

Genetic relation between antibody response and faecal shedding of MAP in dairy cattle

L.C.M. de Haer¹, M.F. Weber² and G. de Jong¹

1: CRV, Arnhem, PO Box 454, 6800 AL Arnhem, the Netherlands
2: GD Animal Health, PO Box 9, 7400 AA Deventer, the Netherlands

Abstract

Paratuberculosis (Johne's disease) is an infectious disease of cattle caused by *Mycobacterium avium* spp. *paratuberculosis* (MAP). Breeding for disease resistance may be performed by selection on antibody response. Antibody levels in milk can be routinely collected on a large scale by using ELISA tests in milk samples. However, the ultimate goal is reduction of faecal shedding of MAP ('golden standard'). Selection on antibody response is only meaningful if it results in reduced shedding. The aim of this study was to estimate the genetic relation between antibody response and faecal shedding, and selection responses were calculated.

Two data sets consisted of results of laboratory tests performed by GD Animal Health on samples from Dutch dairy herds. The first data set (PA1) consisted of 517,672 individual milk samples of 408,459 cows from 5,938 herds tested by ELISA for antibodies against MAP between 2007-2010. The second data set (PA2) consisted of test results of 78,604 individual faecal samples of 52,348 cows from 435 herds. Faecal samples were tested between 1996-2015 by either modified Lowenstein-Jensen culture method, ESP-TREK culture system or qPCR assay.

Heritabilities and genetic solutions for sires were estimated with a sire-maternal grandsire model with random permanent environment effect and with fixed effects herd*year, parity, birthyear and lactation period. In addition, for PA1 a covariable for milk production was included in the model, for PA2 a fixed effect for test method. Sire solutions were used to estimate MACE correlations (with correction for reliability) between PA1 and PA2. Sires with at least 15 daughters on 10 herds per trait were included in the evaluation, resulting in 446 sires for PA1 and 272 sires for PA2.

Heritability for PA1 was 0.05 (0.003), for PA2 0.06 (0.008). Repeatabilities for PA1 and PA2 were respectively 0.42 (0.003) and 0.28 (0.006). The genetic correlation between PA1 and PA2 was 0.81. Heritability and genetic correlation indicated that it is feasible to reduce faecal shedding of MAP by selection for low antibody responses against MAP. By excluding the 10% bulls with lowest breeding values for paratuberculosis, incidence of paratuberculosis will decline from 2.4% to 2.1% and the fraction of contaminated herds will decline from 47% to 38% in The Netherlands. A breeding value of a bull of one genetic standard deviation higher than average, indicating higher resistance against paratuberculosis, will result in 2.8% less daughters tested positive for antibody levels.

Key words: dairy cattle, paratuberculosis, genetic parameters, antibody levels, faecal shedding, breeding

Introduction

Paratuberculosis (Johne's disease) is an infectious disease of cattle caused by *Mycobacterium avium* spp. *paratuberculosis* (MAP). Breeding for disease resistance may be performed by selection on antibody response. The ultimate goal of breeding for disease resistance however is reduction of the transmission of *Mycobacterium avium* spp. *paratuberculosis* (MAP). Selection of breeding stock is attractive only if it results in offspring

with reduced shedding of MAP given exposure to MAP. Genome-wide association studies using different phenotypes to define infection status (serology, faecal culture, tissue culture) resulted in different chromosomes and different chromosomal regions identified as involved in resistance to MAP (Minozzi *et al.*, 2012; Küpper *et al.*, 2014). Studies using an ELISA-based phenotype may identify genes which are involved in the immune response to the agent, whereas faecal culture of tissue culture based phenotypes will identify genetic loci involved

in the persistence of MAP infection, the development of a granulomatous enteritis and the release of MAP into faeces (Küpper *et al.*, 2014). Thus, it was unknown whether selection against ELISA-positivity results in offspring more resistant to (progression of) the infection, rather than in offspring that is unable to mount an ELISA response given infection (Barkema *et al.*, 2017). Therefore, the aim of this study was to estimate the genetic relation between MAP-specific antibody response and faecal shedding.

Material and Methods

Data

Data were provided by the GD Animal Health and were based on a nation-wide survey. Only records of cows with at least 75% HF genes were included. Two data sets were available to estimate the genetic correlation between antibody response (PA1) and faecal shedding (PA2). Herd numbers, cow identification numbers and pedigree were anonymised. Only sire identification numbers could be traced back to non anonymised numbers.

The first data set (Set 1) consisted of individual milk samples that were taken during the routine milk sampling scheme in the period 2007-2010. A herd was sampled once per year or once per two year. All samples were tested for antibodies specific for Johne's disease using a commercially available ELISA kit (ELISA Paratuberculosis Antibody screening, Institut Pourquier, Montpellier, France) according to the instructions of the manufacturer. Results were expressed as percentage of the sample to positive ratio (*S/P*), calculated as $100 \times [\text{the optical density (OD) value of the sample} - \text{the OD value of the negative control}] / [\text{the OD value of the positive control} - \text{the OD value of the negative control}]$ (van Weering *et al.*, 2007). Results were transformed to the natural logarithm $\ln(S/P + 50)$ to approximate the normal distribution. Animals could have repeated records across years on antibody levels. Data were selected from herd-year combinations with at least two positive ELISA results and at least 20 cows sampled.

The second data set (Set 2) consisted of individual faecal samples collected during the period 1996-2015 and tested by either modified Lowenstein-Jensen culture method, ESP-TREK culture system or qPCR assay. Results were given as positive, negative or missing. Repeated measurements within cows and across years

were possible. For PA2 herd-year combinations with at least one positive faecal sample were selected. Further, within herd-year at least 80% of the animals should be tested with a minimum of 20 animals.

In both data sets records were combined with lactation data and pedigree to estimate genetic parameters.

Method

Heritabilities and genetic solutions for sires were estimated with a sire-maternal grandsire model with random permanent environment effect and with fixed effects herd-year, parity, birth year and lactation period. In addition, for PA1 a covariable for milk production was included in the model, for PA2 a fixed effect for test method.

The following sire-maternal grandsire model (van Hulzen, 2011) was used to estimate the genetic effect on PA1 with ASReml (Gilmour *et al.*, 2006):

$$Y_{ijklmnop} = \mu + HY_i + P_j + BY_k + LS_l + b \times M_m + \text{sire}_n + \text{mgs}_o + \text{perm}_p + \text{error}_{ijklmnopq} \quad (1)$$

Where:

$Y_{ijklmnop}$ = Milk ELISA test result (PA1)

μ = mean

HY_i = fixed effect of herd-year

P_j = fixed effect of parity

BY_k = fixed effect year of birth

LS_l = fixed effect stage of lactation

M_m = covariable daily milk production

sire_n = random effect of sire

mgs_o = random maternal grandsire effect

perm_p = random permanent environment effect

$\text{error}_{ijklmnopq}$ = random error

Each parity was defined as a separate class, parity 6 or more was set to 6.

Birthyears of 1993 or less were set to 1993.

Stage of lactation was divided into 6 groups determined by days in milk: 1: less than 15 days; 2: 15-84 days; 3: 85-195 days; 4: 197-308 days; 5: 309-420 days, 6 : more than 420 days.

For the covariable daily milk yield a linear, quadratic and cubic term were used. Daily milk yields of less than 1 kg or more than 130 kg were set to missing.

A comparable sire-maternal grandsire model was used to estimate the genetic effect on PA2 with ASReml (Gilmour *et al.*, 2006):

$$Y_{ijklmnopq} = \mu + HY_i + P_j + BY_k + LS_l + T_m + \text{sire}_n + \text{mgs}_o + \text{perm}_p + \text{error}_{ijklmnopq} \quad (2)$$

Where:

- $Y_{ijklmnopq}$ = faecal score (PA2)
 μ = mean
 HY_i = fixed effect of herd-year
 P_j = fixed effect of parity
 BY_k = fixed effect year of birth
 LS_l = fixed effect stage of lactation.
 T_m = fixed effect for faeces test
 sire_n = random effect of sire
 mgs_o = random maternal grandsire effect
 perm_p = random permanent environment effect
 $\text{error}_{ijklmnopq}$ = random error

Faeces were analysed by LJ culture, ESP culture, Taqman PCR, or KaspRT PCR test.

Variance components were used to estimate heritability, according to the following formula:

$$h^2 = 4\sigma_s^2 / \sigma_p^2$$

$$\sigma_p^2 = (\sigma_s^2 + \sigma_{\text{mgs}}^2) + \sigma_{\text{perm}}^2 + \sigma_e^2$$

Where:

- h^2 = heritability
 σ_s^2 = sire variance
 σ_{mgs}^2 = variance of maternal grand sire = $1/4\sigma_s^2$
 σ_p^2 = phenotypic variance
 σ_{perm}^2 = perm. environmental variance
 σ_e^2 = error variance

Repeatability was calculated as:

$$\text{Rep} = (\sigma_s^2 + \sigma_{\text{mgs}}^2 + \sigma_{\text{perm}}^2) / (\sigma_s^2 + \sigma_{\text{mgs}}^2 + \sigma_{\text{perm}}^2 + \sigma_e^2)$$

Sire solutions were used to estimate correlations between PA1 and PA2 with the method of Multiple Across Country Evaluation (MACE) (Interbull, 2016). This method accounts for variation in reliability of the sire solutions for the two traits.

Results

Only animals with a test result for ELISA and/or faeces analysis were included in the data sets. This resulted in 517,672 individual milk samples of 408,459 cows from 5,938 dairy

herds tested by ELISA for antibodies against MAP in Set 1. Set 2 consisted of test results of 78,604 individual faecal samples of 52,348 cows from 435 cattle herds.

In table 1 the distribution over fixed effects for PA1 and PA2 is shown. Most observations for PA1 and PA2 were from cows in parity 1, 2 or 3. For PA2 also faecal samples were taken from virgin heifers. Most animals were in lactation stage 3 or 4: from 85 to 308 days in milk.

The stage of lactation for PA2 was determined as number of days between sampling date and previous calving date. If the previous calving date was unknown, stage of lactation was set to -9. The LJ culture faecal test method is most frequently used compared to the other tests.

Table 1. Number of observations for parity (par) and stage of lactation (lact.stage) for PA1 and PA2 and for faeces test for PA2 (test: 1=LJ, 2=Pcr2, 3=Pcr3, 4=ESP).

trait	PA1		PA2		
	par	lact. stage	par	lact. stage	test
0			6982		
1	148880	17319	17723	1312	58457
2	130977	91202	17962	6436	10906
3	96787	153026	13186	9663	6426
4	63671	158257	9343	8459	2815
5	38118	68452	5848	5634	
6	39239	29416	7560	2327	
-9				44773	

Estimated heritabilities for antibody response to paratuberculosis (PA1) was 0.05 and for faecal shedding (PA2) was 0.06 (Table 2). The standard errors were low, indicating a heritability that is significantly different from zero.

The repeatability, which indicates the correlation between records of one animal, is 0.42 for PA1 with standard error of 0.003. For PA2 the repeatability is lower (0.28, s.e. 0.006). This can be explained, as ELISA tests are routinely performed, but faeces tests are merely performed on animals that will be treated or that have been treated, with a higher chance of changing results.

Table 2. Sire variance (σ_s^2), genetic variance (σ_g^2), permanent environmental variance (σ_{perm}^2), phenotypic variance (σ_p^2), repeatability (rep) and heritability (h^2) for antibody response specific for Johne's disease (PA1) and for faecal shedding (PA2). Standard errors are between brackets.

Trait	σ_s^2	σ_g^2	σ_{perm}^2	σ_p^2	rep	h^2
PA1	0.001	0.004	0.033	0.081	0.418	0.052 (0.003) (0.003)
PA2	0.001	0.005	0.021	0.081	0.281	0.063 (0.006) (0.008)

Sires with at least 15 daughters on 10 herds per trait were included in the MACE evaluation, resulting in 446 sires for PA1 and 272 sires for PA2, with an overlap of 242 sires. The genetic correlation between PA1 and PA2 was 0.81.

Discussion

The heritabilities for PA1 and PA2 are 0.05 and 0.06. They are comparable, although the traits are measured in different ways: antibody levels in milk or bacterial secretion in faeces. In literature heritabilities of 0.05 to 0.15 are reported for antibody levels against MAP in serum (Berry *et al.*, 2010; Berry *et al.*, 2011), and 0.03 to 0.10 for antibody levels against MAP in milk (Van Hulzen *et al.*, 2011).

This study showed a high genetic correlation between PA1 (milk ELISA reading) and PA2 (faecal shedding) of 0.81. This means that selection of sires on a breeding value for high resistance (low ELISA response) is likely to result in less offspring that become infectious. The genetic standard deviation for antibody response is 0.063. From this value it can be derived that selecting a bull with a breeding value of one genetic standard deviation higher than average will result in 2.8% less daughters tested positive for antibody levels. In the study of Van Hulzen *et al.* (2011) potential results of breeding for disease resistance in The Netherlands are shown. By excluding the 10% bulls with lowest breeding values for paratuberculosis, incidence of paratuberculosis could decline from 2.4% to 2.1% at animal level and the fraction of contaminated herds could decline from 47% to 38%.

The genetic variation and genetic correlation indicate that it is feasible to reduce faecal shedding of MAP by selection for low antibody responses against MAP. If no selection is

performed to reduce faecal shedding, susceptibility to Johne's disease may increase by a correlated response to selection for other traits (Bermingham *et al.*, 2010; Berry *et al.*, 2010).

Conclusion

In conclusion, the genetic variation of the milk ELISA response and faecal shedding and the high genetic correlation between those two traits mean that selection of sires based on a high breeding value for milk ELISA results (high resistance) may result in offspring that are less likely to become infectious if they are exposed to MAP. Therefore, such a selection of sires can be a useful tool to control MAP, in addition to preventive management measures and test-and-cull programmes in cattle herds.

Acknowledgements

We gratefully acknowledge GD Animal Health for providing the data and supporting the research and Zuivel NL for their financial support.

References

- Barkema, H.W., Orsel, K., Nielsen, S.S., Koets, A.P., Rutten, V.P.M.G., Bannantine, J.P., Keefe, G.P., Kelton, D.F., Wells, S.J., Whittington, R.J., Mackintosh, C.G., Manning, E.J., Weber, M.F., Heuer, C., Forde, T.L., Ritter, C., Roche, S., Corbett, C.S., Wolf, R., Griebel, P.J., Kastelic, J.P., De, B.J., 2017. Knowledge gaps that hamper prevention and control of *Mycobacterium avium* subspecies paratuberculosis infection. *Transbound. Emerg. Dis.*
- Bermingham, M.L., More, S.J., Good, M., Cromie, A.R., Higgins, I.M. Higgins and Berry, D.P., 2010. Genetic correlations between measures of *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian dairy cows. *Journal of Dairy Science* 93, 5413-5422.
- Berry D.P., Good, M., Mullowney, P., Cromie, A.R. and More, S.J., 2010. Genetic variation in serological response to *Mycobacterium avium* subspecies paratuberculosis and its association with performance in Irish Holstein-Friesian dairy cows. *Livestock Science* 131, 102-107.

- Berry, D.P., Bermingham, M.L., Good M. and More, S.J., 2011. Genetics of animal health and disease in cattle. *Irish Veterinary Journal* 64, 5.
- Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and R. Thompson. 2006. ASReml User Guide Release 2.0 VSN International Ltd., Hemel Hempstead, HP1 1ES, UK.
- Interbull, 2016. MACE programme (Multiple-trait Across country Evaluation). Code of Practice, Chapter 5., http://www.interbull.org/ib/cop_chap5).
- Küpper, J., Brandt, H., Donat, K. and Erhardt, G., 2014. Phenotype definition is a main point in genome-wide association studies for bovine *Mycobacterium avium* ssp. *paratuberculosis* infection status. *Animal*. 8, 1586-1593.
- Minozzi, G., Williams, J.L., Stella, A., Strozzi, F., Luini, M., Settles, M.L., Taylor, J.F., Whitlock, R.H., Zanella, R. and Neiberghs, H.L., 2012. Meta-analysis of Meta-analysis of two genome-wide association studies of bovine paratuberculosis. *PLoS. ONE* 7(3), e32578
- Van Hulzen, K.J.E., Nielsen, M., Koets, A.P., de Jong, G., van Arendonk, J.A.M. and Heuven, H.C.M., 2011. Effect of herd prevalence on heritability estimates of antibody response to *Mycobacterium avium* subspecies *paratuberculosis*. *Journal of Dairy Science* 94, 992-997.
- Van Weering, H., van Schaik, G., van der Meulen, A., Waal, M., Franken, P. and van Maanen, K., 2007. Diagnostic performance of the Pourquier ELISA for detection of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in individual milk and bulk milk samples of dairy herds. *Veterinary Microbiology* 125, 49–58.