. Variance components, and breed and environmental effects, for resistance to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea, and the other diseases were estimated by fitting a Weibull, sire-dam frailty model to the times until first diagnosis for each disease category (after Ducrocq and Casella, 1996). In the Weibull, sire-dam frailty model, the hazard function for time, t, from the start of the performance test until first diagnosis of pig i, conditional on random effects, is given by $\lambda_i(t \mid \mathbf{u}) = \lambda_0(t)\cdot\exp\{\mathbf{x'}_i(t)\mathbf{b} + \mathbf{z}_i'(t)\mathbf{u}\}$, where the baseline hazard function, $\lambda_0(t)$, is assumed to be Weibull distributed [i.e. $\lambda\rho(\lambda t)^{\rho-1}$], $\mathbf{x'}_i(t)$ and $\mathbf{z}_i'(t)$ are vectors of time-independent and/or time-dependent covariates with associated fixed (**b**) and random (**u**) effects, and λ and ρ are parameters of the Weibull distribution. The hazard for time until first diagnosis of pig i, describes the instantaneous probability (i.e. risk) of it being diagnosed at time t, conditional upon it not being diagnosed up to t. Modelling the hazard of the pigs accounted for the performance test of the pigs (i.e. period of risk) varying from 7 to 123 days and enabled censored observations (i.e. pigs that were not diagnosed during the performance test) and time-dependent effects to be included in the analyses.

The Weibull, sire-dam frailty model fitted to the times until first diagnosis for each disease category was:

$$\lambda_i(t \mid \mathbf{v},\mathbf{a}) = \lambda_0(t)\cdot\exp\{\tau_j(t) + \varpi_k + \delta_l + \kappa_m + \varphi_n(t) + \eta(t)\beta_1 + \eta^2(t)\beta_2 + \theta_q + \int(a_{si} + a_{di})\} \quad (1)$$

with equation symbols defined as follows.

$\tau_j(t)$ = time-dependent piece-wise constant function, which changed when pig i was in the jth pre-defined period of the performance test. The j pre-defined periods differed when the model was fitted to any clinical or subclinical disease (i.e. periods were between days 1-6, 7-16, 17-57, and ≥58), lameness (days 1-6, 7-17, 18-73, and ≥74), respiratory diseases (days 1-6, 7-17, 18-45, and ≥46), diarrhoea (days 1-6, 7-15, and ≥16), and the other diseases (days 1-6, 7-17, 18-65, and ≥66). By fitting the piece-wise constant function, the Weibull distribution was an appropriate fit of $\lambda_0(t)$ over the course of the performance test.

ϖ_k = time-independent fixed effect of the kth breed of pig i (k = Duroc, Yorkshire, Landrace, Hampshire).

δ_l = time-independent fixed effect of the lth breeding farm on which pig i was bred (l = 1, ..., 49). The effect of breeding farm was only included when the model was fitted to any clinical or subclinical disease, lameness, diarrhoea, and the other diseases.

κ_m = time-independent fixed effect of the mth stall group in which pig i was performance tested (m = 1, ... , 134).

$\varphi_n(t)$ = time-dependent fixed effect of the nth treatment level (n = non-treated, treated). The risk of pig i being diagnosed was expected to be lower for the period it was preventively treated or treated for another disease category. Pig i was considered treated on the days of treatment and for 2 days following treatment. On all other days, it was considered non-treated. The effect of treatment level was only included when the model was fitted to respiratory diseases and diarrhoea.

$\eta(t)$ = time-dependent regression effect of the number of pigs (excluding pig i) within the same stall or pen group as pig i that were diagnosed with the disease category being analysed. The risk of pig i being diagnosed on each day of the performance test was expected to increase with the number of diagnosed pigs. For respiratory diseases, it was the number of pigs in the same stall as pig i that were diagnosed each day, while for lameness, diarrhoea and the other diseases it was the number of pigs in the same pen. The pigs were considered to be infectious for a different period of time relative to their day of diagnosis when the model was fitted to lameness (i.e. between 1 day prior and 4 days after diagnosis), respiratory diseases (1 day prior and 2 days after), diarrhoea and the other diseases (1 day prior and 3 days after). For any clinical or subclinical disease, the number of pigs diagnosed each day was the summation of the number of pigs diagnosed with lameness, respiratory diseases, diarrhoea and the other diseases.

β_1 = regression coefficient associated with $\eta(t)$. $\eta^2(t)$ = square of $\eta(t)$. The square of the number of diagnosed pigs was only included when the model was fitted to any clinical or subclinical disease and respiratory diseases.

β_2 = regression coefficient associated with $\eta^2(t)$.

θ_q = time-independent random effect of the qth pen group in which pig i was performance tested (q = 1, ... , 1053). The vector of pen group effects, $\mathbf{v} = (\theta_1, \ldots,$

$\theta_{1053})'$, was assumed to be iid and follow a log-gamma distribution (~log-gamma(γ,γ), where γ is the parameter of the log-gamma distribution).

$\frac{1}{2}(a_{si} + a_{di})$ = time-independent random effect, where a_{si} and a_{di} are the breeding values of sire, *s*, and dam, *d*, of pig *i*. The vector of sire and dam breeding values, $\mathbf{a} = (a_1, \ldots, a_{8136})'$, was assumed to follow a normal distribution (~$N(0, A\sigma_a^2)$, where A is the numerator relationship matrix).

In this model, $\exp\{\theta_q + \frac{1}{2}(a_{si} + a_{di})\}$ is the frailty variable of pig *i*, while the log-frailty variable is $\theta_q + \frac{1}{2}(a_{si} + a_{di})$. The model was fitted using 'The Survival Kit' developed by Ducrocq and Sölkner (1998).

Model (1) was fitted to each disease category after the following preliminary analyses.

Validation of the Weibull baseline hazard assumption and establishment of the *j* pre-defined periods of the performance test (i.e. when including the time-dependent piece-wise constant function, $\tau_j(t)$) were carried out empirically (Kalbfleisch and Prentice, 1980). Specifically, a semi-parametric Cox model (Cox, 1972) was fitted to the times until first diagnosis. The model included the fixed and regression effects included in model (1) (i.e. excluded the random pen group effects and random breeding values of the sires and dams) and the distribution of $\lambda_0(t)$ was not specified [i.e. $\lambda_0(t)$ was arbitrarily defined]. Solving for the fixed and regression effects enabled $\hat{\lambda}_0(t)$ and the interrelated baseline survival function, $\hat{S}_0(t)$, to be evaluated. A graphical test for the suitability of a Weibull distribution (i.e. plot of $\ln[-\ln[\hat{S}_0(t)]]$ against $\ln(t)$ produces a straight line) demonstrated that the Weibull distribution was an appropriate fit of $\lambda_0(t)$ when the performance test was divided into the *j* pre-defined periods.

The fixed and regression effects included in model (1) were arrived at through backward elimination of non-significant effects (Myers, 1989) from a Weibull model. The Weibull model included all available fixed and regression effects (i.e. piece-wise constant function, breed, breeding farm, stall group, treatment level, and number and square of the number of diagnosed pigs), but no random effects. The test criterion was the difference in −2lnL (L = likelihood) between a full and a reduced model, where the full model was the Weibull model following each round of elimination, and the reduced model was the full model following each round of elimination with the effect being assessed removed. The critical χ^2-value for elimination was $\chi^2_{\upsilon w}$ with $P > 0{\cdot}10$, where $\chi^2_{\upsilon w}$ is the χ^2-value of the *w*th effect with υ d.f. The χ^2-values and associated d.f. for significant fixed and regression effects (i.e. fixed and regression effects included in model (1)) are presented in Table 2. These values were obtained for each effect as the difference in −2lnL when the effect was removed from the Weibull model after non-significant fixed and regression effects had been eliminated.

Pig *i* was considered treated for 2 days following treatment (i.e. when including the fixed effect of treatment level, $\varphi_n(t)$, in model (1) fitted to respiratory diseases and diarrhoea) for two reasons. First, veterinarians working at Bøgildgård advised that the pigs be considered treated for 2 days following treatment. Second, when treatment level was included in a Weibull model fitted to the times until first diagnosis (i.e. model (1) without the random pen group effects and random breeding values of the sires and dams), considering pig *i*

Table 2 *χ^2-values and associated d.f. for fixed and regression effects included in a Weibull model fitted to time until growing pigs were first diagnosed with five categories of clinical and subclinical disease during performance test. The χ^2-values and d.f. were obtained for each effect as the difference in −2ln(Likelihood) between a full and a reduced model, where the full model was the Weibull model that included significant fixed and regression effects, and the reduced model was the full model with the effect being assessed removed. The disease categories are any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases. The χ^2-values are significant (P < 0·10) for all effects*

	Any disease		Lameness		Respiratory		Diarrhoea		Other	
	d.f.	χ^2	d.f.	χ^2	d.f.†	χ^2	d.f.†	χ^2	d.f.†	χ^2
Piece-wise constant	3	434·6	3	275·0	3	41·9	2	110·3	3	174·3
Breed	3	78·2	3	25·0	3	116·0	3	7·5	3	38·2
Breeding farm	48	66·6	48	63·3			45	68·9	48	80·7
Stall group	133	593·3	133	311·8	97	589·4	96	568·8	129	583·8
Treatment					1	7·2	1	30·4		
No. diagnosed	1	186·0	1	177·0	1	240·1	1	27·3	1	14·8
(No. diagnosed)^2	1	39·0			1	104·4				

† Some levels of the fixed breeding farm and stall group effects were not estimable for respiratory diseases, diarrhoea, and the other diseases because no pigs in these levels were diagnosed.

treated for 2 days following treatment resulted in a lower −2lnL than when pig *i* was considered treated for other lengths of time following treatment (i.e. 0, 1, 3, 4, and 5 days). The number of days a pig was considered infectious relative to its day of diagnosis (i.e. when including the regression effect of number and the square of the number of diagnosed pigs, $\eta(t)$ and $\eta^2(t)$) was arrived at in a similar manner.

Additive genetic variation. Additive genetic variation was estimated as the variance of log-frailty associated with the random breeding values of the sires and dams (σ_a^2). A heritability was estimated by noting that model (1) is also a log-linear model when no time-dependent effects are included (cf. Kalbfleisch and Prentice, 1980; Ducrocq and Casella, 1996). Hence, the heritability was presented for the time until diagnosis on the logarithmic-time scale as if there were no time-dependent effects. It was calculated as:

$$h^2_{\log} = \frac{\sigma_a^2}{\frac{1}{2}\sigma_a^2 + \psi^{(1)}(\gamma) + \pi^2/6}$$

(after Ducrocq and Casella, 1996), where $\psi^{(1)}(\gamma)$ is the variance of log-frailty associated with the random pen group effects, $\psi^{(1)}(.)$ is a trigamma function, and $\pi^2/6$ is the error variance of an extreme value distribution. Other definitions of heritability for Weibull distributed traits have also been proposed (e.g. Korsgaard *et al.*, 1999; Yazdi *et al.*, 2000).

To illustrate the additive genetic variation for resistance to clinical and subclinical diseases in the nucleus breeding population, predicted survival functions were plotted for resistance to any clinical or subclinical disease. Specifically, a predicted survival function of pigs expected from mating a sire and dam with high resistance to any clinical or subclinical disease was plotted alongside a predicted survival function of pigs expected from mating a sire and dam with low resistance. The survival functions were obtained using solutions to model (1) fitted to any clinical or subclinical disease. The sires and dams were chosen after ranking them by predicted breeding values. The sires and dams with high resistance were chosen from the 10th percentile, while the sires and dams with low resistance were chosen from the 90th percentile. Two assumptions were made. First, the pigs were assumed to be from the same breed, and performance tested under identical environmental conditions (i.e. fixed, regression, and random effects were the same, with the exception of the predicted breeding values of the sires and dams). Second, the risk of a diagnosed disease was assumed to be high (i.e. there were always diagnosed pigs in the same stall and/or pen group).

Correlation among predicted breeding values. Genetic correlations among the resistances to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea, and the other diseases were approximated as product-moment correlations among the predicted breeding values (i.e. solutions to model (1)) of sires with more than 40 offspring. Sires with more than 40 offspring were chosen for two reasons. First, product-moment correlations of the same sign and similar magnitude were obtained when correlating the predicted breeding values of all sires and dams, and when correlating the predicted breeding values of sires with more than 10, 20, and 30 offspring. Second, product-moment correlations obtained using sires with more than 40 offspring were considered more reliable approximations of the genetic correlations than the product-moment correlations using sires (and dams) with less than 40 offspring.

Breed and environmental effects. The levels of the breed and environmental effects are presented for each disease category as their relative effects on the hazard. Specifically, the effects of the Duroc, Yorkshire, Landrace, and Hampshire breeds on the hazard are presented relative to the Duroc breed as $\exp\{\varpi_k - \varpi_{\text{Duroc}}\}$, where ϖ_k is the effect of the Duroc, Yorkshire, Landrace, or Hampshire breeds, and ϖ_{Duroc} is the effect of the Duroc breed. The effect of treatment level is presented as $\exp\{\varphi_{\text{treated}} - \varphi_{\text{untreated}}\}$, where φ_{treated} and $\varphi_{\text{untreated}}$ are the treatment and non-treatment effects. Differences among the breed and treatment level effects were tested for significance by χ^2-test. That is, the difference between the *u*th and *v*th breeds or treatment levels was tested by fitting model (1) with the *u*th breed or treatment level effect constrained to zero. The difference was significant ($P < 0{\cdot}05$) when $\chi_v^2 > 3{\cdot}8$, where χ_v^2 is the χ^2-value of the *v*th breed or treatment level effect with 1 d.f.

For the breeding farm and stall group effects, a range is presented by ranking the effects and calculating $\exp\{\alpha_{\text{high}} - \alpha_{\text{low}}\}$, where α_{high} is the mean of the highest 25th percentile of effects and α_{low} is mean of the lowest 25th percentile. The effect of the number of diagnosed pigs on the hazard was presented by plotting the function $f(\eta) = \exp\{\eta\beta_1 + \eta^2\beta_2\}$ against η, where η is the number of diagnosed pigs, and β_1 and β_2 are the regression coefficients associated with η and η^2.

Results

Incidence of disease

A total of 3 256 (24·0%) of the 13 551 pigs were diagnosed with at least one clinical or subclinical

Table 3 *Number of growing pigs (from a total of 13 551) that were diagnosed for five categories of clinical and subclinical diseases, the average number of days taken for those pigs that were diagnosed for each category of disease to be diagnosed, and estimates of variance components. The disease categories are any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases. The variance components are additive genetic variance ($\sigma_a^2 \pm$ standard error) and pen group variance ($\psi^{(1)}(\gamma)$) for log-frailty. A heritabilty (h^2_{log}) for resistance to each disease category is presented for the time until diagnosis on the logarithmic-time scale*

	No. diagnosed	Days to first diagnosis	σ_a^2	$\psi^{(1)}(\gamma)$	h^2_{log}
Any disease	3256	39	0·18 ± 0·05	0·01	0·10
Lameness	1351	46	0·29 ± 0·11	0·04	0·16
Respiratory	697	55	0·24 ± 0·16	0·21	0·12
Diarrhoea	428	28	0·30 ± 0·27	0·10	0·16
Other	1174	33	0·34 ± 0·15	0·00	0·19

disease during the performance test (Table 3). In turn, 10·0% of the pigs were diagnosed with lameness, 5·1% with respiratory diseases, 3·2% with diarrhoea, and 8·7% with the other diseases. Of those pigs that were diagnosed, the average time of first diagnosis with any clinical or subclinical disease was day 39 of the performance test, and between days 28 and 55 for lameness, respiratory diseases, diarrhoea and the other diseases. Only 376 pigs were diagnosed with more than one disease category (i.e. 357 pigs were diagnosed with two disease categories, while 19 were diagnosed with three).

Kaplan-Meier estimate of the survival function

The daily rate (i.e. proportion) of pigs first diagnosed was highest between days 7 and 18 (approx.) of the performance test (Figure 3). For each disease category, the daily rate of pigs first diagnosed between days 7 and 18 was 1·5 to 2·5 times higher than the rate between days 1 and 6, and 1·5 to 5·0 times higher than the rate after day 18. However, there were differences among the disease categories during the latter stages of the performance test. For any clinical or subclinical disease (after day 57), lameness (day 73), and respiratory diseases (day 45), the daily rate of pigs first diagnosed increased again to levels that approximated the rate between days 7 and 18. By comparison, there was a only a small increase in the rate for the other diseases after day 65 and there was no increase for diarrhoea during the latter stages of the performance test.

Genetic variation

Additive genetic variation for log-frailty was detected for resistance to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and the other diseases (Table 3). Further, the additive genetic variance and heritability for resistance to any clinical or subclinical disease (0·18 and 0·10) were lower than the additive genetic variances and heritabilities for resistance to lameness, respiratory diseases, diarrhoea and the other diseases (0·24 to 0·34, 0·12 to 0·19).

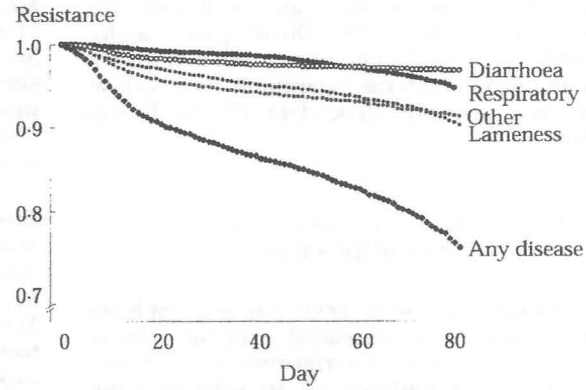

Figure 3 Kaplan-Meier estimate of the survival function for first diagnosis of growing pigs with any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases during performance test. The survival functions represent resistance of the pigs (i.e. proportion not diagnosed on each day of the performance test).

As an illustration of the additive genetic variation, there was a large difference in the predicted survival functions of pigs expected from mating sires and dams with high and low resistance to any clinical or subclinical disease (Figure 4). Approximately half of the pigs from the sire and dam with high resistance were predicted to be diagnosed with any clinical or subclinical disease during the performance test. On the other hand, approximately 70% of the pigs from the sire and dam with low resistance were predicted to be diagnosed.

Correlation among predicted breeding values

There were generally favourable correlations among the predicted breeding values for resistance to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and the other diseases (Table 4). Correlations between the predicted breeding values for any clinical or subclinical disease and the predicted breeding values for lameness, respiratory diseases, diarrhoea and the other diseases were

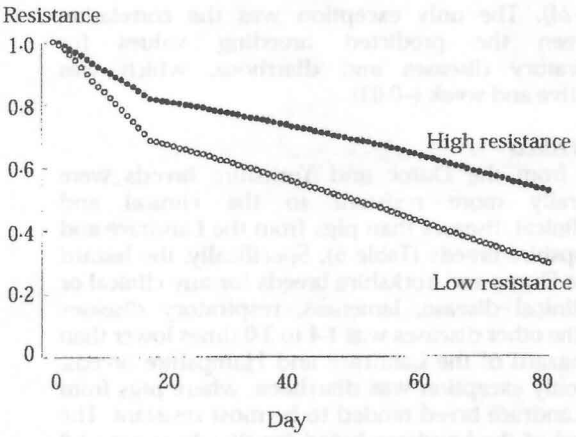

Figure 4 Predicted survival function of growing pigs expected from mating a sire and dam with high resistance to any clinical or subclinical disease plotted alongside the predicted function of pigs expected from mating a sire and dam with low resistance. The survival functions represent resistance of the pigs (i.e. proportion not diagnosed on each day of the performance test).

Table 4 *Product-moment correlations among predicted breeding values for resistance of growing pigs to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases*

	Lameness	Respiratory	Diarrhoea	Other
Any disease	0·65	0·52	0·32	0·59
Lameness		0·24	0·08	0·04
Respiratory			−0·03	0·12
Diarrhoea				0·05

positive and moderately large (0·32 to 0·65). By contrast, correlations among the predicted breeding values for lameness, respiratory diseases, diarrhoea, and the other diseases were positive but weak (0·04 to 0·24). The only exception was the correlation between the predicted breeding values for respiratory diseases and diarrhoea, which was negative and weak (–0·03).

Breed effects

Pigs from the Duroc and Yorkshire breeds were generally more resistant to the clinical and subclinical diseases than pigs from the Landrace and Hampshire breeds (Table 5). Specifically, the hazard of the Duroc and Yorkshire breeds for any clinical or subclinical disease, lameness, respiratory diseases and the other diseases was 1·4 to 3·0 times lower than the hazard of the Landrace and Hampshire breeds. The only exception was diarrhoea, where pigs from the Landrace breed tended to be most resistant. The hazard of the Landrace breed for diarrhoea was 1·8 and 2·2 times lower than the hazard of the Yorkshire and Hampshire breeds. However, the hazard of the Duroc breed was not significantly higher than that of the Landrace breed and the hazards of the Duroc, Yorkshire, and Hampshire breeds were not significantly different.

Environmental effects

There was a large range among the breeding farm and stall group effects. For the breeding farm effects, there was a 1·6-fold difference between the hazard of the highest and lowest 25th percentile means for any clinical or subclinical disease. A larger difference was found for lameness (2·0-fold difference), diarrhoea (3·4) and the other diseases (2·3). Similarly, for the stall group effects, there was a 3·7-fold difference between the hazard of the highest and lowest 25th percentile means for any clinical or subclinical disease and a larger difference for lameness (4·1), respiratory diseases (11·4), diarrhoea (11·2), and the other diseases (6·8).

The hazard for respiratory diseases and diarrhoea was 1·6 and 7·0 times lower ($P < 0·05$) when the pigs

Table 5 *Effect of Duroc, Yorkshire, Landrace and Hampshire breeds on the hazard for time until diagnosis of growing pigs with five categories of clinical and subclinical diseases during performance test. The disease categories are any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases. The effect of each breed on the hazard is presented relative to the Duroc breed*†

	Any disease	Lameness	Respiratory	Diarrhoea‡	Other
Duroc	1·00^{a}	1·00^{a}	1·00^{a}	1·00^{ab}	1·00^{a}
Yorkshire	1·05^{a}	0·77^{a}	1·15^{a}	1·25^{a}	1·17^{ab}
Landrace	1·52^{b}	1·37^{b}	2·07^{b}	0·63^{b}	2·16^{c}
Hampshire	1·92^{b}	1·57^{b}	2·99^{b}	1·44^{a}	1·66^{bc}

† Values in the same column with different superscripts are significantly different ($P < 0·05$).
‡ Differences between the Landrace and Yorkshire breeds ($P = 0·06$), and the Landrace and Hampshire breeds ($P = 0·08$), were approaching significance for diarrhoea.

were preventively treated or treated for another disease category.

The hazard for any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and the other diseases increased with the number of diagnosed pigs in the stall and/or pen groups (Figure 5). However, there were two distinct differences among the disease categories. First, the effect of the number of diagnosed pigs on the hazard was largest for lameness, for which the hazard was increased 29-fold when six pigs in a pen were diagnosed with lameness. Diagnosed pigs increased the hazard by up to 11-fold for respiratory diseases and by up to between five- and six-fold for any clinical or subclinical disease, diarrhoea, and the other diseases. Second, there was a diminishing effect on the hazard for any clinical or subclinical disease and respiratory diseases as the number of diagnosed pigs in a stall increased, and the effect on the hazard reached a plateau (i.e. as the number of diagnosed pigs increased, the effect of an additional diagnosed pig on the hazard was lower than the effect of the previous diagnosed pig). A diminishing effect was not apparent for lameness, diarrhoea, and the other diseases. Instead, for these disease categories, there was an increasing effect on the hazard as the number of diagnosed pigs in a pen increased (i.e. as the number of diagnosed pigs increased, the effect of an additional diagnosed pig on the hazard was higher than the effect of the previous diagnosed pig).

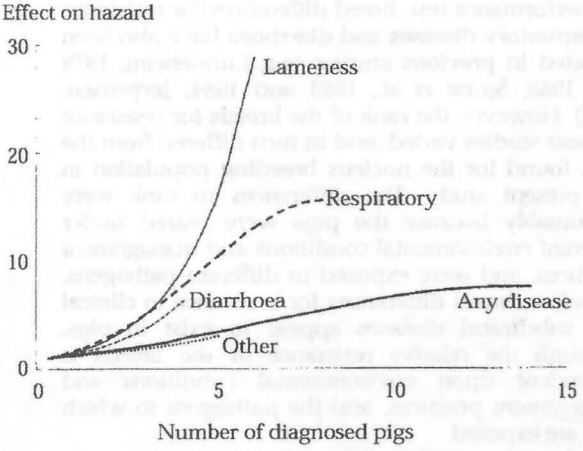

Figure 5 Effect of number of diagnosed pigs on the hazard for time until diagnosis of growing pigs with five categories of clinical and subclinical diseases during performance test. The disease categories are any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and other diseases. The effect of the number of diagnosed pigs on the hazard was estimated by $f(\eta) = \exp\{\eta\beta_1 + \eta^2\beta_2\}$, where η is the number of diagnosed pigs, and β_1 and β_2 are the regression coefficients associated with η and η^2.

The pen group variation for log-frailty was larger for respiratory diseases (0·21) and diarrhoea (0·10) than for any clinical or subclinical disease, lameness and the other diseases (0·00 to 0·04) (Table 3).

Discussion

This study established that additive genetic variation for resistance to clinical and subclinical diseases exists in growing pigs. Additive genetic variation was detected for resistance to any clinical or subclinical disease, lameness, respiratory diseases, diarrhoea and the other diseases and there were generally favourable correlations among the predicted breeding values for resistance to these disease categories. These results indicate that selection of pigs for resistance to clinical and subclinical diseases could be successful.

The additive genetic variation detected in the nucleus breeding population indicates that there were genes within the population that conveyed resistance to the pathogens encountered during the production test. However, the pathogens encountered, and the immunological mechanisms controlled by these genes to resist infection, remain unclear. All forms of micro-organisms (i.e. bacteria, virus, and protozoa) may have been encountered, and the mechanisms of resistance could have involved all aspects of the immune system (i.e. innate and/or acquired immunity). Despite this uncertainty, there was presumably greater variation in the pathogens encountered among disease categories than there was within the categories. For this reason, it was not surprising to find that the additive genetic variation and heritability for resistance to any clinical or subclinical disease were lower than the additive genetic variation and heritabilities for the resistances to lameness, respiratory diseases, diarrhoea and the other diseases.

Favourable and moderately-strong correlations were found between the predicted breeding values for resistance to any clinical or subclinical disease and the predicted breeding values for lameness, respiratory diseases, diarrhoea and the other diseases. These correlations existed because resistance to any clinical or subclinical disease was a composite of the resistances to lameness, respiratory diseases, diarrhoea and the other diseases and because of the generally favourable correlations among the predicted breeding values for lameness, respiratory diseases, diarrhoea and the other diseases. This result could have particular implications for selective breeding programmes with

pigs. In such programmes, selection may need only be placed on resistance to any clinical or subclinical disease for there to be simultaneous and favourable responses in the resistance of the pigs to lameness, respiratory diseases, diarrhoea and the other diseases.

The correlations among the predicted breeding values are approximations of the genetic correlations and should be interpreted accordingly for two reasons. First, strong assumptions were made when calculating these approximations, most notably independence among residual terms. This assumption was unlikely to hold, given that the same pigs were used to assess resistance to each disease category. Second, the predicted breeding values were estimated with uncertainty, suggesting that the correlations among the predicted breeding values underestimated the genetic correlations. Despite these drawbacks, while multivariate Weibull frailty models remain undeveloped (Ducrocq, 1999), *ad hoc* methods, such as correlations among predicted breeding values, remain the only alternative to approximate genetic correlations.

Assessing resistance to clinical and subclinical disease has the drawback that the outcome of infection can be caused by many different pathogens and much of the variation for resistance among pigs is due to environmental factors. A complementary approach to increase the reliability of resistance estimates could involve indirect, multitrait selection for phenotypes reflecting variation in the immunocompetence of the pigs (e.g. antibody and cell-mediated immune response). Such phenotypes would need to be well defined, accurately measured, heritable and highly correlated with the incidence of clinical and subclinical disease. As yet, no suitable phenotypes have been identified, although pigs performance tested at Bøgildgård are currently being tested to identify such phenotypes.

There are two areas that require consideration before resistance to clinical and subclinical diseases is included in breeding programmes for pigs. First, although there were generally favourable correlations among the predicted breeding values for each of the disease categories, it may be unrealistic to hope to achieve resistance to all forms of disease. Diseases differ in their aetiologies, each requiring a different mechanism of immunity on the part of the pigs to prevent infection and there is evidence to suggest that some of these mechanisms of immunity may be negatively intercorrelated (cf. Biozzi *et al.*, 1982). Second, during selection of pigs resistant to a pathogen, the pathogen is likely to evolve to survive in the pig (Nicholas, 1987). Increased resistance in the pathogen may offset at least some of the progress made in the resistance of the pigs. These two considerations are certain to make selective breeding for resistance challenging.

The Kaplan-Meier estimate of the survival function demonstrated that the daily rate of pigs first diagnosed for each disease category was highest between days 7 and 18 (approx.) of the performance test. This period followed transfer of the pigs from the acclimatization pens to the test facility, where pigs from different acclimatization pens were mixed together in the same pen group. Such a practice could have increased the risk of diagnosed disease in two ways. First, both transfer to a new environment and mixing of pigs are stressors that have a detrimental effect on the immunocompetence of pigs (Curtis and Backstrom, 1992). Second, the mixing of pigs could have increased the risk of infection by bringing together pigs from acclimatization pens where pathogens were encountered, and pigs from acclimatization pens where such pathogens were not present.

Pigs from Duroc and Yorkshire breeds were more resistant to the clinical and subclinical diseases than pigs from the Landrace and Hampshire breeds. The only exception was diarrhoea, where pigs from the Landrace breed tended to be most resistant. These findings suggest that there were genes specific to Duroc and Yorkshire breeds that provided greater resistance against the pathogens encountered during the performance test. Breed differences for resistance to respiratory diseases and diarrhoea have also been reported in previous studies (e.g. Lundeheim, 1979 and 1988; Straw *et al.*, 1983 and 1984; Jørgensen, 1992). However, the rank of the breeds for resistance in these studies varied, and in turn differed from the rank found for the nucleus breeding population in the present study. The differences in rank were presumably because the pigs were reared under different environmental conditions and management practices, and were exposed to different pathogens. Therefore, breed differences for resistance to clinical and subclinical diseases appear to exist in pigs, although the relative resistance of the breeds is dependent upon environmental conditions and management practices, and the pathogens to which they are exposed.

The most important environmental effects affecting the hazard for each disease category were the breeding farm (with the exception of respiratory diseases) and stall group effects, and the number of diagnosed pigs in the stall and/or pen groups. The breeding farm effects were important presumably because pigs from different breeding farms differed

in immune status (i.e. pigs bred on different breeding farms were exposed to different pathogens, causing variation for resistance to specific pathogens among the farms). The stall group effects and the number of diagnosed pigs were important as they represent exposure of the pigs to pathogens during the performance test.

The increase in hazard with the number of diagnosed pigs in the stall and/or pen groups is of particular interest for breeding programmes for resistance. It indicates that a successful breeding programme for resistance would not only reduce the number of diagnosed pigs at any time but simultaneously reduce the risk of susceptible pigs being diagnosed (cf. Knap and Bishop, 2000). In this way, breeding for resistance may have a dramatic impact on the incidence of clinical and subclinical diseases at the population level, whereby the population could carry a sizeable proportion of susceptible pigs without the risk of disease outbreak.

The additive genetic variation for resistance to clinical and subclinical diseases detected in this study demonstrates that selective breeding could be successful, providing a complementary approach to disease control in pig production. Pigs selectively bred for resistance may provide additional benefits by reducing the reliance on current methods of disease control, in particular, vaccines, medicines, and animal culling.

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S and I represent the proportion of the population susceptible and infectious, respectively. U represents the proportion dead and recovered, with the number dead dependent on the specific case fatality rates given in the data sets. The contacts of cases are divided into the following classes: E_n the number of untraced latent individuals in the population, E_i the number of traced latent contacts, and C_i the number of traced uninfected contacts. The final class of contacts are those untraced and uninfected and so effectively remaining in S. Q represents the proportion in quarantine and V the proportion protected by vaccination. The average rate at which latent individuals become infectious[13,21] is $\alpha = (\text{latency period})^{-1} = 0.0685\ \text{days}^{-1}$ and the rate at which infectious individuals in the community recover or die[22] is $\gamma = (\text{infectious period})^{-1} = 0.116\ \text{days}^{-1}$. Two states of quarantine are defined: the first for the traced contacts successfully vaccinated and released into the community at a rate χ_1, and the second for the infectious cases, which enter U at a rate χ_2. Different vaccine efficacies are assumed for those uninfected, ϵ_1, and infected, ϵ_2. The proportion of contacts found through contact tracing is ρ and the daily rate at which infectious individuals enter quarantine from the community is θ. The proportion of contacts infected is defined as φ. The rate at which potentially infected contacts occur is defined as β, as in equation (2), and N is the size of the population in which the epidemic occurs.

$$\beta = \frac{R_0\gamma}{\varphi N} \tag{2}$$

Additional assumptions are that no transmission occurs from those quarantined, dead or recovered and the background mortality rate was assumed to be negligible over the time periods examined.

For the Boston, Burford, Warrington and Chester data sets, $\rho = \theta = 0$, which effectively reduces equations (1) above to a simple SEIR model[9]. Intervention parameters were only required when equations (1) was fitted to the data from Kosovo. Here, interventions were implemented 31 days after the onset of symptoms in the index case[1] with the associated parameters shown in Table 2. The number of potentially infected contacts per case was determined as 50 (ref. 1). Values of R_0 were derived for each outbreak by minimizing the mean square error between the mortality data and the predictions of mortality from the model, while applying the outbreak-specific case fatality rates to U and adjusting R_0 and time of onset of symptoms in the index case. In the case of Kosovo, equations (1) were fitted more simply to the reported number of cases rather than deaths. All the other parameters required for equations (1) were obtained independently from the published source(s) given in Table 1. For epidemics in London, R_0 was roughly calculated from the interepidemic interval, $T = 2\pi[L(D + D')/(R_0 - 1)]^{1/2}$, where L is life expectancy between 1840 and 1870 adjusted for excess births over deaths, equal to 25 years, and $D + D'$ is latent + infectious period, equal to 0.063 years[9].

Estimation of current vaccination coverage

Given that smallpox vaccination ceased in industrialized countries in the mid to late 1970s (ref. 13), a crude estimate of the immunity of the contemporary UK population was calculated, on the basis of 50% having been vaccinated as infants up to 1972, and estimating that about 60% of these would be alive today from current population statistics. Of these only about 60% would still be protected by the vaccinations done on average 50 years previously, calculated by extrapolating from data on secondary attack rates, which increased from 4 to 12% over 10 years following vaccination[23]. This suggests that the level of herd immunity may be about 18%, which will continue to decrease with time.

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Competing interests statement

The authors declare that they have no competing financial interests.

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Imperfect vaccines and the evolution of pathogen virulence

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Vaccines rarely provide full protection from disease. Nevertheless, partially effective (imperfect) vaccines may be used to protect both individuals and whole populations[1–3]. We studied the potential impact of different types of imperfect vaccines on the evolution of pathogen virulence (induced host mortality) and the consequences for public health. Here we show that vaccines designed to reduce pathogen growth rate and/or toxicity diminish selection against virulent pathogens. The subsequent evolution leads to higher levels of intrinsic virulence and hence to more severe disease in unvaccinated individuals. This evolution can erode any population-wide benefits such that overall mortality rates are unaffected, or even increase, with the level of vaccination coverage. In contrast, infection-blocking vaccines induce no such effects, and can even select for lower virulence. These findings have policy implications for the development and use of vaccines that are not expected to provide full immunity, such as candidate vaccines for malaria[4].

Previous studies on the evolution of vaccine resistance have focused on the spread of 'escape' mutants that display epitopes different to those in the vaccine, thereby escaping immune recognition[5–7]—this has already happened for polio[8] and hepatitis B[9]. New vaccines may eventually get around this problem by, for example, targeting conserved epitopes or multiple epitopes simultaneously. Here we study an alternative counter-adaptation to vaccination involving pathogen life-history traits, namely virulence (induced host mortality) and transmission rate. To address this issue we incorporated standard evolutionary theory for virulence evolution[10–12] into an epidemiological framework[1].

We begin with an analysis of the evolution of parasite virulence

(the disease-induced mortality rate of the host) in a homogeneous host population. We do this by studying the ability of a rare mutant, with virulence denoted α^*, to invade a population of resident parasites with virulence α (the asterisk distinguishes the mutant's trait from the resident's). The evolutionarily stable (ES) pathogen virulence can be found by maximizing the mutant pathogen's $R_0[\alpha^*,\alpha]$ at $\alpha = \alpha^*$. (See equation (1) below.) When the host population is homogeneous and has reached epidemiological equilibrium (as denoted by the circumflex accent ('hat' symbol) throughout), the expression for the mutant's fitness is given by the expected number of secondary cases produced by a single host infected by this mutant over its entire infectious period[10–13]:

$$R_0[\alpha^*, \alpha] = \frac{\beta^*(\hat{x} + \sigma\hat{y})}{\delta + \alpha^* + \chi^* + \sigma\hat{h}} \tag{1}$$

where x and y are the densities of uninfected and infected hosts, respectively, β is the pathogen's transmission rate, $h = \beta y$ is the rate at which hosts acquire new infections (termed 'the force of infection'), χ is the pathogen's clearance rate (rate at which the host becomes non-infectious), δ is the host's natural mortality rate, and σ is the efficiency with which the pathogen invades an already infected host (superinfection) relative to invading an uninfected host[13,14]. The superinfection parameter, σ, can also be modelled as a function of virulence[13], but here, for simplicity, we assume it to be a constant. It is assumed that superinfecting parasites immediately replace the strain already present in the host: thus σh is the rate at which the pathogen is cleared from the host due to arrival of another strain. Note that $\hat{h}$ is determined by the resident pathogen strain. Thus, by setting the density of infected hosts to zero, we recover the classical definition of R_0, which allows us to tell whether the mutant pathogen can invade a fully susceptible host population[1].

Here we assume, as in classical models of the evolution of virulence[10–14], that the pathogen fitness function in equation (1) includes trade-offs involving pathogen virulence—that is, virulence has beneficial, pleiotropic effects on other pathogen life-history traits that offset the fitness cost of host death (which prematurely ends the infectious period). Two types of virulence benefits have been proposed. First, transmission rate is assumed to be an increasing function of pathogen virulence. Second, clearance rate is assumed to be slower with higher virulence. The net result of these negative and positive influences on pathogen fitness is that there is an intermediate optimum level of virulence that maximizes fitness. Although there are few data testing these assumed fitness relationships in pathogens, they are generally supportive[10,15,16]. The exact nature of these relationships will depend on the biology of each particular host–pathogen interaction, but here we define these trade-offs in simple forms by:

$$\begin{aligned} \beta &= \beta[\alpha] = b_1\alpha^{b_2} \\ \chi &= \chi[\alpha] = c_1\alpha^{-c_2} \end{aligned} \tag{2}$$

where the coefficients with subscripts are constants that determine the shape of the trade-offs, and hence the value of α that maximizes fitness.

The question now is how does host immunity (or 'resistance') change the optimum virulence relative to that in a completely non-immune ('susceptible') host population? Still assuming a homogeneous host population, we consider four different forms of immunity, with efficacies denoted r_1, r_2, r_3 and r_4, which independently affect different stages of the pathogen's life cycle (Fig. 1). The first is anti-infection immunity, which decreases the probability that a host becomes infected. The second is anti-growth-rate immunity, which directly reduces virulence and concomitantly affects transmission rate and host recovery. The third is transmission-blocking immunity, which only decreases parasite transmission. The fourth is anti-toxin immunity which directly reduces virulence but, contrary to anti-growth-rate immunity, does not affect parasite transmission and host recovery rates. This yields:

$$\begin{aligned} h' &= (1 - r_1)\beta' y' \\ \alpha' &= (1 - r_2)(1 - r_4)\alpha \\ \beta' &= (1 - r_3)\beta[(1 - r_2)\alpha] \\ \chi' &= \chi[(1 - r_2)\alpha] \end{aligned} \tag{3}$$

where the prime pertains to immune hosts. Assuming that only the trade-off between virulence and transmission is operating (clearance rate, χ, is a constant), yields the ES virulence:

$$\alpha^* = \frac{b_2(\delta + \chi + \sigma h(1 - r_1)(1 - r_3))}{(1 - b_2)(1 - r_2)(1 - r_4)} \tag{4}$$

Note that, throughout, virulence is measured as induced host mortality in susceptible (non-immune) hosts. Equation (4) implies that anti-growth-rate and anti-toxin immunity (modelled by r_2 and r_4) always select for higher virulence. This is because they reduce the risk of host death and hence selection against more virulent mutants. Indeed, evolution will restore the virulence observed in a uniform population of resistant hosts, as well as the force of infection, to that observed in a uniform population of susceptible hosts by increasing intrinsic virulence (that is, virulence as measured in susceptible hosts). Thus a pathogen following a strategy that would generate optimal virulence in a resistant host will induce a higher-than-optimal virulence in a susceptible host[17]. In contrast, anti-infection (r_1) and transmission-blocking (r_3) immunity select for lower virulence whenever there is superinfection, and leave it unchanged otherwise. They act indirectly on the evolution of parasite virulence via the force of infection through their effects on the rate at which an infection is prematurely ended by the arrival of a superinfecting pathogen[14,17]. Although equation (4) defines the ES virulence as a function of the force of infection, which is itself a function of virulence, the results discussed above can be rigorously proven using implicit differentiation analysis (not shown).

If we alternatively assume that there is only a trade-off between virulence and recovery rate, the ES virulence is:

$$\alpha^* = \frac{(c_1 c_2/(1 - r_4))^{\frac{1}{1+c_2}}}{1 - r_2} \tag{5}$$

In this case, anti-growth and anti-toxin immunity increase virulence, and the other two forms of immunity have no effect. When both trade-offs are included, the conclusions derived from equation

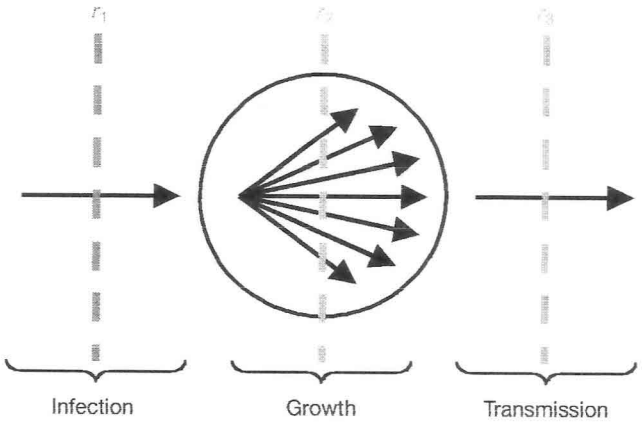

Figure 1 Schematic representation of the action of different types of host resistance at different stages of the pathogen's life cycle. r_1, anti-infection resistance; r_2, anti-growth-rate resistance; r_3, transmission-blocking resistance. A fourth type of resistance—anti-toxin resistance, r_4—is not shown because it only acts upon host death rates.

reduced transmission due to the direct effect of the vaccine and increased transmission through the evolution of higher virulence in vaccinated hosts. Anti-toxin vaccines may be even worse. Because these vaccines do not reduce transmission rate, increased vaccination coverage can increase pathogen prevalence above pre-vaccination levels.

The above evolutionary analysis assumes that hosts and pathogens are in population dynamic equilibrium. Extensive numerical simulations indicate that our model generates simple dynamics of rapid approaches to a stable point equilibrium. Many diseases, however, have more complex epidemiological dynamics, which may exhibit cycles or chaotic behaviour. Even if there is a point attractor, the transient dynamics following vaccination may be so pronounced or so long-lasting that an analysis based on equilibrium conditions may be irrelevant. In these situations, the selective pressures would vary both in space (from one host to another) and time, and the evolutionary analysis would need to take into account the effects of such variability on the invasion exponent of a mutant parasite[20].

Although a single epidemiological equilibrium exists, the evolutionary analysis of our model revealed that there could be different evolutionary outcomes depending on the initial conditions. Such evolutionary bistability emerges when the parasite can take an evolutionary route that leads to specialization on either susceptible or resistant hosts (leading to low or high virulence, respectively). However, our predictions regarding the effects of imperfect vaccines are not qualitatively altered by evolutionary bistability. This result has potentially important implications for the evolution of specialization[21,22] and for the evolution of multihost pathogens[23], but further exploration of this phenomenon falls outside the scope of the present Letter.

Can the above general theory for a virtual pathogen contribute to the rational design of vaccines against real pathogens such as malaria parasites? Current efforts to develop a malaria vaccine are focused on three different stages of the parasite's life cycle—the pre-erythrocytic stages (sporozoites and liver-stage parasites), asexual blood-stage parasites (merozoites and infected erythrocytes) and the mosquito-stage parasites (gametocytes, gametes, ookinetes)[4]. Immunity against these three stages corresponds to the anti-infection, anti-growth-rate and transmission-blocking forms of resistance studied here. Anti-toxin malaria vaccines are also being explored[24]. Using a modified form of the general model to incorporate two important features of malaria epidemiology—naturally acquired immunity and vector transmission (see Supplementary Information)—we evaluated the public health consequences of using various vaccines.

The model was parameterized using values typical of year-round endemic *Plasmodium falciparum* malaria in a high transmission area. Figure 3a–e shows that, as for the general model, the malaria model predicts that anti-growth-rate and anti-toxin vaccines select for higher virulence, while anti-infection vaccines select for lower parasite virulence. In the malaria model, however, transmission-blocking vaccines may favour slightly higher virulence (Fig. 3c). This vaccine reduces transmission and, consequently, the reproduc-

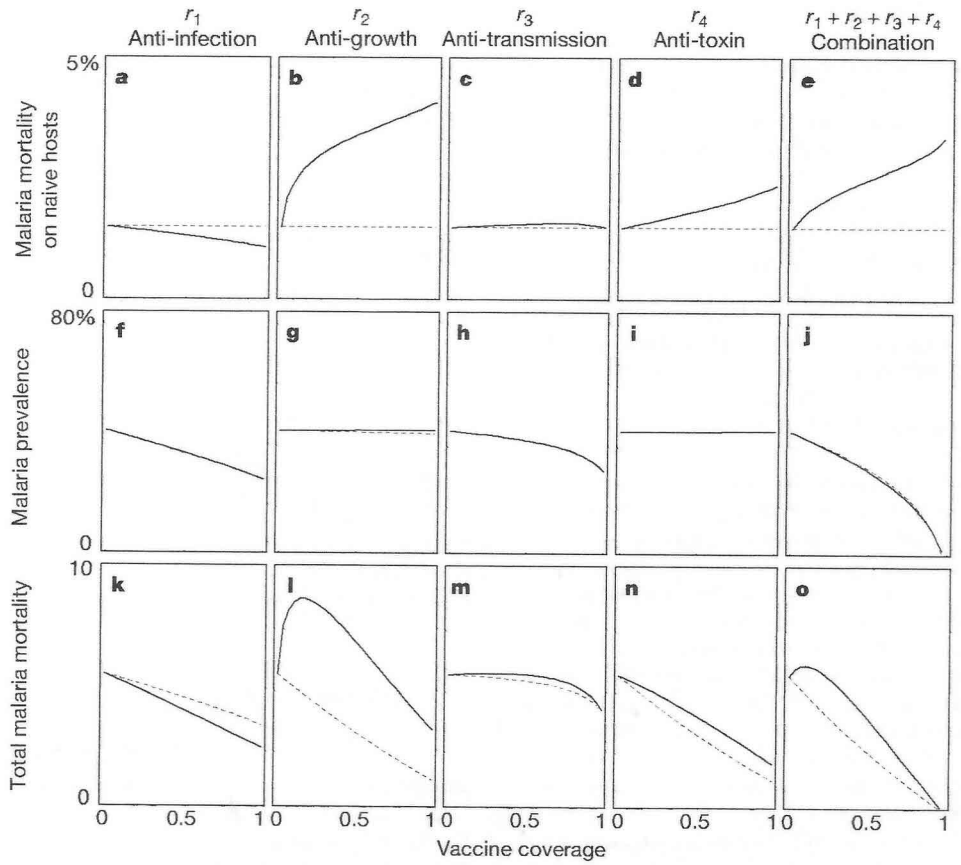

Figure 3 Predicted effects of anti-malaria vaccination coverage on virulence. **a–e**, The probability of dying due to malaria in naive hosts, $\alpha_N/(\alpha_N + \chi_N + \delta)$. **f–j**, the prevalence of malaria ($y_N + y_I + y_V$). **k–o**, The total (population-wide) disease-induced mortality (population average virulence weighted by prevalence of the different host types given in number of deaths per thousand per year). These results are for an example of endemic malaria; predictions are shown not allowing (dashed line) and allowing (full line) parasite evolution. We show the effects of single type vaccines and a combination vaccine under the assumption that the different types of vaccines have the same level of efficacy as that provided by natural immunity: $r_1 = \rho_1 = 0.8$, $r_2 = \rho_2 = 0.8$, $r_3 = \rho_3 = 0.8$, $r_4 = \rho_4 = 0.8$. See Supplementary Information for symbol definition and further details on the malaria model.

(4) do not qualitatively change.

What, then, if the pathogen faces a heterogeneous population of susceptible and resistant hosts, as would happen if a vaccination programme was implemented? On the one hand, we have shown that the consequences of vaccinating individual hosts is to select for higher levels of intrinsic virulence in the case of anti-growth-rate and anti-toxin vaccines. On the other hand, immunized hosts will transmit less, die less and recover more quickly than non-immune hosts, thus reducing the overall level of disease in the population. We now allow this epidemiology to feed back into the pathogen's virulence evolution, and vice versa, in order to determine the overall impact of vaccination programmes on the health of the population. The epidemiological model that we used was a modified version of the standard susceptible-infected model[1] with two classes of hosts—those that are fully susceptible to the pathogen, and those that are partially immune. In addition, we assume a continuous vaccination procedure which provides imperfect but life-long immunity. It is written as:

$$\begin{aligned} dx/dt &= (1-f)\lambda - (\delta + h)x + \chi y \\ dx'/dt &= f\lambda - (\delta + h')x' + \chi' y' \\ dy/dt &= hx - (\delta + \alpha + \chi)y \\ dy'/dt &= h'x' - (\delta + \alpha' + \chi')y' \end{aligned} \qquad (6)$$

where λ is a constant rate of flow (which covers both reproduction and immigration) of uninfected hosts into the population, among which a fraction f are resistant, that is, vaccinated, and the forces of infection on susceptible and resistant hosts become $h = \beta y + \beta' y'$ and $h' = (1 - r_1)h$, respectively. Note that there are no terms for superinfection in equation (6) as these cancel out. For simplicity, we assume that resistant hosts do not lose immunity to become susceptible, and, except by vaccination, susceptible hosts do not acquire immunity. This latter assumption is relaxed in our malaria example below.

As for the simple homogeneous case, the ES parasite virulence is found by maximizing at $\alpha = \alpha^*$:

$$R_0[\alpha^*, \alpha] = \frac{\beta^*(\hat{x} + \sigma\hat{y})}{\delta + \alpha^* + \chi^* + \sigma\hat{h}} + \frac{\beta'^*(1 - r_1)(\hat{x}' + \sigma\hat{y}')}{\delta + \alpha'^* + \chi'^* + \sigma\hat{h}'} \qquad (7)$$

which can be seen as a weighted average of the per-host transmission factors[18] on susceptible and resistant hosts (see Supplementary Information). Equations (6) and (7) can then be solved jointly to yield the ES virulence and population prevalence of disease once evolution has occurred.

A numerical example shows that as the efficacy of anti-growth-rate and anti-toxin vaccines increases, there is a marked increase in virulence (Fig. 2a). An exception occurs for very high efficacy anti-growth-rate vaccines, because the contribution to fitness from vaccinated individuals becomes very small (see Supplementary Information). ES virulence always increases with the efficacy of anti-toxin vaccines, because this type of vaccine removes the cost of virulence (increased mortality) without affecting its benefit (increased transmission). Consequently, the fitness contribution of vaccinated hosts always increases with the efficacy of an anti-toxin vaccine. In contrast, as the efficacy of anti-infection and anti-transmission vaccines increases, pathogens will evolve lower levels of virulence if superinfection occurs (Fig. 2a). This is because these vaccines reduce superinfection rates. When pathogens are less likely to be competitively excluded, the benefits of keeping the host alive are greater. The reduction in virulence is weaker for transmission-blocking vaccines than for infection-blocking vaccines because, with transmission-blocking vaccines, vaccinated hosts are fully susceptible to infection. Consequently, the force of infection is higher and this selects for higher virulence levels. When vaccines are fully effective ('perfect'), all except the anti-toxin vaccines share the same ES virulence. When superinfection occurs, this level falls below the virulence reached when vaccines are never used (or are totally ineffective). This is because, with a perfect vaccine, vaccinated hosts do not transmit the disease and, through the decrease of the force of infection, serve to indirectly favour lower virulence[17,19]. A perfect anti-toxin vaccine (a vaccine that completely removes the deleterious effects of the parasite on its host) does not decrease transmission and always selects for extreme intrinsic virulence (Fig. 2a).

In addition to virulence consequences, vaccination changes disease prevalence. Figure 2b shows that as vaccination coverage increases, anti-infection and anti-transmission vaccines always reduce disease prevalence when pathogen evolution occurs, and can sometimes eliminate the disease. Anti-growth-rate vaccines, on the other hand, have hardly any effect on prevalence because a balance between two forces that act in different directions—

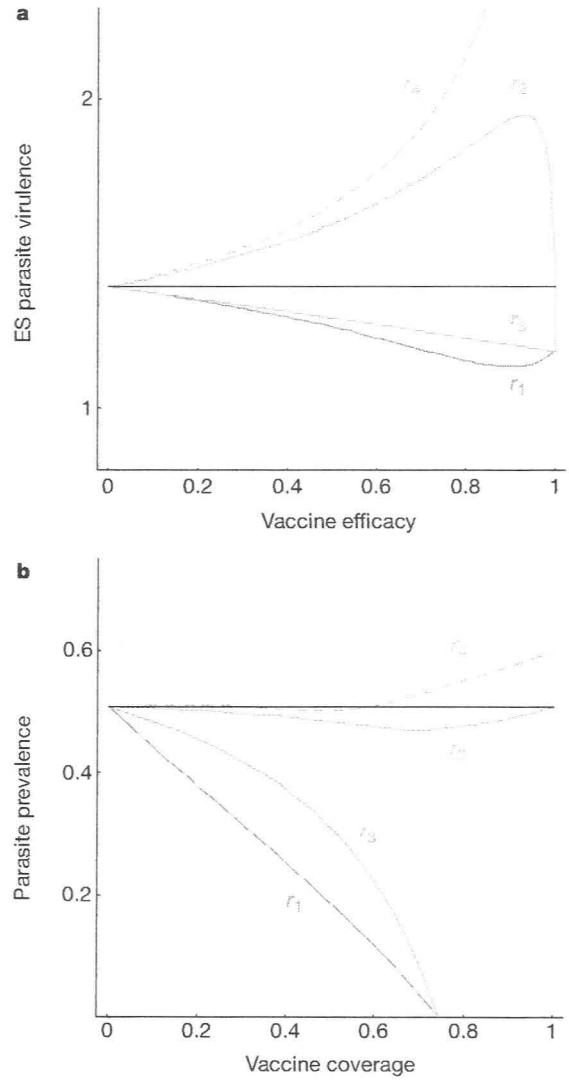

Figure 2 Evolutionary and epidemiological consequences of using different types of vaccines. The different coloured solid curves represent the first three types of vaccines (see Fig. 1), and the red dashed line is for anti-toxin vaccines. **a**, Evolutionarily stable (ES) parasite virulence (measured on susceptible hosts) plotted against the efficacy of the vaccine. **b**, Parasite prevalence (fraction of infected hosts) plotted against the proportion of vaccinated hosts. The horizontal black lines show the outcome in the absence of vaccination. In **a**, $b_1 = 0.5$ and $f = 0.2$ (see text for definitions of b_1 and f). In **b**, $b_1 = 0.2$ and efficacy (r_1, r_2, r_3 or r_4) is 0.9. Other parameter values (see text for definitions) are: $\lambda = 25$, $\delta = 1$, $\sigma = 1$, $b_2 = 0.2$, $c_1 = 0$ (that is, no relationship between recovery rate and virulence).

tive value of parasites infecting vaccinated hosts. In this case, evolution becomes mainly driven by the selective pressures occurring in naturally immune hosts. This explains the increase in virulence in spite of the indirect effect of superinfection acting in the opposite direction (equation (4)). We also examined the evolutionary consequences of a combination of different types of vaccines. The use of a vaccine combining the four different types also favours higher pathogen virulence despite the beneficial effect of anti-infection vaccines (Fig. 3e).

With or without evolution of the pathogen, vaccination is expected to affect the prevalence of malaria. As in the general model (Fig. 2b), the use of anti-infection and transmission-blocking vaccines reduces the force of infection and consequently malaria prevalence (Fig. 3f, h). In contrast, anti-growth-rate and anti-toxin vaccines have hardly any effect on prevalence (Fig. 3g, i). A combination vaccine, via the anti-infection and transmission-blocking effects, is the most efficient in reducing malaria prevalence, and could even lead to eradication for extreme vaccine coverage (Fig. 3j).

The total number of deaths due to malaria depends on both malaria virulence and on the prevalence of infection in the different types of hosts. Figure 3k–o presents the consequences of vaccination at the level of the whole host population. In the absence of pathogen evolution, not surprisingly, all the different types of vaccines decrease the total disease mortality. However, with anti-growth-rate, anti-toxin and transmission-blocking vaccines, evolution towards higher virulence (Fig. 3b–d) erodes the overall benefits of vaccination (Fig. 3l–n). In contrast, when anti-infection vaccines are used, evolution towards lower virulence (Fig. 3a) may increase the population-level benefits of vaccination (Fig. 3k). At high vaccination coverage, a vaccine that incorporates all four types of vaccines would be the most efficient, even when evolution occurs (Fig. 3o). This result supports the development of multivalent, multi-stage vaccines which, it is hoped, will provide greater overall protection than single-target vaccines[4]. Our finding that anti-infection vaccines may have favourable effects on virulence evolution also strongly supports the use of other partially effective control methods (for example, bed nets, mosquito control) to enhance the long-term benefits of vaccination.

How long might such virulence evolution take? Given that genetic variation exists for pathogen virulence[10,15,16,25,26], one might expect that, like the evolution of vaccine and drug resistance, the evolution of virulence would occur on timescales that are relevant to public health (decades or less). As an explicit example, we used the malaria model to track the spread of a virulence mutant through time following the start of a vaccination campaign. At 90% vaccine coverage with an anti-growth-rate vaccine of 80% efficacy, it took 38 years for a mutant more than twice as virulent to increase from 1% to 50%, after which it spread towards fixation very rapidly (see Supplementary Information). Numerical simulations, however, indicate that the speed of invasion is very sensitive to the shape of the trade-off function. Accurate predictions of both the invasion dynamics of a virulence mutant and of the evolutionary outcome require further investigation of the shape of these trade-off functions.

Purely epidemiological models have demonstrated that vaccines which are protective for individuals in clinical trials can nonetheless generate unwelcome consequences for a population as a whole[2,3]. Our incorporation of evolution into the analysis shows that clinically detrimental or beneficial evolution can also occur. The direction of virulence evolution depends critically on the type of vaccine, with several types promoting evolution that increases mortality risk to individual hosts. However, virulence management (an application of darwinian medicine[27,28]) requires specific models to answer questions regarding specific biological systems. Using malaria as an example, we found that the wide-scale use of even reasonably effective anti-growth-rate and transmission-blocking vaccines may ultimately do little to relieve community disease levels in malaria-endemic areas. Moreover, the evolution prompted by anti-growth-rate and anti-toxin vaccines can substantially increase the risk for non-immune individuals, such as unvaccinated children and non-immune travellers. The widespread use of such vaccines thus raises difficult ethical issues. Nevertheless, it is probable that anti-disease vaccines (anti-growth-rate and anti-toxin) will be used widely for their short-term beneficial effect at the individual level.

Like drug resistance, the clinically detrimental evolution that we are discussing here will occur on timescales longer than those of clinical trials. Marked increases in virulence of some viral diseases have already followed widespread use of anti-growth-rate vaccines in the chicken industry[29]. When human populations become uncontrolled experimental systems, we recommend that at the very least, intrinsic virulence of the pathogen population (or more realistically, putative virulence determinants such as *in vitro* multiplication rates[30]) be closely monitored. □

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Competing interests statement

The authors declare that they have no competing financial interests.

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Drosophila Toll is activated by Gram-positive bacteria through a circulating peptidoglycan recognition protein

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Microbial infection activates two distinct intracellular signalling cascades in the immune-responsive fat body of *Drosophila*[1,2]. Gram-positive bacteria and fungi predominantly induce the Toll signalling pathway, whereas Gram-negative bacteria activate the Imd pathway[3,4]. Loss-of-function mutants in either pathway reduce the resistance to corresponding infections[4,5]. Genetic screens have identified a range of genes involved in these intracellular signalling cascades[6–12], but how they are activated by microbial infection is largely unknown. Activation of the transmembrane receptor Toll requires a proteolytically cleaved form of an extracellular cytokine-like polypeptide, Spätzle[13], suggesting that Toll does not itself function as a *bona fide* recognition receptor of microbial patterns. This is in apparent contrast with the mammalian Toll-like receptors[14] and raises the question of which host molecules actually recognize microbial patterns to activate Toll through Spätzle. Here we present a mutation that blocks Toll activation by Gram-positive bacteria and significantly decreases resistance to this type of infection. The mutation *semmelweis* (*seml*) inactivates the gene encoding a peptidoglycan recognition protein (PGRP-SA). Interestingly, *seml* does not affect Toll activation by fungal infection, indicating the existence of a distinct recognition system for fungi to activate the Toll pathway.

To isolate new genes implicated in the *Drosophila* immune response, we screened the first chromosome for mutations that inactivate genes involved in the control of the challenge-induced expression of the antimicrobial peptides Diptericin and Drosomycin. *diptericin* expression is controlled by the Imd pathway, whereas induction of *drosomycin* is dependent on the Toll pathway[4,5]. From 2,500 independent lines, we isolated a mutant line in which the ability to express *drosomycin* in response to infection by Gram-positive bacteria (*Micrococcus luteus*, *Streptococcus faecalis*, *Bacillus thuringiensis*) was abolished or severely reduced (Fig. 1a and data not shown). We named this mutation *semmelweis* (*seml*) after Ignaz Philipp Semmelweis, a Hungarian physician who was a pioneer in the field of antiseptic treatments and discovered the cause of puerperal fever[15]. Expression of *diptericin* by Gram-negative bacteria (*Escherichia coli*, *Erwinia carotovora carotovora* and *Enterobacter cloacae*; Fig. 1b and data not shown) was unaffected in *seml* mutant flies but abolished in two mutants of the Imd pathway[7,8], *key* and *dredd* (Fig. 1b). These phenotypes of *seml* in response to challenges by various microorganisms are similar to those of loss-of-function mutants of the Toll pathway[5,6] (*spz*, *Dif*) but distinct from mutations affecting the Imd pathway.

In addition to its Toll-dependent induction by Gram-positive bacteria, *drosomycin* expression in the fat body is also activated by fungal infection in a Toll-mediated process[5]. When challenging *seml* flies with the entomopathogenic fungus *Beauveria bassiana*, we noted that *drosomycin* expression was wild type, in contrast to the results obtained with *spz* and *Dif* mutants, in which fungal induction of *drosomycin* was nearly abolished (Fig. 1c). *seml* is therefore the first described *Drosophila* mutation that specifically impairs the Toll-dependent induction of *drosomycin* by Gram-positive bacteria without affecting that induced by fungal infection.

We next analysed the resistance of *seml* mutants to infections by various microorganisms. We compared the data with those obtained with *spz* and *key* mutants. The results (Fig. 2) show that *seml* mutants are highly susceptible to Gram-positive infection (*Bacillus megaterium*, *S. faecalis*), but are as resistant as wild-type flies to fungal (*B. bassiana*) and Gram-negative bacterial infection (*E. coli*, *E. c. carotovora*). As expected, in these experiments *key* flies were susceptible only to Gram-negative infection[8], and *spz* mutants both to fungal and Gram-positive infections[5].

The similarities between the antibacterial responses in *seml*, *spz* and *Dif* mutants prompted us to study the epistatic relationship between *seml* and the Toll pathway components. We first tested whether *seml* was genetically upstream or downstream of *Toll*, using the $Toll^{10b}$ gain-of-function allele, which leads to a challenge-independent expression of *drosomycin*. The levels of *drosomycin* transcription in $Toll^{10b}$ and *seml*; $Toll^{10b}$ flies were similar (Fig. 3a), indicating that *seml* is genetically upstream of *Toll*. We used the same strategy to analyse the relationship between *seml* and the serine protease inhibitor *nec*. A loss-of-function mutation in the *nec* gene results in challenge-independent expression of *drosomycin* and in the formation of melanotic spots on the cuticle[13]. Both phenotypes are mediated by *spz* and *Toll*. Both *nec* and *seml*; *nec* flies express *drosomycin* in the absence of immune challenge and exhibit melanotic spots (Fig. 3b, c). These results suggest that the *seml* mutation inactivates a protein acting upstream of the Toll receptor and of the protease cascade necessary to activate its ligand Spz through proteolytic cleavage. In contrast to Toll-pathway mutants, homozygous *seml* females are fertile. Therefore *seml* does not seem

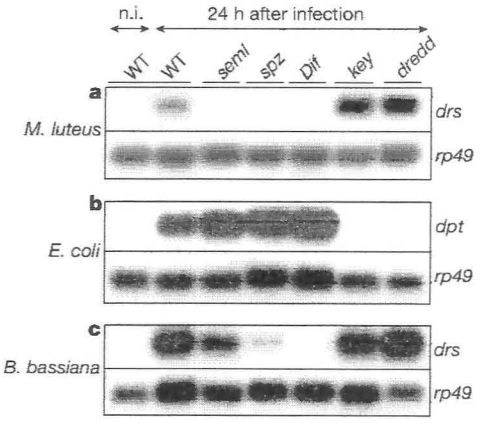

Figure 1 Expression of antimicrobial peptides in different mutant backgrounds after infection by fungi, Gram-positive or Gram-negative bacteria. Northern blots were performed with total RNA from wild type (WT) flies, *seml* mutant flies, or flies mutant for genes in the Toll signalling pathway (spz^{rm7}, Dif^{2}) and in the Imd pathway (key^{4}, $dredd^{D55}$). The flies were infected with *M. luteus* (**a**), *E. coli* (**b**) or *B. bassiana* (**c**) and incubated for 24 h at 25 °C before RNA preparation. *rp49* is used as an RNA loading control. n.i., not induced; *drs*, *drosomycin*; *dpt*, *diptericin*.

SUPPLEMENTARY INFORMATION FOR

"IMPERFECT VACCINES AND THE EVOLUTION OF PATHOGEN VIRULENCE"

PARASITE VIRULENCE EVOLUTION IN A HETEROGENEOUS HOST POPULATION

1. Derivation of evolutionarily stable virulence

The dynamics of a resident and a mutant parasite (the asterisk refers to the mutant strain) competing in a heterogeneous host population with two types of hosts (the prime refers to resistant hosts) is given by (see main text and Fig. 4 for details):

$$
\begin{aligned}
\mathrm{d}x/\mathrm{d}t &= (1-f)\lambda - \left(\delta + h + h^*\right)x + \chi y + \chi^* y^* \\
\mathrm{d}x'/\mathrm{d}t &= f\lambda - \left(\delta + h' + h'^*\right)x' + \chi' y' + \chi'^* y'^* \\
\mathrm{d}y/\mathrm{d}t &= h\left(x + \sigma\left(y + y^*\right)\right) - \left(\delta + \alpha + \chi + \sigma\left(h + h^*\right)\right)y \\
\mathrm{d}y'/\mathrm{d}t &= h'\left(x' + \sigma\left(y' + y'^*\right)\right) - \left(\delta + \alpha' + \chi' + \sigma\left(h' + h'^*\right)\right)y' \\
\mathrm{d}y^*/\mathrm{d}t &= h^*\left(x + \sigma\left(y + y^*\right)\right) - \left(\delta + \alpha^* + \chi^* + \sigma\left(h + h^*\right)\right)y^* \\
\mathrm{d}y'^*/\mathrm{d}t &= h'^*\left(x' + \sigma\left(y' + y'^*\right)\right) - \left(\delta + \alpha'^* + \chi'^* + \sigma\left(h' + h'^*\right)\right)y'^*
\end{aligned}
$$

with

$$\lambda = \delta + \alpha y + \alpha' y' + \alpha^* y^* + \alpha'^* y'^* .$$

The direction of evolution and, ultimately the evolutionarily stable (ES) virulence, can be derived from the analysis of the fate of a rare mutant. When the mutant is rare we can neglect its effect on the resident dynamics and focus on its own dynamics which, in matrix form, is given by[1]:

$$\begin{pmatrix} \mathrm{d}y^*/\mathrm{d}t \\ \mathrm{d}y'^*/\mathrm{d}t \end{pmatrix} = \mathbf{A}^* \begin{pmatrix} y^* \\ y'^* \end{pmatrix}$$

with

$$\mathbf{A}^* = \begin{pmatrix} \beta^* N - \left(\delta + \alpha^* + \chi^* + \sigma h\right) & \beta'^* N \\ \beta^* N' & \beta'^* N' - \left(\delta + \alpha'^* + \chi'^* + \sigma h'\right) \end{pmatrix}$$

where $N = x + \sigma y$ and $N' = (1 - r_1)(x' + \sigma y')$. There are several ways to find the ES parasite virulence from this model. One is to maximise ε, the dominant eigenvalue of $\mathbf{A}^*$, which is the standard measure of fitness for a rare mutant (the rate of invasion when the resident population has reached an equilibrium)[2]. It is known that, in populations at demographic equilibrium, correct results can also be obtained by maximising the basic reproductive ratio of the mutant strategy, given by equation 7 in the text[2].

2. Reproductive values and the strength of selection in different hosts

These maximisations must be carried out numerically. As both a check on the numerical calculations, and as a means of gaining insight into their results, a further approach is useful. Maximising the dominant eigenvalue of **A*** is equivalent to maximising the following function:

$$w[\alpha^*, \alpha] = \mathbf{v} \cdot \mathbf{A} \cdot \mathbf{u}$$

where $\mathbf{v}$ and $\mathbf{u}$ are the left and right eigenvectors of $\mathbf{A}$ (which is the same as **A***, but with the resident's parameters):

$$\mathbf{v} \propto \left[\frac{\beta}{\delta + \alpha + \chi} \quad \frac{\beta'}{\delta + \alpha' + \chi'} \right] \text{ and } \mathbf{u}^{\mathsf{T}} \propto \left[\frac{N}{\delta + \alpha + \chi} \quad \frac{N'}{\delta + \alpha' + \chi'} \right].$$

These two eigenvectors are, respectively, the individual reproductive values and the frequencies of the two classes of resident parasite infections (i.e. the infections of susceptible or resistant hosts). The product of these two quantities gives the class reproductive values[3] of the two types of parasites. This analysis of reproductive values is particularly insightful since it provides the appropriate fitness weights associated with the selective forces acting in the different types of hosts.

For instance, the non-monotonic effect of the efficacy, r_2, of the anti-growth rate vaccine on the ES virulence (Fig. 2a) can be explained with the help of class reproductive values of parasites

infecting different types of hosts. Note that the reproductive value of parasites in vaccinated hosts is proportional to $\beta[(1-r_2)\alpha]$. When r_2 is low, individual reproductive values are very similar and the direction of evolution at the scale of the whole host population (with both susceptible and vaccinated hosts) is mainly governed by the relative frequencies of the two classes of parasite infections. In this situation, since an increase in vaccine efficacy selects for higher virulence in vaccinated hosts (equations 4 and 5), this yields an increase in the ES virulence. However, when r_2 is high, the reproductive value of parasites infecting vaccinated hosts can be very low (when $r_2 \to 1$, $\beta' \to 0$). This means that, for very efficient vaccines, parasites infecting vaccinated hosts do not contribute to the future of the parasite population. Even though selection for increased virulence is very strong in those hosts, the selective pressures acting in susceptible hosts drive the evolution of the parasite. This explains the drop in ES virulence as $r_2 \to 1$.

MALARIA MODEL

Here we describe (1) the epidemiological model for malaria under vaccination, allowing for natural immunity and vector transmission, (2) the evolutionary dynamics of a virulence mutant, and (3) the derivation of the evolutionarily stable virulence.

1. Epidemiology

We assume there are three types of hosts - naïve, naturally immune and vaccinated, denoted by subscripts *N, I* and *V*, respectively - which are infected by a single (resident) strain of parasite. Hosts slowly acquire natural immunity through repeated infections and do not lose this immunity once acquired. This natural immunity comprises all four types and is imperfect, i.e. less than 100% effective. Vaccination with a single vaccine type is assumed to immediately confer the same level of immunity as natural immunity, but only for the type of immunity stimulated by the vaccine. We further assume that the dynamics of the infection processes of the vector happen on a relatively fast time scale, so that the vector dynamics are captured in the equilibrium fraction of infected mosquitoes[4,5]. Finally, we assume constant host population size yielding the following dynamical equations, with variables and parameters described below:

$$
\begin{aligned}
\mathrm{d}x_N/\mathrm{d}t &= \lambda(1-f) + \chi_N(1-\phi)y_N - (\delta + h_N)x_N \\
\mathrm{d}x_I/\mathrm{d}t &= \chi_I y_I + \phi\chi_N y_N + \phi\chi_V y_V - (\delta + h_I)x_I \\
\mathrm{d}x_V/\mathrm{d}t &= \lambda f + \chi_V(1-\phi)y_V - (\delta + h_V)x_V \\
\mathrm{d}y_N/\mathrm{d}t &= h_N x_N - (\delta + \alpha_N + \chi_N)y_N \\
\mathrm{d}y_I/\mathrm{d}t &= h_I x_I - (\delta + \alpha_I + \chi_I)y_I \\
\mathrm{d}y_V/\mathrm{d}t &= h_V x_V - (\delta + \alpha_V + \chi_V)y_V
\end{aligned}
$$

Note that, as in equation 6 in the main text, there are no superinfection terms because they cancel out.

1.1. Variables

x_N, x_I, x_V : proportions of uninfected hosts

y_N, y_I, y_V : proportions of infected hosts

$\alpha_N, \alpha_I, \alpha_V$: parasite virulence where $\alpha_I = (1-\rho_2)(1-\rho_4)\alpha_N$ and $\alpha_V = (1-r_2)(1-r_4)\alpha_N$

$\beta_N, \beta_I, \beta_V$: parasite transmission probabilities (infectivity from infected humans to uninfected mosquitoes) where $\beta_N = \beta[\alpha_N]$ (see below), $\beta_I = (1-\rho_3)\beta[\alpha_I]$ and $\beta_V = (1-r_3)\beta[\alpha_V]$

h_N, h_I, h_V : forces of infection where $h_N = abm\hat{z}$, $h_I = (1-\rho_1)h_N$ and $h_V = (1-r_1)h_N$

$\hat{z}$: equilibrium proportion of infectious mosquitoes, given by

$$\hat{z} = \frac{a(\beta_N y_N + \beta_I y_I + \beta_V y_V)}{\mu + a(\beta_N y_N + \beta_I y_I + \beta_V y_V)} e^{-\mu\tau}$$

λ : growth/immigration rate of the host population, given by

$$\lambda = \delta + \alpha_N y_N + \alpha_I y_I + \alpha_V y_V$$

1.2. Parameters (assumed values in parentheses based on endemic malaria in a high transmission area[6-10] with rates given on an annual basis)

$\rho_1, \rho_2, \rho_3, \rho_4$: resistance of naturally immune hosts (0.8)

r_1, r_2, r_3, r_4 : vaccine efficacy for different types of vaccines (0.8). We note that this is substantially higher than most current candidate vaccines.

χ_N, χ_I, χ_V : recovery rates where $\chi_N = \chi_V = 1$, $\chi_I = 7$.

f : vaccination coverage (0 to 1)

ϕ : fraction of the recovering individuals that become naturally immune (0.03)

δ : natural host death rate (0.02)

a : biting rate on humans by a single mosquito (120)

b : infectivity of infected mosquitoes (0.1)

m : number of female mosquitoes per human host (5)

μ : mortality rate of the mosquito (infected or not) (50)

τ : latent period in the mosquito (0.04)

σ : susceptibility to superinfection relative to an uninfected host (0.1). Competitive suppression can occur in malaria[11] and superinfection events have been directly observed in the field[12,13].

2. Invasion dynamics of a mutant

The invasion dynamics of a virulent mutant can be described by the following system of differential equations:

$$\begin{aligned}
dx_N/dt &= \lambda(1-f)+(1-\phi)\left(\chi_N y_N + \chi_N^* y_N^*\right)-\left(\delta + h_N + h_N^*\right)x_N \\
dx_I/dt &= \chi_I y_I + \chi_I^* y_I^* + \phi\left(\chi_N y_N + \chi_V y_V + \chi_N^* y_N^* + \chi_V^* y_V^*\right)-\left(\delta + h_I + h_I^*\right)x_I \\
dx_V/dt &= \lambda f + (1-\phi)\left(\chi_V y_V + \chi_V^* y_V^*\right)-\left(\delta + h_V + h_V^*\right)x_V
\end{aligned}$$

$$\begin{aligned}
dy_N/dt &= h_N\left(x_N + \sigma\left(y_N + y_N^*\right)\right)-\left(\delta + \alpha_N + \chi_N + \sigma\left(h_N + h_N^*\right)\right)y_N \\
dy_I/dt &= h_I\left(x_I + \sigma\left(y_I + y_I^*\right)\right)-\left(\delta + \alpha_I + \chi_I + \sigma\left(h_I + h_I^*\right)\right)y_I \\
dy_V/dt &= h_V\left(x_V + \sigma\left(y_V + y_V^*\right)\right)-\left(\delta + \alpha_V + \chi_V + \sigma\left(h_V + h_V^*\right)\right)y_V
\end{aligned}$$

$$
\begin{aligned}
\mathrm{d}y_N^*/\mathrm{d}t &= h_N^*\left(x_N + \sigma\left(y_N + y_N^*\right)\right) - \left(\delta + \alpha_N^* + \chi_N^* + \sigma\left(h_N + h_N^*\right)\right)y_N^* \\
\mathrm{d}y_I^*/\mathrm{d}t &= h_I^*\left(x_I + \sigma\left(y_I + y_I^*\right)\right) - \left(\delta + \alpha_I^* + \chi_I^* + \sigma\left(h_I + h_I^*\right)\right)y_I^* \\
\mathrm{d}y_V^*/\mathrm{d}t &= h_V^*\left(x_V + \sigma\left(y_V + y_V^*\right)\right) - \left(\delta + \alpha_V^* + \chi_V^* + \sigma\left(h_V + h_V^*\right)\right)y_V^*
\end{aligned}
$$

with

$$
\begin{aligned}
&\lambda = \delta + \alpha_N y_N + \alpha_I y_I + \alpha_V y_V + \alpha_N^* y_N^* + \alpha_I^* y_I^* + \alpha_V^* y_V^* \\
&h_N = abm\hat{z}, \quad h_I = (1-\rho_1)h_N, \quad h_V = (1-r_1)h_N \\
&h_N^* = abm\hat{z}^*, \quad h_I^* = (1-\rho_1)h_N^*, \quad h_V^* = (1-r_1)h_N^* \\
&\alpha_I = (1-\rho_2)(1-\rho_4)\alpha_N, \quad \alpha_V = (1-r_2)(1-r_4)\alpha_N \\
&\alpha_I^* = (1-\rho_2)(1-\rho_4)\alpha_N^*, \quad \alpha_V^* = (1-r_2)(1-r_4)\alpha_N^*
\end{aligned}
$$

As we assume that the dynamics of the infection processes of the vector happen on a relatively fast time scale, the equilibrium fractions of infected mosquitoes with the resident strain or with the mutant strain are:

$$
\begin{aligned}
\hat{z} &= \frac{a\left(\beta_N y_N + \beta_I y_I + \beta_V y_V\right)}{\mu + a\left(\beta_N y_N + \beta_I y_I + \beta_V y_V + \beta_N^* y_N^* + \beta_I^* y_I^* + \beta_V^* y_V^*\right)}e^{-\mu\tau} \\
\hat{z}^* &= \frac{a\left(\beta_N^* y_N^* + \beta_I^* y_I^* + \beta_V^* y_V^*\right)}{\mu + a\left(\beta_N y_N + \beta_I y_I + \beta_V y_V + \beta_N^* y_N^* + \beta_I^* y_I^* + \beta_V^* y_V^*\right)}e^{-\mu\tau}
\end{aligned}
$$

The above system of equations can be used to follow the invasion of the parasite population by a virulence mutant after the start of a vaccination campaign (Fig. 5).

3. Derivation of evolutionarily stable virulence

As in the simpler model presented earlier, the long-term evolutionary outcome of the parasite population (the evolutionarily stable virulence) can be derived from the analysis of the invasion of a rare mutant when the resident strategy settles at an epidemiological equilibrium. The

dynamics of a rare mutant strain appearing in a population of the resident parasite can be put in matrix form:

$$\begin{pmatrix} \mathrm{d}y_N^*/\mathrm{d}t \\ \mathrm{d}y_I^*/\mathrm{d}t \\ \mathrm{d}y_V^*/\mathrm{d}t \end{pmatrix} = A^* \begin{pmatrix} y_N^* \\ y_I^* \\ y_V^* \end{pmatrix}$$

with

$$A^* = \begin{pmatrix} c\beta_N^*\hat{N} - \left(\delta + \alpha_N^* + \chi_N^* + \sigma h_N\right) & c\beta_I^*\hat{N} & c\beta_V^*\hat{N} \\ c\beta_N^*\hat{I} & c\beta_I^*\hat{I} - \left(\delta + \alpha_I^* + \chi_I^* + \sigma h_I\right) & c\beta_V^*\hat{I} \\ c\beta_N^*\hat{V} & c\beta_I^*\hat{V} & c\beta_V^*\hat{V} - \left(\delta + \alpha_V^* + \chi_V^* + \sigma h_V\right) \end{pmatrix}$$

and

$$\hat{N} = \hat{x}_N + \sigma\,\hat{y}_N$$
$$\hat{I} = (1-\rho_1)(\hat{x}_I + \sigma\,\hat{y}_I)$$
$$\hat{V} = (1-r_1)(\hat{x}_V + \sigma\,\hat{y}_V)$$

where the hat indicates the equilibrium densities of resident hosts.

$h_N^* = abm\frac{a}{\mu}\left(\beta_N^* y_N^* + \beta_I^* y_I^* + \beta_V^* y_V^*\right)e^{-\mu\tau} = c\left(\beta_N^* y_N^* + \beta_I^* y_I^* + \beta_V^* y_V^*\right)$ is the force of infection of the mutant on naïve hosts (with $c = bma^2e^{-\mu\tau}/\mu$) and $h_I^* = (1-\rho_1)h_N^*$ and $h_V^* = (1-r_1)h_N^*$ are the forces of infection of the mutant on immune and vaccinated hosts. Note that we can neglect the terms in y^* in the denominator of $\hat{z}^*$ because the mutant is assumed to be rare.

Analysis proceeds as with the general model yielding the following expression for the fitness of the virulence mutant:

$$R_0\left[\alpha_N^*, \alpha_N\right] = \frac{\beta_N^*\hat{N}}{\delta + \alpha_N^* + \chi_N^* + \sigma\hat{h}_N} + \frac{\beta_I^*\hat{I}}{\delta + \alpha_I^* + \chi_I^* + \sigma\hat{h}_I} + \frac{\beta_V^*\hat{V}}{\delta + \alpha_V^* + \chi_V^* + \sigma\hat{h}_V}.$$

We present a numerical example (Fig. 3) with only a trade-off between virulence and transmission given by:

$$\beta_N = \beta[\alpha_N] = \frac{1}{1.2 + 5e^{-1000\,\alpha_N}}.$$

However, adding a trade-off between virulence and recovery does not qualitatively affect our conclusions. The choice of the particular shape of the trade-off that we used was based on the assumption that observed levels of malaria mortality in non-immune individual[5] in endemic areas[14,15] are at the parasite's ES virulence ($\alpha_N \approx 0.015$). There are, however, many different trade-off functions which may yield realistic values of the ES virulence.

Our model yields the following values for other relevant variables before vaccination: $h_N = 3.5$, $\beta_N = 0.83$, $\beta_I = 0.14$ and the fraction of infected hosts that die due to malaria as 1.4% and 0.04% in naïve and naturally immune hosts, respectively[14].

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Relationships Among Severity and Duration of Clinical Mastitis and Sire Transmitting Abilities for Somatic Cell Score, Udder Type Traits, Productive Life, and Protein Yield

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ABSTRACT

The objective of this study was to determine the relationships among severity and duration of clinical mastitis during first and second lactation and sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. Recording of clinical episodes began at first parturition for 1704 Holstein cows (in six Pennsylvania herds and one Nebraska herd) and continued into second lactation for 1055 of these cows. A total of 456 cows (sired by 168 bulls) had at least one clinical episode during first lactation, and 230 cows (sired by 100 bulls) had at least one clinical episode during second lactation. A severity code from 1 (normal milk) to 5 (acute systemic mastitis) was assigned daily (for up to 30 d after detection) to all quarters that had clinical mastitis. Only the severity codes for the first clinical episode to occur during first and second lactation are considered here. The initial and maximum severity codes, as well as the natural logarithms of both the sum of severity codes that were above normal (> 1) and the total days severity codes were above normal were regressed on herd (a classification variable), age at first calving, days in milk at clinical detection, and sire transmitting abilities taken one at a time. Linear and nonlinear effects were estimated for sire transmitting abilities. Separate analyses were conducted on dependent variables that considered severity and duration of clinical mastitis from: all organisms, coagulase-negative staphylococci, coliform species, streptococci other than *Streptococcus agalactiae*, and the most common environmental organisms (coliform species and streptococci other than *Streptococcus agalactiae*). Daughters of sires that transmit the lowest somatic cell score had the least severe and shortest clinical episodes from environmental organisms during first lactation. Selection for lower somatic cell score may reduce the severity and duration of clinical episodes from environmental organisms during first lactation.
(**Key words:** severity and duration of clinical mastitis, somatic cell score, productive life, udder type traits)

Abbreviation key: **CNS** = coagulase-negative staphylococci, **ISC** = initial severity code, **LOGDAYS** = natural logarithm of the total days severity codes were above normal in the 30 d after detection, **LOGSUM** = natural logarithm of the sum of severity codes that were above normal in the 30 d following detection, **MSC** = maximum severity code in the 30 d after detection, **PL** = productive life, **SNA** = streptococci other than *Streptococcus agalactiae*, **STA** = standardized transmitting abilities.

INTRODUCTION

Approximately 10% of the total value of milk sales made by US dairy farms (nearly $2 billion dollars) is lost to mastitis each year (National Mastitis Council, 1996). Although mastitis from contagious organisms (especially *Streptococcus agalactiae*) has been reduced by improvements in management, economic losses due to mastitis will continue because the causative organisms in the dairy cow's environment cannot be eradicated (National Mastitis Council, 1996). In addition, an unfavorable (positive) genetic correlation exists between mastitis and milk yield (Schmidt and Van Vleck, 1965; Emanuelson et al., 1988; Shook, 1993; Rogers et al., 1998), indicating that economic losses may increase. The increased susceptibility to mastitis accompanying selection for milk yield suggests that selection for resistance to mastitis is needed. Optimal selection for resistance to mastitis (under current economic conditions) would slow, rather than halt, the rate of increase in genetic susceptibility to mastitis (Strandberg and Shook, 1989; Rogers, 1993).

Direct selection for resistance to mastitis is not possible because records of clinical mastitis occurrence are not readily available for most US dairy cattle. Traits being considered for indirect selection for resistance to

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mastitis include SCS, udder type traits, and productive life (**PL**).

Approximately 80% of the cows in the national DHIA milk recording program, representing 40% of all US dairy cows, have milk SCC recorded once a month (Shook, 1993). The primary cause of elevated SCC is the presence of mastitis causing organisms in the udder (Harmon, 1994). Furthermore, higher SCS (a logarithmic transformation of SCC) is genetically associated with higher occurrence of clinical mastitis (Coffey et al., 1986; Emanuelson et al., 1988; Philipsson et al., 1995; Rogers et al., 1998; Nash et al., 2000). These findings indicate that selection for lower SCS may reduce the incidence of clinical mastitis. However, the impact of selection for lower SCS on the severity and duration of clinical episodes is unknown. Therefore, the objective of this study was to determine the relationships among severity and duration of daughter clinical mastitis during first and second lactation and sire transmitting abilities for SCS, udder type traits, PL, and protein yield.

MATERIALS AND METHODS

Data

Recording of clinical episodes began at first parturition for 1704 cows in seven herds (six in Pennsylvania, one in Nebraska) and continued into second lactation for 1055 of these cows in six of the seven herds. Clinical episodes were recorded from May, 1991, through December, 1995. Recording of clinical episodes began at first parturition because the health history of multiparous cows may be unknown, and multiparous daughters of a sire are not a random sample of daughters from that sire because it is likely that some have been culled.

Herdsmen collected milk samples from all quarters that had clinical mastitis. Quarter samples were collected when clinical mastitis was first observed. All quarter samples were frozen and transported weekly to diagnostic laboratories (one in Pennsylvania and one in Nebraska) for culturing following procedures described previously (Wanner et al., 1998).

Herdsmen also assigned a severity code daily to all quarters that had clinical mastitis. Daily assignment of a severity code began when clinical mastitis was first detected and continued until the milk and cow returned to normal or 30 d had elapsed. If a quarter returned to normal and subsequently became abnormal within 30 d after clinical mastitis was first detected, daily assignment of a severity code resumed.

Research technicians taught herdsmen the techniques to use to avoid contamination when collecting milk samples. They also taught herdsmen how to visually identify and classify clinical mastitis in order to avoid inconsistent assignment of severity codes across herds. In addition, research technicians made weekly visits to the Pennsylvania herds to ensure that the study protocol was being followed.

One of five severity codes could be assigned by herdsmen. A severity code of 1 indicated normal milk and quarter, which allowed recording that a previously clinical quarter had returned to normal. A severity code of 2 indicated a normal quarter with questionably normal milk. A severity code of 3 indicated abnormal milk (definite clots or flakes) but little or no swelling of the quarter (subacute clinical mastitis). A severity code of 4 indicated abnormal milk and a swollen quarter (acute clinical mastitis). A severity code of 5 indicated abnormal milk, swollen quarter, and a physically ill cow (acute systemic mastitis). A cow was considered to have clinical mastitis if a severity code of 2 or higher was recorded.

Four measures of the severity and duration of clinical mastitis were developed from these severity codes. The initial severity code (**ISC**) and the maximum severity code in the 30 d following detection (**MSC**) were two of the measures. The third measure was the natural logarithm of the sum of severity codes that were above normal (>1) in the 30 d following detection (**LOGSUM**). The fourth measure was the natural logarithm of the total days severity codes were above normal in the 30 d following detection (**LOGDAYS**). The logarithmic transformation was used to normalize the last two measures.

Only the severity and duration of the first clinical episode to occur during first and second lactation are considered here. To be considered, the first clinical episode of a lactation had to occur before 365 DIM had elapsed.

Analyses

The four measures of severity and duration of clinical mastitis (ISC, MSC, LOGSUM, and LOGDAYS) were regressed on herd (a classification variable), age at first calving, DIM at clinical detection, and sire transmitting abilities for SCS, udder type traits, PL, and protein yield taken one at a time. Linear and quadratic effects were estimated for each of the transmitting abilities using the GLM procedure of SAS (SAS Inst., Inc., 1995). Cubic effects were also estimated for PTA for SCS.

First and second lactation data were analyzed separately. Furthermore, separate analyses were conducted on dependent variables that considered severity and duration of clinical mastitis from: all organisms, coagulase-negative staphylococci (**CNS**), coliform species, streptococci other than *Streptococcus agalactiae* (**SNA**), and the most common environmental organisms (coli-

form species and SNA). Clinical mastitis from all organisms contained all initial clinical episodes during a lactation, including those that had missing or contaminated quarter samples or no detectable organism growth. Organism (CNS, coliform species, SNA, *Staphylococcus aureus*, other organisms, no growth, missing quarter sample, or contaminated quarter sample) was included as an explanatory variable in the regression model when the dependent variables considered severity and duration of clinical mastitis from all organisms. Severity and duration of clinical mastitis from CNS during second lactation were not analyzed separately because its incidence was too low.

When a cow had clinical mastitis in multiple quarters on the same day, each quarter that had clinical mastitis was considered a separate episode. Each quarter that had mastitis on the same day was considered a clinical episode because mastitis causing organisms entered each quarter separately. If a cow had clinical mastitis from a particular organism in multiple quarters on the same day, the quarter that had the highest sum of severity codes that were above normal was considered to be the first clinical episode of the lactation. If a cow had clinical mastitis from different organisms in multiple quarters on the same day, each quarter infected with a different organism was considered to be the first clinical episode of the lactation from that organism. This was done only when the severity and duration of clinical mastitis from different organism groups were analyzed separately.

Two organisms were detected in 32 quarters (7% of those that had the first clinical episode) during first lactation and 15 quarters (7%) during second lactation. These quarters were considered to have a clinical episode from each organism when the severity and duration of clinical mastitis from different organism groups were analyzed separately.

When the dependent variables were regressed on STA for udder type traits, data from 11 first-lactation cows and four second-lactation cows were excluded because STA for udder type traits were not available for their sires. When the dependent variables were regressed on STA for teat length, data from 12 first-lactation cows and five second-lactation cows were excluded because STA for teat length were not available for their sires.

RESULTS AND DISCUSSION

Data

A total of 456 of 1704 cows (27%) had at least one clinical episode during first lactation. In addition, 230 of 1055 cows (22%) had at least one clinical episode during second lactation. Eighty-four of these cows had clinical mastitis during first lactation. The relationships among clinical mastitis incidence during first and second lactation in the cooperating herds and sire transmitting abilities for SCS, udder type traits, PL, and protein yield are presented elsewhere (Nash et al., 2000).

Five of the cooperating herds were commercial herds (all in Pennsylvania) and two were university research herds. The total number of first- and second-lactation cows contributed by each herd, the number that had clinical mastitis, and the frequency of mastitis by lactation in each herd are in Table 1. Herd 5 had the lowest incidence of clinical mastitis during first and second lactation. Herd 5 was the only herd to routinely give all heifers an intramammary antibiotic infusion in each quarter 30 d before the expected calving date. All herds routinely postdipped and administered dry cow therapy.

Table 1 also contains the number of cows in each herd that had clinical mastitis caused by the most prevalent organism groups. Although there was considerable variation in clinical mastitis incidence between herds, the proportion of clinical episodes caused by each organism group was similar.

The efficacy of therapies used to treat the initial clinical episodes in five of the seven herds (all in Pennsylvania) has been studied (Sischo et al., 1995). For this study, therapies were categorized as follows: no antibiotics used, treatment with intramammary beta-lactams, treatment with intramammary ceftiofur, and miscellaneous therapy. Intramammary ceftiofur was the most commonly used mastitis therapy (used in 39% of cases). No antibiotics were used in 14% of cases. Only one farm administered some form of antibiotic to all cows that had clinical mastitis. Mastitis therapy had no effect on the number of days until milk and quarter returned to normal (severity code = 1).

Mastitis was the second and fourth most common reason for culling cows during first and second lactation, respectively. However, fewer than 3% of first- and second-lactation cows were culled due to mastitis.

The measures of severity and duration of the first clinical episode during first and second lactation are summarized in Table 2 for all herds by organism group. Approximately 37, 39, 20, and 4% of the severity codes assigned to the initial clinical episode during first lactation were severity codes 2, 3, 4, and 5, respectively. Approximately 34, 41, 19, and 6% of the severity codes assigned to the initial clinical episode during second lactation were severity codes 2, 3, 4, and 5, respectively.

Table 2 also contains the number of cows that had clinical mastitis caused by the most prevalent organism groups. Most of the initial clinical episodes during first or second lactation were caused by SNA or coliform

Table 1. Total first- and second-lactation cows per herd, the number that had clinical mastitis, and the frequency of mastitis in each herd.

	Herd							
	1	2	3	4	5	6	7	Totals
First lactation								
Total cows	170	49	136	226	753	211	159	1704
Cows that had mastitis	90	7	54	101	80	63	61	456
Frequency	0.53	0.14	0.40	0.45	0.11	0.30	0.38	0.27
Cows that had mastitis by organism group								
SNA[1]	29	2	20	26	25	18	25	145
Frequency	0.32	0.29	0.37	0.26	0.31	0.29	0.41	0.32
Coliform species	19	1	16	17	7	15	6	81
Frequency	0.21	0.14	0.30	0.17	0.09	0.24	0.10	0.18
CNS[2]	15	1	11	24	22	11	1	85
Frequency	0.17	0.14	0.20	0.24	0.28	0.17	0.02	0.19
Environmental organisms[3]	45	2	36	39	32	33	31	218
Frequency	0.50	0.29	0.67	0.39	0.40	0.52	0.51	0.48
Second lactation								
Total cows	110	0[4]	73	98	614	100	60	1055
Cows that had mastitis	43		23	45	75	28	16	230
Mastitis frequency	0.39		0.32	0.46	0.12	0.28	0.27	0.22
Cows that had mastitis by organism group								
SNA[1]	9		10	18	28	6	5	76
Frequency	0.21		0.43	0.40	0.37	0.21	0.31	0.33
Coliform species	5		3	8	17	6	0	39
Frequency	0.12		0.13	0.18	0.23	0.21	0.00	0.17
CNS[2]	2		0	3	5	1	1	12
Frequency	0.05		0.00	0.7	0.07	0.04	0.06	0.05
Environmental organisms[3]	14		13	25	44	11	5	112
Frequency	0.33		0.57	0.56	0.59	0.39	0.31	0.49

[1]SNA = Streptococci other than *Streptococcus agalactiae*.

[2]CNS = Coagulase-negative staphylococci.

[3]Coliform species and streptococci other than *Streptococcus agalactiae*.

[4]Herd 2 was sold before clinical episodes during second lactation could be recorded.

species (*Escherichia coli* and *Klebsiella* species). Few of the initial clinical episodes were caused by contagious organisms. *Staphylococcus aureus* was detected in the first clinical episode experienced by 4 and 6% of the cows that had clinical mastitis during first and second lactation, respectively. *Streptococcus agalactiae* was detected in the first clinical episode experienced by approximately 1% of the cows that had clinical mastitis during first or second lactation.

No organism growth was detected in the milk samples collected from the first clinical episode experienced by 19% of the cows that had clinical mastitis during first or second lactation. Milk samples were missing (not collected) for the first clinical episode experienced by 10 and 13% of the cows that had clinical mastitis during first and second lactation, respectively. Milk samples were classified as contaminated for the first clinical episode experienced by 6 and 3% of the cows that had clinical mastitis during first and second lactations, respectively. Severity codes were recorded for cows with missing or contaminated quarter samples. Therefore, data from these cows were used in the analyses. Nash (1999) concluded that the incidence of clinical mastitis during first and second lactations and the types and relative proportions of organisms detected in quarters that had clinical mastitis were similar to those in other studies.

The first clinical episode usually occurred early in each lactation. The mean DIM at detection was 65 d for the first clinical episode during first lactation. The first clinical episode was detected within 1 mo after calving for 250 of the 456 cows (55%) that had clinical mastitis during first lactation. One hundred and seventy-seven of these cows had their first clinical episode within 1 wk after calving. During second lactation, the mean DIM at detection was 89 d for the first clinical episode. The first clinical episode was detected within 3 mo after calving for 134 of the 230 cows (58%) that had clinical mastitis during second lactation. Eighty-four of these cows had their first clinical episode within 1 mo after calving.

Mean length of lactation was 283 and 264 d for cows that had clinical mastitis during first and second lactations, respectively. Approximately 78% of the cows that had clinical mastitis during first lactation and 85% of the cows that had clinical mastitis during second lactation were milked fewer than 365 d. Mean length of lactation was 301 d for first-lactation cows and 273

Table 2. Measures of severity and duration of the first clinical mastitis episode during first and second lactations by organism group.

Severity and duration measure by organism group	First lactation					Second lactation				
	Cows	Mean	SD	Minimum	Maximum	Cows	Mean	SD	Minimum	Maximum
ISC[1]										
All organisms	456	3.14	0.76	2	5	230	3.33	0.79	2	5
SNA[2]	145	3.23	0.71	2	5	76	3.20	0.73	2	5
Coliform species	81	3.40	0.75	2	5	39	3.72	0.79	2	5
CNS[3]	85	2.96	0.63	2	5	12	3.33	0.65	2	4
Environmental organisms[4]	218	3.27	0.72	2	5	112	3.39	0.79	2	5
MSC[1]										
All organisms		3.24	0.79	2	5		3.42	0.80	2	5
SNA[2]		3.34	0.75	2	5		3.34	0.70	2	5
Coliform species		3.51	0.79	2	5		3.77	0.81	2	5
CNS[3]		3.04	0.70	2	5		3.33	0.65	2	4
Environmental organisms[4]		3.39	0.76	2	5		3.50	0.76	2	5
Sum of severity codes that were above normal										
All organisms		17.75	18.84	2	128		19.30	18.72	2	117
SNA[2]		22.08	24.02	2	128		19.45	15.37	2	90
Coliform species		17.52	15.78	2	79		24.41	18.16	5	82
CNS[3]		10.99	10.25	2	58		11.33	6.79	2	30
Environmental organisms[4]		20.65	21.84	2	128		20.88	16.19	2	90
LOGSUM[1]										
All organisms		2.45	0.93	0.69	4.85		2.58	0.89	0.69	4.76
SNA[2]		2.68	0.91	0.69	4.85		2.70	0.76	0.69	4.50
Coliform species		2.53	0.82	0.69	4.37		2.92	0.77	1.61	4.41
CNS[3]		2.08	0.80	0.69	4.06		2.26	0.65	0.69	3.40
Environmental organisms[4]		2.63	0.89	0.69	4.85		2.77	0.77	0.69	4.50
Total days severity codes were above normal										
All organisms		6.11	5.70	1	30		6.50	5.51	1	30
SNA[2]		7.49	6.92	1	30		7.01	5.00	1	30
Coliform species		5.70	4.72	1	30		7.85	5.24	2	25
CNS[3]		4.08	3.76	1	27		4.00	2.22	1	10
Environmental organisms[4]		6.92	6.36	1	30		7.19	4.97	1	30
LOGDAYS[1]										
All organisms		1.47	0.83	0	3.40		1.56	0.80	0	3.40
SNA[2]		1.67	0.83	0	3.40		1.73	0.69	0	3.40
Coliform species		1.47	0.73	0	3.40		1.84	0.69	0.69	3.22
CNS[3]		1.13	0.73	0	3.30		1.25	0.55	0	2.30
Environmental organisms[4]		1.61	0.81	0	3.40		1.75	0.69	0	3.40

[1]ISC = Initial severity code, LOGDAYS = natural logarithm of the total days severity codes were above normal in the 30 d following detection, LOGSUM = natural logarithm of the sum of severity codes that were above normal in the 30 d following detection, MSC = maximum severity code in the 30 d following detection.

[2]SNA = Streptococci other than *Streptococcus agalactiae*.

[3]CNS = Coagulase-negative staphylococci.

[4]Combines all cows that had mastitis from environmental organisms: coliform species and streptococci other than *Streptococcus agalactiae*.

d for second-lactation cows that did not have clinical mastitis. Approximately 76% of the cows that did not have clinical mastitis during first lactation and 86% of the cows that did not have clinical mastitis during second lactation were milked fewer than 365 d.

Actual or projected 305-d milk yield was available from DHIA records for 441 cows that had clinical mastitis during first lactation and 230 cows that had clinical mastitis during second lactation. Mean 305-d milk yield was 8074 kg for first lactation (range was 3215 to 11,629 kg) and 9357 kg for second lactation (range was 3669 to 13,023 kg). Actual or projected 305-d milk yield was available from DHIA records for 1223 first-lactation cows and 810 second-lactation cows that did not have clinical mastitis. Mean 305-d milk yield was 8575 kg for first lactation (range: 2257 to 12,351 kg) and 9958 kg for second lactation (range: 1194 to 15,246 kg).

Lactation average SCS was available from DHIA records for 286 cows (in five herds) that had clinical mastitis during first lactation and 125 cows (in four herds) that had clinical mastitis during second lactation. Mean lactation average SCS was 3.29 for first lactation (range was 0.30 to 8.50) and 3.39 for second lactation (range was 0.20 to 8.40). Lactation average SCS was available from DHIA records for 445 first-lactation cows (in five herds) and 223 second-lactation cows (in four herds) that did not have clinical mastitis. Mean lactation average SCS was 2.41 for first lactation (range was 0.00

Table 4. Predicted and standardized transmitting abilities for sires of Holstein cows that had at least one clinical mastitis episode during second lactation and sires that did not have any daughters that had clinical mastitis during second lactation.

Transmitting abilities	N	Mean	SD	Minimum	Maximum
Sires of cows that had clinical mastitis					
SCS (log_2)	100	3.24	0.204	2.76	3.72
Protein (kg)	100	7.11	9.14	−24.0	27.2
Productive life	100	0.369	1.24	−3.00	2.70
Fore udder attachment	96[1]	0.0116	1.13	−2.53	3.20
Rear udder height	96	0.438	1.04	−2.01	3.15
Rear udder width	96	0.370	0.98	−1.61	2.84
Udder cleft	96	0.188	1.10	−3.59	2.21
Udder depth	96	−0.0689	1.30	−4.16	2.85
Front teat placement	96	0.113	1.27	−3.15	3.38
Teat length	95[2]	0.0889	1.35	−3.30	4.31
Sires that did not have any daughters that had clinical mastitis					
SCS (log_2)	66	3.21	0.225	2.81	3.88
Protein (kg)	66	7.00	10.3	−33.1	29.9
Productive life	66	0.320	1.29	−3.80	3.20
Fore udder attachment	63[3]	−0.0598	1.27	−3.13	2.66
Rear udder height	63	0.0254	1.12	−2.65	2.66
Rear udder width	63	0.0717	1.08	−2.69	2.48
Udder cleft	63	0.0344	1.26	−2.83	3.54
Udder depth	63	−0.337	1.54	−4.36	2.93
Front teat placement	63	−0.0160	1.44	−3.29	3.40
Teat length	63	0.0956	1.44	−3.85	3.61

[1]The standardized transmitting abilities for udder type were not available for four sires.

[2]The standardized transmitting abilities for teat length were not available for five sires.

[3]The standardized transmitting abilities for udder type were not available for three sires.

cal episodes during first lactation (Table 5). In addition, the linear effect was not a significant predictor of ISC, MSC, LOGSUM, or LOGDAYS for clinical episodes during second lactation (Table 5).

The quadratic effect of PTA for SCS was a significant ($P \leq 0.10$) predictor of LOGSUM for clinical episodes from SNA during first lactation (Table 6). As shown in Figure 1, the quadratic regression line (includes linear and quadratic effects) was concave, with the peak occurring near the mean PTA for SCS. The cubic effect of PTA for SCS (regression coefficients and standard errors not shown) was a significant predictor of ISC for

Table 5. Linear regression of measures of severity and duration of the first clinical mastitis episode during first and second lactation on sire PTA for SCS by organism group.

Severity and duration measure[4]	Lactation	All organisms		CNS[1]		Coliform species		SNA[2]		Environmental organisms[3]	
		b-value[5]	SE	b-value	SE	b-value	SE	b-value	SE	b-value	SE
ISC	1	0.196	0.171	0.177	0.386	0.818†	0.417	0.447	0.330	0.502*	0.251
	2	−0.0707	0.264			0.639	0.713	−0.537	0.456	−0.208	0.396
MSC	1	0.139	0.179	−0.0759	0.427	0.995*	0.437	0.306	0.346	0.454†	0.266
	2	−0.00710	0.273			0.716	0.731	−0.285	0.475	0.00410	0.392
LOGSUM	1	−0.0306	0.192	−0.521	0.410	0.711	0.456	0.0306	0.383	0.131	0.288
	2	−0.329	0.261			−0.111	0.677	−0.468	0.460	−0.290	0.364
LOGDAYS	1	−0.0535	0.171	−0.568	0.371	0.451	0.401	−0.0365	0.351	0.0177	0.260
	2	−0.304	0.233			−0.265	0.592	−0.375	0.413	−0.249	0.324

[1]CNS = Coagulase-negative staphylococci.

[2]SNA = Streptococci other than *Streptococcus agalactiae*.

[3]Environmental organisms = coliform species and streptococci other than *Streptococcus agalactiae*.

[4]ISC = Initial severity code, LOGDAYS = natural logarithm of the total days severity codes were above normal in the 30 d after detection, LOGSUM = natural logarithm of the sum of severity codes that were above normal in the 30 d after detection, MSC = maximum severity code in the 30 d following detection.

[5]b-value = Regression coefficient.

†$P \leq 0.10$.

*$P \leq 0.05$.

Table 6. Quadratic regression of measures of severity and duration of the first clinical mastitis episode during first and second lactation on sire transmitting abilities by organism group.[1]

Transmitting abilities	Linear[2]	Quadratic[3]	Organism group[4]	Severity and duration measure[5]	Lactation
SCS	17.3†	–2.64†	SNA	LOGSUM	1
Productive life	0.0718	–0.0731†	Coliform species	LOGSUM	1
	0.0540	–0.0678*	Coliform species	LOGDAYS	1
Udder depth	–0.0272	–0.0497*	Coliform species	LOGDAYS	1
Front teat placement	0.0729†	–0.0459†	All organisms	LOGSUM	2
Teat length	–0.0996*	0.0438*	SNA	ISC	1
	–0.101*	0.0449*	SNA	MSC	1

[1]Results shown for only those sire transmitting abilities that were significant ($P \leq 0.10$) predictors. The quadratic effect of each transmitting ability was tested for significance the same number of times (36) as presented in Table 5 for PTA for SCS.

[2]Linear = Regression coefficient for the linear effect.

[3]Quadratic = Regression coefficient for the quadratic effect.

[4]CNS = Coagulase-negative staphylococci, environmental organisms = coliform species and streptococci other than *Streptococcus agalactiae*, SNA = Streptococci other than *Streptococcus agalactiae*.

[5]ISC = Initial severity code, LOGDAYS = natural logarithm of the total days severity codes were above normal in the 30 d after detection, LOGSUM = natural logarithm of the sum of severity codes that were above normal in the 30 d after detection, MSC = maximum severity code in the 30 d after detection.

†$P \leq 0.10$.

*$P \leq 0.05$.

Table 7. Linear regression of measures of severity and duration of the first clinical mastitis episode during first and second lactations on sire transmitting abilities by organism group.[1]

Transmitting abilities	b-value[2]	SE	Organism group[3]	Severity and duration measure[4]	Lactation
Protein	–0.0320†	0.0179	Coliform species	LOGDAYS	2
Fore udder attachment	–0.105*	0.0486	All organisms	ISC	2
	–0.225†	0.133	Coliform species	ISC	2
	0.0999†	0.0512	SNA	MSC	1
	–0.122*	0.0499	All organisms	MSC	2
	–0.128†	0.0763	Environmental organisms	MSC	2
	0.100†	0.0574	CNS	LOGDAYS	1
Udder depth	0.0989*	0.0404	SNA	MSC	1
	–0.0721†	0.0425	All organisms	MSC	2
	0.104†	0.0581	CNS	LOGSUM	1
	0.102†	0.0526	CNS	LOGDAYS	1
Front teat placement	0.0984*	0.0482	SNA	MSC	1
	0.143†	0.0718	Coliform species	LOGSUM	1
	0.0862*	0.0420	Environmental organisms	LOGSUM	1
	0.128*	0.0621	Coliform species	LOGDAYS	1
	0.0705†	0.0381	Environmental organisms	LOGDAYS	1
Teat length	–0.145†	0.0803	Coliform species	MSC	1

[1]Results shown for only those sire transmitting abilities that were significant ($P \leq 0.10$) predictors. Transmitting abilities for traits other than SCS were each tested for significance the same number of times (36) as presented in Table 5 for PTA for SCS.

[2]b-value = Regression coefficient.

[3]CNS = Coagulase-negative staphylococci, environmental organisms = coliform species and streptococci other than *Streptococcus agalactiae*, SNA = Streptococci other than *Streptococcus agalactiae*.

[4]ISC = Initial severity code, LOGDAYS = natural logarithm of the total days severity codes were above normal in the 30 d following detection, LOGSUM = natural logarithm of the sum of severity codes that were above normal in the 30 d following detection, MSC = maximum severity code in the 30 d following detection.

†$P \leq 0.10$.

*$P \leq 0.05$.

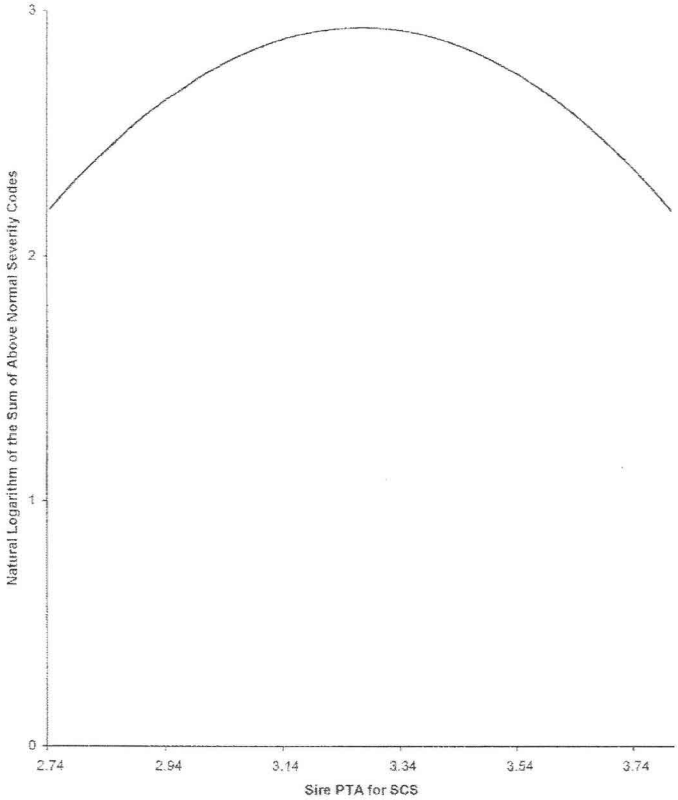

Figure 1. Quadratic regression of the natural logarithm of the sum of severity codes that were above normal (> 1) in the 30 d after detection of the first clinical episode caused by streptococci other than *Streptococcus agalactiae* during first lactation on sire PTA for SCS.

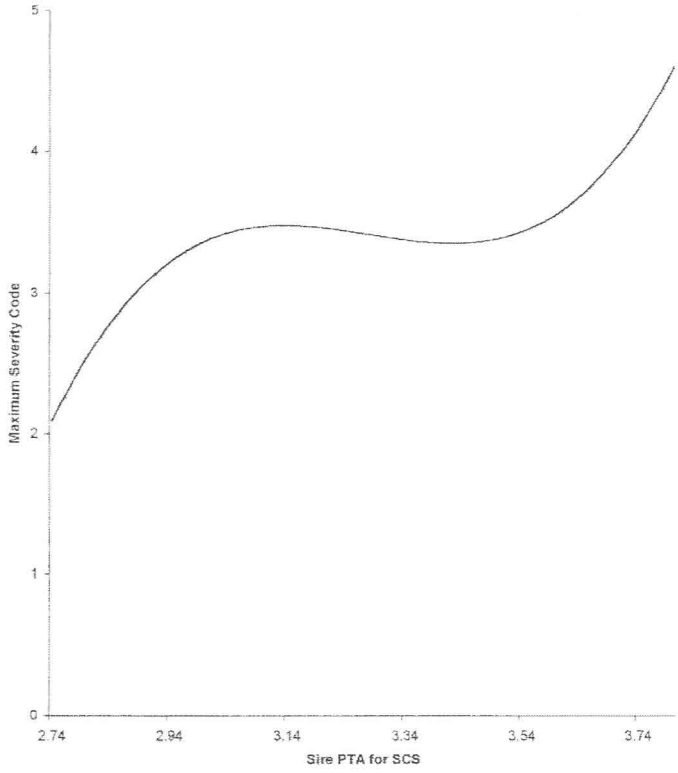

Figure 2. Cubic regression of the maximum severity code in the 30 d after detection of the first clinical episode caused by the most common environmental organisms during first lactation on sire PTA for SCS.

clinical episodes from all organisms, CNS, and the most common environmental organisms during first lactation. In addition, the cubic effect was a significant predictor of MSC for clinical episodes from all organisms, SNA, and the most common environmental organisms during first lactation. The cubic effect of PTA for SCS was also a significant predictor of LOGSUM for clinical episodes from SNA and the most common environmental organisms during first lactation. Finally, the cubic effect of PTA for SCS was a significant predictor of LOGDAYS for clinical episodes from the most common environmental organisms during first lactation. Figure 2 presents the cubic regression line for MSC for clinical episodes from the most common environmental organisms during first lactation. As shown in Figure 2, the cubic regression line was sigmoid, with the nadir occurring at the lowest PTA for SCS. The cubic regression lines for all other measures of severity and duration were similarly shaped. The shape of the quadratic and cubic regression lines indicates that daughters of sires that transmit the lowest SCS had the lowest ISC, MSC, LOGSUM, and LOGDAYS for clinical episodes during first lactation.

These findings do not support the theory that selection for the lowest SCS will result in dairy cattle that are unable to respond to infection. If such were the case, the lowest SCS would be associated with more severe, longer lasting clinical episodes, and an intermediate SCS would provide optimal resistance to mastitis. This theory stems from the results of experimental challenge studies that indicated that elevated SCC before infusion protects against infection by mastitis-causing organisms (Kehrli and Shuster, 1994; Schukken et al., 1994).

Results of studies that examined the association between the occurrence of mastitis and SCS also refute the theory that selection for the lowest SCS will result in dairy cattle that are unable to respond to infection. Philipsson et al. (1995) regressed genetic evaluations for the occurrence of clinical mastitis on SCS evaluations and found no evidence of a nonlinear effect. Rogers et al. (1998) detected a quadratic effect, which indicated that sires with the lowest genetic evaluations for SCS also had the most favorable evaluations for the occurrence of clinical mastitis. Research conducted on a population that included the cows used in the current study

concluded that daughters of sires that transmit the lowest SCS had the least IMI at first parturition and the lowest number of clinical episodes per lactation (Nash, 1999; Nash et al., 2000).

Elevated SCC due to mastitis from environmental organisms may not be detected by monthly SCC measurement (the current practice in the US) because mastitis from these organisms is generally of shorter duration than mastitis from contagious organisms (National Mastitis Council, 1996). Therefore, it has been hypothesized that selection for lower SCS may not improve resistance to mastitis from environmental organisms (Shook, 1993). In the current study though, daughters of sires that transmit higher SCS had higher ISC, MSC, LOGSUM, and LOGDAYS for clinical episodes from environmental organisms during first lactation. These results indicate that selection for lower SCS may lessen the severity and shorten the duration of clinical mastitis caused by environmental organisms during first lactation. Research conducted on a population which included some of the cows used in the current study suggests that selection for lower SCS may also reduce the incidence of both IMI at first parturition and clinical mastitis caused by environmental organisms (Nash, 1999; Nash et al., 2000).

The effect of selection for lower SCS on the severity and duration of clinical mastitis from other organisms (including contagious) could not be predicted because clinical episodes caused by these organisms were not prevalent in this study. Furthermore, the severity and duration of clinical mastitis from environmental organisms (as measured here) may have been more variable than the severity and duration of clinical mastitis from other organisms. If this were the case, the severity and duration of clinical mastitis from environmental organisms may have been more likely to be associated with sire transmitting abilities for SCS.

It is hypothesized that daughters of sires that transmit higher SCS may have more severe, longer lasting clinical episodes from environmental organisms because their immune systems are dysfunctional and therefore incapable of preventing mastitis-causing organisms from surviving or multiplying. Daughters of sires that transmit higher SCS may also have more severe, longer lasting clinical episodes from environmental organisms because their udder conformation increases exposure to mastitis causing organisms or fails to limit the number that gain entry to the mammary gland.

STA for udder type traits. The linear effect of the STA for fore udder attachment was a significant ($P \leq 0.10$) predictor of ISC for clinical episodes from all organisms and coliform species during second lactation (Table 7). The significant regression coefficients were negative, indicating that daughters of sires that transmit strongly attached fore udders had lower ISC for clinical episodes from all organisms and coliform species during second lactation.

The linear effect of the STA for fore udder attachment was also a significant predictor of MSC for clinical episodes from SNA during first lactation, all organisms during second lactation, and the most common environmental organisms during second lactation (Table 7). The significant regression coefficients were positive when MSC for clinical episodes during first lactation were considered, and negative when MSC for clinical episodes during second lactation were considered. These results indicate that daughters of sires that transmit strongly attached fore udders had higher MSC for clinical episodes from SNA during first lactation and lower MSC for clinical episodes from all organisms and the most common environmental organisms during second lactation.

The linear effect of the STA for fore udder attachment was also a significant predictor of LOGDAYS for clinical episodes from CNS during first lactation (Table 7). The regression coefficient was positive, indicating that daughters of sires that transmit strongly attached fore udders had higher LOGDAYS for clinical episodes from CNS during first lactation.

The linear effect of the STA for udder depth was a significant predictor of MSC for clinical episodes from SNA during first lactation and all organisms during second lactation (Table 7). The significant regression coefficients were positive when MSC for clinical episodes during first lactation were considered, and negative when MSC for clinical episodes during second lactation were considered. These results indicate that daughters of sires that transmit shallower udders had higher MSC for clinical episodes from SNA during first lactation and lower MSC for clinical episodes from all organisms during second lactation.

The linear effect of the STA for udder depth was also a significant predictor of LOGSUM and LOGDAYS for clinical episodes from CNS during first lactation (Table 7). The significant regression coefficients were positive, indicating that daughters of sires that transmit shallower udders had higher LOGSUM and LOGDAYS for clinical episodes from CNS during first lactation.

The quadratic effect of the STA for udder depth was a significant predictor of LOGDAYS for clinical episodes from coliform species during first lactation (Table 6). The regression coefficient was negative, indicating that LOGDAYS for clinical episodes from coliform species decreased at an increasing rate during first lactation among daughters of sires that transmit shallower udders.

The linear effect of the STA for front teat placement was a significant predictor of MSC for clinical episodes from SNA during first lactation (Table 7). The regression coefficient was positive, indicating that daughters of sires that transmit closely spaced front teats had higher MSC for clinical episodes from SNA during first lactation.

The linear effect of the STA for front teat placement was also a significant predictor of LOGSUM and LOGDAYS for clinical episodes from coliform species and the most common environmental organisms during first lactation (Table 7). In addition, the quadratic effect of the STA for front teat placement was a significant predictor of LOGSUM for clinical episodes from all organisms during second lactation (Table 6). The significant regression coefficients for the linear effect were positive, indicating that daughters of sires that transmit closely spaced front teats had higher LOGSUM and LOGDAYS for clinical episodes from coliform species and the most common environmental organisms during first lactation. The quadratic regression line indicated that LOGSUM for clinical episodes during second lactation increased at a decreasing rate among daughters of sires that transmit closely spaced front teats.

The linear effect of the STA for teat length was a significant predictor of MSC for clinical episodes from coliform species during first lactation (Table 7). In addition, the quadratic effect of the STA for teat length was a significant predictor of ISC and MSC for clinical episodes from SNA during first lactation (Table 6). The regression coefficient for the linear effect was negative, indicating that daughters of sires that transmit longer teats had lower MSC for clinical episodes from coliform species during first lactation. The quadratic regression lines indicated that ISC and MSC for clinical episodes from SNA during first lactation increased at an increasing rate among daughters of sires that transmit shorter teats.

In general, variations in the severity and duration of daughter clinical mastitis were not consistently associated with variation in sire transmitting abilities for udder type traits. However, strongly attached fore udders, shallower udders, closely spaced front teats, and shorter teats have been associated with lower SCS and reduced incidence of clinical mastitis (Seykora and McDaniel, 1986; Schutz et al., 1993; Lund et al., 1994; Rogers et al., 1998; Nash et al., 2000). These findings indicate that variation in udder conformation is associated with variation in exposure to mastitis causing organisms and their entry into the mammary gland, rather than variation in severity and duration of clinical episodes.

PTA for PL. The linear effect of PTA for PL was not a significant ($P > 0.10$) predictor of ISC, MSC, LOGSUM, or LOGDAYS for clinical episodes during first or second lactation. However, the quadratic effect of PTA for PL was a significant predictor of LOGSUM and LOGDAYS for clinical episodes from coliform species during first lactation (Table 6). The significant regression coefficients for the quadratic effect were negative, indicating that LOGSUM and LOGDAYS for clinical episodes from coliform species decreased at a increasing rate during first lactation among daughters of sires that transmit longer PL. These results indicate that daughters of sires that transmit long PL had lower LOGSUM and LOGDAYS for clinical episodes from coliform species during first lactation. Lower SCS, decreased clinical mastitis, and less IMI at first parturition have also been associated with longer PL (Weigel et al., 1997; Rogers et al., 1998; Nash, 1999; Nash et al., 2000).

PTA for protein yield. The linear effect of the PTA for protein was a significant ($P \leq 0.10$) predictor of LOGDAYS for clinical episodes from coliform species during second lactation (Table 7). The regression coefficient was negative, indicating that daughters of sires that transmit higher protein yield had lower LOGDAYS for clinical episodes from coliform species during second lactation. However, lower, not higher, yield has been associated with reduced incidence of clinical mastitis and lower SCS (Schmidt and Van Vleck, 1965; Wilton et al., 1972; Emanuelson et al., 1988; Weller et al., 1992; Rogers et al., 1998).

CONCLUSIONS

Daughters of sires that transmit higher SCS had more severe, longer lasting clinical episodes from environmental organisms during first lactation (only the first clinical episode during first and second lactations were considered in this study). In addition, the severity and duration of clinical episodes caused by environmental organisms during first lactation were nonlinearly associated with PTA for SCS. However, daughters of sires that transmit the lowest SCS had the least severe, shortest lasting clinical episodes from environmental organisms during first lactation. Therefore, selection for lower SCS may reduce the severity and duration of clinical episodes caused by environmental organisms during first lactation without diminishing the ability to respond to infection.

Variation in the severity and duration of daughter clinical episodes were not consistently associated with variation in sire transmitting abilities for udder type traits. However, daughters of sires that transmit longer productive life had less severe, shorter lasting clinical episodes from coliform species during first lactation. This indicates that selection for longer productive life

may reduce the severity and duration of clinical episodes from coliform species during first lactation.

The number of significant ($P \leq 0.10$) regressions of measures of severity and duration on sire transmitting abilities were low (e.g., 4 of 36 linear regressions on PTA for SCS were significant). Therefore, these results should be interpreted with caution.

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12

Escherichia coli and *Salmonella* Diarrhoea in Pigs

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Summary

Diarrhoea due to bacterial infection is a problem mainly in young growing animals, including pigs. Among the bacteria that cause diarrhoea are various strains of *Escherichia coli* and *Salmonella*. Considerable genetic variation in resistance and susceptibility has been found for both neonatal diarrhoea caused by *E. coli* carrying K88 fimbriae and post-weaning diarrhoea and oedema disease due to *E. coli* strains with F18 fimbriae, and the loci for both types of 'receptors' have been mapped. In mice, resistance to *Salmonella* infections is associated with the antimicrobial activity of macrophages and is linked with polymorphism in the *Nramp1* gene. The gene has been identified in several species, including the pig, but data are so far lacking concerning the association between polymorphism in the porcine gene and resistance and susceptibility to *Salmonella* infection.

The rapid development in molecular genetics has given us detailed genome maps and the tools to identify and study individual genes. This means that in the near future we may be able to determine the genotype of individual animals and to study the association between 'disease resistance' genes and production traits. This information is needed before we can include 'disease genes' in breeding programmes.

Introduction

Diarrhoea (scours) is a common problem in animal production, affecting mostly the young growing animal. Despite considerable interherd differences, large field studies have reported that a high average frequency (6–7%) of all litters born are affected with diarrhoea pre-weaning (Svensmark *et al.*, 1989a) as well as post-weaning (Svensmark *et al.*, 1989b). In another large study the mortality due to scours pre-weaning has been reported as 2.7% of the piglets born, representing 11.9% of the total mortality during that period (Nielsen *et*

al., 1974). According to Svensmark *et al.* (1989a), piglets that had experienced pre-weaning diarrhoea reached 25 kg liveweight 2 days later than others, corresponding to a decreased productivity of 3%. Further, the risk for developing diarrhoea post-weaning increased if the piglets had experienced gastrointestinal disorders during the suckling period, and the risk of dying before reaching 25 kg body weight was increased fourfold for piglets having post-weaning diarrhoea (Svensmark *et al.*, 1989b).

The routine use of feed additives (antibiotics) has been prohibited in Swedish pig production since 1986. Initially this led to an increased frequency of post-weaning diarrhoea and to decreased productivity of the piglets (Robertsson and Lundeheim, 1994). Thus, specific management and hygiene demands were required to prevent disease outbreaks in Sweden. The total mortality among piglets was 14.6% pre-weaning and 3.4% post-weaning in Swedish conventional herds during 1992, figures that were reduced to 7.0% and 0.1%, respectively, in specific pathogen free (SPF) herds (Wallgren, 1993). These differences, and the large interherd differences obtained between the conventional herds, demonstrate a large influence of management and environment on health status and productivity of piglets.

During the neonatal period, scours is generally associated with one pathogen, commonly *E. coli* or *Clostridium perfringens*. Among older piglets, various infectious agents may cause diarrhoea, among them bacteria such as *E. coli* and *Salmonella*. However, viruses and protozoa also contribute to the clinical status. Diarrhoea is thus a multifactorial disease where the outcome of an infection is due to many factors and their interactions. The genetic make-up of the bacteria, determining various virulence factors such as fimbriae enabling adherence to intestinal mucosa and enterotoxin production, is essential for the pathogenity of the bacteria. Although management and housing routines influence the frequency and severity of scours, the genotype of the pig also has a large impact on resistance and susceptibility to clinical infection. This chapter will deal mainly with the genetic resistance of pigs to bacterial infections leading to diarrhoea, focusing on *E. coli* and *Salmonella* infections.

E. coli Diarrhoea

The ability of enteropathogenic (EPEC) or enterotoxigenic (ETEC) *E. coli* to adhere to the brush borders of enterocytes is fundamental for the initiation of the infection. Attachment of pathogenic bacteria to the mucosa of the small intestine is mediated by distinct surface antigens, called pili or fimbriae (Duguid and Anderson, 1967). Several fimbrial adhesins have been identified both in animal and human EPEC/ETEC strains. In the pig, strains expressing fimbriae of F4 (K88), F5 (K99), F6 (987P) or F41 types dominate during the neonatal period (Söderlind *et al.*, 1982; Brinton *et al.*, 1983; Gonzáles *et al.*, 1995), while strains expressing other types of fimbriae such as F18ab (F107), F18ac (2134P) and Av24 are found during the post-weaning period (Bertschinger *et al.*, 1990; Nagy *et al.*, 1992, 1996; Hide *et al.*, 1995; Kennan *et al.*, 1995).

Neonatal diarrhoea

Much neonatal diarrhoea is due to infections with *E. coli* strains possessing fimbriae of the F4 (K88) type. The frequency of K88 amongst enterotoxigenic strains isolated in various countries differs somewhat, but these strains mostly dominate (Söderlind and Möllby, 1978; Söderlind *et al.*, 1982, 1988; Brinton *et al.*, 1983).

Three antigenic variants, *ab*, *ac* and *ad*, have been identified for the K88 fimbriae, all containing a common a-type antigen (Ørskov *et al.*, 1964; Guiné and Jansen, 1979). The K88 fimbriae adhere to specific receptors on the intestinal cell brush borders. Early studies showed that the K88ac receptor contained a variety of sugar molecules, such as D-galactoside (Kearns and Gibbons, 1979; Sellwood, 1980), *N*-acetylglucoseamine, *N*-acetylgalactoseamine and D-galactoseamine (Sellwood, 1984). A later study has described the K88ac receptor as a mucin-type sialoglycoprotein (Erickson *et al.*, 1994). Glycoproteins of 210 and 240 kDa binding K88ab and K88ac, but not K88ad, fimbriae have been identified (Seignole *et al.*, 1994; Billey *et al.*, 1998). Another glycoprotein (74 kDa) belonging to the transferrin family that binds *in vitro* to K88ab fimbriae has been detected (Grange and Mouricout, 1996). However, K88ac and K88ad fimbriae did not bind to this intestinal transferrin.

Detection of receptor phenotype

Identification of the receptor phenotype of pigs can be performed by examining the adhesion of *E. coli* K88-positive bacteria to intestinal cell brush borders *in vitro* (Sellwood *et al.*, 1975). A variant of the assay, in which whole enterocytes instead of brush borders are used, has also been described (Rapacz and Hasler-Rapacz, 1986). Mostly, the intestinal specimens have been sampled after slaughter and the enterocytes have been collected by gently rubbing or scraping a segment of the intestine. The technique has also been performed on specimens from intestinal biopsies (Snodgrass *et al.*, 1981). To shorten the assay time, an enzyme immunoassay and an ELISA (enzyme-linked immunosorbent assay) have been developed (Chandler *et al.*, 1986; Valpotic *et al.*, 1989).

Testing potential breeding animals by intestinal biopsies or test matings are costly and cumbersome. Nor can any of the described assays differentiate between pigs carrying one or two copies of the receptor allele, i.e. distinguish between heterozygous and homozygous animals. Identification of the gene coding for the receptor structure will make direct typing of breeding animals possible and the genotype of individual animals can then be determined. Several laboratories are currently performing research towards this goal.

Inheritance of receptor phenotype

A genetic influence on resistance to ETEC was described as long as 30 years ago (Sweeney, 1968) and a Mendelian inheritance with a dominant receptor allele was later found for *E. coli* K88ac (Sellwood *et al.*, 1975; Gibbons *et al.*, 1977). One locus coding for both the K88ab and K88ac receptors was first suggested (Rapacz and Hasler-Rapacz, 1986; Bijlsma and Bouw, 1987), but later studies suggested two closely linked loci (Guérin *et al.*, 1993; Edfors-Lilja *et al.*, 1995).

The inheritance of the receptor for K88ad has been less clear and in several studies a weak adherence phenotype has been identified (Rapacz and Hasler-Rapacz, 1986; Bijlsma and Bouw, 1987; Hu, 1988). Studies by Hu *et al.* (1993) suggest that there are two receptors for K88ad, a high-affinity and a low-affinity receptor, both allelic to the K88ab and K88ac receptor(s).

Strong linkage disequilibrium between the *K88abR* and *K88acR* loci, with very few pigs positive for *K88abR* and negative for *K88acR*, has been found in most breeds studied so far (Bijlsma *et al.*, 1982; Edfors-Lilja *et al.*, 1986, 1995; Rapacz and Hasler-Rapacz, 1986; Hu *et al.*, 1993; Baker *et al.*, 1997). However, in one recent study a somewhat higher frequency of the K88abR$^+$–K88ac$^-$ phenotype was found in the Hampshire breed, i.e. four of 24 tested pigs (Baker *et al.*, 1997). With a recombination distance of 1–2%, one would expect to find the recombinant haplotypes *K88abR*$^+$–*K88acR*$^-$ and *K88abR*$^-$–*K88acR*$^+$ occurring at high frequency in some breeds. As this is not the case, there are two possible explanations for the strong association: (i) that haplotypes either positive for both *K88abR* and *K88acR* or negative for both are favoured by selection; or (ii) that the recombination frequency was overestimated in studies that suggest two loci, by typing errors or incomplete penetrance. The K88ab and K88ac proteins differ only slightly in amino acid composition (Gaastra *et al.*, 1979). The finding that the antibody response is not variant specific (Bijlsma *et al.*, 1987) might explain the linkage disequilibrium, as discussed by Ollivier and Renjifo (1991).

The receptor(s) for K88ab and K88ac have been determined in newborn as well as adult pigs. In contrast, it has been found that the weak adhesion phenotype for K88ad cannot be detected in pigs after the age of approximately 16 weeks (Hu *et al.*, 1993). A similar age influence has also been found for the adhesion of *E. coli* carrying K99 (Runnels *et al.*, 1980) and 987P fimbriae (Dean-Nystrom, 1995). A variation in amount of K88 receptor along the length of the small intestine has been reported (Chandler *et al.*, 1994).

Chromosomal localization and candidate genes

In humans, the P blood group constitutes the adhesion factor for urinary tract infections with pathogenic *E. coli* (Källenius *et al.*, 1980; Svensson *et al.*, 1983). Linkage studies between blood group loci and the K88 receptor have also been performed, and a weak linkage between the L blood group locus and the K88 receptor was found (Vögeli *et al.*, 1992). However, linkage had also been suggested between the transferrin locus and the K88ac receptor (Gibbons *et al.*, 1977; Guérin *et al.*, 1993). The *TF* and *EAL* loci have been assigned conclusively to two different chromosomes, the q31 band on chromosome 13 (Chowdhary *et al.*, 1993) and the q arm of chromosome 4 (Marklund *et al.*, 1993). The establishment of detailed linkage maps in the pig (Rohrer *et al.*, 1994; Archibald *et al.*, 1995; Marklund *et al.*, 1996) has improved the opportunities to map the receptor. The localization of the gene for the K88ac receptor to chromosome 13 has been confirmed in this way, and the locus for the receptor has been localized 7.4 cM proximal to the transferrin locus (Edfors-Lilja *et al.*, 1995). This chromosomal region is homologous to human chromosome 3 and, using comparative mapping, research is in progress to map candidate genes in this region. Ten human chromosome 3 genes have been assigned to porcine chromosome 13 (Van Pouke *et al.*, 1997; Peelman, 1998a). Further mapping indicates that the K88ac receptor locus is localized terminal to the transferrin locus, and one marker showing no recombination with the K88ac receptor locus has been mapped (Peelman, 1998b). Radiation hybrid mapping (Alexander *et al.*, 1998) and intestinal cDNA libraries (Winterø *et al.*, 1996) are other tools currently used to identify markers close to the receptor gene and possibly the causative gene itself.

Selection for the receptor phenotype – performance of sows and fattening pigs

The newborn pig is dependent on the mothering capacity of the sow, which includes the provision of antibodies in the colostrum and milk. Sows lacking the receptor produce low levels of antibodies to K88 after natural exposure or oral vaccination (Sellwood, 1979, 1982; Bijlsma *et al.*, 1987). A small but significantly higher IgG response has been found in receptor-positive pigs 3 weeks after intramuscular immunization, suggesting that the immunization acted as a booster dose in receptor-positive pigs (Edfors-Lilja *et al.*, 1995). This confirms earlier observations where pigs possessing receptors for K88ab and K88ac had a more pronounced IgG response to K88 after subcutaneous immunization than did pigs lacking the receptors (Edfors-Lilja *et al.*, unpublished observations).

Although the receptors mediate increased susceptibility to neonatal *E. coli* diarrhoea, the function and significance of the receptors on a more basic level is not known. A low frequency of pigs possessing the receptor has been identified in breeds not selected for increased growth. No receptor phenotype pigs were identified in the Chinese Meishan breed (Chappuis *et al.*, 1984; Michaels *et al.*, 1994), while a low frequency of the receptor phenotype was found in the Chinese Minzu breed (Michaels *et al.*, 1994). In another study, weak adhesion to intestinal cells, but with no correlation with virulence, was found for Chinese Meishan pigs (Bertin and Duchet-Suchaux, 1991). Both European wild boars that were used as parents in a reference pedigree for gene mapping lacked the receptor (Edfors-Lilja *et al.*, 1995).

Post-weaning diarrhoea

Diarrhoea in the older pig is often associated with strains other than those causing neonatal diarrhoea. The change in diet at weaning and some nutritional components are thought to predispose to diarrhoea and oedema disease. The frequency of these problems differs largely between countries and populations, but breed differences have also been observed. In Switzerland, oedema disease and post-weaning diarrhoea are responsible for considerable economic losses (Bertschinger *et al.*, 1992) and can also be a problem in adult pigs (Sydler *et al.*, 1996). Oedema disease is rarely seen in pigs in Australia, but a majority of strains isolated from pigs with post-weaning diarrhoea were positive for F107 fimbriae (Hide *et al.*, 1995). Similar results have been found in Denmark (Ojeniyi *et al.*, 1992), where several outbreaks of oedema disease have

been reported recently (Jorsal *et al.*, 1996). In Sweden, oedema disease has a low frequency, but almost 50% of *E. coli* strains collected from pigs with post-weaning diarrhoea contained the gene for a major subunit of the F107 fimbriae (Kennan *et al.*, 1995).

The F107 fimbriae belong to a group of related adhesins named F18 (Imberechts *et al.*, 1994). Like the K88 fimbriae, the F18 possesses a common antigenic variant *a*, and two variant-specific determinants, *b* and *c* (Rippinger *et al.*, 1995). Another adhesion group is the F17 family (Bertin *et al.*, 1996) which includes *E. coli* strains associated with bovine diarrhoea and human urinary tract infections (Martin *et al.*, 1997).

Detection and inheritance of receptor phenotype

A genetic influence on the frequency of post-weaning diarrhoea and oedema disease was described 30 years ago (Smith and Halls, 1968) and has since been confirmed (Bertschinger *et al.*, 1986). After the development of the adherence assay for identification of K88 receptor phenotype pigs, similar studies were performed to identify pigs resistant to oedema disease and post-weaning diarrhoea. Genetic studies have shown that susceptibility to colonization by F18ab-positive *E. coli* is dominantly inherited (Bertschinger *et al.*, 1993). Further studies have mapped the locus for the F18ab receptor to chromosome 6, close to the genes for blood group system S and the calcium release channel, CRC (Vögeli *et al.*, 1996). Two α (1, 2)-fucosyltransferase genes (*FUT1* and *FUT2*) are closely linked to the S and F18 receptor loci and a polymorphism in *FUT1* co-segregates with *E. coli* F18 adhesion (Meijerink *et al.*, 1997).

Salmonella Diarrhoea

Salmonella infections are an important human health problem in many countries. Swine, poultry, cattle and seafood are important carriers (Wilcock and Schwartz, 1992). There are over 2 million cases of meat and poultry food-borne disease in humans in the USA per year, at a cost approaching US$1.4 billion. Most of this disease is attributed to *Salmonella* and *Campylobacter* infections (Menning, 1988). The most frequently reported *Salmonella* serotype is *S. typhimurium*. In addition to the economic impact of salmonellosis on the human population, it is also an economic disease of swine resulting in lost income to the pork industry. Data by Fedorka-Cray *et al.* (1994) suggest that two types of disease syndromes appear to occur after infection; clinical disease within 48 hours and a subclinical syndrome that may be important in establishing a carrier state.

In Sweden, an official control programme with respect to *Salmonella* spp. has been running since 1961 and was last revised in 1995 (Anonymous, 1995). Infected farms are subject to restrictions including a total ban of movements of animals, with the exception for transportation to sanitary slaughter. Regular controls are performed at the abattoirs, aiming to detect a prevalence of infection at 5% with a confidence level of 95%. The control is based on bacteriological examinations from five ileo-caecal lymph nodes per animal and from surface swabs of approximately 1400 cm² per animal. Further, feed plants are controlled with respect to *Salmonella*. Regardless of the source, all isolations of *Salmonella* made have to be reported (Eld *et al.*, 1991; Malmqvist *et al.*, 1995). Together these measurements have accomplished low prevalences of animals positive to *Salmonella* at slaughter, i.e. well below 1% among broilers (Wierup *et al.*, 1992) as well as among ruminants and pigs (Wahlström *et al.*, 1997).

Genetic resistance

In chickens, differences between inbred strains in resistance to various serotypes of *Salmonella*, including *S. typhimurium* and *S. enteritidis*, have been described (Bumstead and Barrow, 1988, 1993). In these studies, birds were challenged orally, intramuscularly and intravenously, suggesting that resistance is not a function of adherence to epithelial cells. It was also shown that the resistance was inherited as a dominant autosomal trait and that it was not linked to the major histocompatibility complex (MHC). Further studies with *S. enteritidis* have confirmed these chicken strain differences in resistance and susceptibility (Guillot *et al.*, 1995; Protais *et al.*, 1996). In a recent linkage study, a region on chicken chromosome 5 has been identified that accounts for more than 50% of the difference in resistance between two chicken lines (Mariani *et al.*, 1998). This region corresponds to part of mouse chromosome 12 and human chromosome 14, regions that so far contain no mapped genes likely to contribute to resistance.

In mice, a high level of resistance to *S. typhimurium* infection and other facultative intracellular bacteria is determined primarily by the *Ity/Lsh/Bcg* gene (Skamene *et al.*, 1982; Lissner *et al.*, 1983). This gene was later identified as *Nramp*, natural resistance-associated macrophage protein gene (Vidal *et al.*, 1993). Nramp is a family of integral membrane proteins that have been identified in several species, including *Drosophila*, plants and yeast (Cellier *et al.*, 1995). The function is not known, but it has been suggested that the Nramp polypeptides are part of a group of transporters or channels spanning the plasma membrane. The Nramp1 protein seems to have many effects in regulating macrophage activation, including respiratory burst activity, synthesis of nitric oxide synthase, antigen processing, MHC class II molecule expression and regulation of production and release of the cytokines TNF-α and IL-1β (reviewed by Blackwell, 1996).

The *Nramp1* gene has been identified and assigned in mice and humans to chromosomes 1 and 2, in chickens to chromosome 7 (Girard-Santosuosso *et al.*, 1997) and in sheep to chromosome 2 (Pitel *et al.*, 1994, 1995). A second gene, *Nramp2*, has been identified in humans and mice and localized to chromosomes 15 and 12, respectively (Gruenheid *et al.*, 1995). In the pig, a full-length cDNA of *Nramp1* was recently sequenced (Tuggle *et al.*, 1997). The gene has been assigned to chromosome 15 and a small population study revealed large allele frequency differences among breeds (Sun *et al.*, 1998). Association between polymorphism and resistance or susceptibility to *Salmonella* infection is rather well documented in mice (Blackwell, 1996). In humans, data suggest

an association between polymorphism in the *Nramp1* promoter region and susceptibility to rheumatoid arthritis. Linkage studies to determine whether this polymorphism also contributes to infectious disease susceptibility are in progress (Blackwell, 1996).

Conclusions

Diarrhoea due to bacterial infections is a problem in pig production, both with regard to the loss in productivity and also from a human health view, as the pig can act as a carrier of human infections. Genetic resistance to neonatal diarrhoea caused by *E. coli* carrying K88 fimbriae has been known for many years. Also, genetic resistance to post-weaning diarrhoea or oedema disease due to *E. coli* with F18 fimbriae has been identified, and the loci for both types of 'receptors' have been mapped. The receptor for *E. coli* K88, as well as the receptor for uropathogenic *E. coli*, contains various carbohydrate molecules. The nature of the F18 receptor is not yet known, but a close linkage with two α-fucosyltransferase genes has been found.

Resistance to *Salmonella* infections is not associated with 'receptor' molecules, but with the antimicrobial activity of macrophages. In mice, this resistance is linked with polymorphism in the *Nramp1* gene. The gene has been identified in several species including the pig, but data are so far lacking for any association with resistance or susceptibility to *Salmonella* infection. The rapid development in molecular genetics has given us detailed genome maps and the tools to identify and study individual genes. This means that, in the near future we may be able to select breeding animals of preferred genotype. How to decide which genotype to select? Some data suggest that pig populations not selected for growth have a low frequency of the K88 receptor, but we do not yet have enough results to know whether receptor-phenotype pigs grow faster. Even fewer studies concerning the influence of the F18 receptor on production traits have been reported. As regards *Salmonella* infections, it has been suggested that good resistance might increase the frequency of autoimmune diseases.

In conclusion, we know far more about genetic resistance to bacteria caused diarrhoea than we did some years ago. The development of DNA-based tests will enable us to determine the genotype of individual animals, and hence it will also be possible to study association between 'disease genes' and production traits. Thus, in the near future we will have the knowledge to identify and select the preferred genotypes.

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Ticks and Tick-borne diseases: Background information

Tick (*Boophilus microplus*) worry and tick-borne diseases cause considerable production losses throughout tropical and subtropical cattle production systems, although they are generally ranked below helminth and several other infections in terms of economic impact. Tick-borne diseases include anaplasmosis, babesiosis, cowdriosis, theileria and East Coast fever. McLeod and Kristjanson (1999) provide estimates of the economic costs due to production losses and mortality. For example, in Australia the total annual costs have been estimated at $US20.8m, $4.0m in Indonesia, $24m in Zimbabwe, $57.6m in South Africa, $132.6m in Kenya and $355.4m in India.

Selection of individual animals with enhanced tick resistance for breeding, based simply on tick counts, can quickly lead to reduced tick infestation. Published heritabilities of tick numbers are generally moderate to high (e.g. 0.2 – 0.8(!)). Frisch *et al.* (2000) demonstrated that with as little as 6 years of continuous selection, a nucleus herd could be created that has tick burdens sufficiently low to be of little consequence. Often, indigenous adapted breeds show considerably greater resistance to, and tolerance of, ticks and tick-borne diseases than exotic introduced breeds.

Mechanisms of tick resistance include avoidance, grooming behaviour, skin thickness (& other skin characteristics), and immune response. The impact of immune responses vary, ranging from simple rejection of the tick, reduced engorged weight of all instars, reduced number or viability of eggs, to death of the tick on the host. Genetic differences to tick-borne diseases have yet to be satisfactorily dissected into resistance to the tick (the vector) or resistance to the disease *per se*.

Frisch *et al.* (2000) costed the within-herd benefits of selection *vs.* the benefits of using acaricides for controlling ticks. Offsetting acaricide costs against production benefits, there was a net annual benefit of AUD$4.5/animal. Offsetting data collection and processing costs against the production benefits from genetic selection, costs were higher than benefits for the first 10 years, subsequently returns were greater than costs and cumulated annually thereafter. In addition to these calculations, no acaricide treatment is required after 10 years of continuous selection, increasing the financial benefits and reducing future problems of acaricide resistance. In these calculations, the nucleus breeding herd bears the full cost of data recording. When the nucleus is used to breed sires for widespread use, the value of resistant sires would far exceed the relatively small cost incurred over the 10 years required to produce them. Moreover, acaricides costs are incurred throughout the industry, whereas genetic selection costs are incurred predominantly in the nucleus herds.

Simple selection does not assume high-level technological input. A gene conferring essentially 100% resistance in some genetic backgrounds is thought to exist, through data inference (Frisch, 1999), although genetic markers still need to be identified for this gene. Once identified, this gene could be used to considerable effect in various situations through crossbreeding programmes that use genetic markers to ensure retention of the gene. The benefits, but also the costs, of this approach are potentially considerably greater than those described above.

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Breeding for Disease Resistance - Uniting Genetics and Epidemiology

Montpellier, August 12-14, 2002

Programme

Monday 12

08 h 15 - 08 h 30	Introduction to the course
08 h 30 - 09 h 30	Lecture: Introduction to disease biology and disease genetics
09 h 45 - 10 h 45	Lecture: Introduction to epidemic modelling
10 h 45 - 11 h 15	*Coffee break*
11 h 15 - 12 h 15	Lecture: Genetic-epidemiological models applied to macroparasites
13 h 30 - 14 h 00	Each participant introduces him(hir)-self
14 h 00 - 15 h 00	Lecture: Genetic-epidemiological models applied to microparasites
15 h 15 - 17 h 45	Group session: Literature appraisal **(A)** – *including coffee break* –

Tuesday 13

08 h 30 - 09 h 30	Lecture: Generalised immunity, continuous challenge scenarios and impact of immune activation upon performance
09 h 45 - 10 h 45	Lecture: Mastitis example
10 h 45 - 11 h 15	*Coffee break*
11 h 15 - 12 h 15	Lecture: Co-evolution theory
14 h 00 - 15 h 30	Group Session: Co-evolution workshop **(B)**
15 h 30 - 16 h 00	*Coffee break*
16 h 00 - 17 h 30	Group Session: Disease case study workshop **(C)**

Wednesday 14

08 h 30 - 09 h 30	Lecture: Further applications of epidemic models to animal breeding: advanced genetic management and biodiversity
09 h 45 - 10 h 45	Lecture: Nematode example
10 h 45 - 11 h 15	*Coffee break*
11 h 15 - 12 h 15	Lecture: Data analysis
14 h 00 - 17 h 30	Group Session: Theory to practice **(D)** – *including coffee break* – - Research strategies - Data collection - Breeding programme design & implementation.

Group Sessions

A. Literature Appraisal
Several papers will be circulated to participants prior to the workshop. They are encouraged to read them prior to coming. During the session participants will split into several groups, to discuss and appraise the papers according to specified criteria. From each group a nominated person will present the paper to all participants and lead the interactive discussion.

B. Co-evolution Workshop
Parasite co-evolution is a critically important area for scientists with an interest in disease genetics. The participants will split into two groups; one group will consider macroparasite evolution, the other group will consider microparasite evolution. The distinction between macroparasites and microparasites is necessary because of differences in generation lengths, transmission pathways and host-parasite relationships, all of which affect potential co-evolution rates. Guidance will be given on how to consider the issues. Once again, feedback will be made to all participants.

C. Case Study Workshop
For several diseases there are major opportunities to combine genetics and epidemiology to advance the opportunities for breeding for disease resistance. Using knowledge gained in the course, groups will consider specific diseases and:

- Define the genetic and epidemiological issues
- Formulate modelling strategies to address these issues
- Determine how the outputs from the modelling approach aids in the control or management of the disease problem (by both genetic and non-genetic means)

D. Theory to Practice: Interactive Sessions
By defining general concepts and applying them to specific examples, participants will address:

- Research strategies
- Data collection
- Breeding programme design & implementation