Within Herd Test Prevalence affects Genetic Variation in Antibody Response to *Mycobacterium avium* subspecies *paratuberculosis* in Milk of Dutch Holstein-Friesians

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Introduction

Johne's disease, also known as paratuberculosis, is characterized by granulomatous lesions in the distal part of the ileum. Ileal lesions elicit a deterioration of nutrient uptake and therefore animals suffering from Johne's disease show weight loss, diarrhea and reduced milk production. Because most infected animals are not able to clear the infection, Johne's disease has a substantial economical impact on farms where the disease is endemic (Kreeger, 1991). Classical control strategies on the farm are based on hygiene and culling of infected animals but eradication of the causative agent, *Mycobacterium avium* subspecies *paratuberculosis* (MAP), has been shown to be difficult or even impossible. New approaches to address Johne's disease are needed. One approach is genetic selection for animals resistant to disease. Earlier research showed heritabilities of susceptibility to Johne's disease of 0.060 to 0.159 (Koets *et al.*, 2000; Mortensen *et al.*, 2004; Gonda *et al.*, 2006; Hinger *et al.*, 2007). Large variation in estimated heritability in those studies was due to: 1) differences in incidence of Johne's disease in the research populations; 2) differences in sample sizes; 3) the use of different statistical methods, and 4) differences in diagnostic methods. However, data used in earlier studies were limited; population-wide screening is missing and the effect of incidence on genetic parameter estimation has still to be established. Therefore, the objective of this study was to estimate genetic parameters for the presence of a MAP specific antibody response in milk of Dutch Holstein-Friesian cows using subsets of data based on within herd test prevalence levels.

Material and methods

Data. Milk samples were collected from lactating cows during the routine milk production scheme. Upon decision of the farmer, milk samples could be sent to the Dutch Health Service, to test for antibodies specific for Johne's disease using an ELISA test. All samples

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were tested using a commercially available ELISA kit (Institut Pourquier, ELISA Paratuberculosis Antibody screening) according to the instructions of the manufacturer. Pedigrees and milk production records of the animals were provided by the Dutch cattle improvement (CRV, Arnhem, the Netherlands).

To investigate the possible effect of within herd test prevalence on genetic parameter estimation, variance components were estimated for 4 subsets of the data based on within herd test prevalence: 1) the complete dataset, 2) herds with at least 2 positive tested animals, 3) animals herds with a within herd test prevalence of at least 5% and 4) herds with a within herd test prevalence of at least 10%. To classify the number of test positive animals per herd, a minimal ELISA test result of 25% on the animal level was used.

Statistical analyses. The log-transformed ELISA test results of individual animals were implemented in the model as linear response variable. Variance components and heritabilities were estimated for the 4 subpopulations using a sire-maternal grandsire model with fixed effects for parity, year of birth, lactation stage and herd; a covariate for milk yield at test day; and random effects for sire, maternal grandsire and error.

Cross-validation. The accuracy of breeding value estimation depends largely on 1) the heritability of a trait and 2) the number of progeny of a sire in the dataset. Because the heritability as well as the number of sire progeny varied over the different subsets of data, cross-validation was applied to determine which of the subsets of data produced the most accurate estimated breeding values. For each dataset the following procedure was applied:
i) 20% of the phenotypes of each herd were set on missing, which resulted in 5 subsets each containing 80% of the data. In this way, each herd was once removed from the data, but each fixed effect class was present in each of the subsets.
ii) breeding values were estimated for all sires based on the remaining 80% of data in the corresponding dataset using the linear model.
iii) Breeding values for 20% of the observations that were excluded were estimated as follows: $ebv_{cow} = 1/2 \times ebv_{sire} + 1/4 \times ebv_{mgs}$. This procedure was repeated 5 times to obtain an estimated breeding value for each animal in the dataset.
iv) The final step was to calculate the correlation coefficient between the estimated breeding values of each animal when it was excluded and the observed phenotypes.

Additionally, subpopulations containing only herds with a specific prevalence were defined and correlation coefficients were calculated between estimated breeding values from one subpopulation and observed phenotypes from the remaining subpopulations to investigate the ability of subpopulations to predict phenotypes in the other subpopulations.

Potential genetic gain. To show how much potential progress could be made by single trait selection, the average within herd prevalence and number of positive tested animals in the complete dataset were calculated. Subsequently, the estimated sire breeding values were used to select the 10% sires most susceptible to Johne's disease according to our definition. Progeny of those sires were removed from the data and the average within herd test prevalence and number of positive tested animals were recalculated. The effect of sire

selection on the distribution of herds over various within herd test prevalence intervals was also examined.

Results and discussion

Intra-herd heritability. In 2008, there were 20,750 dairy farms in the Netherlands of which 12,077 herds including 684,364 animals were implemented in the data (58%). Prevalence of infection as measured by a positive ELISA test in milk demonstrated that of the 12,077 herds participating in the program, 6,438 herds were free of test positive animals and 1,712 herds had 1 positive tested animal. In 2,153 herds, at least 2 test positive animals were detected but the within herd test prevalence remained lower than 5%. In 1,232 herds, a within herd test prevalence between 5% and 10% was found whereas 542 herds had a within herd test prevalence of at least 10%.

Genetic parameters for susceptibility to Johne's disease were estimated in 4 subsets of the data based on within herd test prevalence to investigate the effect of within herd test prevalence (Table 1). The estimated heritability ranged from 9.7% for herds with a minimum test prevalence of 10% to 3.1% for the complete dataset. This suggests that an increasing within herd test prevalence goes together with a rise in exposure to the causative agent. At that moment, genetic variation can be observed that is invisible in populations with a within herd test prevalence (close to) zero.

Using an ELISA in milk facilitates in testing a complete dairy sector for antibodies against paratuberculosis but the sensitivity of the test makes it impossible to find all infected animals. In this study, we assume that due to within herd prevalence restrictions, the large sample size and therefore a high number of progeny per sire, the low sensitivity of the ELISA test in milk has little or no influence on the estimation of genetic parameters.

Table 1: Sire variance $\left(\sigma_s^2\right)$, residual variance $\left(\sigma_e^2\right)$, and intra-herd heritability $\left(h^2\right)$with standard errors between brackets for the presence of a MAP specific antibody response in milk of Dutch Holstein-Friesian cows in populations differing in within herd test prevalence.

Dataset	# animals	# herds	# sires	σ_s^2	σ_e^2	h^2 (SE)[1]
1) Complete	684,364	12,077	9,870	0.215^{E}-3	0.273^{E}-1	0.031 (0.002)
2) At least 2 pos. tested animals in herd	265,290	3,927	7,021	0.606^{E}-3	0.578^{E}-1	0.041 (0.004)
3) Only prev. => 5%	104,382	1,774	4,570	0.150^{E}-2	0.984^{E}-1	0.060 (0.006)
4) Only prev. => 10%	28,916	542	1,851	0.383^{E}-2	0.153	0.097 (0.014)

[1] $\sigma_P^2 = \left[\left(\sigma_s^2 + 1/4\,\sigma_s^2\right) + \sigma_e^2\right]; h^2 = 4\sigma_s^2 / \sigma_P^2.$

Cross-validation. Cross-validation analysis showed that breeding value estimation based on dataset 2 (herds with at least 2 positive tested animals) performed best when correlating estimated breeding values from this subpopulation to observed phenotypes from other

subpopulations. Therefore, it is optimal to estimate breeding values based on herds with at least 2 positive tested animals and a heritability of 4.1%.

Potential genetic gain. The average within herd test prevalence decreased from 4.4% to 3.2% when progeny of 10% of sires that were most susceptible to Johne's disease based on their estimated breeding values were removed from the data. More remarkable was the decrease in number of positives in the total population due to sire selection: from 30,882 positives in the complete dataset, to 10,474 positives in the dataset with 10% sire selection (a decrease of 66% positive tested animals in the population whereas the number of animals in the data decreased with 25% respectively). At a sire selection level of 10%, the amount of herds free of test positive animals increased from 53% to 62%.

Conclusions

Genetic differences were observed between cows in the presence of a MAP specific antibody response in milk. This study shows that it is optimal to estimate breeding values based on herds with at least 2 positive tested animals and hence, a heritability of 4.1%. Although heritability is low, breeding for disease resistance can contribute to a more effective control of Johne's disease.

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