Genetic Variation of Susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* Infection in Dairy Cattle


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ABSTRACT

Paratuberculosis is an infectious disease that is not easily amenable to classical control methods such as treatment and vaccination. Experimental animal models suggest that there could be genetic factors responsible for susceptibility or resistance to infection with the causative agent, *Mycobacterium avium* subsp. *paratuberculosis*. The aim of this study was to estimate genetic variation in susceptibility to paratuberculosis in Dutch dairy cattle. Data collected during a vaccination trial, conducted from 1984 to 1994, was used. A total of 3020 cows, with complete pedigree records and infection status at slaughter, were available for analysis. A standard polygenic statistical probit model was used to estimate heritabilities. The estimated heritability of susceptibility to *M. avium* subsp. *paratuberculosis* infection was 0.06 for the overall population. In the subpopulation of vaccinated animals the estimated heritability was 0.09. Other calculations based on the model used in this study argue against a prominent role for vertical transmission.

Because the establishment of genetic variation is one of the first steps towards the exploration of the possible use of selection for genetic improvement, the present study provides evidence for the presence of genetic variation in the susceptibility of cattle to paratuberculosis. Because the economic impact of the disease is substantial, the development and application of genetic tools, along with other control methods, could be instrumental in the eradication of paratuberculosis. (Key words: genetic variation, paratuberculosis, susceptibility, cattle)

INTRODUCTION

Paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis*, is a chronic progressive disease of the small intestine of ruminants in which the organism is able to survive inside macrophages. In cattle, infection with *M. avium* subsp. *paratuberculosis* occurs mainly in calves (Chiodini et al., 1984; Kreeger, 1991; Sweeney, 1996) via oral uptake of the bacteria from the environment or objects with fecal contamination. Some of the animals are able to effectively clear the infection; others, however, become chronically infected (Chiodini et al., 1984). These animals shed the bacteria in their feces intermittently and thus contribute to the spread of the infection. A limited number of these infected animals will progress to a clinical stage as adult animals. Vaccination of young animals with either heat killed or live whole bacterin vaccines results in a marked reduction of the number of animals with clinical disease, but vaccination does not prevent infection and shedding (Goodger et al., 1996; Larsen et al., 1978; van Schaik et al., 1996; Wilesmith, 1982).

Diagnosis of the disease poses problems because the tests available lack sensitivity, specificity, or both (van der Giessen, 1993). Moreover, vaccination does not reduce the number of infected animals (Wentink et al., 1994), and therefore control of the disease tends to be cumbersome. To control and eradicate paratuberculosis, the available diagnostic tools and farm management changes should be combined (Kreeger, 1991).

Research on genetic variation in susceptibility may aid in understanding the pathogenesis of this disease, and lead to the identification of disease resistance markers. Consequently, selection may be used as an additional tool for control of paratuberculosis. In murine models for intracellular infections, such as tubercu-
losis and paratuberculosis, a genetic component in host susceptibility has been shown (Blackwell et al., 1994; Frelier et al., 1990; Veazey et al., 1995). However, only a limited amount of data is available on the role of genetic components in the susceptibility of cattle to infection with M. avium subsp. paratuberculosis. Withers (1959) indicated that some breeds were more susceptible than others. However, these findings were later considered to be related to the relative proportion of those breeds in a certain region (Chiodini et al., 1984). Only one report in the late fifties indicated that there appeared to be some familial susceptibility in certain female breeding lines (Hole and Maclay, 1959).

The aim of the present study was to investigate genetic variation of susceptibility to infection with M. avium subsp. paratuberculosis in cattle using field data. Data from a 10-yr paratuberculosis-vaccination trial of the Dutch Animal Health Service was linked to pedigree information from the Dutch Cattle Syndicate to estimate heritability of susceptibility of cattle to M. avium subsp. paratuberculosis infection.

**MATERIALS AND METHODS**

**Data**

The study was based on data from a vaccination trial conducted by the Animal Health Service of the Netherlands from 1984 to 1994. Twenty farms, each with at least 50 cows and at least 5% clinical cases of paratuberculosis per year (prior to 1984), were included in the trial. The vaccination trial started in 1984, and all calves born on the farms from 1984 on were vaccinated once, before 30 d of age, with a heat-killed water-in-oil emulsion vaccine. On some farms, the animals born before the commencement of the vaccination trial were vaccinated after 30 d of age as described in detail by Kalis et al. (1991) and van Schaik et al. (1996).

During the trial period, all animals culled from the participating herds were slaughtered and subjected to clinical, microbiological, and histological examinations to establish their paratuberculosis infection status. During the trial period only postmortem tests were conducted, so farmers had no information about the infection status of animals present on the farm. The postmortem tests included bacterial culture of samples obtained from the intestinal tissues and lymph nodes, histopathological investigation of the tissues, and direct microscopic evaluation of Ziehl-Neelsen stained specimens. Additionally, the records of the data set included the following information: farm number, cow registration number, date of birth, date of slaughter, and vaccination status.

**Data Editing**

The infection status of individual cases was evaluated with the results of the postmortem examinations. An animal positive in one of the aforementioned tests was considered infected with M. avium subsp. paratuberculosis, an animal negative in all tests was considered uninfected. In total, 6405 records were available with animals born between 1972 and 1996. No accurate assessment of farm prevalence could be made for the earlier and later birth years due to left- and right-sided censoring. After examination of the data, we concluded that sufficient accuracy could be obtained to estimate the prevalence in the years 1982 to 1989 (included). For these 8 birth years, at least 50 animals per farm had entered the data set with a total of 4245 observations. Prevalence was estimated in retrospect on the within-herd level, per month of birth, by assuming that cattle with a positive infection status for M. avium subsp. paratuberculosis at the time of slaughter acquired the infection as young calves (Chiodini et al., 1984; Payne et al., 1961). Cases were used for prevalence estimation when both the date of birth and the date of culling as well as the infection status were known. An animal was considered present in a particular month when the date of birth was in or prior to that month, and the date of culling was after that month. Subsequently, farm-specific and time-related prevalence was estimated by dividing the number of infected animals by the total number of animals present on the farm in each month of the aforementioned period. In total, the records of 3614 animals were used to estimate prevalence. The pedigrees of animals were made available by the Dutch Cattle Syndicate. Animals were included in the data set when at least the father and the mother were traceable. The final size of the available data set decreased to 3020 observations due to untraceable pedigree records. The number of known generations of ancestors for these observations varied from 1 to 10.

**Data Subsets**

From the total data set (n = 3020) three data subsets were constructed. Vaccinated (either young or old vaccinated, 74.8%) and nonvaccinated animals (25.2%) were separated in two subdata sets. Due to the nature of the trial, the data set with vaccinated animals contained generally younger animals. A third data subset was created by extracting those animals of which a mother phenotype (infected or not infected) was also available. This data set also contained relatively young animals, and with over 98% vaccinated animals, it therefore was largely a subset of the vaccinated subset. In Table 1, the numbers of observations in each data set and the number of known parents that could be retrieved from
Table 1. Numerical composition of the four data subsets used for mixed model probit analysis.

<table>
<thead>
<tr>
<th>Data set</th>
<th>Cases (n)</th>
<th>Fathers (n)</th>
<th>Mothers (n)</th>
<th>Other Ancestors (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All data</td>
<td>3020</td>
<td>586</td>
<td>2259</td>
<td>4430</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>2260</td>
<td>466</td>
<td>1753</td>
<td>4597</td>
</tr>
<tr>
<td>Nonvaccinated</td>
<td>760</td>
<td>195</td>
<td>670</td>
<td>2316</td>
</tr>
<tr>
<td>With mother phenotypes</td>
<td>805</td>
<td>249</td>
<td>644</td>
<td>3729</td>
</tr>
</tbody>
</table>

For the different data sets (Data set) the number of cases is given (cases (n)). The amount of pedigree data that could be retrieved is indicated by the number of parental records, broken down to the number of fathers (Fathers (n)), number of mothers (Mothers (n)) and number of other ancestors (Other Ancestors (n)).

Related to the design of this study, a clear difference existed in the distribution of cases from the subsets of vaccinated and nonvaccinated animals (Figure 1). From the start of the vaccination experiment in 1984, almost all newborn calves were vaccinated on the 20 farms. Thus, a sharp increase in the number of vaccinated animals was seen, concomitant with a sharp decrease in the number of nonvaccinated animals. As a consequence, the population susceptible to infection with *M. avium* subsp. *paratuberculosis* changed between 1983 and 1985 from a predominantly nonvaccinated to a vaccinated population.

To estimate the heritability of susceptibility, we assumed that in the complete data set (All data) resistance to infection was the same trait in all vaccination classes. In the data subsets, resistance to infection was analyzed separately for vaccinated and nonvaccinated animals, and, therefore, was not forced to be the same trait.

### Statistical Analysis

Student’s *t* test was used to compare the mean age at culling of the infected and noninfected animals.

Data on infection status was analyzed with a probit mixed model (also called threshold model) using a “full pedigree sire- and dam model,” using (only) parental genetic effects as estimated transmitting ability (ETA). A so-called “animal model” was not used because it was found to lead to different estimates in cases in which the two models should be equivalent, and we considered results from the “animal model” to be at least dubious. The reduced animal model, which is very close to our sire and dam model with relationships, was not used because it has a more complex error variance structure which is not easily specified in available software packages for nonlinear models. In the mixed model probit analysis used here, the genetic effects were included by modeling random sire and dam effects, and accounting for relationships between sires and dams when available. The model can be expressed as:

$$
Pr (Y_{ijkl} = 1) = \Phi (\mu + S_i + D_j + bP_{ijkl} + V_k + M_l + E_{ijkl})
$$

Where:

- $Pr (Y_{ijkl} = 1)$ = probability to become infected (of observation $ijkl$), with infection scored as 0 (not infected) or 1 (infected),
- $\Phi (...) =$ probit link, the cumulative normal distribution function,
- $\mu =$ model intercept,
- $S_i =$ random sire effect,
- $D_j =$ random dam effect,
- $P_{ijkl} =$ prevalence (continuous fraction between 0 to 1) associated with observation $Y_{ijkl}$,
- $b =$ regression coefficient,
- $V_k =$ fixed effect of vaccination status $k$ [3 classes (vaccinated as calf, vaccinated at older age, nonvaccinated; for combined data only)],
- $M_l =$ fixed effect of mother phenotype $l$ [2 classes: mother infected, mother not infected; for “with known maternal phenotype” data only], and
- $E_{ijkl} =$ random residual effect.

The model included a regression on farm prevalence in the month an animal was born. Further accounting for farm-time effects (e.g., herd-year-season) was therefore deemed redundant, especially since the effect of exposure (i.e., prevalence at month of birth) was considered more relevant than a fixed effect for herd-year-
Figure 2. Estimated mean farm prevalence between 1982 and 1989. The apparent mean farm prevalence of infection (+ 1 × SD) calculated among herds within month-year, is illustrated as a decimal fraction summarized per year.

The vaccination experiment to 19% in 1989. Analysis of variance showed the presence of significant differences between the farms.

Heritability Estimates

Heritability estimates in the complete data and in the three data subsets ranged from < 0.01 to 0.09. The virtual absence of heritability was found in the data subset of nonvaccinated animals; the highest heritability (0.09) was found in the data subset with vaccinated animals (Table 2). In the complete data set, which contained a mix of vaccinated and nonvaccinated animals, an intermediate heritability was found (0.06). In the data set with known maternal phenotypes, a heritability was found of 0.08. Additional data analysis, using only the sire contribution in estimating heritability, produced essentially identical estimates of genetic components (data not shown).

Analysis of Relative Risks of Fathers

Estimates of the sire effects in the complete data set evaluated with the estimated heritability of 0.06 were transformed to relative risks for their descendents at a population mean of 30% infection. In Figure 3, the frequency distribution of the relative risk of fathers (n = 1761) to give infected offspring is depicted. The relative risk ranged between 0.75 and 1.23, when the data of the 1761 sires were evaluated. The limits of the 95% confidence interval were at relative risk 0.905 and 1.052, respectively, encompassing 1585 sires.

Estimates of Model Effects

Estimates of model effects are presented in Table 3 for the complete data set and in Table 4 for the “with known maternal phenotypes” data set. Estimates were obtained including genetic effects in the model, and using heritabilities as estimated. In the complete data set, vaccination class was included, and results show a

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Table 2. Estimates of heritability of susceptibility of dairy cattle to infection with Mycobacterium avium subsp. paratuberculosis, based on mixed model probit analysis.

<table>
<thead>
<tr>
<th>Data</th>
<th>Heritability1</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All data</td>
<td>0.06</td>
<td>0.037</td>
<td>†</td>
</tr>
<tr>
<td>Vaccinated animals</td>
<td>0.09</td>
<td>0.050</td>
<td>*</td>
</tr>
<tr>
<td>Nonvaccinated animals</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With mother phenotypes</td>
<td>0.08</td>
<td>0.095</td>
<td>NS</td>
</tr>
</tbody>
</table>

1As two parental effects were included in the model, heritability $h^2 = 4\sigma_g^2/(1 + 2\sigma_g^2)$.

†P ≤ 0.10.

*P ≤ 0.05.

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higher risk for nonvaccinated animals ($P = 0.001$) and no significant difference between young and old vaccinated animals. The model effects correspond to a 20% infection probability for vaccinated animals and a 33% infection probability for nonvaccinated animals. The regression on farm prevalence is significant ($P < 0.001$) and positive, indicating that a higher farm prevalence in the month of birth was associated with a higher risk to become infected.

For the model estimates (Table 4), farm prevalence and mother phenotype (Infected or Not Infected) were included, besides the genetic effects. Model estimates showed a similar and significant ($P < 0.001$) effect of farm prevalence. The mother phenotype revealed a risk decrease when mothers were infected ($P = 0.04$); these effects correspond to an 18% infection probability for offspring from an infected mother and a 24% infection probability for offspring from a noninfected mother with a within herd prevalence of infection of 30%. This difference in infection probability can also be observed in the raw data (Table 5) with 19% infection probability for offspring from an infected mother and a 22% infection probability for offspring from a noninfected mother; however, in that case, without correction for prevalence and genetic effects, the observed difference of 3% is not significant.

### DISCUSSION

The estimated heritabilities of the susceptibility of cattle to *M. avium* subsp. *paratuberculosis* infection (up to 0.09) are comparable to many other disease traits (Philipsson et al., 1980; Simianer et al., 1991). For instance, heritability estimates for first-lactation cows in Sweden over two breeds were 0.07 for ketosis, 0.14 for teat injury, 0.07 for mastitis, 0.1 for diseases of the feet and legs, and 0.06 for the sum of all diseases (Philipsson et al., 1980). The applied threshold model (mixed model probit) analysis, modeling ETA for all parents, provides an “approximate animal model.” This is an improvement over the often employed sire- or sire-maternal grandsire models in threshold model analysis, yet remaining computationally feasible. According to R. L. Quaas (personal communication), the discrepancy of this model with that of the ideal true animal model would be larger for low heritabilities and for few prog-

### Table 3. Estimates of infection probability for the complete data set.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate (%)</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>26.4</td>
<td>2.225</td>
<td>***</td>
</tr>
<tr>
<td>Prevalence</td>
<td>0.91</td>
<td>0.098</td>
<td>***</td>
</tr>
<tr>
<td>Vaccination class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not</td>
<td>10.8</td>
<td>3.465</td>
<td>***</td>
</tr>
<tr>
<td>Young</td>
<td>0.16</td>
<td>2.848</td>
<td>NS</td>
</tr>
<tr>
<td>Old</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

1Estimates of infection probability are on an underlying Probit scale transformed back to a percentage scale with a mean prevalence of 29%. The effect of prevalence is indicated as the estimated increase in infection probability per 1% increase in prevalence. Vaccination class “old” was arbitrarily set to zero.

### Table 4. Estimates of effects for the data subset “with known maternal phenotype.”

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate (%)</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>21.2</td>
<td>2.532</td>
<td>***</td>
</tr>
<tr>
<td>Prevalence</td>
<td>1.08</td>
<td>0.218</td>
<td></td>
</tr>
<tr>
<td>Mother phenotype class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>-6.48</td>
<td>3.648</td>
<td>*</td>
</tr>
<tr>
<td>Not infected</td>
<td>0</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

1Estimates of infection probability are on an underlying Probit scale transformed back to a percentage scale with a mean prevalence of 29%. The effect of prevalence is indicated as the estimated increase in infection probability per 1% increase in prevalence. Mother phenotype “Not infected” was arbitrarily set to zero.

### Table 5. Infection phenotype distribution for the sub data set “with known maternal phenotype.”

<table>
<thead>
<tr>
<th>Infected mother</th>
<th>Noninfected mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected daughter</td>
<td>50 (19.38)</td>
</tr>
<tr>
<td>Noninfected daughter</td>
<td>208 (80.62)</td>
</tr>
<tr>
<td></td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>805</td>
</tr>
</tbody>
</table>

| Infected daughter   | 171                |
| Noninfected daughter| 634                |

1Indicated are the number of infected or non-infected daughters born from either infected or non-infected mothers. The numbers in brackets indicate the percentage of offspring that was infected or non-infected.
eny per parent, but with much progeny information, as is the case in this study, it is likely to be minor.

The heritability as estimated for the different sub-data sets suggest the presence of genetic differences especially among vaccinated animals (estimated heritability 0.09). On the one hand, this could imply that susceptibility to infection is a different trait in vaccinated and nonvaccinated animals. Because vaccination is the introduction of bacterial antigens (in adjuvant) in tissue different from natural infection, this may have significant consequences for the subsequent immune response due to alternative activation of antigen presenting cells in the different microenvironments in which the response is initiated due to usage of different genes (Goerdt and Orfanos, 1999). On the other hand, the low heritability found in nonvaccinated animals may be related to the study design. Data regarding the nonvaccinated animals is based on the postmortem examination of cows born predominantly in 1982 and 1983; afterwards all newborn animals were vaccinated. This implies that the group of nonvaccinated animals is small, less information regarding infection status of parents of these animals is available, and the two groups come from periods with limited overlap. It therefore cannot be excluded that genetic effects also play a role in a nonvaccinated population.

A number of environmental factors such as housing of calves directly after calving (Collins et al., 1994), newborn calf care, and manure handling (Goodger et al., 1996) have been reported to be significantly associated with the prevalence of paratuberculosis. Those, and other environmental, or nongenetic, factors that may play a role are summarized in the prevalence variable in our model. In this particular case, prevalence indicates the combined effects, either being risk increasing or decreasing, of all nongenetic effects that play a direct role in the farm dynamics of *M. avium* subsp. *paratuberculosis* infection. The heritability estimate in the known maternal phenotype sub-data set (0.08), which is very close to the estimation of the vaccinated group (0.09), implies that genetic differences remain, also after correction for the maternal phenotype. This last analysis especially indicates that a fair comparison has been made between the sire progeny groups, which were the main source of information in this analysis. The status of the mother is also related to the risk of vertical transmission. Vertical transmission can occur in utero because the organism can be isolated from fetuses of (heavily) infected mothers in a number of cases (Seitz et al., 1989; Sweeney, 1996). Colostrum, from which the organism can also be isolated in some cases, is another potential source for vertical transmission (Streeter et al., 1995; Sweeney et al., 1992; Taylor et al., 1981). However, in this population, infected vaccinated moth-
ers have fewer infected offspring than vaccinated, non-infected mothers. Hence, there appears to be no dominant role for vertical transmission of the infection from mother to offspring in this population. This observation, besides accounting for prevalence in the model, also argues in favor of the presence of genetic differences between animals in relation to their susceptibility to infection with *M. avium* subsp. *paratuberculosis* since strong vertical transmission would interfere with estimating (maternal) genetic influences as they cannot be separated in that case. According to the analysis presented, comparing vaccinated and nonvaccinated animals, vaccination as such reduces the risk of infection, although infection remains possible. Because vaccination also strongly reduces the occurrence of clinical disease (van Schaik et al., 1996), which is accompanied by severe fecal shedding of the organism, vaccination could remain valuable as one of several control methods to be applied.

In general, the genetic control of disease susceptibility and resistance is polygenic, and several QTL will be responsible for the genetic component of variation in individual resistance to infectious disease (Wilkie and Mallard, 1999). Candidate genes involved in QTL may be the bovine major histocompatibility antigens (BoLA) (Newman et al., 1996: Wilkie and Mallard, 1999) and genes involved in innate resistance, such as natural resistance associated with macrophage growth (*Nrap*). The Nramp gene has been shown to be linked to resistance to mycobacterial infection, including murine models of paratuberculosis (Blackwell et al., 1994; Chandler, 1961; Frelier et al., 1990; Veazey et al., 1995). The bovine equivalent of the Nramp gene has been isolated and based on its homology with the murine gene (Feng et al., 1996), may have similar functions. The search for polymorphisms in the Nramp gene might be an interesting path to attempt to identify genetic markers that could be used in culling programs.

**CONCLUSIONS**

Although no previous work is available for comparison, the present finding does imply that there is significant genetic variation in the susceptibility of cattle to paratuberculosis. The results may be typical for disease traits, as heritabilities are relatively low, indicating that traditional selection would be inefficient, but still “significant” in the sense that important differences between animals can be found in the extremes of the population. In such a case, the search for polymorphisms of individual genes affecting resistance could lead to the identification of genetic markers that could be used in culling programs.
Furthermore, the observed differences in estimated heritability between vaccinated and nonvaccinated animals warrants further investigation with respect to future efforts of developing vaccines against bovine paratuberculosis.

Note Added in Proof
This paper is dedicated to the memory of Grima Aduniga, our fellow scientist and friend, who died July 18, 2000, at age 35 due to a tragic traffic accident in his country, Ethiopia.

REFERENCES


