Genetic variation in paratuberculosis in dairy populations

Printing of this thesis was financially supported by
Stichting Mesdag-Zuivelfonds NLTO (Drachten),
Nederlandse Zuivel Organisatie (Zoetermeer), and
ELDA ICT & Services B.V. (Rijen)

ISBN 978-90-393-5873-3
Printed by GVO drukkers & vormgevers B.V. | Ponsen & Looijen
Cover design by www.BasBoerman.nl

Genetic variation in paratuberculosis in dairy populations

Genetische variatie in paratuberculose in melkvee populaties
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 6 december 2012 des middags te 12.45 uur

door

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geboren op 30 januari 1981 te Arnhem

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Contents

Chapter 1

General introduction

This thesis aims to contribute to control of Johne's disease by investigating genetic variation in the pathogen and studying genetic variation in host susceptibility. In this chapter, Johne's disease will be introduced in terms of causative agent, course of infection, diagnostic test available for disease detection and control strategies to reduce the impact of infection. The documented genetic variation in susceptibility to Johne's disease in dairy cattle and dairy goats will be shortly reviewed. Finally, the objective and outline of this thesis are presented.

JOHNE'S DISEASE IN COWS AND GOATS

Johne's disease, also known as paratuberculosis, is caused by oral uptake of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from the environment. MAP causes granulomatous lesions in the distal part of the ileum, mainly in domestic and wild ruminants. Ileal lesions limit sufficient nutrient uptake and in cows, infection may lead to weight loss, decreased milk production and diarrhea. In goats, clinical symptoms are weight loss, decreased milk production, a rough hair coat and a peeling skin.
Chapter 4 shows that in the Netherlands in 2008, prevalence of a positive ELISA test in milk in Holstein-Friesian cows was 46.7% on the herd level and 2.4% on the animal level. Prevalence of Johne's disease in dairy goat herds is unknown but suspected to be higher than in cattle herds based on clinical and routine pathological observations. Although infection has been described mainly in domestic and wild ruminants worldwide, Beard et al. (2001) showed that also foxes, rabbits, crows and stoats can be infected with MAP.

MYCOBACTERIUM AVIUM SUBSPECIES *PARATUBERCULOSIS*

MAP is a small (0.5×1.5 micron), gram-positive, and acid fast bacterium. MAP is not able to grow and multiply in the environment because of its inability to produce mycobactin (a chemical needed to transport iron). MAP is only able to multiply inside macrophages where it uses iron from its host. Although MAP needs a macrophage for replication, MAP is able to survive outside the host up to 250 days in spiked slurry, water and urine samples depending on the temperature (Larsen et al., 1956; Jorgensen, 1977). Survival time of MAP was even

longer in naturally contaminated soil and grass, up to 55 weeks after removing the ruminants in dry fully shaded locations (Whittington et al, 2004).

Sequencing the whole genome of a bovine MAP isolate (Li et al., 2005) led to the development of PCR-based methods based on the detection of genetic elements called Variable-Number Tandem Repeats and Mycobacterial Interspersed Repetitive Units (Bull et al., 2003; Overduin et al., 2004; Romano et al., 2005; Thibault et al., 2007). These genetic elements represent one of the rare categories of polymorphic structures in the highly monomorphic MAP genome. A better understanding of the biodiversity of MAP offers more insight in the transmission within and between herds (chapter 2; Möbius et al., 2008; Pradhan et al., 2011) and between species (Stevenson et al., 2009) and therefore may contribute to control of disease.

COURSE OF INFECTION

Disease susceptibility is highest in newborn animals and decreases with age (Windsor and Whittington, 2010). Susceptible animals can be infected by ingesting contaminated colostrum or milk or through contact with a contaminated environment (Chiodini et al., 1984). Current findings show that also the inhalation of bioaerosols containing viable MAP (such as dust) should be considered as a potential infection route (Eisenberg et al., 2012).

The course of infection with Johne's disease can be represented by stages. In cows, infected animals enter a transiently infected stage (van Roermund et al., 2007). In this stage, calves shed small amounts of fecal MAP in the environment. Until now, the transiently infected stage has not been described for goats. After the transiently infected stage, animals enter a long, latent stage of disease with no detectable MAP excretion. After the latent stage, animals enter a subclinical stage of disease where MAP shedding occurs intermittently. Only a small proportion of the subclinically infected animals reach the clinical stage of disease. As animals in the transiently infected, subclinical and clinical stage of disease shed MAP in the environment, only these animals contribute to new infections (Figure 1).

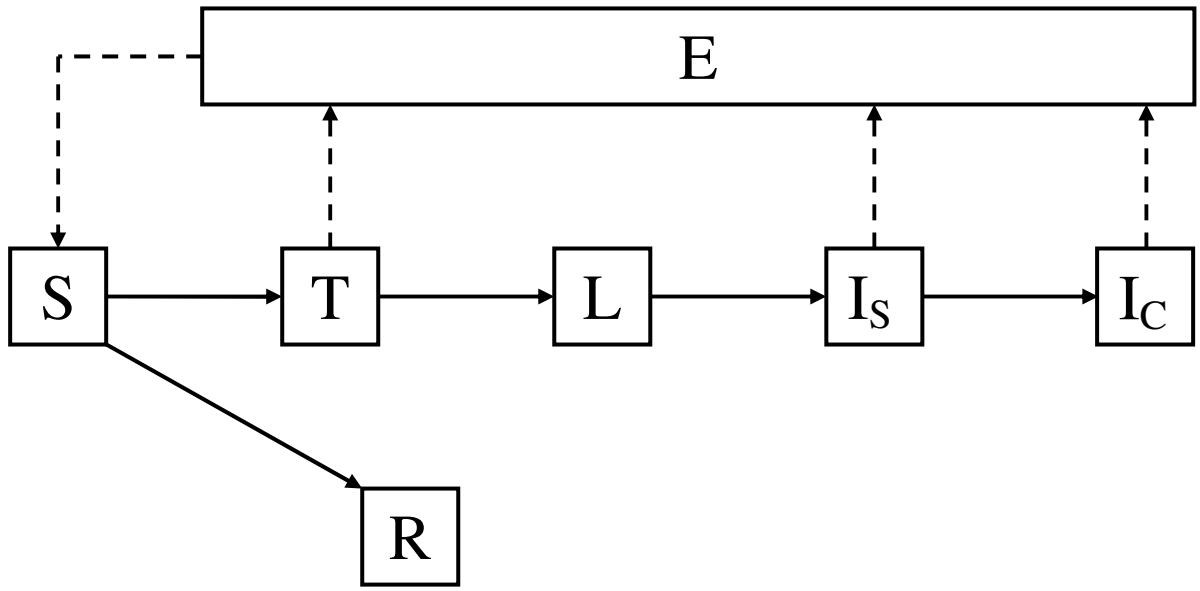

Figure 1. Schematic representation of the Johne's disease course of infection. Susceptible animals (S) could be infected through contact with a contaminated environment (E) or remain uninfected (R). Infected animals first enter a transiently infected stage (T) (this stage has only been described for cows), followed by a long, latent stage of disease (L) and a subclinical stage of disease (I_S). Only a small proportion of the subclinically infected animals reach the clinical stage of disease (I_C). As animals in the transiently infected, subclinical and clinical stage of disease shed MAP in the environment, only these animals contribute to new infections (indicated by broken arrows).

DIAGNOSTIC TESTS

Diagnosis of Johne's disease can be divided in tests used to detect the infectious agent and tests used to detect the MAP specific immune response in the host.

Indicators based on infectious agent detection

Tests used to detect MAP in fecal samples, by culture and by PCR, have high specificity (98-100%) and a stage of disease dependent sensitivity. The sensitivity is relatively high for animals in the clinical stage of disease (around 70%) because in this stage MAP is frequently transmitted via the feces. The sensitivity is low for animals in the subclinical stage of disease (around 25%) because in this stage animals shed MAP intermittently (Nielsen and Toft, 2008; Whitlock et al., 2000).

Indicators based on immune response

The test that is most commonly used to detect a MAP specific immune response is an absorbed ELISA to detect MAP specific antibodies (humoral response) in serum and milk samples. The specificity is high however, the sensitivity of the absorbed ELISA is low for detecting animals in the subclinical stage of disease (around 15%), and increases with advancing stage of disease (70% in clinically infected animals) (Nielsen and Toft, 2008).

Besides the absorbed ELISA to detect a MAP specific antibody response (humoral response), there are tests that detect the cell-mediated response (tuberculin skin test, interferon gamma assay). Tests that detect the cell-mediated response are not routinely used for diagnosis of Johne's disease. The tests require complex logistics, are labor intensive, expensive and there are concerns about the specificity (Santema et al., 2011).

CONTROL STRATEGIES

Infection with Johne's disease leads to economic losses due to lower milk production, decrease in slaughter value and higher animal replacement (Johnson-Ifearulundu et al., 1999). In addition, studies detecting highly significant differences in the occurrence of MAP in individuals with Crohn's disease versus controls (Bull et al., 2003; Naser et al., 2004) and studies showing abnormal distributions in the incidence of Crohn's disease indicating an effect of environmental exposure (Green et al., 2006; Loftus et al., 2007) have led to concern that Johne's disease may be a zoonosis.

Classical control strategies to reduce the impact of MAP from infected farms are: 1) management restrictions to reduce MAP transmission; 2) test and cull strategies to reduce the sources of infection; and 3) vaccination to decrease the susceptibility of the young stock. Management restrictions to reduce MAP transmission focus mainly on avoiding contact of young, susceptible stock with infected animals like separation of progeny from dams immediately after birth. For test and cull strategies to reduce the sources of infection, an absorbed ELISA test is most commonly used because of its simplicity and low cost. Test and cull is the principal control strategy for bovine Johne's disease in the Netherlands. Farmers obliged to test milk of their cows for antibodies specific to Johne's disease at least bi-annually. Positives in the absorbed ELISA may be validated by fecal PCR and after

confirmation, positive cows must be culled, followed by annual re-testing. The principal control strategy for caprine Johne's disease in the Netherlands is vaccination. Nevertheless, despite the effectiveness of the vaccine in reducing the number of clinical cases on the farm, continuous shedding of MAP is suspected and may be a source for new infections. In Norway, a vaccination programme was initiated in 1967 after several years of unsuccessful efforts to eradicate paratuberculosis in goats. The efficacy of the vaccine was judged mainly by post mortem examination of vaccinated and unvaccinated goats in the period 1967-82 and results showed that MAP vaccination offers a high degree of protection to infection (Saxegaard et al., 1985). In Australia, MAP vaccination of Merino flocks was usually effective in reducing prevalence of shedding but the response to vaccination in the different flocks was variable (Dhand et al., 2012). Bastida and Juste (2011) summarized results of vaccination experiments and showed that MAP vaccination performed well in reducing production, epidemiological and pathogenetic effects in cows, sheep and goats.

Classical control strategies mainly reduce transmission of MAP through an adjustment of animal contact structure and lowering the infectious doses in the environment. However, eradication of MAP has been shown to be difficult (Beyerbach et al., 2001) and additional approaches to contribute to control of Johne's disease are needed. One additional approach is selective breeding for animals less susceptible to Johne's disease.

GENETIC VARIATION AND HERITABILITY IN HOST SUSCEPTIBILITY

In dairy cattle, a number of studies estimated genetic variation and heritability for susceptibility to Johne's disease. Genetic variation was shown to exist and heritability estimates for susceptibility to Johne's disease reported in literature range from 0.03 to 0.23 (chapter 4; Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; Küpper et al., 2012). Although the presence of genetic variation involved in susceptibility to Johne's disease in cows has been demonstrated, the understanding of genes contributing to the genetic variance is far from complete. Research revealed contributions to Johne's disease susceptibility from polymorphisms in candidate genes *SLC11A1* (Pinedo et al., 2009b; Ruiz-Larranaga et al., 2010), *TLR1* (Mucha et al., 2009, *TLR2* (Mucha et al., 2009; Koets et al., 2010), *TLR4* (Mucha et al., 2009), *CARD15*/*NOD2* Pinedo et al., 2009a) and

PGLYRP1 (Pant et al., 2011); a quantitative trait locus (QTL) on chromosome 20 (Gonda et al., 2007); regions on chromosomes 3, 9 (Settles et al., 2009) and 12 (Minozzi et al., 2010); a set of 51 SNP covering several chromosomes that could be used as predictor of a MAP susceptibility breeding value in Holstein cattle (Kirkpatrick et al., 2010; and genomic regions on chromosome 1, 5, 6, 7, 10, 11, and 14 (Pant et al., 2010). Zanella et al. (2010 & 2011) revealed contributions to Johne's disease tolerance from regions on chromosomes 1, 2, 6 and 15.

In goats, Singh et al. (2009) found differences in susceptibility to Johne's disease between five native breeds in two agro-climates in India. Differences in susceptibility were determined by examination of body weight, morbidity, and mortality due to Johne's disease, fecal shedding of MAP, and gross and microscopic lesions in target tissues. In another study, goats were classified as resistant and susceptible based on clinical signs, microscopic examination, fecal culture, ELISA and diagnostic PCR. Analysis of the polymorphism in the exon-2 of the caprine major histocompatibility complex Class II *DRB* gene in the susceptible and resistant goats showed association with susceptibility to Johne's disease (Singh et al., 2012).

OBJECTIVE AND OUTLINE OF THIS THESIS

The aim of this thesis was to contribute to control of Johne's disease by investigating genetic variation in the pathogen and studying genetic variation in host susceptibility.

Genetic variation in the pathogen

A better understanding of the biodiversity of MAP offers more insight in the epidemiology of Johne's disease and therefore may contribute to control of disease. Chapter 2 describes genetic variation in bovine MAP isolates between and within herds in the Netherlands using combined Mycobacterial Interspersed Repetitive Units and Variable-Number Tandem Repeats typing to determine if multiple strains of MAP can coexist on farms with endemic MAP infection.

Genetic variation in host susceptibility

Non-zero heritability indicates that part of the phenotypic variation in the population is due to genetics. It also implies that susceptibility to Johne's disease can be potentially changed by means of genetic selection. Chapter 3 describes genetic variation and heritability estimates of antibody response specific for MAP in milk of Dutch Holstein-Friesian cows. Chapter 4 describes a genome-wide association approach aiming to increase understanding of genes contributing to genetic variance as estimated in chapter 3. Chapter 5 describes genetic variation and heritability in infection status as determined by a specific antibody response against MAP in milk of Dutch dairy goats. Chapter 6 describes a model study on the effect of genetic selection for less susceptibility on on-farm prevalence of Johne's disease in dairy cattle.

Chapter 2

Different *Mycobacterium avium* subspecies *paratuberculosis* MIRU–VNTR patterns coexist within cattle herds

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Veterinary Microbiology (2011) 148:419–424.

ABSTRACT

A better understanding of the biodiversity of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) offers more insight in the epidemiology of paratuberculosis and therefore may contribute to the control of the disease. The aim of this study was to investigate the genetic diversity in bovine MAP isolates using PCR-based methods detecting genetic elements called Variable-Number Tandem Repeats (VNTR) and Mycobacterial Interspersed Repetitive Units (MIRU) to determine if multiple MAP strains can coexist on farms with endemic MAP infection.

For 52 temporal isolates originating from infected cattle from 32 commercial dairy herds with known trading history, MIRU–VNTR analysis was applied at 10 loci of which six showed variation. Within the group of 52 isolates, 17 different MIRU–VNTR patterns were detected. One MIRU–VNTR pattern was found in 29 isolates, one pattern in four isolates, one pattern in three isolates, two times one MIRU–VNTR pattern was found occurring in two isolates, and 12 patterns were found only once. Eleven herds provided multiple isolates. In five herds a single MIRU–VNTR pattern was detected among multiple isolates whereas in six herds more than one pattern was found.

This study confirms that between dairy farms as well as within dairy farms, infected animals shed MAP with different MIRU–VNTR patterns. Analysis of trading history and age within herds indicated that cows born within the same birth cohort can be infected with MAP strains exhibiting variations in the number of MIRU–VNTR repeats. These data indicate that such multiple genotypes of MAP can coexist within one herd.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis*, MIRU–VNTR analysis, dairy cattle.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease and MAP causes a chronic granulomatous inflammation of the distal part of the ileum mainly affecting ruminants. Johne's disease is an infectious disease that causes serious animal health problems and has a substantial economical impact on farms where the disease is endemic (Kreeger, 1991). No treatment for the disease exists and controlling is difficult due to its long latent period (Gonda et al., 2007). Strain typing may be a useful additional tool to create a better understanding of the epidemiology of the disease and may contribute to control of Johne's disease.

Sequencing the whole genome of a bovine MAP isolate (Li et al., 2005) led to the development of PCR-based methods based on detection of genetic elements called Variable-Number Tandem Repeats (VNTR) and Mycobacterial Interspersed Repetitive Units (MIRU). Repetitive genetic elements represent one of the rare categories of polymorphic structures in the genomes of highly monomorphic species and are therefore frequently used as markers for differentiation of pathogens associated with human or animal diseases, also for MAP (Bull et al., 2003; Overduin et al., 2004; Romano et al., 2005; Thibault et al., 2007). The aim of this study was to investigate the genetic variation in bovine MAP isolates between and within herds using combined MIRU–VNTR typing to determine if multiple strains of MAP can coexist on farms with endemic MAP infection.

MATERIALS AND METHODS

Animals

Fecal samples of 52 MAP infected dairy cows from 32 herds with endemic MAP infection located across the Netherlands were available for this research. Twenty-one herds provided a single isolate and 11 herds provided two to four isolates. Animal disease status was based on seroreactivity in an absorbed ELISA for bovine Johne's disease expressed as the sample to positive ratio (*S/P*) calculated according to instructions provided by the manufacturer (Institut Pourquier, Montpellier, France) and fecal culture at the Veterinary Health Service

Laboratories (Deventer, The Netherlands) performed in spring 2007. For each animal, age (date of birth) and trading history was available.

Collection and cultivation of bacteria

Fecal shedding of bacteria of the 52 cows was confirmed in spring 2007 by liquid culture in the automated TREK-DS paraJEM ESP culture system (Cleveland, USA) according to instructions provided by the manufacturer.

DNA extraction

DNA for PCR amplifications was obtained by isolating genomic DNA from 1ml of the culture medium using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega Corporation, Madison, USA). The isolates were identified as MAP based on PCR on mycobacterial DNA for the MAP specific IS*900* insertion sequence (Hruska et al., 2005). In addition, the absence of *Mycobacterium avium* subspecies *avium* (MAA) and *Mycobacterium avium* subspecies *hominissuis* (MAH) was identified by PCR on mycobacterial DNA for the specific insertion sequences IS*901* (Inglis et al., 2003) and IS*1245* (Guerrero et al., 1995).

MIRU and VNTR typing

Typing was performed by amplification of a combination of four MIRUs and six VNTRs identified in earlier studies (Bull et al., 2003; Thibault et al., 2007). MIRU-1, MIRU-4 as identified by Bull et al. (2003) were used with primer sequences and PCR conditions as described by Möbius et al. (2008) and VNTR-32, MIRU-2 (alias MIRU-292), MIRU-3 (alias MIRU-X3 and MIRU-1658), VNTR-25, VNTR-3, VNTR-7, VNTR-10 and VNTR-47 were used with primer sequences and PCR conditions as described by Thibault et al. (2007).

The published genomic sequence of MAP strain K10 (GenBank accession number AE16958; reference sequence NC_002944) was used to identify the regions amplified by the given primer sequences. Expected number of tandem repeats and size of tandem repeats at each locus were identified by implementation of the regions in Tandem Repeats Finder software of the Laboratory for Biocomputing and Informatics, Boston University (http://tandem.bu.edu/trf/trf.html), under the default settings of the program. The size of the

amplification product was analyzed on a 1.5% agarose gel (Invitrogen, Carlsbad, USA), compared with the expected product length, and converted into a numerical code which referred to the copy numbers of the repetitions.

Discriminatory power

The discriminatory index (DI) of Hunter and Gaston (1988) was used as numerical index for the discriminatory power of the typing method according to the following formula:

$$DI = 1 - \left[\frac{1}{N(N-1)} \sum_{j=1}^{s} n_j \left(n_j - 1 \right) \right],$$

where N is the total number of isolates in the typing scheme, s is the total number of distinct patterns discriminated by the typing method and strategy, and n_j is the number of isolates belonging to the jth pattern.

RESULTS

MIRU–VNTR typing patterns

For this study, MIRU–VNTR analysis was applied at 10 loci to genotype a panel of 52 MAP isolates originating from 32 herds dairy herds in the Netherlands. Four out of 10 loci showed variation in the number of repeats found in the genome of the 52 isolates (MIRU-2, MIRU-3, VNTR-7 and VNTR-10). MIRU-1 showed three repeats in all isolates but one and VNTR-32 showed nine repeats in 46 isolates but no amplification product could be detected in six isolates. MIRU-4, VNTR-25, VNTR-3 and VNTR-47 did not show variation and were excluded from further analysis. Within the group of 52 isolates, 17 different MIRU–VNTR patterns were detected. One pattern was found in 29 isolates, one pattern in four isolates, one pattern in three isolates, one pattern occurred in two isolates, this happened twice, and 12 patterns were found only once (Table 1).

In addition, Table 1 shows for each pattern the occurrence in the different herds and (average) time to detection of a sample to become positive in the automated TREK-DS paraJEM ESP culture system (Cleveland, USA). The relation between age in months and ELISA *S/P* is depicted in Figure 1. MIRU–VNTR patterns are randomly distributed over different ages. MIRU-VNTR patterns N, O and Q are always related to a low ELISA *S/P*.

Table 1. Results of MIRU-VNTR typing analysis of 52 *Mycobacterium avium* subspecies *paratuberculosis* isolates from 32 Dutch herds with endemic MAP infection.

MIRU-VNTR pattern	Herd no.	No. of isolates	Geographic distribution in the Netherlands	No. of repeats at loci[a]						Time to detection[g,h]
				MIRU-1	MIRU-2 (alias MIRU-292)	MIRU-3 (alias MIRU-X3 and MIRU-1658)	VNTR-7[b]	VNTR-10[c]	VNTR-32[d]	
A	29	1	North	3	3	1	2	2	9	16
B	21	1	North	3	3	2	1	2	9	22
C	7,9,30	3	North, East, West	3	3	2	2	1	9	17
D	1-19	29	North, East, West, South	3	3	2	2	2	9	14
E[f]	24	1	North	3	3	2	2	1+2	9	14
F	25	1	North	3	3	2	4	2	9	17
G[f]	23	1	Unknown	3	3	2	4	1+2	9	Unknown
H[f]	21	1	North	3	3	2	1+2	2	9	17
I[f]	22	1	Unknown	3	3	2	1+3	2	9	22
J[f]	6	1	Unknown	3	3	2	2+4	2	9	15
K	28	1	South	3	3	3	2	2	9	14

L	20,26	4	North	3	4	2	2	2	9	15
M[e]	27	1	East	3	3	2	2	1	N.A.	13
N[e]	5,31	2	North, East	3	3	2	2	2	N.A.	20
O[e,f]	6	2	Unknown	3	3	2	2+4	2	N.A.	17
P[e]	32	1	North	3	4	2	1	2	N.A.	14
Q	8	1	East	1	4	2	2	2	9	13

[a]If the partial repeats at VNTR-32 and VNTR-10 are significant proportions of the entire repeat it is considered as a whole repeat, as done previously in MAP (Castellanos et al., 2010).
[b]Amplicon sizes for the partial repeats at VNTR-7: 1.1 TR (interpreted as 1): 181 bp; 2.1 TR (interpreted as 2): 203 bp; 3.1 TR (interpreted as 3): 225 bp; 4.1 TR (interpreted as 4): 247 bp.
[c]Amplicon sizes for the partial repeats at VNTR-10: 0.9 TR (interpreted as 1): 248 bp; 1.9 TR (interpreted as 2): 303 bp.
[d]Amplicon sizes for the partial repeats at VNTR-32: 8.6 TR (interpreted as 9): 300 bp.
[e]N.A.: no amplification product detected at VNTR-32.
[f]Identification of two repeats at a single locus.
[g]Time to detection of positive sample in days after the start of the incubation period; average time to detection if number of isolates of specific pattern > 1.
[h]No statistic evidence available of a correlation between MIRU-VNTR pattern and the time to detection of a positive sample after the start of the incubation period.

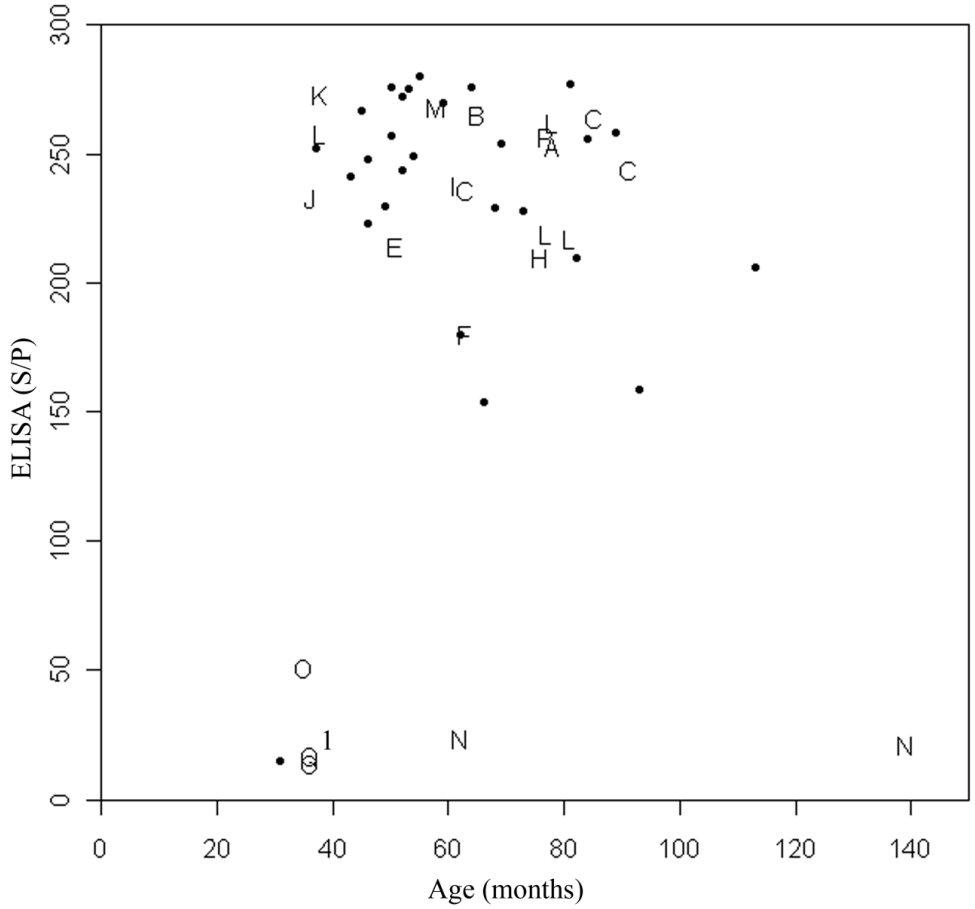

Figure 1. Age in months of 49 animals included in this study versus their ELISA test result expressed as sample to positive ratio (*S/P*). The letters indicate the different MIRU-VNTR patterns found in the *M. avium* subspecies *paratuberculosis* isolates of the animals as shown in Table 1. ELISA *S/P* of two animals with MIRU-VNTR pattern D and one animal with MIRU-VNTR pattern G were unknown. MIRU-VNTR pattern D is denoted by a dot.
[1]MIRU-VNTR patterns O and Q.

Locus specific polymorphisms

Table 2 shows the allelic distribution at the different loci among the MAP isolates typed in this study. The number of polymorphisms found varied between two (MIRU-1, VNTR-32, MIRU-2) and six (VNTR-7). In pattern E, G, H, I, J and O, two repeats were found at a single locus. Presumably, pattern E is a co-infection of patterns D and C, pattern H is a co-infection of patterns D and B and pattern J is a co-infection of patterns D and F. In patterns G and O, the double repeats found at a single locus appeared to be a combination of repeats existing in the population whereas in pattern I, the double repeats appeared to be a combination of an existing repeat in this population and one repeat that has not been described previously.

Table 2. Allelic distribution among *Mycobacterium avium* subspecies *paratuberculosis* isolates from 32 Dutch herds with endemic MAP infection determined by MIRU-VNTR analysis.

No. of isolates with the following TR copy no.:	Locus					
	MIRU-1	MIRU-2 (alias MIRU-292)	MIRU-3 (alias MIRU-X3 and MIRU-1658)	VNTR-7	VNTR-10	VNTR-32
N.A.[a]						6
1	1		1	4	6	
2			50	48	48	
3	51	46	1	1		
4		6		5		
8						46
Sum[b]	52	52	52	58	54	52

[a]N.A.: no amplification product detected.
[b]Sum of no. of isolates with specific TR copy can be more than number of isolates typed in this study because of the detection of two repeats at one locus.

Within herd variation

Eleven herds provided multiple isolates. In five herds only isolates of a single MIRU–VNTR pattern were found whereas in six herds more than one pattern was found (Table 3). Analysis of trading history revealed that none of the animals changed herds between birth and the time of study.

Herd 5 showed coexistence of three times pattern D and pattern N in cows of different ages. MIRU–VNTR pattern D differs from N at one locus. Herd 6 showed coexistence of MIRU–VNTR patterns D, J and O in animals born in the same birth cohort and therefore those animals were likely infected in the same period that indicates coexistence of strains at a farm. Patterns J and O showed two repeats at VNTR-7. The two repeats appeared to be a combination repeats observed in pattern D and another repeat existing in the population. In herd 21 we found coexistence of closely related MIRU–VNTR patterns H and B. Similar as in MIRU–VNTR patterns J and O, pattern H showed two repeats at VNTR-7, which appeared to

be a combination of the repetitive unit found in pattern B and another repetitive unit existing in the population, which suggests co-infection of an animal by two strains.

Discriminatory power

The MIRU–VNTR typing method applied in this study subdivided the 52 isolates originating from 32 herds into 17 genetically distinguishable patterns. The discriminatory index of this typing technique resulted in 0.65. Note that patterns where double repeats occurred at a locus were not included in calculating the discriminatory index (Allix-Beguec et al., 2008; Romero et al., 2008) and only epidemiologically unrelated strains can be taken into account, thus identical isolates from a farm were counted as one (Möbius et al., 2008; Castellanos et al., 2010). Hence, the calculation of discriminatory power was based on 32 isolates originating from 27 herds.

Table 3. Eleven herds with multiple infected animals, age of the animals in months and MIRU-VNTR pattern of the corresponding *Mycobacterium avium* subspecies *paratuberculosis* isolates.

Herd no.[a]	No. of isolates	Age in months at time of study	MIRU-VNTR pattern
1	1	43	D
	2	52	D
	3	89	D
	4	93	D
2	1	49	D
	2	53	D
3	1	45	D
	2	52	D
	3	64	D
4	1	46	D
	2	73	D
	3	82	D
5	1	37	D
	2	59	D
	3	66	D

	4	139	N
6	1	31	D
	2	35	O
	3	36	J
	4	36	O
7	1	63	C
	2	81	D
8	1	36	Q
	2	50	D
9	1	50	D
	2	91	C
20	1	77	L
	2	78	L
	3	81	L
21	1	65	B
	2	76	H

[a]Herd no. corresponds with herd no. in Table 1.

DISCUSSION

This study shows MAP isolates from cattle having different MIRU–VNTR patterns indicating the presence of different MAP strains between and within Dutch dairy herds. The temporal isolates were obtained from herds with a wide geographical distribution on a national scale. The cows observed in this study were not traded after birth. However, as the isolates were not randomly collected from the Netherlands conclusions about distribution of the different MIRU–VNTR patterns across the Netherlands could not be drawn.

The discriminatory index of the typing method used in this study was 0.65. MIRU–VNTR typing is a fast method for molecular epidemiological studies of MAP but combination of this method with other methods may improve the discriminatory index.

All animals in this study were in relatively advanced stages of infection. The height of the ELISA *S/P* of an animal and time to detection of a sample to become positive in the culture

system may be influenced by the strain that causes infection. Figure 1 showed that generally, MIRU–VNTR patterns are distributed over different ages and related to a high ELISA *S/P*. MIRU–VNTR patterns N, O and Q are always related to a low ELISA *S/P*. Isolates with MIRU–VNTR pattern O originated from animals of the same age and one herd; hence this can be an age effect, herd effect as well as a strain effect. Isolates with MIRU–VNTR pattern N did not originate from animals with the same age and were obtained from two herds; this may indicate a strain effect. As suggested previously by Bull et al. (2003) and Castellanos et al. (2010): whether the patterns are associated with more severe disease phenotypes awaits future typing studies e.g. isolates from culture positive but ELISA negative cattle.

One dominant MIRU–VNTR pattern was widely distributed. We hypothesize that the dominant pattern transmits more efficiently between animals or has a higher ability to cause disease in an animal in contrast to other patterns. On the other hand, in six herds that provided multiple isolates more than one pattern was found, and five of these showed coexistence of the dominant pattern with at least one other pattern. This result is in accordance with other studies which indicate that isolates exhibit multiple MIRU–VNTR patterns in a spatially restricted environment, in this case a farm, at a given time (Möbius et al., 2008; Sevilla et al., 2008; Castellanos et al., 2010).

Analysis of trading history indicated that the existence of multiple strains in one herd did not occur due to introduction of the studied cattle to the herd. Analysis of age indicated that animals born on the same farm and even within the same birth cohort can be infected with different MIRU–VNTR patterns. Together these data indicate that multiple MIRU–VNTR patterns can coexist in one herd at a given time and infect different animals. In three of six herds providing multiple isolates coexistence of closely related patterns was observed and this may be an indication that patterns develop over time given the age differences of infected animals.

Six out of 10 loci showed variation in the number of polymorphisms at the MIRU–VNTR loci tested. We conclude that most MIRU–VNTR typed polymorphisms were found in multiple studies although the exact number of repeats per locus varied between studies (Bull et al., 2003; Thibault et al., 2007; Möbius et al., 2008; El-Sayed et al., 2009; Stevenson et al., 2009; Radomski et al., 2010). Supply et al. (2003) stated that the relative degree of genetic information carried by the different *Mycobacterium tuberculosis* loci is globally conserved

but may slightly vary locally in subpopulations or by species. Our findings suggest that this is the case in MAP also.

Exceptional cases were patterns E, G, H, I, J and O, which represented seven of the 52 isolates. At VNTR-7 and VNTR-10 double repetitive units were found that appeared to be a combination of single repetitive units observed in other patterns, where the overlapping pattern may indicate the presence of two strains. The potential co-infection was described for *Mycobacterium bovis* infections (Michel et al., 2008; Romero et al., 2008) and MAP infections (Sevilla et al., 2007; Castellanos et al., 2010). Mixed infection is rarely described in humans and has been associated with high exposure to tuberculosis or super infection (García de Viedma et al., 2003).

CONCLUSIONS

Despite the high level of genetic homogeneity of *Mycobacterium avium* subspecies *paratuberculosis*, MIRU–VNTR based typing was sensitive enough to discriminate isolates on the between and within herd level. Analysis of trading history and age showed that multiple MAP patterns can coexist in one herd at a given time and infect or may co-infect different animals.

ACKNOWLEDGMENTS

The authors would like to thank Ing. Marina Bouman for her valuable technical support.

Chapter 3

Genetic variation for infection status as determined by a specific antibody response against *Mycobacterium avium* subspecies *paratuberculosis* in milk of Dutch dairy goats

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Journal of Dairy Science (2012) 95:6145–6151.

ABSTRACT

Classical control strategies based on management restrictions to reduce transmission, culling of infected goats and vaccination have not been able to eradicate Johne's disease from infected herds. Selective breeding for less susceptibility to disease may be a useful additional tool to contribute to control of the disease. The aim of this study was to estimate genetic variation and heritability for infection status as determined by a specific antibody response against *Mycobacterium avium* subspecies *paratuberculosis* in milk of Dutch dairy goats. Milk samples from 950 goats were tested for antibodies specific to Johne's disease by an ELISA test on five consecutive test days with a time interval of around three months. Test results were coded as infected or not infected according to the instructions of the manufacturer. Heritability of infection status was estimated for three data sets to determine the impact of repeated sampling: only test results obtained on first test day (first-test); the maximum test result of each animal obtained on one of the five test days (max-test); and all test results per animal with a maximum of five consecutive samplings (all-test). Data set first-test and max-test were analyzed with a sire model with fixed effects for year of birth and stage of lactation, and random effects for sire and error. For data set all-test an additional permanent environment effect was included in the model. The estimated heritability on the underlying scale ranged from 0.12 in data set first-test, to 0.09 in data set max-test, to 0.07 in data set all-test.

Keywords: Johne's disease, ELISA, repeated sampling, dairy goat.

INTRODUCTION

Johne's disease, also known as paratuberculosis, is caused by oral uptake of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from the environment. MAP causes granulomatous lesions in the distal part of the ileum in domestic and wild ruminants. In goats, ileal lesions limit sufficient nutrient uptake leading to weight loss, a rough hair coat and skin peeling. In the Dutch Holstein-Friesian dairy cattle population, prevalence of Johne's disease based on a positive ELISA test in milk was 2.4% in 2008 (van Hulzen et al., 2011). Prevalence of Johne's disease in dairy goat herds is unknown but suspected to be higher than in cattle herds based on clinical and routine pathological observations.

Classical control strategies to eradicate MAP from infected goat farms are: 1) management restrictions to reduce MAP transmission; 2) test and cull strategies to reduce the sources of infection; and 3) vaccination to decrease the susceptibility of the young stock. Management restrictions to reduce MAP transmission focus mainly on avoiding contact of young, susceptible stock with infected animals like separation of kids from dams immediately after birth. For test and cull strategies to reduce the sources of infection, an ELISA test is most commonly used because of its simplicity and low cost. In cows, the ELISA test is considered to be highly specific, but of low sensitivity (Whitlock et al., 2000). This is due to the fact that the humoral response, as measured by an ELISA test, is associated with later stages of disease. Moreover, when animals reach the advanced stage of infection in which they show a humoral response, the response may be intermittent. Antibody detection by an ELISA test, even in animals in advanced stages of disease, may therefore be unreliable. Repeated testing of animals over a longer time period can be applied to increase the probability of antibody detection in infected animals and to reduce the sources of infection. Vaccination is the principal control strategy for caprine Johne's disease in the Netherlands. Nevertheless, despite the effectiveness of the vaccine in reducing the number of clinical cases on the farm, continuous shedding of MAP is suspected and may be a source for new infections. In Norway, a vaccination programme was initiated in 1967 after several years of unsuccessful efforts to eradicate paratuberculosis in goats. The efficacy of the vaccine was judged mainly by post mortem examination of vaccinated and unvaccinated goats in the period 1967-82 and results showed that MAP vaccination offers a high degree of protection to infection

(Saxegaard et al., 1985). In Australia, MAP vaccination of Merino flocks was usually effective in reducing prevalence of shedding but the response to vaccination in the different flocks was variable (Dhand et al., 2012). Bastida and Juste (2011) summarized results of vaccination experiments and showed that MAP vaccination performed well in reducing production, epidemiological and pathogenetic effects in cows, sheep and goats. Although these classical control strategies are able to reduce the rate of infection considerably, eradication of MAP has been shown to be difficult and additional approaches to address Johne's disease are needed. One additional approach is selective breeding for animals less susceptible to Johne's disease.

In cows, the heritability of susceptibility to Johne's disease ranged from 0.03 to 0.23 (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; van Hulzen et al., 2011; Küpper et al., 2012). In goats, Singh et al. (2009) found differences in susceptibility to Johne's disease between five native breeds in two agro-climates in India. Differences in susceptibility were determined by examination of body weight, morbidity, and mortality due to Johne's disease, fecal shedding of MAP, and gross and microscopic lesions in target tissues. In the study of Singh et al. (2012), goats were classified as resistant and susceptible based on clinical signs, microscopic examination, fecal culture, ELISA and diagnostic PCR. Analysis of the polymorphism in the exon-2 of the caprine major histocompatibility complex Class II *DRB* gene in the susceptible and resistant goats showed association with susceptibility to Johne's disease. However, a quantification of the genetic variation within a dairy goat population, by using phenotypes for susceptibility to Johne's disease and pedigree information, is lacking.

The objective of this study was to estimate genetic variation and heritability for infection status as determined by a specific antibody response against MAP in milk of Dutch dairy goats.

MATERIALS AND METHODS

Animals

Nine hundred fifty goats originating fromonenon-vaccinated herd with endemic MAP infection were included in the study. Goats were selected based on age (at least two years old

at time of selection) and known sire. Selected goats originated from 56 sires and 847 dams, and were mainly purebreds of the Dutch White milking goat breed, which is closely related to Saanen. Pedigrees and milk production records of the animals were provided by ELDA ICT & Services BV (Rijen, the Netherlands). For each goat, sire pedigree information of at least three generations was available.

Samples

From May 2009 until June 2010, milk samples were collected during five consecutive samplings with a time interval of around three months (Figure 1). Table 1 shows the number of goats tested on the five consecutive samplings. During the study period, goats were culled based on positive test results in a MAP ELISA test, illness or low milk production which decreased the number of tested goats at later test days. The number of tested goats on test day 2 is less compared with test day 3 due to technical problems with electronic animal identification.

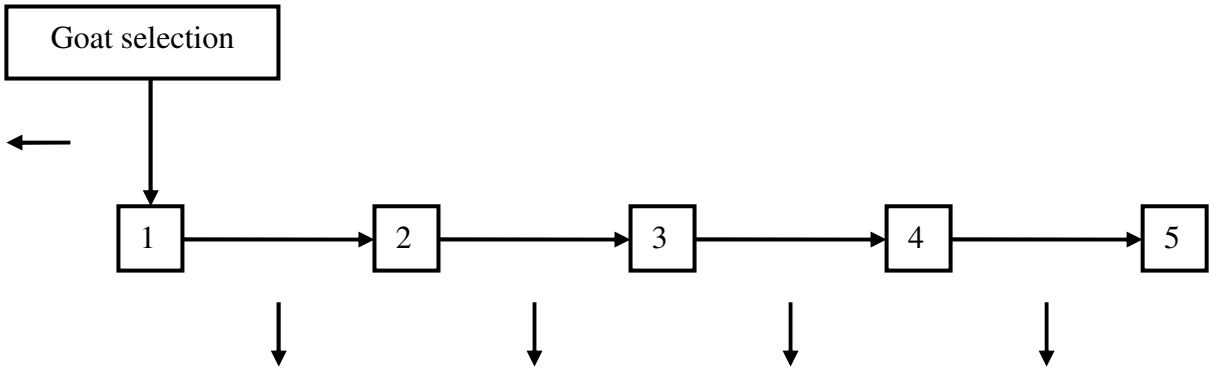

Figure 1. Sampling flow chart: at the start of the study, 950 goats were selected based on age and known sire. Numbers 1 to 5 represent single test days with a time interval of around three months. Culling during the research period (indicated by left-pointing arrow and downward-pointing arrows) was due to (1) selection of animals a few months before start data collection; (2) decision of farmer based on low production or disease or (3) decision of farmer based on positive ELISA.

Antibody detection

All samples were tested 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 by 100 × [the optical density value (OD value) of the sample – the OD value of the negative control] / [the OD value of the positive control – the OD value of the negative control] and coded binary with a cut-off of 40, as recommended by the manufacturer.
Until now, no official validation for the use of an ELISA test on milk samples of goats is available. In this study, the use of an ELISA test in milk was preferred because logistic advantages that allow testing of large number of goats for infection with Johne's disease. To be able to assess the correlation between ELISA test results in serum and in milk, 62 paired serum and milk samples from goats at risk for paratuberculosis were pre-tested.

Impact of repeated measures

To quantify the effect of repeated measures, genetic parameters were estimated on three subsets of the collected data: test results obtained on first test day (first-test); the maximum test result of each animal obtained on one of its test days (max-test); and all test results per animal with a maximum of five consecutive samplings (all-test). All data sets were edited to meet the minimum requirement of five daughters per sire. In total, 950 animals were included in data set max-test and all-test and of these, 878 animals were included in data set first-test.

Statistical analysis

Variance components for the genetic effect for data sets first-test and max-test were estimated using a sire model with a logit link in ASReml (Gilmour et al., 2009):

$$Y_{ijk} = \mu + YB_i + LS_j + sire_k + e_{ijk} . \qquad [1]$$

For data set all-test a repeated measures sire model with a logit link was used:

$$Y_{ijkl} = \mu + YB_i + LS_j + sire_k + pe_l + e_{ijkl} , \qquad [2]$$

where $Y_{ijk(l)}$ is the binary ELISA test result of the individual goat; μ is the general mean; YB_i is the fixed effect of the *i*th year of birth (*i*=2007, 2006, 2005 and ≤2004); and LS_j is the fixed effect of the *j*th stage of lactation in days (*j*= 0-50, 51-100, 100-200, 201-450, and ≥451). $sire_k$ is the random genetic effect of the *k*th sire; pe_l is the random effect of the *l*th animal (permanent environment) and $e_{ijk(l)}$ is the random residual component. The following distributional assumptions were made for the random effects:

$$\mathbf{sire} \sim N(0, \mathbf{A}\sigma_s^2) , \qquad [3]$$

$$\mathbf{pe} \sim N(0, \mathbf{I}\sigma^2_{pe}), \text{ only for model 2, and} \qquad [4]$$

where **sire** is the vector included in the sire additive genetic effect and **pe** is the vector included in the animal effect (permanent environment effect). **A** is the sire relationship matrix, σ^2_s represents the sire variance, **I** is the identity matrix, σ^2_{pe} represents the permanent environmental variance, and σ^2_e represents the residual variance which was fixed at $\pi^2/3$. Variance components were used to estimate the heritability (h^2) on the underlying scale, which was defined as follows: $h^2 = \sigma^2_a/\sigma^2_p$. Additive genetic variation (σ^2_a) was defined as $4\times\sigma^2_s$. Phenotypic variation (σ^2_p) was defined as $\sigma^2_s + \sigma^2_e$ for model 1 and as $\sigma^2_s + \sigma^2_{pe} + \sigma^2_e$ for model 2.

To test the hypothesis that the heritability is significantly different from zero, a likelihood-ratio test (LRT) was performed as twice the difference in log-likelihoods between the full model (model 1 and 2) and the reduced model without a random sire effect. For a single variance component, the theoretical asymptotic distribution of the LRT is a mixture of χ^2 variates, where the mixing probabilities are 0.5, one with zero degrees of freedom and the other with one degree of freedom that has a 5% critical value of 2.71 (Gilmour et al., 2009).

Model comparison

To maximize genetic response for the trait of interest, superior breeding stock for the trait of interest must be accurately identified and selected. Model comparison was used to assess the consistency of results provided by: model 1 including first-test data; model 1 including the max-test data; and model 2 including all-test data. Estimated breeding values of 56 sires were compared using Spearman rank correlation coefficients in R (package 2.90, www.r-project.org).

RESULTS

Correlation between ELISA in serum versus milk

To assess the correlation between ELISA test results in serum and in milk, 62 paired serum and milk samples from goats at risk for paratuberculosis were pre-tested. Out of 62 goats, 54 goats tested negatively in serum and milk and six goats tested positively in serum and milk.

One goat tested negatively in serum and positively in milk and one goat tested positively in serum and negatively in milk.

Prevalence

Prevalence of infection in the selected group as measured by a positive ELISA test in milk was 6% on the first test day (51 out of 878 goats tested positive). Although goats were culled based on positive test results in the ELISA test during the study period, test prevalence remained quite stable: 4% on test day 2 (25 out of 668), 6% on test day 3 (43 out of 712), 8% on test day 4 (47 out of 621), and 2% on test day 5 (10 out of 552). Prevalence of infection in the data set containing the maximum test result of each animal obtained on one out of five test days (max-test) was 0.13. Prevalence of infection in the data set containing all test results per animal with a maximum of five consecutive samplings (all-test) was 0.05.

Genetic variation and heritability

Genetic variation and heritability were estimated for infection status as measured by MAP specific antibody response in Dutch dairy goats using three sets of data (Table 1). Additive genetic variance was largest in data set first-test, followed by data set all-test and data set max-test. The estimated heritability on the underlying scale ranged from 0.12 in data set first-test, to 0.09 in data set max-test, to 0.07 in data set all-test. Only for first-test and max-test, estimated heritability differed significantly from zero ($P < 0.05$).

Model comparison

Spearman rank correlation coefficients were calculated between breeding values estimated from three different combinations of input data set and model and are shown in table 2. The largest correlation was between max-test and all-test (0.90). Correlations between first-test and max-test, and first-test and all-test were lower, 0.61 and 0.68 respectively.

Table 1. Variance components and heritability (with standard error in parentheses) for infection status as determined by a *Mycobacterium avium* subspecies *paratuberculosis* specific antibody response in milk of Dutch dairy goats for data set first-test (test results obtained on test day 1), data set max-test (the maximum test result of each animal obtained on one of its five test days) and data set all-test (all test results per animal with a maximum of five consecutive samplings).

Data set	Variance components[1]					h^2
	σ_s^2	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2	
first-test[2]	0.10	0.40	-	3.29	3.39	0.12 (0.23)
max-test[2]	0.08	0.31	-	3.29	3.37	0.09 (0.13)
all-test	0.09	0.36	2.17	3.29	5.55	0.07 (0.09)

[1] σ_s^2 = sire variance; σ_a^2 = additive genetic variance; σ_{pe}^2 = permanent environmental variance; σ_e^2 = residual variance; σ_p^2 = phenotypic variance.

[2]Estimated heritability differed significantly from zero (P<0.05).

Table 2. Spearman rank correlation coefficients between 56 sire breeding values estimated from model 1 including test results obtained on first test day (first-test); model 1 including the maximum test result of each animal obtained on 1 of its test days (max-test); and model 2 including all test results per animal with a maximum of five consecutive samplings (all-test).

	Model 1		Model 2
	First-test	Max-test	All-test
First-test	1	0.61	0.68
Max-test	-	1	0.90
All-test	-	-	1

DISCUSSION

This study estimated genetic variation and heritability for infection status as determined by a specific antibody response against MAP in milk in one dairy goat herd in the Netherlands in order to investigate if genetic selection for less susceptibility to Johne's disease can contribute to control of disease.

Pre-testing of paired serum and milk samples from 62 goats at risk for paratuberculosis showed a high correlation in infection status as determined by an ELISA. Another study estimated the concordance between paired serum and milk of dairy goats at four stages of

lactation and showed only a significant difference between the proportion of positive serum samples and milk in late lactation. In late lactation milk testing may outperform serological testing (Aggelidou et al., 2011). Both studies show high correlation between ELISA test results in serum and milk and therefore, an ELISA test in milk was selected.

The sensitivity of the ELISA test in milk of goats is unknown but expected to be low. Since the humoral response, as measured by an ELISA test, is associated with later stages of disease, the probability of antibody detection in an infected goat increases with age. To increase the probability of detecting the humoral response, only goats of at least two years of age were included in the study and repeated testing was applied up to five times with an interval of around three months. Repeated testing would allow the calculation of repeatability. However, (technical) calculation of repeatability does not provide any biological information for this data. This is mainly due to the low sensitivity of the test combined with the potential change in antibody titer of infected animals over the one year period.

Goats with a MAP specific antibody response above a defined threshold were considered to be infected. Since the process of infection is said to take place at early age and antibody detection develops in later stages of disease, only goats of at least two years of age were included in this study to increase the probability of detecting infection. The factors age, stage of lactation, parity and number of kiddings during study period were considered for correction in the models by backwards elimination. Age and stage of lactation appeared to have a significant effect ($P<0.05$) on infection status as determined by a MAP specific antibody response in all data sets.

For cows in the Netherlands, prevalence of a positive ELISA test in milk was 2.4% on the animal level and 46.7% on the herd level in 2008 (van Hulzen et al., 2011). Although the prevalence of infection on the goat level was expected to be higher than in cows based on clinical and routine pathological observations, prevalence was measured in a selected group within one herd. The intensive use of vaccination in the general Dutch goat population hampers the estimation of the true prevalence, both on the animal level and herd level.

According to the test prevalence in the different data sets, additive genetic variation was expected to be comparable in first-test and all-test, but additive genetic variation was expected to be highest in max-test. No confounding occurred between the genetic effect and year of birth or stage of lactation. The F-value for year of birth was highest in first-test and estimates

for the different year of birth categories showed low values for the youngest goats included in the study (year of birth 2007). In max-test and all-test, the F-values for year of birth were lower and estimates for the different year of birth categories were much more comparable. This indicates that in the first-test data set the probability of antibody detection in the youngest infected goats was much lower, even though goats were selected based on age to exclude goats in early stages of disease without a detectable humoral response. Repeated testing over a one year period increased the probability of antibody detection of the goats of at least two years of age at the start of the study.

Standard errors of heritability estimates were large (0.09 to 0.23). The standard error of the heritability is useful as an indicator of the precision of the heritability estimate but it is better to use a LRT as a formal test of a component. In contrast to first-test and max-test, the estimated heritability was not significantly different from zero ($P < 0.05$) according to the LRT for all-test. Confounding between the sire effect and the permanent environment effect was observed and resulted in a non-significant heritability for all-test.

Low spearman rank correlation coefficients between first-test and max-test, and first-test and all-test indicate that changes in EBV ranking are to be expected when comparing selection candidates originating from analysis of the first-test data set with selection candidates originating from analysis using the repeated measures. This is most likely due to an improvement in disease phenotype when using repeated measures over time, combined with age clustered sires. As mentioned earlier, testing at the first time point (first-test) provides positive tests mainly for older goats that are more likely to be in advanced stages of disease. As a result, differences between sires in genetic capacity are observed but in reality younger sires do not have test positive progeny yet and are therefore classified as resistant. Data showed that susceptible sires with only two year old progeny at the start of the study were classified as resistant in the analysis of first-test and classified as susceptible in the analysis of max-test and all-test.

The high spearman rank correlation coefficient between the max-test and all-test data set indicates that sires were ranked nearly the same when using the maximum test result of each animal obtained on one of the five test days or all test results per animal with a maximum of five consecutive samplings. However, to our opinion, estimation of genetic variation and heritability in this study should preferably be based on the maximum ELISA test result of

each animal obtained by multiple testing over a longer (1 year) time period to reduce the number of false-negative results included in the analysis.

In this study, genetic variation for infection status as determined by a MAP specific antibody response was quantified in Dutch dairy goats. However, knowledge of genes contributing to genetic variance of susceptibility to caprine Johne's disease is still very limited. In the study of Singh et al. (2012), analysis of the polymorphism in the exon-2 of the major histocompatibility complex Class II *DRB* gene showed association with susceptibility to caprine Johne's disease. As Johne's disease progression contains different stages, a variety of immunological mechanisms may be used to combat infection which suggests involvement of a large number of genes in susceptibility to Johne's disease, each having a small effect.

Potential for genetic improvement of susceptibility to Johne's disease has been shown however, genetic variation and heritability were estimated on herd level and displayed on an underlying scale. Among others, goats included in this study were selected based on known sire. In the Netherlands, pedigree registration is mainly applied when using artificial insemination and as a result, numerous selected goats originated from billy goats that are used for artificial insemination on a national scale. Also, in terms of management factors to reduce transmission of Johne's disease (separate rearing of kids, culling of diseased animals) the farm is representative for Dutch dairy farms. Even though the existence of genetic variation on a national scale is still unknown and difficult to estimate because pedigree registration is not common and vaccination hampers diagnosis, genetic variation for susceptibility to caprine Johne's disease on a national scale is expected to be comparable to estimates in this study. Genetic variation and heritability in Dutch dairy cows were in range with the present study, heritability estimates varied from 0.04 to 0.10 depending on the herd test prevalence (van Hulzen et al., 2011). Further research should include the effect of selection for goats less susceptible to Johne's disease on other (production) traits.

CONCLUSIONS

Differences were observed between goats in their susceptibility to infection with Johne's disease as determined by a specific antibody response against MAP. For this reason, it seems feasible to breed for animals less susceptible to disease to contribute to a more effective

control of Johne's disease. Estimated heritability varied between 0.07 and 0.12 in different subsets of data. Spearman rank correlation coefficients showed that changes in EBV ranking are to be expected when comparing selection candidates originating from the different subsets of data. This difference is most likely due to an improvement in disease phenotype when using repeated measures over a longer time.

ACKNOWLEDGMENTS

The authors thank the farmer for participation in the study, ELDA ICT & Services B.V. (Rijen, the Netherlands) for supplied pedigree information, milk production records and support where needed, and Marina Bouman (Utrecth University, Utrecht, the Netherlands) for her assistance and guidance in the lab. Many thanks to Judith Dijcker, Wiebren Santema, Gerrit Koop, Susanne Eisenberg, Marina Bouman, Boris Schmid, Lindert Benedictus, Sanne Slot, Manouk Vrieling, Thijs Wessels, and Gerrit Oosterhuis (all of Utrecht University) for their excellent assistance during the sampling days.

Chapter 4

Effect of herd prevalence on heritability estimates of antibody response to *Mycobacterium avium* subspecies *paratuberculosis*

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Journal of Dairy Science (2011) 94:992-997.

ABSTRACT

Worldwide, classical control strategies based on hygiene and culling of infected animals have been implemented to eradicate Johne's disease. Breeding for disease resistance may be a useful additional tool to control the disease. The aim of this study was to estimate genetic parameters for the presence of a *Mycobacterium avium* subspecies *paratuberculosis* specific antibody response in milk of Dutch Holstein-Friesian cows using subsets of data based on within herd test prevalence.

The analyzed data set consisted of milk samples of 684,364 animals from 12,077 herds collected during the routine milk production scheme. Milk samples were tested for antibodies specific against Johne's disease by an ELISA test. Heritability estimates were calculated for four different subsets of data to determine the sensitivity of heritability for within herd test prevalence. Results expressed as percentage of the sample to positive ratio were analyzed with 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.

The estimated heritability ranged from 0.031 for the complete data set to 0.097 for herds with a test prevalence of at least 10%. Cross-validation was applied to determine which of the subsets of data produced the most accurate estimated breeding values. Results showed that for genetic selection to contribute to disease control, breeding values were estimated most accurately from herds with at least two positive tested animals. In this subset the heritability was 0.041.

Keywords: Johne's disease, heritability, ELISA, dairy cow.

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, reduced milk production, and eventually they die. The causative agent of Johne's disease is *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Most animals infected with MAP are not able to clear the infection and as a result, 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 management measures to improve hygiene and culling of infected animals but eradication of MAP has been shown to be difficult (Beyerbach et al., 2001). Additional approaches to address Johne's disease are needed. One approach is genetic selection for animals resistant to Johne's disease.

Earlier research showed heritability estimates 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; Attalla et al., 2010). Large variation in estimated heritability in these studies could be due to: 1) differences in incidence of Johne's disease in the research populations, 0.077 (Gonda et al., 2006) versus 0.310 (Koets et al., 2000) before the start of a vaccination trial; 2) differences in sample sizes, 4,524 animals (Hinger et al., 2007) versus 21,514 animals (Attalla et al., 2010); 3) differences in the statistical methods used in the analysis, a binary trait versus linear trait or a sire model versus an animal model, and 4) differences in diagnostic methods, determining infection status based on clinical inspection after slaughter (Koets et al., 2000) versus determining infection status based on ELISA to detect a MAP specific antibodies in milk or blood (Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007). Information from population-wide screening is missing and knowledge of the effect of within herd exposure on estimates of heritability is limited.

The objective of this study was to estimate genetic parameters for the presence of a MAP specific antibody response in milk based on population-wide screening of the Holstein-Friesian cows in the Netherlands. Further, the impact of within herd exposure on estimate of heritability will be quantified using subsets of data based on within herd test prevalence.

Finally, opportunities to reduce test prevalence of Johne's disease by selective breeding will be quantified.

MATERIALS AND METHODS

Data

Milk samples were collected from lactating cows during the routine milk production scheme. Collection, transportation and storage of milk samples were accomplished as described by Van Hulzen et al. (2009). From January until December 2008, milk samples could be sent to the Dutch Animal Health service upon the decision of the farmer to test for antibodies specific for Johne's disease using an ELISA. Testing individual cows with the ELISA was financially supported to stimulate Dutch dairy farmers to participate in the program. Pedigrees and milk production records of the animals were provided by the Dutch cattle improvement (CRV, Arnhem, the Netherlands). The data set was edited to meet the requirements of at least 20 animals per herd and a minimum of five daughters plus granddaughters per sire were met. Only cows with at least 75% Holstein-Friesian genes were included. In 2008, 20,750 dairy farms were present in the Netherlands (CRV, 2009) of which 12,077 herds with 684,364 animals were included in the data (58%).

Antibody detection

All samples were tested 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 by 100 × (the optical density value (OD value) of the sample – the OD value of the negative control) / (the OD value of the positive control – the OD value of the negative control; Van Weering et al., 2007).

Classification of within herd test prevalence

In order to quantify the effect of within exposure on genetic parameter estimation, estimates of variance components were calculated for four subsets of data based on levels of within herd test prevalence: 1) the complete data set, 2) herds with at least two positive tested

animals, 3) 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 define the various subsets of within herd test prevalence each individual animal needed to be classified as positive or negative. Cut-off value used for a positive test result was 25% *S/P*, which was lower than the cut-off value recommended by the manufacturer at 40% *S/P*. Because of our lowered cut-off value for the individual animal we increased the sensitivity, but also increased the likelihood of a false-positive result. Therefore, only herds with at least two positive tested animals were considered to have MAP present on the farm (data set 2).

Statistical analysis

Variance components for the genetic effect were estimated using a sire-maternal grandsire model in ASReml (Gilmour et al., 2006):

$$\ln(Y_{ijklmno} + 50 = \mu + P_i + YB_j + LS_k + b_1 MY_l + HERD_m + sire_n + mgs_o + e_{ijklmno},$$

where $Y_{ijklmno}$ is the ELISA test result of the individual animal. The ELISA test result used in the model is the log-transformed (percentage *S/P* + 50) to approach the normal distribution. μ is the general mean. P_i is the fixed effect of the *i*th parity (*i* = 1, 2, 3, 4, 5 or >5), YB_j is the fixed effect of the *j*th year of birth (*j* = 1993<, 1993 to 2007 as separate classes), LS_k is the fixed effect of the *k*th stage of lactation with six classes: week 1 and 2, week 3-12, week 13-28, week 29-44, week 45-60 and >60 weeks, MY_l is the *l*th milk yield on test day, b_1 is the regression coefficient for MY_l, $HERD_m$ is the fixed effect of the *m*th herd, $sire_n$ is the random effect of the *n*th sire, mgs_o is the random effect of the *o*th maternal grandsire and $e_{ijklmno}$ is the random residual component. The following distributional assumptions were made for the random effects:

$$\mathbf{sire} \sim N(0, \mathbf{A}\sigma_s^2),$$

$$\mathbf{mgs} \sim N(0, \mathbf{A}\frac{1}{4}\sigma_s^2),$$

$$\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2),$$

where **sire** and **mgs** are the vectors included in the sire additive genetic effects and **e** is the vector of residual effects, **A** is the sire relationship matrix, σ_s^2 represents the sire variance, **I** is the identity matrix and σ_e^2 represents the residual variance. Variance components were used to estimate the heritability (h^2), which was defined as follows:

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

where σ_p^2 is the phenotypic variance:

$$\sigma_p^2 = (\sigma_s^2 + \frac{1}{4}\sigma_s^2) + \sigma_e^2.$$

Cross-validation

Heritability as well as the number of progeny per sire varied over the different subsets of data and cross-validation was applied to determine which of the subsets of data produced the most accurate estimated breeding values. Accuracy was estimated by determination of the correlation between predicted breeding values and observed phenotypes using the following steps: a) for each data set, 20% of the phenotypes of each herd were set as missing, which resulted in a subset containing 80% of the data of each herd. This process was non-randomly repeated five times. In this way, each observation was missing once. b) Sire and maternal grandsire breeding values were predicted by the remaining 80% of data in the corresponding data set using the linear mixed model. c) Breeding values for 20% of the cows that were set as missing were estimated as follows: $ebv_{cow} = \frac{1}{2}ebv_{sire} + \frac{1}{4}ebv_{mgs}$. This procedure was repeated five times to obtain an estimated breeding value for each animal in the data set. The final step (d) was to calculate the correlation coefficient between the predicted breeding values and the observed phenotypes of cows. Correlation coefficients were also expressed as percentage of maximum correlation which was calculated as: $\sqrt{\frac{\sigma_s^2 + 1/4\,\sigma_s^2}{\sigma_p^2}}$.

Additionally, correlation coefficients were calculated between predicted breeding values from one subset of data based on levels of within herd test prevalence and observed phenotypes from the remaining three subsets of data based on levels of within herd test prevalence to

investigate the ability of each subset of data to predict observed phenotypes in the other subsets.

Potential genetic gain

To determine how much potential genetic progress could be made by single trait selection, the number of positive tested animals and number of herds without test positive animals in the complete data set were calculated. Subsequently, the estimated sire breeding values were used to eliminate 10% sires most susceptible to Johne's disease. Progeny of those sires were removed from the data and the number of positive tested animals and number of herds without test positive animals were calculated in the reduced data set. The same procedure was repeated for the elimination of 20% sires most susceptible to Johne's disease based on their EBV.

RESULTS

Within herd test prevalence

Prevalence of infection as measured by a positive ELISA test in milk according to our test interpretation demonstrated that of the 12,077 herds participating in the program, 6,438 herds no test positive animals and 1,712 herds contained one test positive animal. In 2,153 herds, at least two 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%.

Heritability

Genetic parameters for susceptibility to Johne's disease were estimated in four different sets of data (Table 1). The estimated heritability ranged from 0.031 for the complete data set to 0.097 for herds with a minimum test prevalence of 10% (data set 4). Table 1 shows an increase in phenotypic variance concurrently with an increase in within herd test prevalence. This can be explained by a relative increase in number of high ELISA test results. Additionally, the heritability increased which indicated a relatively larger increase in genetic variance compared to the phenotypic variance.

Table 1. Sire variance (σ_s^2), residual variance (σ_e^2), and within-herd heritability (h^2) with standard errors between brackets for the presence of a MAP specific antibody response in complete data set and subsets based on within herd test prevalence.

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

[1] $\sigma_p^2 = (\sigma_s^2 + \frac{1}{4}\sigma_s^2) + \sigma_e^2$; $h^2 = 4\sigma_s^2 / \sigma_p^2$.

Cross-validation

The ability of the different subsets of data to predict observed phenotypes in the specific data set itself increased with increasing within herd test prevalence (Table 2; diagonal values). Higher heritability seemed more important than the number of progeny per sire to obtain accurate breeding values.

The ability of the different subsets of data based on levels of within herd test prevalence to predict observed phenotypes in the other subsets of data is depicted in Table 2 (off-diagonal values). Breeding values predicted by data set 1 provided high correlation coefficients with observed phenotypes from data set 1 and 2. Breeding values predicted by data set 2 provided high correlation coefficients with observed phenotypes from data set 2, 3 and 4. Breeding values predicted by data set 3 provided high correlations with observed phenotypes from data set 3 and 4. Breeding values predicted by data set 4 only provided a high correlation with observed phenotypes from data set 4.

Table 2. Correlations between breeding values predicted by complete data set and subsets based on within herd test prevalence and observed phenotypes in the data sets with between brackets the correlations expressed as percentage of maximum correlation.

Data set[1]			Observed phenotypes from data set			
	n	h^2	1	2	3	4
1	684,364	0.031	0.072 (73)	0.099 (87)	0.128 (93)	0.157 (90)
2	265,290	0.041	0.059 (60)	0.096 (84)	0.130 (95)	0.174 (100)
3	104,382	0.060	0.048 (49)	0.082 (72)	0.130 (95)	0.178 (>100)
4	28,916	0.097	0.037 (37)	0.066 (58)	0.104 (76)	0.187 (>100)
Maximum correlation	-	-	0,099	0,114	0,137	0,174

[1]Breeding values were estimated based on different data sets: 1) the complete data set (no restrictions regarding within herd test prevalence), 2) herds with at least two positive tested animals, 3) herds with a within herd test prevalence of at least 5% and 4) herds with a within herd test prevalence of at least 10%.

Potential genetic gain

In Table 3, a decrease in number of test positive animals due to sire elimination can be observed. The complete data set contained 16,627 test positive animals whereas in the data set with 10% sire elimination 11,438 test positive animals were included. After implementation of 20% sire elimination 10,112 test positive animals were left in the data set. In other words, this theoretical approach to determine how much genetic progress could be made by single trait selection showed by 10% sire elimination a reduction in test positive animals of 31% whereas the number of animals in the data set reduced with 21%. Twenty percent sire elimination showed an additional reduction in test positive animals of 8% whereas the number of animals in the data set reduced by 6%.

Table 3 shows that 6,438 out of 12,077 herds had a within herd test prevalence of zero. When progeny of 10% of sires which were most susceptible to Johne's disease based on their estimated breeding values were eliminated from the data, 7,248 herds out of 12,074 had a within herd test prevalence of zero. Elimination of progeny of 20% of the most susceptible sires from the data resulted in 7,576 herds out of 12,073 having a within herd test prevalence of zero.

DISCUSSION

This study estimated genetic parameters for the presence of a MAP specific antibody response in milk of Dutch Holstein-Friesian cows. Besides the genetic capacity of the animal to resist infection to Johne's disease, an important environmental factor for infection is the degree of exposure to MAP (Gonda et al., 2006). A high level of exposure increases the likelihood to become infected and therefore, a high level of exposure is expected to increase the within herd test prevalence. In this study, subsets of data based on within herd test prevalence were used to investigate the effect of exposure to MAP on genetic parameter estimation. The estimated heritability increased from 0.031 for the complete data set to 0.097 for herds with a minimum within herd test prevalence of 10%. Selection on within herd test prevalence may also induce sire selection. Specific sires may produce susceptible progeny and this will increase the herd prevalence resulting in an underestimation of the heritability. However, this was not the case in this study. In the Netherlands, unproven sires are randomly used for breeding. That this is the case becomes apparent in Table 3: when progeny of 10% and 20% most susceptible sires were removed from the data, the number of herds present in the data hardly declined (from 12,077 to 12,074 to 12,072 herds, respectively).

To obtain reliable genetic parameter estimates, the use of an animal model is preferred over a sire model. A sire model does not account for the genetic merit of the dam. Within a sire model, it is assumed that all dams are of similar genetic merit and this can result in bias in the predicted breeding values if there is preferential mating (Mrode, 2005). Everett et al. (1979) and Schaeffer (1983) showed that including the maternal grandsire of the animal could reduce bias compared with the sire model. In this study, a sire-maternal grandsire model was implemented due to limits on computing resources. Besides, infection with Johne's disease may take place early in life as well as intra-uterine (Whittington and Windsor, 2009). Using an animal model in the case of intra-uterine infection will probably give an overestimation of the heritability because intra-uterine infection is included in the genetic effect whereas the sire-maternal grandsire model accounts for the genetic merit of the dam but excludes the intra-uterine infection effect.

Table 3. Number of test positives and number of herds without test positives 1) without the implementation of sire elimination, 2) with the elimination of progeny of 10% sires most susceptible to Johne's disease based on their estimated breeding values and 3) with the elimination of progeny of 20% sires most susceptible to Johne's disease based on their estimated breeding values.

Variable	1) No sire elimination	2) 10% sire elimination	3) 20% sire elimination
# sires	9,870	8,878	7,885
# animals	684,364	539,892	498,666
# test positives	16,627	11,438	10,112
# herds	12,077	12,074	12,073
# herds without test positives	6,438	7,248	7,576

In the case of Johne's disease, different diagnostic methods can be applied to determine infection status of the animal. Sensitivity of an Elisa test is low, indicating that in contrast to determination of infection status at slaughter, an ELISA test to detect MAP specific antibodies in milk or blood is not necessarily positive when the animal is infected. An ELISA test measures the humoral immune response which is often induced when a late-stage disease type 2-like response, characterized by production of immunoglobulin G1 antibodies, predominates. This shift in predominant immune response is often associated with progression to clinical disease (Stabel, 2000; Coussens, 2004). Using an ELISA test in milk facilitates quick testing in many animals and herds but the sensitivity of the test makes it hard to detect all infected animals. Determination of infection status at slaughter or repeated fecal cultures is more sensitive but has prohibitive costs for sampling and testing. In this study, ELISA in milk enabled the analysis of a large sample size and facilitated the estimation of reliable genetic parameters for the presence of a MAP specific antibody response.

The accuracy of breeding value estimation from the different subsets of data was assessed using cross-validation. Accuracy was estimated by determination of the correlation between predicted breeding values and observed phenotypes. On two occasions the percentage of the maximum correlation exceeded 100 percent. This may be the result of rounding errors and underestimation or overestimation of sire breeding values. Because breeding values predicted based on data set 2 gave high correlations with observed phenotypes in the different subsets of data independent of within herd test prevalence, for selection purposes to reduce the

incidence of paratuberculosis it is optimal to estimate breeding values based on herds with at least two positive tested animals resulting in a heritability of 0.041. Including cows from herds where exposure to MAP is absent will not add information to determine the genetic ability of sires. Including all herds were exposure to MAP is present, in this study defined as a herd with at least two positive tested cows, allows the maximum number of daughters to be included in a sire's proof. Using more daughters is preferred over using only herds with at least 5% or 10% positive tested cows although the heritability in these subsets is higher.

This study showed that elimination of progeny of 10% sires most susceptible to Johne's disease based on their estimated breeding values has a strong decreasing effect on the number of positive tested cows in the population and showed an increase of the number of herds without test positive animals. Additionally, genetic-epidemiological models demonstrated that altering the host genotype for disease resistance will alter the transmission of disease through the population, hence the challenge faced by each animal (MacKenzie and Bishop, 1999). Therefore, effects of selection might be larger than predicted by the quantitative genetic theory (Bishop and Stear, 1997). Results show potential for genetic improvement but additional cost-benefit analysis should precede selection, and should also consider the consequences of selection for resistance on other traits. Mortensen et al. (2004) showed a non-significant negative genetic correlation between daily milk yield and ELISA OD value for antibodies specific against Johne's disease in milk. Attalla et al. (2010) showed statistically significant negative correlations of productive life and net merit with sire breeding values for OD value which suggest that selection for productive life and net merit will result in cows genetically more resistant to disease. Implementation of estimated breeding values of sires for susceptibility to Johne's disease in the breeding program can be done in several ways: a) by breeding organizations for their approval decisions; b) by farmers for their mating decisions; c) in combination with other disease breeding values to create a general resistance parameter; and d) in combination with other sustainability parameters to create a general sustainability parameter.

CONCLUSIONS

Differences were observed between cows in their genetic ability to produce antibodies against Johne's disease. This study shows that it is optimal to estimate breeding values based on herds with at least two positive tested animals which resulted in a heritability of 0.041. Although heritability is low, breeding for disease resistance to contribute to a more effective control of Johne's disease seems feasible.

ACKNOWLEDGMENTS

This project was supported by the 'Steering Committee Paratuberculosis and Salmonellosis' which includes members of the Dutch Dairy Board (PZ, Zoetermeer, the Netherlands), Dutch Dairy Association (NZO, Zoetermeer, the Netherlands) and the Dutch organization for Agriculture and Horticulture (LTO Nederland, Den Haag, the Netherlands). The authors thank the farmers for participating in the program, the 'Steering Committee Paratuberculosis and Salmonellosis' for putting the data at our disposal, the Dutch cattle improvement (CRV, Arnhem, the Netherlands) for supplying pedigrees, milk production records and an informative work environment, and Dr. Piter Bijma (ABGC, Wageningen, the Netherlands) for his valuable contribution to the cross-validation analysis.

Chapter 5

Genome-wide association study to identify chromosomal regions associated with antibody response to *Mycobacterium avium* subspecies *paratuberculosis* in milk of Dutch Holstein-Friesians

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Journal of Dairy Science (2012) 95:2740-2748.

ABSTRACT

Heritability of susceptibility to Johne's disease in cattle has been shown to vary from 0.041 to 0.159. Although the presence of genetic variation involved in susceptibility to Johne's disease has been demonstrated, the understanding of genes contributing to the genetic variance is far from complete. The objective of this study was to contribute to further understanding of genetic variation involved in susceptibility to Johne's disease by identifying associated chromosomal regions using a genome-wide association approach.

Log-transformed ELISA test results of 265,290 individual Holstein-Friesian cows from 3,927 herds from the Netherlands were analyzed to obtain sire estimated breeding values for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) specific antibody response in milk using a sire-maternal grandsire model with fixed effects for parity, year of birth, lactation stage, and herd; a covariate for milk yield on test day; and random effects for sire, maternal grandsire and error. For 192 sires with estimated breeding values with a minimum reliability of 70%, SNP typing was conducted by a multiple SNP analysis with a random polygenic effect fitting 37,869 SNP simultaneously.

Five SNP associated with MAP specific antibody response in milk were identified distributed over four chromosomal regions (chromosome 4, 15, 18 and 28). Thirteen putative SNP associated with MAP specific antibody response in milk were identified distributed over 10 chromosomes (chromosome 4, 14, 16, 18, 19, 20, 21, 26, 27, 29). This knowledge contributes to the current understanding of genetic variation involved in Johne's disease susceptibility and facilitates control of Johne's disease and improvement of health status by breeding.

Keywords: genome-wide association approach, Johne's disease susceptibility, ELISA, dairy cow.

INTRODUCTION

Johne's disease, also known as paratuberculosis, occurs mainly in ruminants and is caused by the oral uptake of the causative agent *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from the environment. Johne's disease is characterized by granulomatous lesions in the distal part of the ileum. Ileal lesions elicit the deterioration of nutrient uptake and animals suffering from clinical Johne's disease show weight loss, diarrhea and reduced milk production. Most animals infected with Johne's disease are not able to clear the infection. As a result, Johne's disease has a substantial economical impact on farms where the disease is endemic (Weber, 2006). Classical control strategies on the farm are based on management measures to improve hygiene and culling of infected animals but eradication of MAP from an infected herd has been shown to be difficult (Beyerbach et al., 2001). Additional approaches to address Johne's disease are needed. One approach is genetic selection for animals that are less susceptible to Johne's disease.

Earlier research showed a heritability of susceptibility to Johne's disease in cattle ranging from 0.041 to 0.159 (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; van Hulzen et al., 2011). Non-zero heritability indicates that part of the phenotypic variation in the population is due to genetics. It also implies that susceptibility to Johne's disease can be successfully changed by means of genetic selection. Although the presence of genetic variation involved in susceptibility to Johne's disease has been demonstrated, the understanding of genes contributing to the genetic variance is far from complete. Research revealed contributions to Johne's disease susceptibility from polymorphisms in candidate genes *SLC11A1* (Pinedo et al., 2009b; Ruiz-Larranaga et al., 2010), *TLR1* (Mucha et al., 2009), *TLR2* (Mucha et al., 2009; Koets et al., 2010), *TLR4* (Mucha et al., 2009), *CARD15*/*NOD2* (Pinedo et al., 2009a) and *PGLYRP1* (Pant et al., 2011); a quantitative trait locus (QTL) on chromosome 20 (Gonda et al., 2007); regions on chromosomes 3, 9 (Settles et al., 2009) and 12 (Minozzi et al., 2010); a set of 51 SNP covering several chromosomes that could be used as predictor of a MAP susceptibility breeding value in Holstein cattle (Kirkpatrick et al., 2010); and genomic regions on chromosome 1, 5, 6, 7, 10, 11, and 14 (Pant et al., 2010). Zanella et al. (2010 & 2011)

revealed contributions to Johne's disease tolerance from regions on chromosomes 1, 2, 6 and 15.

The objective of this study was to contribute to further understanding of genetic variation in susceptibility to Johne's disease. A genome-wide association (GWA) approach was selected to perform a multiple SNP analysis fitting 37,869 SNP simultaneously in order to identify chromosomal regions associated with MAP specific antibody response in milk of Dutch Holstein-Friesians. Genes located in the associated chromosomal regions were discussed with respect to their potential role in Johne's disease susceptibility.

MATERIALS AND METHODS

Animals

Milk samples were collected from lactating cows during the routine milk production scheme. Collection, transportation and storage of milk samples was described by van Hulzen et al. (2009). From January until December 2008, milk samples were sent to the Dutch Animal Health service (Deventer, the Netherlands) based on the decision of the farmer to test for antibodies specific for Johne's disease. Antibody testing was financially supported to stimulate Dutch dairy farmers to participate in the program. The raw data set contained over a million records. Pedigrees and milk production records of the animals were provided by the Dutch cattle improvement organisation (CRV, Arnhem, the Netherlands). The data set was edited to meet the minimum of 20 animals per herd and the minimum of five daughters plus granddaughters per sire. Only cows with at least 75% Holstein-Friesian genes were retained. Data from 265,290 cows from 3,927 herds with least two test positive animals for Johne's disease were used for breeding value estimation (van Hulzen et al., 2011).

Phenotype

All samples were tested 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 × [the optical density (OD) value of the sample – the OD value of the

negative control] / [the OD value of the positive control – the OD value of the negative control].

Log-transformed *S/P* ratios of the individual cows were analyzed to obtain sire estimated breeding values (EBV) for MAP specific antibody response in milk using a sire-maternal grandsire model in ASReml (Gilmour et al., 2006) with fixed effects for parity, year of birth, lactation stage, and herd; a covariate for milk yield on test day; and random effects for sire, maternal grandsire and error. Van Hulzen et al. (2011) provides a detailed description of the model and presents results of quantitative genetic analysis of the data used in this study. The quantitative genetic analysis resulted in EBV for 2,702 sires. Reliabilities of EBV were calculated as $\frac{n}{n+k}$, where n is the number of daughters and $k = \frac{1}{1/4\, h^2} - 1$ with $h^2 = 0.041$ (van Hulzen et al., 2011). Of the available 2,702 EBV, 192 EBV had a reliability of at least 70% and were used for further GWA analysis. Figure 1 shows the frequency distribution of 192 sire EBV used for GWA analysis.

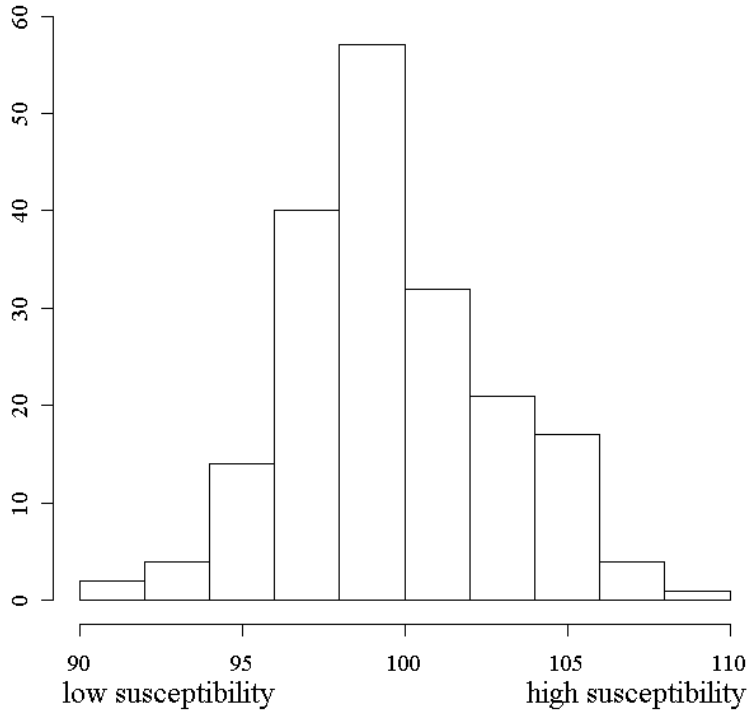

Figure 1. Histogram of breeding values of 192 sires for MAP specific antibody response in milk estimated on ELISA test results of 684,364 cows from 12,077 herds.

Sire EBV were de-regressed to account for (1) shrinkage of EBV using Best Linear Unbiased Prediction (BLUP) estimates and (2) differences in shrinkage of estimated breeding values of sires due to a differences in number of daughters used to calculate BLUP EBV (Thomsen et al., 2001; Garrick et al., 2009; Guo et al., 2010). Deregressed proofs (*DRP*) based on estimated daughter contributions (*EDC*) were calculated using the following formula (VanRaden et al., 2009).

$$DRP = PA + (EBV - PA) \times (\frac{EDC_{parents+progeny}}{EDC_{progeny}}),$$

where *PA* is the parent average. *EDC* were derived from the reliabilities of the EBV using the following formula (Mäntysaari et al., 2010):

$$EDC_{parents+progeny} = \frac{k \times REL_{EBV}}{1 - REL_{EBV}},$$

$$EDC_{parents} = \frac{k \times REL_{PA}}{1 - REL_{PA}}$$

$$EDC_{progeny} = EDC_{parents+progeny} - EDC_{parents},$$

where REL_{EBV} is the reliability of the EBV and REL_{PA} is the reliability of the expected value of the animal (parent average).

DNA extraction and genotyping

Genomic DNA was extracted from sperm with a Qiagen M48 robotic station using the MagAttract DNA Mini M48 kit (Qiagen, Venlo, the Netherlands) according to the instructions of the manufacturer. DNA concentration was determined using picogreen (Quand-iT PicoGreen, Life Technologies, Grand Island, New York, USA) according to the instructions of the manufacturer.

Single nucleotide polymorphism genotypes used were provided by Dutch cattle improvement organisation (CRV, Arnhem, the Netherlands). Genotypes for 37,869 SNP were available, of which 464 SNP were not mapped to any of the 29 chromosomes. The SNP were mapped using the bovine genome assembly Btau_4.0. Following quality control checks, the SNP retained had a high call rate (>95%), minor allele frequency (MAF) of at minimum 0.025% and differed 0.15 units from their expectation not to be in Hardy-Weinberg disequilibrium.

Multiple SNP analysis

The multiple SNP analysis was performed using the following model (Meuwissen and Goddard, 2004):

$$Y_i = \mu + \sum_{j=1}^{37869}(q_{ij1} + q_{ij2})v_j + sire_i + e_i \ ,$$

where Y_i is the *DRP* of the individual sire *i*, μ is the general mean, v_j is the scale parameter of the QTL effect of the SNP at putative QTL position *j*, q_{ij1} (q_{ij2}) is the size of the QTL effect for the paternal (maternal) allele of sire *i* at SNP *j* drawn from a standard normal distribution $N(0,1)$, $sire_i$ is the random polygenic effect of sire *i* and e_i is the random residual component. The following assumptions were made for the random effects:

$$\mathbf{sire} \sim N(0, \mathbf{A}\sigma_s^2),$$

$$\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2),$$

where **sire** is the vector included in the polygenic effects, **e** is the vector of the residual effects, **A** is the relationship matrix, σ_a^2 is the polygenic variance, **I** is the identity matrix and σ_e^2 represents the residual variance. The prior mixture distribution was:

$$v_j \sim \begin{cases} N(0, \sigma_v^2); \text{ with probability } \pi_0 \\ N(0, \frac{\sigma_v^2}{100}); \text{ with probability } \pi_1 = 1 - \pi_0 \end{cases},$$

where the 'null' distribution modeled the majority of SNP with (virtually) no effect using the prior setting $\pi_0 = \frac{29}{37869} = 0.001$, assuming 29 additive and independent QTL affecting the trait across the 29 chromosomes. The second distribution modeled SNP with large effects using the prior setting $\pi_1 = 1 - 0.001 = 0.999$. The variance of v_j, σ_v^2, was sampled from a scaled inverse chi-square distribution with a prior variance. This prior variance was calculated as the additive genetic variance, divided by 29 (Calus et al., 2009). Unequal variances were assumed across SNP.

The presence of a QTL at SNP *j* was sampled from a Bernoulli distribution with probability equal to $\frac{P(v_j|\sigma_v^2) \times \Pr_j}{P(v_j|\sigma_v^2) \times \Pr_j + P(v_j|\frac{\sigma_v^2}{100}) \times (1 - \Pr_j)}$, where $P(v_j|\sigma_v^2)$ is the probability of v_j from

$N(0,\sigma_v^2)$, i.e. $\frac{1}{\sqrt{2\pi\sigma_v^2}} e^{-\frac{v_j^2}{2\sigma_v^2}}$, and $\Pr_j$ is the prior probability of the presence of a QTL at SNP *j*. More details on the prior distributions and the full conditional distributions can be found in (Meuwissen and Goddard, 2004) and (Calus et al., 2008). The multiple SNP analysis was performed using a Markov chain Monte Carlo method using Gibbs sampling to obtain posterior estimates for all the effects in the model. The Gibbs sampler ran for 100,000 iterations and 10,000 iterations were removed as burn-in. In total, four chains were performed.

Significance threshold

Bayes Factors (BF) were calculated to determine the threshold on the posterior probabilities for the selection of significant associations by the following formula:

$$BF = \frac{(\hat{p}_i/(1-\hat{p}_i)}{(\pi_1/\pi_0)},$$

where the estimated posterior probability was denoted by $\hat{p}_i$ and prior probabilities by π_0 and π_1. Guidelines of Kass and Raftery (1995) were used to judge BF: a value above 3.2 is "substantial", a value above 10 is "strong", and a value above 100 is "decisive".

Linkage disequilibrium analysis

Linkage disequilibrium (LD) was calculated according to Hill and Robertson (1968) for the SNP found to be strongly associated to MAP specific antibody response in milk (BF>10) in this study with 200 surrounding SNP. The SNP found to be associated was located in the middle. SNP were considered to be linked at a degree of LD (r^2) of at least 0.7.

Variance explained by SNP

The proportion of genetic variance explained by a SNP was calculated using the following formula assuming no dominance:

$$\sigma_{SNP}^2 = 2 \times p \times q \times a^2,$$

where *p* was the allele frequency of the first allele of the SNP, *q* was the allele frequency of the second allele of the SNP and *a* was the allele substitution effect. The allele substitution effect was calculated by the difference in estimated effects of both alleles ($v_j \times q_{ij1} - v_j \times q_{ij2}$).

The proportion of genetic variance was calculated for the SNP found to be strongly associated to MAP specific antibody response in milk (BF>10) in this study itself and for the SNP with 50 surrounding SNP. The SNP found to be associated was located in the middle.

RESULTS

GWA analysis identified four chromosomal regions strongly associated with MAP specific antibody response in milk on four chromosomes (Table 1). Genome-wide plots displaying the BF of four separate chains and average BF over four chains for different positions on the genome are shown in figure 2.

Two SNP showed association with MAP specific antibody response in milk in four chains. On chromosome 4, ss66537488 at 51425144 bp showed association with an average BF of 81.5 (min=32.9, max=110.0). On chromosome 18, ss61532938 at 22546064 bp showed association with an average BF of 21.8 (min=16.9, max=27.0).

Sixteen SNP showed association with MAP specific antibody response in milk in two out of four chains. Although not significantly associated in all four chains of the analysis, the chromosomal region around the SNP may be in association with the trait of interest. Multiple linked SNP in one association area are drawn randomly from the 'null' distribution of small effects or from the second distribution with SNP of large effects randomly which can cause differences in estimates between chains. Single SNP may not show association in all the chains and may not seem significant but when a group of linked SNP is considered the association area may often be found to have a high probability. Linkage disequilibrium analysis showed that of 16 putative associations, three sets of SNP strongly associated with MAP specific antibody response in milk could be identified located in two chromosomal regions. First, on chromosome 15, ss61536244 at 60257450 bp showed association with an average BF of 8.0 (min=1.7, max=15.3) and ss61536232 at 60260849 bp showed association with an average BF of 14.7 (min=0.8, max=42.8). The degree of linkage disequilibrium (r^2) between ss61536244 and ss61536232 was 0.7. Significant linkage disequilibrium was also identified for ss61536244 and ss61536232 with ss61536234 at 60257030 bp, ss61536246 at 60257679 bp and ss61531353 at 60733532; r^2 = 0.7). Apparently, genetic variation of the chromosomal region associated with MAP specific antibody response in milk was not

attributed to ss61536244 or ss61536232 in all four chains but genetic variation was attributed to a set of five SNP that were in high linkage disequilibrium indicating a chromosomal region associated with MAP specific antibody response in milk on chromosome 15. Second, on chromosome 28, rs41648898 at 11839054 bp showed association with an average BF of 7.2 (min=1.0, max=15.6). Significant linkage disequilibrium was identified for rs41648898 with rs41648897 at 11823511 bp and rs41648896 at 11823594 bp (r^2=0.8), rs41648895 at 11823663 bp and rs41648899 at 11838800 bp (r^2=0.9). Equally to chromosome 15, genetic variation of the chromosomal region associated with MAP specific antibody response in milk was not attributed to rs41648898 in all four chains but genetic variation was attributed to a set of three SNP that were linked indicating a chromosomal region associated with MAP specific antibody response in milk on chromosome 28. The remaining 13 SNP being putative associations are shown in Table 2. In total, SNP found to be strongly associated with MAP specific antibody response in milk explained 0.021% of the genetic variation averaged over four chains.

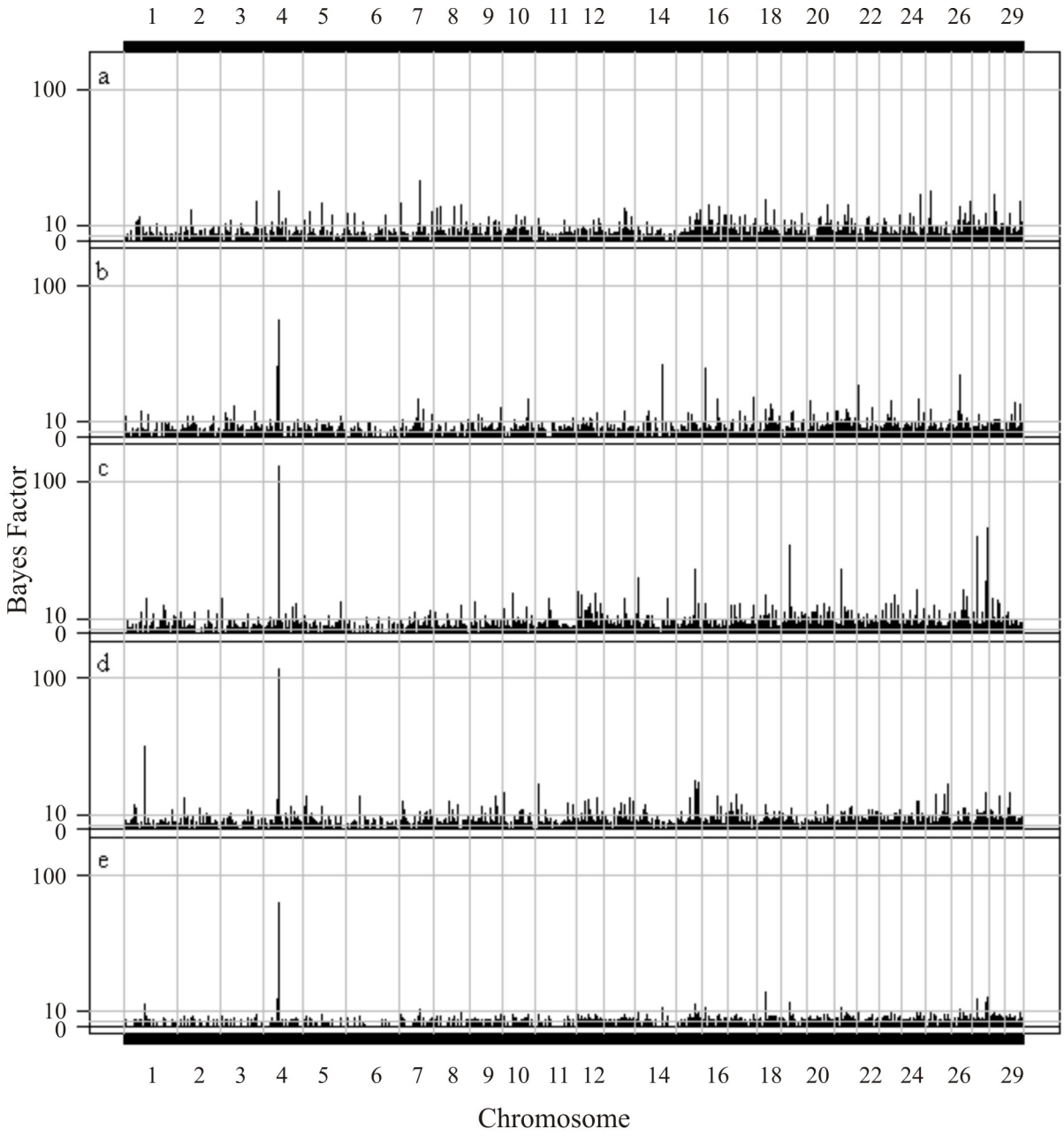

Figure 2. Genome-wide plot of Bayes Factors for an association of loci with *Mycobacterium avium* subspecies *paratuberculosis* specific antibody response in milk. Deregressed proofs of sires with a minimum reliability of 70% were used as response variable and 29 additive and independent QTL were assumed to affect the trait across the chromosomes. The Gibbs sampler ran for 100,000 iterations and 10,000 iterations were removed as burn-in. In total, four chains were performed represented by sub graphs a, b, c, and d. The average Bayes Factor over four chains is represented by sub graph e.

Table 1. SNP found to be associated with MAP specific antibody response in milk, with position (bp) on the chromosome (BTA), SNP id, Bayes Factor, proportion of variance explained by the SNP (σ^2_{SNP}) and 50 adjacent SNP together with the length of the chromosome segment covered and genes found in 1Mbp region.

BTA	Pos (bp)	SNP id[1]	Bayes Factor[2]	σ^2_{SNP}				Genes found in 1Mbp region
				1[3]	50[3,4]	Length of region (bp)	Length of chromosome (bp)	
4	51425144	ss66537488	81.5	1.78E-02	1.98E-02	2513939	124012344	*DLD*, *Q28899*, *LAMB4*, *Q6Q146*, *PNPLA8*, *LOC784535*, *Q2YDK7*, *Q17QP5*, *EPDR1*
15	60257030 - 60733532	ss61536243, ss61536244, ss61536246, ss61536232, ss61531353	-	-	8.71E-04	3999824	84598801	*KCNA4*, *FSHB*, *LOC787432*, *A5PJ77*, *CK046*
18	22546064	ss61532938	21.8	6.83E-04	1.11E-03	23010241	66118950	*A2VDX5*, *IRX5*

28	11823511 - 11839054	rs41648897, rs41648896, rs41648898, rs41648895, rs41648899	-	-	4.81E-04	3658610	45855377	*ACM3*, *Q3SX15*, *ENSBTAG00000018960*, *ZNF25*, *ZNF334*, *LOC534200*, *ENSBTAG00000013592*, *BMS1*, *RET*, *CSGALNACT*, *Q5E9S7*

[1]SNP id as displayed by NCBI dbSNP database.
[2]Average Bayes Factor over four chains.
[3] σ^2_{SNP} is expressed as percentage of additive genetic variance and averaged over four chains.
[4]The SNP found to be associated with MAP specific antibody response in milk was located in the middle.

DISCUSSION

Numerous studies have been carried out to increase the understanding of genetic variation involved in susceptibility and resistance to Johne's disease. Although the presence of genetic variation in the trait of interest has been demonstrated, the identification of genes contributing to the genetic variance is far from complete. Different approaches have been applied to identify genes and chromosomal regions including testing of candidate genes and linkage analysis using low density markers. Following the publication of the bovine genome sequence and the development of the high density SNP chip, GWA analysis revealed information on a number of genomic regions involved in susceptibility to Johne's disease (Gonda et al., 2007; Settles et al., 2009; Kirkpatrick et al., 2010; Minozzi et al., 2010; Pant et al., 2010). Our study showed four chromosomal regions associated with susceptibility to Johne's disease on chromosome 4, 15, 18 and 28 and 13 chromosomal regions putatively associated with Johne's disease susceptibility on chromosome 4, 14, 16, 18, 19, 20, 21, 26, 27 and 29. Putative associations usually occur in situations where: (1) QTL effects are small and therefore difficult to identify; (2) multiple linked SNP in one association area are drawn randomly from the 'null' distribution of small effects or from the second distribution with SNP of large effects randomly which can cause differences in estimates between chains; and (3) a trait displays low heritability. In this study, we applied multiple chains of GWA analysis and linkage analysis in an attempt to create a cleared picture. This helped in some instances but it was not able to eliminate putative associations completely. Further research involving additional data is needed to achieve that goal. Comparison of results with GWA studies previously conducted showed one region in common: ss61487847 and ss86337081 on chromosome 27 (46630699 bp and 46767632 bp) were found only 1.3 Mbp from a SNP found by Minozzi et al., 2010. Little congruence with other studies may be due to the low heritability of the trait, the analysis of different populations and the use of different markers. Furthermore, Johne's disease progression within an animal contains multiple stages: (1) infection of cells by MAP, (2) a cell-mediated response restricts the expansion of MAP and (3) for those animals that progress to Johne's disease, the cell-mediated response is replaced by the humoral response. The different stages involved in the development of Johne's disease and the way the phenotype is defined may also reflect the different chromosomal regions

found to be associated with Johne's disease susceptibility. In our study, Johne's disease susceptibility is defined as having MAP specific antibodies in milk whereas in the other studies susceptibility was defined as having MAP specific antibodies in serum and/or fecal culture of MAP or tissue culture of MAP which may all reflect another stage of disease. Zanella et al. (2010 & 2011) identified loci associated with tolerance to Johne's disease. Tolerance was defined as a cow's fitness at a given level of MAP infection intensity. Moreover, in contrast to other GWA studies on Johne's disease susceptibility (Gonda et al., 2007; Settles et al., 2009; Kirkpatrick et al., 2010; Minozzi et al., 2010; Pant et al., 2010), a multiple SNP GWA approach was used. With multiple SNP analysis, there is simultaneous adjustment for all the genetic variation that is captured by all SNP. This reduces the residual genetic variance, which is expected to result in higher power to detect other SNP associated with the trait of interest. This increase in power is similar to the principles of multiple QTL mapping (Jansen, 1993) and can lead to the detection of new chromosomal regions associated with the trait of interest, which might not be detected in a single SNP analysis and may explain differences found between studies. Additionally, two simulation studies (Sillanpaa and Arjas, 1998; Uleberg and Meuwissen, 2007) showed that confidence interval became smaller when information from all QTL positions was used in multiple QTL analysis compared to single QTL analysis.

The sensitivity of the ELISA used in this study is low, especially for cows in early stages of disease. Low test sensitivity influences the number of infected cows detected by the test. Improvement of diagnosis by ELISA can be achieved by multiple testing. In this study, DRP of sires with a minimum reliability of 70% were used as input for the GWA analysis. Breeding value estimation was performed for sires based on ELISA test results of large numbers of daughters and therefore, despite the low sensitivity of the ELISA, breeding values of sires reflect differences between sires in susceptibility to paratuberculosis defined as MAP specific antibody response in milk.

Table 2. SNP found to be putatively associated with MAP specific antibody response in milk, with position (bp) on the chromosome (BTA), SNP id and Bayes Factor (minimum and maximum value in parentheses).

BTA	Pos (bp)	SNP id[1]	Bayes factor[2]
4	50973953	ss61554144	17.9 (0.3; 46.5)
14	7018689	rs109291899	7.3 (0.3; 12.2)
16	55936466	ss61508455	7.2 (0.3; 15.3)
18	30565399	ss61529077	6.4 (0.5, 12.4)
19	23716787	rs29012539	6.9 (0.4, 14.2)
20	3680593	rs41580314	5.7 (0.4, 10.9)
21	38012768	ss61485088	8.3 (0.8, 18.1)
21	45214439	rs29010390	8.0 (1.7, 15.7)
26	36889435	ss61549364	6.8 (0.5, 15.4)
27	40240120	rs29015783	15.4 (0.8, 34.2)
27	46630699	ss61487847	10.8 (0.9, 18.1)
27	46767632	ss86337081	27.6 (1.2, 89.5)
29	332626	rs29018684	7.0 (1.2, 13.0)

[1]SNP id as displayed by NCBI dbSNP database.
[2]Average Bayes Factor over four chains.

Nine genes are located within 1 Mbp of the significant SNP on chromosome 4. Genes possibly involved in Johne's disease susceptibility and resistance from a biological point of view are suggested to be *LAMB4* (laminin beta 4) and *DLD* (dihydrolipoamide dehydrogenase). The presence of laminin increased interaction of MAP with epithelial cells in vitro (Lefrancois et al., 2011). Therefore, differences in *LAMB4* genotype in cattle may result in differences in development of infection occurring in the early stages. The *DLD* gene codes for an enzyme called dihydrolipoamide dehydrogenase in humans. Dihydrolipoamide dehydrogenase forms a subunit called the E3 component that is shared by several enzyme complexes. Branched-chain alpha-keto acid dehydrogenase, or BCKD, is one of the enzyme complexes that include the E3 component. The BCKD enzyme complex is responsible for one step in the normal breakdown of three amino acids, leucine, isoleucine, and valine which are obtained from the diet. The breakdown of these amino acids produces energy beneficial for

metabolic processes. In cattle, differences in *DLD* genotype and therefore the presumable ability to utilize nutrients may explain why some animals develop to the clinical stage of Johne's disease and others, despite of intestinal lesions, do not. On 1.7 Mbp (53163592-53207516 bp) of ss66537488 on chromosome 4, the *WNT2* gene is located. *WNT* genes encode small secreted proteins and *WNT* signalling is involved in virtually every aspect of embryonic development and also controls homeostatic self-renewal in a number of adult tissues, including the gut (Clevers, 2006). In animals infected with Johne's disease, MAP has the ability to cause granulomatous lesions in the distal part of the ileum which may suggest a role for the *WNT* gene. Multiple genes are located within 1 Mbp of the significant SNP on chromosomes 15, 18 and 28. However, none of these genes are likely to be candidate genes for involvement in Johne's disease susceptibility based on their function as described in literature.

Five SNP found to be associated with Johne's disease susceptibility explained only a small part of the genetic variation (0.021%) in the population. Results of our study suggest that there are many QTL with a small effect and therefore breeding values estimated based on all SNP simultaneously might be more appropriate for selection to reduce MAP infection in the Holstein cattle population.

CONCLUSIONS

This study provides evidence for chromosomal regions involved in MAP specific antibody response in milk. A multiple SNP GWA approach identified five SNP associated with MAP specific antibody response in milk distributed over four chromosomal regions (chromosome 4, 15, 18 and 28) and thirteen putative chromosomal regions. This knowledge contributes to the current understanding of genetic variation involved in Johne's disease susceptibility and facilitates control of Johne's disease and improvement of health status by breeding.

ACKNOWLEDGMENTS

This project was supported by the 'Steering Committee Paratuberculosis and Salmonellosis' which includes members of the Dutch Dairy Board (PZ), Dutch Dairy Association (NZO) and the Dutch organization for Agriculture and Horticulture (LTO Nederland). The authors thank the farmers for participating in the program, the 'Steering Committee Paratuberculosis and Salmonellosis' for putting the data at our disposal, the Dutch cattle improvement (CRV, Arnhem, the Netherlands) for supplying pedigrees, milk production records and SNP data of individual sires.

Chapter 6

The effect of genetic selection for Johne's disease resistance in dairy cattle: results of a genetic-epidemiological model

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Submitted to Veterinary Research.

ABSTRACT

The objective of this study was to model genetic selection for Johne's disease resistance and to study the effect of different selection strategies on the prevalence in the dairy cattle population. In the Netherlands, a certification-and-surveillance program aiming to reduce sources of infection uses an ELISA test in milk to detect and cull infectious dairy cows in infected herds. To investigate the additional genetic effect of the certification-and-surveillance program, genetic selection was applied to the cows by selection of those cows that test negative for Johne's disease at the point the observations are made (dam selection). To investigate the genetic effect of selection at the sire level, selection of 80% of sires producing the most resistant offspring based on their breeding values was applied (sire selection). Parameters affected by genetic selection were: 1) length of latent period ($1/\gamma_L$); 2) susceptibility, i.e. the number of infectious doses needed to become infected ($1/\beta$); and 3) length of susceptible period ($1/\nu$). The effect of selection on the parameters under selection was translated to an effect in prevalence to assess the contribution of genetic selection to control of Johne's disease. For dam selection, responses to selection for Johne's disease resistance were small, irrespective of the parameter that was affected in genetic selection. For sire selection, responses to selection were much larger for all three parameters under selection. If genetic selection has an effect on one of three parameters under selection, response to selection was largest for the length of the susceptible period ($1/\nu$), followed by the susceptibility ($1/\beta$) and then the length of the latent period ($1/\gamma_L$), irrespective of the selection method applied. Sensitivity analysis of parameters under selection for disease prevalence in the population was applied and results revealed that the ranking of parameters under selection is not sensitive to disease prevalence. This study shows that genetic selection for Johne's disease resistance on the sire level is able to contribute to control of Johne's disease in the long run.

Keywords: Johne's disease, genetic-epidemiological model, selection response, dairy cow.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease. Infection with Johne's disease is characterized by lesions in the distal part of the ileum, mainly seen in ruminants. Ileal lesions hinder an efficient nutrient uptake and cows suffering from Johne's disease show diarrhea, weight loss and a decrease in milk production.

Classical control strategies to eradicate MAP from infected dairy cattle farms are: 1) management restrictions to reduce MAP transmission; and 2) test and cull strategies to reduce the sources of infection. Management restrictions to reduce MAP transmission focus mainly on avoiding contact of susceptible young stock with infected animals by for example separation of calves from dams immediately after birth. For test and cull strategies to reduce the sources of infection, an ELISA test is most commonly used to identify infected cows. In the Netherlands, a certification-and-surveillance program aiming to reduce sources of infection uses an ELISA test in milk to detect and cull infectious dairy cows in infected herds, once every year. The ELISA test is considered to be cheap, highly specific, but of low sensitivity (Whitlock et al., 2000). Antibody detection by an ELISA test, even in cows in advanced stages of disease, may therefore be insensitive. Although management restriction and test and cull strategies are able to reduce the rate of infection considerably, eradication of MAP has been shown to be difficult and additional approaches to control Johne's disease are needed. One additional approach could be genetic selection for cows resistant to Johne's disease.

Earlier research showed heritability estimates of susceptibility to Johne's disease in cattle ranging from 0.03 to 0.23 (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; van Hulzen et al., 2011; Küpper et al., 2012) where susceptibility to disease was defined as having MAP specific antibodies in milk or serum, or positive fecal culture or tissue culture of MAP. Non-zero heritability indicates that part of the phenotypic variation in the population is due to genetic variation between cows. It also implies that susceptibility to Johne's disease can be changed by means of genetic selection. Selection for resistance to an infectious disease has a direct genetic effect, by reducing the chance to become infected when exposed to the pathogen in the environment. In addition, it has an indirect epidemiological effect, because if fewer animals become infected, it reduces

total pathogens in the environment and in this way exposure to pathogen for all animals. To be able to accurately predict the long-term effect of genetic selection for disease resistance, it is therefore necessary to combine the heritability estimates with the epidemiological model, as Nieuwhof et al. (2009) did for footrot in sheep.

The objective of this study was to model genetic selection for Johne's disease resistance and to study the effect on the prevalence in the dairy cattle population using a deterministic genetic-epidemiological model, adapted from the model of Marcé et al. (2011). Different breeding strategies were evaluated. In the first scenario on the selection of dams, the additional genetic effect of the certification-and-surveillance program currently used in the Netherlands was investigated. Phenotypic information as it is collected in the Dutch certification-and-surveillance program was used to select dams based on their genetic merit for resistance to Johne's disease (dam selection). In the second scenario on the selection of sires, the effect of selection to the male side of the breeding program was investigated (sire selection). The effect of selection to the female side (dam selection) in combination with the male side (sire selection) was also investigated (combined selection). Parameters under selection were: 1) length of latent period ($1/\gamma_L$); 2) susceptibility, i.e. the number of infectious doses needed to become infected ($1/\beta$); and 3) length of susceptible period ($1/\nu$). The effect of selection on the parameters under selection was translated to an effect in prevalence to assess the contribution of genetic selection to control of Johne's disease.

MATERIAL AND METHODS

Epidemiological model

The model was adapted from the model described by Marcé et al. (2011) that was used to assess the effect of contact structure on MAP transmission in persistently infected dairy herds. The model was chosen because it was recently published and included an environmental compartment to model infection through contamination in the environment. For the purposes of this study, the model was simplified by reducing the multiple susceptible, transiently infected and environmental compartments to one for each stage (Figure 1).

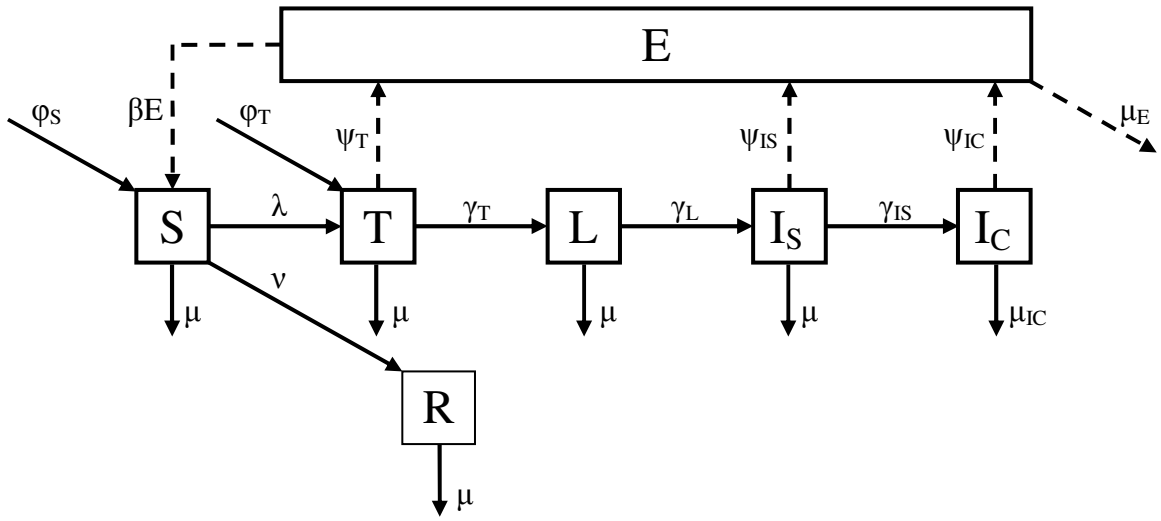

Figure 1. Schematic representation of the path of infection with Johne's disease, where *E* is the environmental compartment and all other compartments represent disease states.

In our model, the host population is divided into susceptible animals (*S*), transiently infected animals (*T*), latently infected animals (*L*), subclinically infected animals (I_S), clinically infected animals (I_C) and resistant animals (*R*). Calves are born either susceptible or transiently infected through vertical transmission. Susceptible animals can become transiently infected through contamination in the environment, but if they do not become infected during early age (first 50 weeks of life), they gain long-lasting immunity to infection (age-dependent susceptibility). The presence and persistence of MAP in the environment is considered by modeling environmental contamination (*E*). Calves (*T*) and adult cows (I_S and I_C) are considered to be infectious, i.e. they shed MAP in their feces and contaminate the environment. The quantity of MAP organisms shed by an animal depends on its infection status (Marcé et al., 2011). The environment (*E*) is measured in units of MAP excreted on a weekly base by a clinically infected cow.

The equations describing the weekly rate of change (in proportions) in each compartment are:

$$dS/dt = \varphi_S - \lambda S - \nu S - \mu S$$
$$dE/dt = \psi_T T + \psi_{I_S} I_S + \psi_{I_C} I_C - \mu_E E$$
$$dT/dt = \varphi_T + \lambda S - \gamma_T T - \mu T$$
$$dL/dt = \gamma_T T - \gamma_L L - \mu L$$
$$dI_S/dt = \gamma_L L - \gamma_{I_S} I_S - \mu I_S$$
$$dI_C/dt = \gamma_{I_S} I_S - \mu_{I_C} I_C$$
$$dR/dt = \nu S - \mu R$$

The parameter λ represents the rate at which susceptible animals become infected ($\lambda = \beta E$). All parameters are defined in Table 1. All parameter values used in the model were adapted from literature, with exception of β. The model was calibrated with β to obtain a start prevalence for Johne's disease infection that reflected an average Dutch herd (0.087).

Losses from the host population. Losses occur as a result of routine culling and sales or deaths. These losses are summarized by the parameter μ, which is similar in all compartments except I_C. For clinically infected animals, losses from the herd are summarized by parameter μ_{I_C}, which includes an increased loss due to additional culling related to clinical Johne's disease.

Births to the host population. Animals in the latently infected (L), subclinically infected (I_S), clinically infected (I_C) and resistant (R) compartments are assumed to give birth to newborn calves. Vertical transmission results in the birth of transiently infected calves, with a probability of 0.15 for latently and subclinically infected cows, and a probability of 0.65 for clinically infected cows (Benedictus et al., 2008; Whittington and Windsor, 2009). Resistant animals are assumed to give birth only to susceptible calves. The overall birth rate is equal to the overall loss rate to ensure a constant population size.

Equilibrium prevalence. Because of our aim of selection of test negative animals, we define prevalence as the proportion of test positives, which are all animals in states I_S and I_C. An equilibrium state is reached when the environmental contamination as well as the proportion of animals in each compartment does not change. In other words, the rates of change for all compartments are all zero, i.e. $dE/dt = dS/dt = dT/dt = dL/dt = dI_S/dt = dI_C/dt = dR/dt = 0$. By programming this in Mathematica 7.0 (Wolfram Research), the equilibrium prevalence (I_S

+ I_C) can be calculated as a function of all parameters included in the model, or a single parameter value can be calculated given all other parameters and the equilibrium prevalence.

Predicting responses to selection

In this study, the epidemiological model described above was used to predict responses to selection (expressed as a reduction in prevalence of test positives) in the dairy cattle population for: 1) genetic selection in cows, i.e. selection of those cows that test negative for Johne's disease at the time point at which the observations are made (dam selection); 2) selection of 80% of sires producing the least susceptible offspring based on their breeding values (sire selection); and 3) selection based on a combination of 1 and 2 (combined selection).

Disease dynamics that were supposed to be affected by genetic selection in this study were: 1) the rate at which latently infected animals become infectious, i.e. selecting animals that have an increased period of latency ($1/\gamma_L$), 2) the transmission rate, i.e. selecting animals that are more resistant to infection, requiring exposure to a greater number of infectious doses before becoming infected ($1/\beta$); and 3) the rate at which susceptible animals become resistant, i.e. selecting animals that have an shorter period of susceptibility ($1/\nu$). Responses in parameters were assessed independent of each other in separate analyses as observed differences in prevalence were due to one of these three. It was assumed that the inverse rates were normally distributed in the population, reflecting a normal distribution of the mean latent period ($1/\gamma_L$ weeks), of the mean number of infectious doses needed to become infected ($1/\beta$ infectious doses), and of the mean age of becoming age-resistant ($1/\nu$ weeks) with constant underlying variances, thus ignoring the Bulmer effect. Start prevalence and heritability of resistance were $p_0 = 0.087$ and $h^2 = 0.10$ respectively.

First round of selection: the threshold model. To obtain knowledge on the parameter under selection ($1/\gamma_L$, $1/\beta$ or $1/\nu$, from now on expressed as θ), the response to selection was calculated with a threshold model assuming a standard normal distribution on an underlying, liability scale. On this scale, the initial prevalence p_0 was translated to liability $x_0 = F^{-1}(p_0)$, with F^{-1} being the inverse standard normal distribution function. The first selection round

results in a new liability x_1, R being the response to selection ($x_1 - x_0$), and new prevalence $p_1 = F(x_1)$. The response to selection was calculated as $R = \frac{ir_{IH}h\sigma_p}{2}$ with

- i as the intensity of selection, which is equal to $\frac{d(F^{-1}(1-p))}{1-p}$, with d being the density function of the standard normal distribution. Prevalence p differs for dam and sire selection. For dam selection, prevalence is defined as the prevalence of test positives for Johne's disease at the time point at which the observations are made. For sire selection, prevalence is defined as the exclusion of 20% of sires producing the most susceptible offspring based on their breeding values (constant over generations).

- r_{IH} as the accuracy of selection, which is different for sire or dam selection. For dam selection, the accuracy of selection is equal to $\sqrt{h^2}$. For sire selection, the accuracy is, among others, dependent of the prevalence in the population as sire breeding values for resistance to Johne's disease are estimated based on test results obtained in the cow population. The accuracy was calculated as follows:

$$r_{IH} = \sqrt{\frac{\frac{1}{4}z^2h^2}{\frac{1}{4}z^2h^2 + \left[p(1-p) - \frac{1}{4}z^2h^2\right]/n}},$$

where p is the prevalence of Johne's disease in the dairy cattle population, n is the average number of progeny of sire in the population (set to 100) and z is equal to $d(F^{-1}(p))$, with d being the density function of the standard normal distribution.

- h as the square root of the heritability, so $h = \sqrt{0.10}$.

- σ_p as the phenotypic standard deviation, equal to 1 by definition, because of the liability scale.

For dam selection as well as sire selection, genetic selection only takes place at the female level or at the male level. Dam or sire provides only 50% of the genetic material to the next generation and therefore the selection response was divided by 2. To predict the effect of genetic selection when applying combined dam and sire selection, calculated selection responses were added and divided by two.

Connecting to the epidemiological model. In the epidemiological model, p_1 was used to calculate θ_1, the value for the trait under selection in the next generation. The response to selection in the first round was thus calculated as $|\theta_1 - \theta_0|$, which was in turn used to calculate the phenotypic standard deviation of the trait under selection (σ_θ) with the standard selection response formula $ir_{IH}h\sigma_\theta = |\theta_1 - \theta_0|$, so that:

$$\sigma_\theta = \frac{|\theta_1 - \theta_0|}{ir_{IH}h},$$

with *i*, r_{IH} and *h* as described above.

Procedure for subsequent rounds of selection. With values for p_1, θ_1, σ_θ and the standard selection response formula ($\theta_2 - \theta_1 = ir_{IH}h\sigma_\theta$), θ_2 was calculated. With the formula for the equilibrium prevalence, the corresponding prevalence in the next generation (p_2) was calculated. This procedure was repeated until the proportion of subclinically and clinically infected animals in the population was less than 0.005 representing a zero prevalence of infection in a herd of 100 cows (less than 0.5 test positive cow).

Output evaluation

To compare the effect of genetic selection for Johne's disease resistance between the different selection methods (dam selection, sire selection and combined) and parameters under selection (length of latent period $1/\gamma_L$, susceptibility $1/\beta$ and length of susceptible period $1/\nu$), we used the number of generations to reach the proportion of subclinically and clinically infected cows of less than 0.005 representing a zero prevalence of infection considering a herd of 100 cows. Furthermore, for sire selection the changes in parameters under selection were studied after one round of selection and after the final round of selection needed to reach a 0.005 prevalence of infection.

Table 1. Parameter definitions and values. All rates are per week.

Parameter	Definition	Value	Source
S	Density of hosts susceptible to infection	-	-
T	Density of transiently infected hosts	-	-
L	Density of latently infected hosts	-	-
I_S	Density of subclinically infected hosts	-	-
I_C	Density of clinically infected hosts	-	-
R	Density of hosts resistant to infection	-	-
E	Density of infectious doses in environment	-	-
φ	Total birth rate	$\mu S + \mu T + \mu L + \mu I_S + \mu_{I_C} I_C + \mu R$	
φ_S	Birth rate of susceptible animals	$\frac{0.85L + 0.85I_S + 0.35I_C + R}{L + I_S + I_C + R}\varphi$	Benedictus et al., 2008; Whittington and Windsor, 2009
φ_T	Birth rate of transiently infected animals from latently and subclinically infectious cows	$\frac{0.15L + 0.15I_S + 0.65I_C}{L + I_S + I_C}\varphi$	Benedictus et al., 2008; Whittington and Windsor, 2009
λ	The rate at which susceptibles become transiently infectious	βE	
β	The rate of transmission	0.175	

γ_T	The rate at which transiently infectious animals become latently infected	0.04	van Roermund et al., 2007
γ_L	The rate at which latently infected animals become subclinically infectious	0.02	Nielsen and Ersboll, 2006; Nielsen, 2008
γ_{I_S}	The rate at which subclinically infectious animals become clinically infectious	0.01	Matthews, 1947
ν	The rate at which susceptibles become resistant	0.02	Marcé et al., 2011
ψ_T	The rate at which an transiently infectious animal sheds infectious doses in environment	0.00003	van Roermund et al., 2007
ψ_{I_S}	The rate at which an subclinically infectious animals sheds infectious doses in environment	0.00026	Rossiter and Burhans, 1996
ψ_{I_C}	The rate at which an clinically infectious animals sheds infectious doses in environment	1	Whittington et al., 2000; Jorgensen, 1982
μ	Death rate	0.005	
μ_{I_C}	The rate at which clinically infectious animals are removed from the farm	0.04	Marcé et al., 2011
μ_E	Outflow of infectious doses from environment	0.40	Jorgensen, 1977; Whittington et al., 2004

RESULTS

Dam selection versus sire selection

For dam selection (i.e. selecting those cows that test negative for Johne's disease at the time point at which the observations are made), responses to selection assuming single trait selection for Johne's disease resistance were small, irrespective of the parameter that was affected by genetic selection (Figure 2). Parameters under selection, length of latent period ($1/\gamma_L$), susceptibility ($1/\beta$) and length of susceptible period ($1/\nu$), needed 218, 146 and 113 generations to reach a 0.005 prevalence of infection.

For sire selection (i.e. selecting 80% of sires producing the least susceptible offspring based on their breeding values), responses to selection for Johne's disease resistance were larger compared to dam selection (Figure 3). As well as for dam selection, differences were observed for parameters under selection to reach a 0.005 prevalence of infection. Length of latent period ($1/\gamma_L$), susceptibility ($1/\beta$) and length of susceptible period ($1/\nu$) required 25, 18 and 15 generations to reach a 0.005 prevalence of infection.

Combined selection resulted in the largest response to selection for Johne's disease resistance (Figure 4). Parameters under selection, length of latent period ($1/\gamma_L$), susceptibility ($1/\beta$) and length of susceptible period ($1/\nu$), needed respectively 22, 16 and 14 generations to reach a 0.005 prevalence of infection.

Parameters under selection

Response to selection assuming single trait selection for Johne's disease resistance was largest for the length of the susceptible period ($1/\nu$), followed by the susceptibility ($1/\beta$) and then the length of the latent period ($1/\gamma_L$), irrespective of the selection method applied.

Table 2 shows change in parameters under selection after one round of sire selection and after the final round of sire selection needed to reach a 0.005 prevalence of infection. The length of the latent period increased from 50 to 52.4 weeks (+4.9%) after one round of selection on $1/\gamma_L$, the number of infectious doses needed to become infected increased from 5.71 to 5.79 doses (+1.2%) after one round of selection on $1/\beta$ and the length of the susceptible period decreased from 50 to 49.3 weeks (-1.5%) after one round of selection on $1/\nu$. Relative change

in parameter under selection after one round of selection was largest for $1/\gamma_L$, followed by $1/\nu$ and then $1/\beta$. To reach a 0.005 prevalence of infection, the value of $1/\gamma_L$ almost doubled in comparison with the starting value, whereas the value of $1/\beta$ and $1/\nu$ showed a 17% difference compared to the starting value. The fraction of total change achieved in first round was largest for the length of the susceptible period ($1/\nu$), followed by the susceptibility ($1/\beta$) and then the length of the latent period ($1/\gamma_L$) which is in agreement with the ranking of parameters in the predicted number of generations to reach a 0.005 prevalence of infection.

Table 2. For sire selection, change in parameters under selection after one round of genetic selection and after the final round of genetic selection needed to reach a 0.005 prevalence of infection. Relative change (expressed in percentages in comparison with start value) is shown between brackets. Parameters under selection are: length of latent period in weeks ($1/\gamma_L$), susceptibility ($1/\beta$) and length of susceptible period in weeks ($1/\nu$).

Parameter under selection	Start value	Value after first round of selection	Value after final round of selection	Fraction of total change achieved in first round	Required number of generations
$1/\gamma_L$	50.0	52.4 (+4.9)	96.5 (+93.0)	0.05	25
$1/\beta$	5.71	5.79 (+1.2)	6.73 (+17.8)	0.07	18
$1/\nu$	50.0	49.3 (-1.5)	41.1 (-17.8)	0.08	15

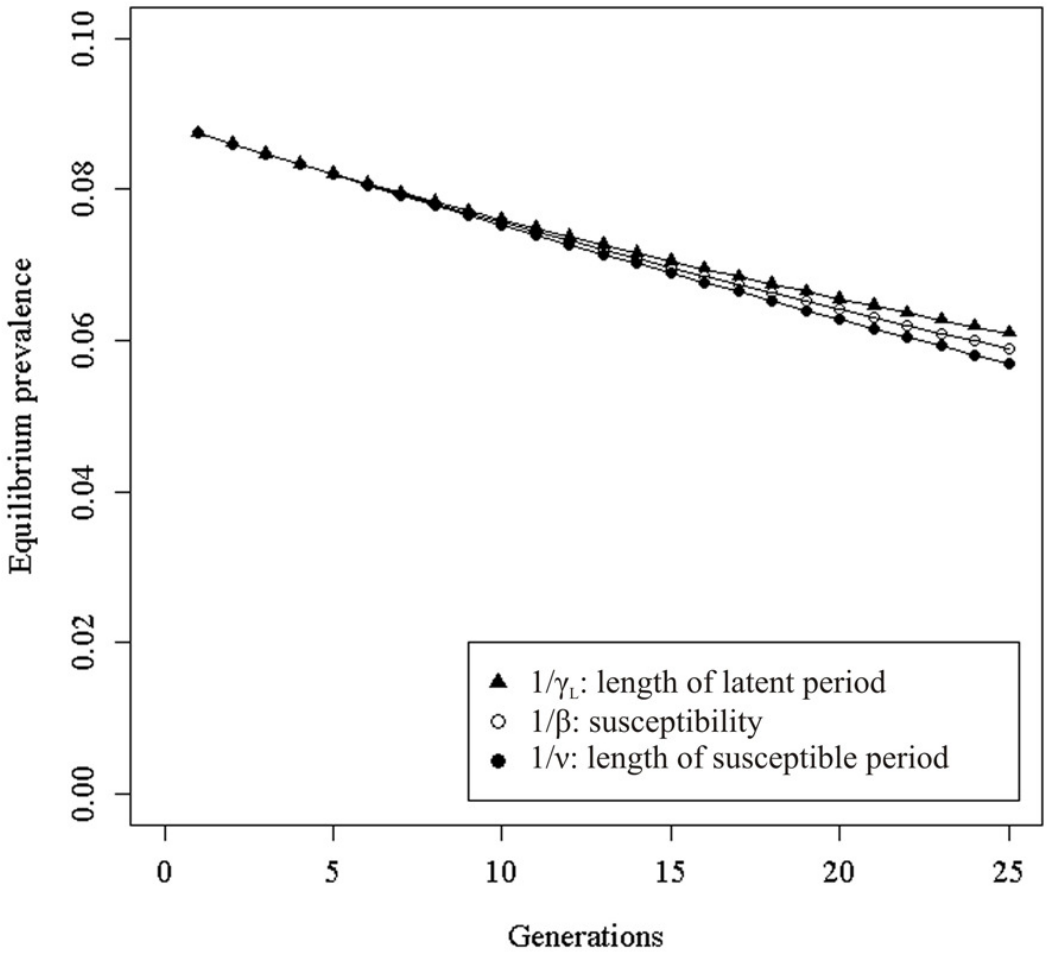

Figure 2. Predicted response to selection for resistance to Johne's disease when selecting those animals that do not show clinical signs at the time point at which the observations are made for the three parameter under selection ($1/\gamma_L$,$1/\beta$ or $1/\nu$). The effect of selection on the parameters under selection was translated to an effect in prevalence (y-axis) to assess the contribution of genetic selection to control of Johne's disease.

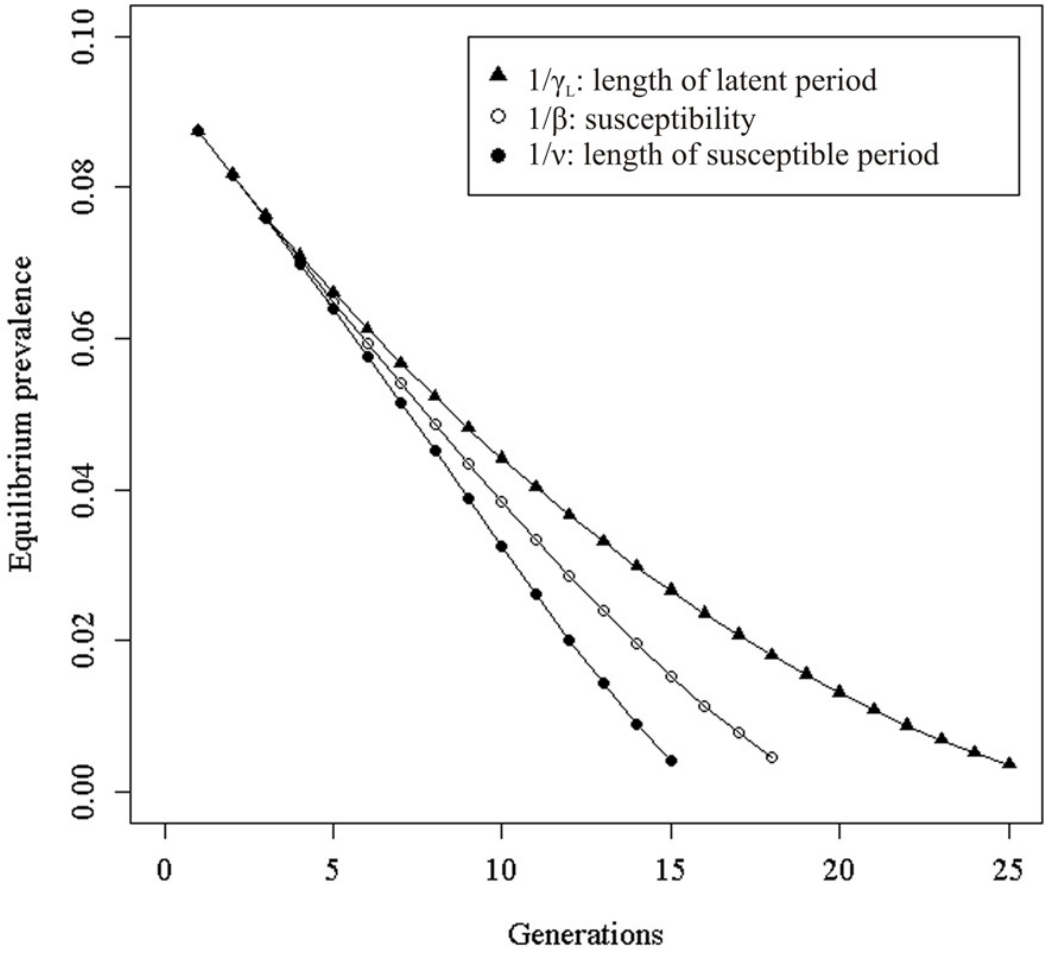

Figure 3. Predicted response to selection for resistance to Johne's disease when selecting 80% of sires producing the least susceptible offspring based on their breeding values for the three parameters under selection ($1/\gamma_L$, $1/\beta$ or $1/\nu$). The effect of selection on the parameters under selection was translated to an effect in prevalence (y-axis) to assess the contribution of genetic selection to control of Johne's disease.

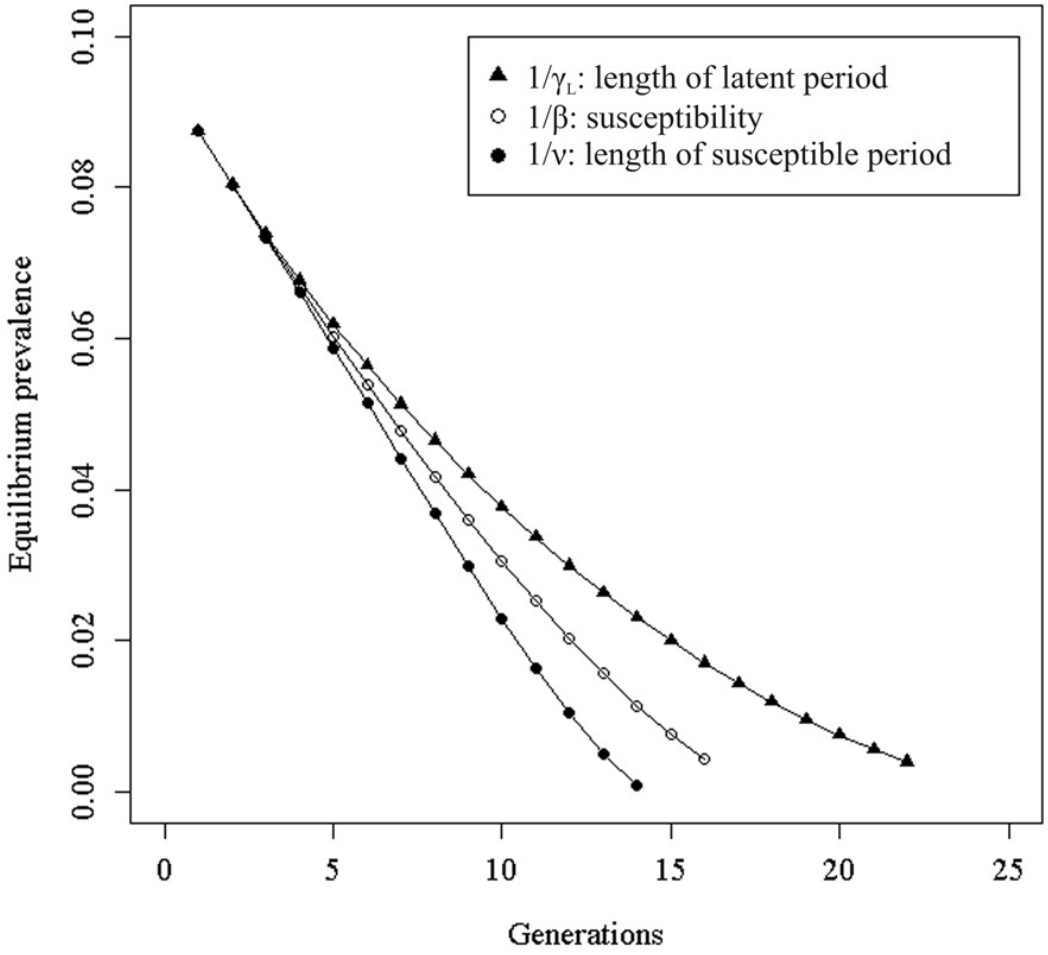

Figure 4. Predicted response to selection for resistance to Johne's disease when selecting those animals that do not show clinical signs at the time point at which the observations are made and 80% of sires producing the least susceptible offspring based on their breeding values for the three parameters under selection ($1/\gamma_L$,$1/\beta$ or $1/\nu$). The effect of selection on the parameters under selection was translated to an effect in prevalence (y-axis) to assess the contribution of genetic selection to control of Johne's disease.

DISCUSSION

The objective of this study was to model genetic selection for Johne's disease resistance and to study the effect of different selection strategies on the prevalence in the dairy cattle population. Selection of those cows that test negative for Johne's disease at the time point at which the observations are made (dam selection), achieved smallest response to selection.

Based on these results, the genetic response resulting from the certification-and-surveillance program currently used in the Netherlands will not substantially contribute to Johne's disease elimination in the Netherlands. Selection of 80% of sires producing the least susceptible offspring based on their breeding values (sire selection) resulted in larger response to selection compared to dam selection. Although the number of generations that is needed to reach a 0.005 prevalence of infection remains substantial, sire selection is able to contribute to control of Johne's disease in the long run.

The large difference in selection response between dam selection and sire selection is mainly due to differences in accuracy of selection and selection intensity. Dam selection as used in this study, only uses information on the cow itself (own performance) to make selection decisions. Consequently, the accuracy of selection low. In this study, sire selection has a much higher accuracy because information from 100 daughters is used to estimate a breeding value for Johne's disease resistance. Also, selection intensity as applied in this study is larger for sire selection, i.e. 80% of sires producing the least susceptible offspring based on their breeding values are selected to produce animals for the next generation. Selection intensity when applying dam selection is equal to 100 minus the prevalence of Johne's disease in the dairy population and has a minimum value of 91.3%.

For dam selection as well as for sire selection, non-linearity in the response in prevalence to selection is caused by a decrease in prevalence. For dam selection, the decrease in prevalence also affected the intensity of selection because the selected proportion of animals in the population is equal to1-p. For sire selection, the decrease in prevalence of disease reduced the accuracy of selection as it is dependent on the prevalence of Johne's disease in the population.

Earlier research showed that genetic variation for resistance to Johne's disease exists (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; van Hulzen et al., 2011; Küpper et al., 2012). Differences in diagnostic methods to determine Johne's disease infection status were used in these studies: clinical inspection after slaughter (Koets et al., 2000), versus an ELISA to detect MAP specific antibodies in milk or serum (Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2008; van Hulzen et al., 2011; Attalla et al., 2010), versus fecal culture (Küpper et al., 2012). However, knowledge of genes and biological pathways involved in resistance to Johne's disease is still incomplete. Therefore, all parameters included in the model were considered for their potential

involvement in the existence of genetic differences between cows in resistance to Johne's disease in this study. To our knowledge, only the length of the latent period ($1/\gamma_L$), susceptibility ($1/\beta$) and the length of the susceptible period ($1/\nu$) may be involved in the existence of genetic differences between cows. Another important infection characteristic that can be different between animals is the rate of shedding of bacteria (Nieuwhof et al., 2009; Doeschl-Wilson et al., 2011). However, this will not affect the chance of a calf to become infected and to progress to the shedding and eventually clinical phase. To achieve lower shedding levels, selection should take place on the shedding level itself.

If genetic variation for resistance to Johne's disease has impact on the length of the latent period ($1/\gamma_L$), response to selection will be slower compared to the susceptibility ($1/\beta$) and the length of the susceptible period ($1/\nu$). As only infected animals are affected by selection on the length of the latent period, the value of this parameter influences only a minor part of the population. All animals except the calves infected through vertical transmission reside in the susceptible compartment for the initial period of their lives and therefore, selection on susceptibility ($1/\beta$) and the length of the susceptible period ($1/\nu$) influence a major part of the population. Additional analysis showed that the ranking of parameters under selection is not sensitive to disease prevalence in the population that is a twice as high and twice as low compared to the initial prevalence used in this study (results not shown).

Epidemiological models usually simplify the disease dynamics. In our epidemiological model, animals in a particular compartment (*S*) all have the same rate at which they are culled (μ) or can progress to the next compartment (*T* or *R*). The assumption of a homogenous group of animals within each compartment is in conflict with the way selection works. The model should ideally include between-animal variation in the rate parameters that describe the transmission of infection, the progress to the resistant, or the progress from latent to shedding compartment. Because the steps in each round of selection are quite small, the approximation that is made in our study is probably justified, but it would be worthwhile to address this issue in more depth in future studies.

The population is assumed to be in an endemic equilibrium in each generation. Considering the slow progression of Johne's disease, one generation is most likely not enough to reach an endemic equilibrium. With dam selection, the genetic progress is made in the dairy cow

population, this means that the effects of selection of subsequent generations overlap, with consequences that are difficult to predict. Selection is likely to be less effective per generation, as the true effect of the selection step is not observed before the next round of selection is taken. However, it may also be more effective in real time, as the cumulative effects of multiple generations may be observed at once. With sire selection, there is no issue of overlapping generations, as genetic progress is actually made outside the dairy cow population. Compared to dam selection, this result in better selection criteria, but it reduces the real time selection response.

Two other features of the model may cause overestimation of the response to selection. First, animals that do not become infected during early age are assumed to gain long-lasting immunity may not be correct. In general, the view that older animals are less susceptible to Johne's disease is quite accepted but the assumption of long-lasting immunity may be too strict. If the susceptible period is longer than modeled in this study and/or animals first show a decrease in susceptibility before gaining long-lasting immunity, response to selection is likely to be different from results in this study. Second, the test used to distinguish the infected from the non-infected animals is assumed to be a perfect test (able to detect all subclinically and clinically infected cows in the population). In reality, the sensitivity of tests used to detect Johne's disease are low and the tests are only able to find only a fraction of the subclinically and clinically infected cows in the population which will also result in a slower response to selection.

CONCLUSIONS

The aim of this study was to model the effect of genetic selection for Johne's disease resistance and to study the effect of different selection strategies on the prevalence of disease in the dairy cattle population. Based on the assumed model and parameters under selection, some conclusions can be drawn. The genetic effect of the current certification-and-surveillance program in the Netherlands is small. Sire selection is able to contribute to control of Johne's disease in the long run. Such a strategy should be used in combination with other control strategies, which are also essential for the collection of information for the breeding program.

ACKNOWLEDGMENTS

The authors would like to thank CRV (Arnhem, the Netherlands) for contributions to previous research on genetic variation in susceptibility to Johne's disease in dairy cattle that forms a fundament for this manuscript. The authors also thank Prof. B. C. Bishop (The Roslin Institute and R(D)SVS, Edinburgh, Scotland, UK) for providing answers to our questions and Dr. P. Bijma (ABGC, Wageningen, the Netherlands) for his valuable contribution to a part of the model analysis.

Chapter 7

General discussion

The aim of this thesis was to contribute to control of Johne's disease by investigating genetic variation in the pathogen and studying genetic variation in host susceptibility. The aim of this chapter is to first summarize the main findings of this thesis, followed by a discussion about the role of genetics within epidemiology research. Finally, the potential contributions of genetic selection for reduced susceptibility of animals to control of Johne´s disease in dairy cows and dairy goats in the Netherlands are discussed.

MAIN FINDINGS OF THIS THESIS

Genetic variation in the pathogen

Chapter 2 shows that, despite the high level of genetic homogeneity of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), MAP isolates from cattle have different MIRU-VNTR patterns. This finding indicates the presence of different MAP strains between and within dairy herds in the Netherlands. Analysis of trading history and age shows that multiple MIRU-VNTR patterns can coexist at a given time and infect or may co-infect different animals.

Genetic variation in host susceptibility

Chapter 3 presents heritability estimates for infection status as determined by a MAP specific antibody response in Dutch dairy goats (binary trait). Heritability estimates range from 0.07 to 0.12 depending on the level of inclusion of diagnostic test information (test value of one test day, maximum test value of multiple test days indicating the maximum concentration of MAP specific antibodies or test values on different days as repeated observations). Differences are also observed in ranking of selection candidates. These differences are most likely due to an improvement in disease phenotype when using repeated measures over a one year period.

Chapter 4 demonstrates that the prevalence of a positive ELISA test in milk in Holstein-Friesian cows in the Netherlands was 2.4% on the animal level and 46.7% on the herd level in 2008. Heritability estimates for MAP specific antibody response (continuous trait) in subsets of this data set based on levels of within-herd test prevalence range from 0.03 to 0.10. Cross-validation analysis shows that to reduce the prevalence of Johne's disease, breeding value estimation should be based on herds with at least two test-positive animals, which results in a heritability of 0.04. Chapter 5 shows results of a genome-wide association study of sire breeding values obtained in chapter 4. This study revealed regions on chromosomes 4, 15, 18 and 28 that are associated with susceptibility to disease as determined by the MAP specific antibody response. Chapter 6 shows the expected effect of (single trait) selection for Johne's disease resistance on the prevalence of disease in a dairy cattle population. The results demonstrate that genetic selection of sires for disease resistance can contribute to Johne´s disease control on the long run.

COMBINING GENETICS AND EPIDEMIOLOGY

Traditional breeding goals for dairy production focus mainly on improving productivity, fertility and functional traits like udder health and longevity. As single trait selection for milk production leads to an increased incidence of diseases in the population (Pryce et al., 1998; Vandorp et al., 1998; Dematawewa and Berger, 1998; Lindhe and Philipsson, 1998; Lund et al., 1999; Rupp and Boichard, 1999; Kadarmideen et al., 2000; Hooijer et al., 2001; Zwald et al., 2004; Hinrichs et al., 2005; Koenig et al., 2005), animal breeders explore the benefits of including health related traits in the breeding program. Reasons to include health related traits in the breeding program are the unfavorable genetic correlation between productivity and disease, the need to improve animal welfare and the need to response to increasing consumer concerns about infectious animal diseases being a threat to human health.

Genetic selection is more and more suggested as additional approach to control of diseases. Genetic improvement of disease resistance is a slow, long-term process, but the results are cumulative and permanent; genetic changes made in one generation remain in future generations, and under a program of continuous improvement, advances in genetic resistance accumulate over generations. Results from two selection experiments in Norwegian dairy cattle demonstrate that considerable genetic improvement can be achieved for resistance to clinical mastitis as well as correlated selection responses in ketosis and retained placenta (Heringstad et al., 2007). In dairy cattle breeding in the Netherlands, traits as resistance to mastitis, claw health and productive life are already included in the breeding program for many years.

For infectious diseases, selection for resistance may have a direct genetic effect, by reducing the chance to become infected when exposed to a pathogen via direct contact or in the environment or improved immunological resistance. In addition, it may have an indirect epidemiological effect by reducing direct and indirect transmission of infection between infected and susceptible animals through reduction of infection pressure. To be able to accurately predict the long-term effect of genetic selection for resistance to infectious diseases, it is therefore necessary to combine both the direct and indirect effects of genetics and epidemiology.

A number of studies implemented genetics within an epidemiological model. MacKenzie and Bishop (2001) demonstrated the benefits of genetic-epidemiological models for quantifying the consequences of selecting animals for a micro parasitic infectious disease. Later on a study from the same group considered the use of disease resistance genes to control the transmission of infection through an animal population using an epidemiological model (Bishop and MacKenzie, 2003). In the same year, Bishop and Stear (2003) developed genetic-epidemiological models to account for host genetics in ruminants and suggested that these models may be used to assess the effect of using genetics to control nematode infections. In the study of Nieuwhof et al. (2009), the indirect epidemiological effect of mass selection for footrot in sheep was quantified by predicting the expected response to selection using a quantitative genetic threshold model versus a genetic-epidemiological model. In chapter 6, elements of the method of Nieuwhof et al. (2009) were used to predict the effect of single trait selection for Johne's disease resistance on the prevalence of disease in the dairy cattle population. Results of this study demonstrated that genetic selection of sires for disease resistance can contribute to Johne´s disease control on the long run.

Several of the above studies accounted for variability in host genotype by implementing the genetic-epidemiological model in a stochastic setting (MacKenzie and Bishop, 2001; Bishop and MacKenzie, 2003; Bishop and Stear, 2003; Nath et al., 2008). In the study described in chapter 6 as well as in the study of Nieuwhof et al. (2009), animals in a particular compartment of the epidemiological model were treated as homogenous, i.e. they all had the same culling rate and probabilities of transition to other compartments. The assumption of a homogenous group of animals within each compartment included in the model ignores genetic variation among animals. Genetic selection exploits the genetic variation among animals to genetically improve the population into the desired direction. Because the steps in each round of selection are quite small, the approximation that is made in our study in chapter 6 is justified, but it would be worthwhile to address this issue in more detail in future studies.

Although the importance of host genetic heterogeneity on disease risk and prevalence has long been recognized by epidemiologists, classical epidemiological studies assume genetic homogeneity among animals. For example, for the use of field data or data obtained from experiments in avian influenza studies, the assumption of genetic homogeneity can sometimes be questioned. The study of Doeschl-Wilson et al. (2011) used a genetic-epidemiological

model to quantify the impact of host genetic diversity on epidemiological characteristics of diseases (in this case transmitted through the environment). They showed that compared to homogenous populations, disease risk and severity are substantially higher in heterogeneous populations indicating the importance of including genetic heterogeneity of populations in epidemiological studies. In addition, for avian influenza as well as for Johne's disease, strain diversity has been shown to exist (chapter 2; Dugan et al., 2008). For Johne's disease, a better understanding of the biodiversity of MAP offers more insight in the transmission within and between herds (Möbius et al., 2008; Pradhan et al., 2011) and between species (Stevenson et al., 2009) and therefore may contribute to control of disease. There is also evidence that suggests a correlation between mycobacterial strain type and course of infection. Two recent studies suggest that different MAP strain types may play a role in polarizing the host immune responses during infection (Janagama et al., 2006; Motiwala et al., 2006). Also, different MAP strains have been found to differ in virulence in experimental infections of deer (O'Brien et al., 2006) and ability to cause pathology in ovine paratuberculosis (Verna et al., 2007). Gollnick et al. (2007) showed that the survival of MAP in bovine monocyte-derived macrophages was not affected by host infection status but by the infecting strain type.

BREEDING FOR JOHNE´S DISEASE RESISTANCE

Animal breeders consider four criteria when deciding what traits to include in the breeding program: 1) the trait of interest must have a reasonably large genetic variation and heritability; 2) the trait of interest must have important economic value; 3) the trait of interest must be measurable at relatively low cost, the measure must be clearly defined and observation consistently recorded according to the definition; and 4) an indicator trait may be used if it has a high genetic correlation with the trait of interest and if it has a lower recording cost, higher heritability, or can be measured earlier in life (Shook, 1989).

In this section, the four criteria will be applied to Johne's disease in dairy cattle and dairy goats. Subsequently sector-specific methods are discussed to implement a breeding program that aims at animals with reduced susceptible to the disease.

1. Genetic variation and heritability

The first criterion to assess the potential of inclusion in the breeding program is that the trait, in this case susceptibility to Johne´s disease, must have a reasonably large genetic variation and heritability.

In dairy goats, genetic variation and heritability were estimated for infection status as determined by a MAP specific antibody response (binary trait). Genetic variation differed significantly from zero and heritability estimates ranged from 0.07 to 0.12 depending on the level of inclusion of diagnostic test information (test value of one test day, maximum test value of multiple test days or test values of repeated test days) (chapter 3). In the Netherlands, pedigree registration in dairy goats is mainly applied when using artificial insemination. In the study as described in chapter 3, numerous selected goats originated from billy goats that are used for artificial insemination on a national scale. Genetic variation and heritability were estimated based on data from one herd only. However, this provides sufficient evidence of genetic variation on a national scale.

In dairy cattle, a number of studies estimated genetic variation and heritability for susceptibility to Johne's disease. Genetic variation was shown to exist and heritability estimates for susceptibility to Johne's disease reported in literature range from 0.03 to 0.23 (chapter 4; Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007;

Attalla et al., 2010; Küpper et al., 2012). Observed differences in heritability estimates were due to: 1) differences in Johne's disease prevalence in the research populations; 2) differences in sample sizes; 3) differences in statistical methods used; and 4) differences in diagnostic methods to determine Johne's disease infection status: clinical inspection after slaughter (Koets et al., 2000), and ELISA to detect MAP specific antibodies in milk or serum (chapter 3 and chapter 4; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2008; Attalla et al., 2010), versus fecal culture (Küpper et al., 2012).

In conclusion, research in dairy goats and dairy cattle showed that for susceptibility to Johne's disease, as defined by a positive result in fecal culture, PCR, tissue culture but also by the absorbed ELISA, genetic variation exist. The heritability of the trait is relatively low.

2. Economic impact

The second criterion to assess the potential of inclusion in the breeding program is that the trait of interest, in this case susceptibility to Johne´s disease, must have important economic value.

For Johne´s disease in dairy cattle and dairy goats, the potential need to include less susceptibility to disease in the breeding program from an economic point of view is threefold. First, infection with Johne's disease leads to economic losses due to lower milk production, decrease in slaughter value and higher animal replacement rates (Johnson-Ifearulundu et al., 1999). In dairy goats, vaccination as principal control strategy in the Netherlands is thought to reduce costs of disease by reducing production losses, epidemiological and pathogenetic effects of disease and in this way costs of caprine Johne's disease (Bastida and Juste, 2011). In dairy cattle, average economic losses on herd level due to subclinical symptoms of Johne's disease are small (Groenendaal et al., 2002). However, economic losses are large in herds that experience a high level of infection. Second, previous studies have led to the concern that Johne´s disease may be a zoonosis. Two studies detected highly significant differences in the occurrence of MAP in individuals with Crohn's disease versus controls (Bull et al., 2003; Naser et al., 2004). Two other studies showed abnormal geographical distributions in the incidence of Crohn's disease indicating an effect of environmental exposure (Green et al., 2006; Loftus et al., 2007). For dairy goats, concerns about Johne's disease being a zoonosis may lead to consumer questions about vaccination as principal control strategy. In dairy

cattle, the awareness in the dairy industry about Johne's disease being a possible threat to human health already resulted in the Dutch certification-and-surveillance program aiming to reduce sources of infection using an ELISA test in milk to detect infectious dairy cows in infected herds, once every year. Economic consequences of Johne's disease increase for infected herds as the program obliges them to cull test positive cows in order to be allowed to produce milk for human consumption. Third, clinical symptoms of Johne's disease result from lesions in the distal part of the ileum causing problems with nutrient intake. Therefore, Johne's disease also has a negative impact on animal welfare. Although this might not have a direct economic effect, the impact on animal welfare needs to be included in the considerations to include a trait in a breeding program.

In conclusion, economic consequences of infection with Johne's disease are substantial in dairy goats and dairy cattle in the Netherlands. The main economic consequences are cost of vaccination in goats and costs of participation in the certification-and-surveillance program in dairy cattle.

3. Trait must be measurable at low cost, clearly defined and consistently recorded

The third criterion to assess the potential of inclusion in the breeding program is that the trait of interest, in this case susceptibility to Johne´s disease must be measurable at relatively low cost, the trait must be clearly defined and observation for the trait consistently recorded according to the definition.

Fecal culture and fecal PCR detect the presence of the causative agent in feces and are commonly applied for diagnosis of infection with Johne´s disease. Although those measures are clearly defined, test sensitivities of fecal culture and fecal PCR are dependent on stage of disease because MAP shedding takes place only at advanced stages of the disease and occurs intermittently. In addition, costs of fecal culture and PCR are relatively high and fecal culture is time-consuming. Postmortem analysis of bacterial cultures from intestinal tissue and lymph nodes and histopathology are also used for diagnosis of infection with Johne´s disease by detecting the presence of the causative agent in tissue. However, these tests are even more expensive and time-consuming compared to fecal culture.

In conclusion, bacteriological diagnosis of MAP infection by fecal culture or PCR is of low sensitivity and measurable at relatively high costs. Therefore, indicator traits should be used to lower costs and increase feasibility of implementation.
Similarly, susceptibility to Johne´s disease cannot be measured easily, cheaply, or consistently, which favors the use of an indicator, as discussed below.

4. Indicator traits

The fourth criterion to assess the potential of inclusion in the breeding program is that for the trait of interest, in this case susceptibility to Johne´s disease, an indicator trait may be used if it has a high genetic correlation with the trait of interest and if it has a lower recording cost, higher heritability, or can be measured earlier in life.
The course of Johne's disease infection can be divided into different stages. The start of infection is the onset of a long latent stage with little to none detectable MAP excretion and no detectable humoral response. Only the cellular response measured by interferon gamma production of mononuclear cells has been shown to be a tool to detect animals in the latent stage of disease (Stabel, 1996). However, the tests require complex logistics, are labor intensive, expensive and there are concerns about the specificity (Santema et al., 2011). Detection of infection remains difficult until cows enter a subclinical stage of disease, during which shedding occurs intermittently and a humoral response starts to develop. The humoral response can be detected by an absorbed ELISA test.
The slow course of infection in combination with limited (known) options for diagnosis makes accurate detection of Johne's disease difficult. To achieve genetic improvement for Johne's disease susceptibility, an absorbed ELISA to detect the humoral response could be a good option to use as indicator trait for Johne's disease infection due to low costs and its ease of recording. Sensitivity of the absorbed ELISA is depends on stage of disease: infection is only detectable from the subclinical stage onwards.
The absorbed ELISA as indicator of an immune response is an example of an indicator trait, similar to somatic cell count for clinical mastitis. For SCC and clinical mastitis, Heringstad et al. (2000) found an average estimated genetic correlation between SCC and clinical mastitis of 0.60 based on literature which indicated a moderate to strong genetic association between SCC and clinical mastitis. Therefore, response to selection on SCC should result in a decrease

in prevalence of clinical mastitis. In Johne's disease, a positive response is to be expected when using an absorbed ELISA when the ELISA test has a clear genetic correlation with susceptibility. However, no estimates are available of the genetic correlation between the ability to mount an antibody response to MAP and infection status.

Roussel et al. (2005) showed that Bos indicus purebreds and crosses had odds ratios 17-fold and 3.5 fold greater than Bos taurus breeds for positive ELISA results however several herds did not show clinical or microbiological evidence of Johne's disease. These findings suggests that in Bos indicus cattle humoral response is more effective to fight infection. On the other hand, genetic selection of animals with a lower absorbed MAP specific ELISA may result in a more generalized selection towards humoral immune response leading to increasing susceptibility for other diseases. However, despite these potential pitfalls, at this moment the MAP specific absorbed ELISA seems the best available indicator trait for genetic selection for resistance to disease in Dutch dairy goats and Dutch dairy cows.

In conclusion, an absorbed ELISA to detect the MAP specific humoral response could be a good option to use as indicator trait for Johne's disease infection. The trait has a reasonably high heritability but until now no estimate of genetic correlation with infection status is available. It is important to monitor that selection for reduced MAP specific ELISA response does not lead to selection of animals with an impaired humoral response. Future research may include finding and implementing other (non-immune) indicator traits that are not dependent on stage of infection and/or can be measured on animals that are not exposed to MAP.

Implementation in the breeding program

In conclusion, the economic consequences of infection with Johne's disease and the existence of genetic variation are good arguments to include breeding for animals less susceptible to Johne's disease in the breeding program. The aim of the following paragraphs is to discuss how genetic selection for animals less susceptible to disease may contribute to control of Johne's disease in the Netherlands. This will be done assuming that a MAP specific absorbed ELISA test will be a sufficient indicator trait for Johne's disease infection.

Low sensitivity of the absorbed ELISA. Under the assumption that an absorbed ELISA can be used as indicator trait for susceptibility to Johne's disease, the low sensitivity of the test would not automatically hinder use for breeding value estimation. On the cow level,

differences in susceptibility to Johne's disease are difficult to observe due to the test sensitivity resulting in numerous false-negatives. However, on the sire level, differences in susceptibility to Johne's disease between progeny groups are likely to be observed. Large daughter groups will enable accurate estimation of genetic differences between sires. The effect of false negatives is expected to cancel out when progeny groups are large enough.

To improve the test sensitivity on the cow level, repeated measures could be applied. However, as shown in chapter 3, only the maximum test value of multiple test days should be included in the genetic analysis as the humoral response measured by an absorbed ELISA may occur intermittently. In addition, the time between the test days should be several months to be effective as Johne's disease progresses slowly.

Dairy goats. In the Netherlands, pedigree registration in dairy goats is mainly applied when using artificial insemination. Breeding value estimation could be implemented for billy goats used for artificial insemination. Breeding values will allow dairy goat farmers with high incidence of Johne's disease to select billy goats with low susceptibility to improve the quality of the herd on the long run. However, the wide use of vaccination hampers diagnosis and thereby the collection of data for estimation of breeding values. It is still impossible to differentiate if absorbed ELISA values measured on vaccinated goats are the result of vaccination or an reaction to infection.

To implement genetic selection for resistance to Johne's disease in the Dutch dairy goat sector, breeding value estimation of sire should be based on information collected on non-vaccinated daughters. In addition to vaccination status, emphasis should be placed on ensuring pedigree information. Registered daughters should be tested from two years onwards because the humoral response starts to develop generally after that age (chapter 3). Selected daughters should be tested repeatedly to improve the sensitivity of the test and only the maximum test value should be used in breeding value estimation.

Genetic selection for resistance to Johne's disease should be seen as an additional approach that contributes to the control of the disease. In the Dutch dairy goat sector, emphasis in disease control should be placed on improving strategies to reduce transmission of MAP. Currently, 40 dairy goat farmers are involved in a project to improve the awareness of farmers for strategies that could help to control the disease and is the onset for developing new ideas.

Dairy cattle. To implement genetic selection for resistance to Johne's disease in the Dutch dairy cows, ELISA test results originating from the Dutch certification-and-surveillance program can be used. These test results were used in the analysis of chapter 4. Population-wide data provides the ability to estimate breeding values of sires with high accuracies. In addition, results in chapter 4 demonstrate that in order to obtain breeding values with high accuracy for susceptibility to Johne's disease, breeding values should be estimated based on as many daughter records as possible but only from infected herds. In the framework of the certification-and-surveillance program, repeated measures become available and therefore the use of only the maximum test value of multiple test days should be considered. As described in chapter 5 sire breeding values could be used as input for a genome-wide association study and provide the opportunity to estimate genomic breeding values. Genomic breeding values offer the opportunity to estimate accurate breeding for sires without progeny information. The accuracy of genomic breeding values depends on the quality of the reference population (Clark et al. 2012). Further research should be done to the size of the reference population needed to be able to estimate genomic breeding values with high accuracies. Once a high quality reference population is developed, genomic breeding value estimation can be applied to for individual animals regardless on stage of infection and exposure to MAP.

Conclusion

The work described and discussed in this thesis indicates that genetic variation dairy cattle and dairy goats may be utilized to improve the control of Johne's disease at the population level in the long run. Suggestions for implementation of resistance to Johne's disease in the breeding program are presented but need to be technically and economically feasible for the dairy industry.

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Nederlandse samenvatting

PARATUBERCULOSE IN KOEIEN EN GEITEN

Paratuberculose is een bacteriële infectie van het maag-darmkanaal dat wordt veroorzaakt door orale opname van *Mycobacterium avium* subspecies *paratuberculose* (MAP) uit de omgeving. De opname van nutriënten uit de voeding verslechtert als gevolg van infectie met MAP. Geïnfecteerde dieren krijgen last van gewichtsverlies, diarree en een afname in productie in het geval van melkvee. Dieren in een gevorderd stadium van de ziekte scheiden MAP uit in hun ontlasting en zijn op deze manier een infectiebron voor gevoelige dieren.
De prevalentie van een positieve MAP specifieke antilichamen test in melkrunderen was 46.7% op bedrijfsniveau en 2.4% op dierniveau in Nederland in 2008. De prevalentie van paratuberculose in melkgeiten is onbekend, maar wordt verondersteld hoger te zijn gebaseerd op klinische en routine pathologische observaties. Infectie met paratuberculose zorgt voor economische verliezen op bedrijfsniveau door een afname in productie, slachtwaarde en een hogere vervanging. Daarnaast heeft infectie met paratuberculose maatschappelijke impact, omdat verschillende studies laten zien dat MAP significant vaker voorkomt in patiënten met de ziekte van Crohn versus controles en daarmee de zorg gewekt wordt dat paratuberculose een zoönose is.
Verschillende studies laten zien dat gevoeligheid voor paratuberculose het hoogste in jonge dieren en afneemt met leeftijd. Gevoelige dieren kunnen worden geïnfecteerd door vervuild colostrum, vervuilde melk of door contact met een vervuilde omgeving. Het verloop van de infectie wordt gekenmerkt door verschillende stadia. Wanneer dieren worden geïnfecteerd op jonge leeftijd komen zij in een latent stadium van de ziekte. De lengte van dit stadium verschilt tussen dieren en wordt gekenmerkt door afwezigheid van de uitscheiding van MAP in de ontlasting. Na het latente stadium volgt een subklinisch stadium waarin MAP met tussenpozen wordt uitgescheiden. Een klein gedeelte van de dieren uit het subklinische stadium bereikt ook het klinische stadium van de ziekte. Omdat alleen dieren in het subklinische en klinische stadium MAP uitscheiden, dragen alleen zij bij aan nieuwe infecties.

CONTROLE VAN PARATUBERCULOSE

Controle strategieën om de impact van paratuberculose op geïnfecteerde bedrijven te verminderen zijn: 1) management maatregelen om de MAP transmissie te verminderen; 2) test en cull strategieën om de infectiebronnen te verminderen; en 3) vaccinatie om de gevoeligheid van jonge dieren te verminderen.
Ondanks de verschillende maatregelen blijkt het in de praktijk zeer moeilijk te zijn om MAP te verwijderen van een geïnfecteerd bedrijf. Zoeken naar maatregelen met een toegevoegde waarde blijft een noodzaak. Het doel van dit proefschrift is om bij te dragen aan het controleren van paratuberculose door het onderzoeken van genetische variatie in het pathogeen (MAP) en de gastheer (rund en geit). Daarnaast is gekeken naar het verwachte effect op het terugdringen van de prevalentie in de rundveepopulatie wanneer gebruik wordt gemaakt van genetische variatie door middel van genetische selectie voor weerstand tegen paratuberculose.

BEVINDINGEN VAN DIT PROEFSCHRIFT

Genetische variatie in het pathogeen

Hoofdstuk 2 laat zien dat ondanks de genetische homogeniteit van de bacterie, MAP isolaten van rundvee kunnen verschillen in genetisch patroon (bepaald aan de hand van MIRU-VNTR analyse). Dit impliceert dat er verschillende MAP kiemen bestaan binnen en tussen bedrijven in Nederland. De analyse van handelsgeschiedenis en leeftijd van het individuele dier laat zien dat meerdere genetische patronen naast elkaar kunnen bestaan en verschillende dieren kunnen infecteren of co-infecteren.

Genetische variatie in de gastheer

Hoofdstuk 3 toont erfelijkheidsgraden voor infectie status, bepaald aan de hand van een MAP specifieke antilichamen respons in melkgeiten (binair kenmerk). De schatters voor de erfelijkheid lopen uiteen van 0.07 tot 0.12 en hangen af van de mate van inclusie van diagnostische informatie (test resultaat van een testdag, het maximale testresultaat van meerdere testdagen of testresultaten van meerdere testdagen als herhaalde waarnemingen).

Verschillen worden waargenomen in de ranking van de selectie kandidaten die hoogstwaarschijnlijk worden veroorzaakt door een verbetering van het ziektefenotype wanneer je gebruik maakt van de herhaalde waarnemingen.

Hoofdstuk 4 laat zien dat de erfelijkheid voor een MAP specifieke antilichamen respons in melkrunderen (continu kenmerk) uiteenloopt van 0.03 to 0.10, in subsets van data gebaseerd op binnen-bedrijfstestprevalentie. Een cross-validatie analyse laat zien dat, om de prevalentie van paratuberculose te verlagen, fokwaardenschattingen gebaseerd moeten zijn op testresultaten verkregen van bedrijven met minimaal twee test positieve dieren, resulterend in een erfelijkheidsgraad van 0.04. Hoofdstuk 5 toont resultaten van een genoomwijde associatie studie waarvoor de stierfokwaarden zijn gebruikt die geschat zijn in hoofdstuk 4. Deze studie toont dat regio's, geassocieerd met een MAP specifieke antilichamen respons, zijn gelegen op chromosoom 4, 15, 18 en 28. Hoofdstuk 6 onderzoekt het verwachte effect van genetische selectie voor weerstand tegen paratuberculose op de prevalentie van de ziekte in de rundveepopulatie. Resultaten laten zien dat genetische variatie voor gevoeligheid voor paratuberculose gebruikt kan worden, om op lange termijn het controleren van paratuberculose op populatieniveau te verbeteren en de prevalentie terug te dringen.

Dankwoord

Dit promotietraject was voor mij een kans: werken op het grensvlak van genetica, epidemiologie en immunologie. De afgelopen vier jaar heb ik ervaren als een uitdaging, leerzaam, vol hoogtepunten en de tijd is omgevlogen. Hiervoor wil ik een aantal mensen bedanken.

Dank aan, …

… mijn begeleiders, voor het bieden van deze kans! Mirjam, wat heb ik je duidelijkheid, analytisch vermogen en vermogen voor het aanbrengen van structuur gewaardeerd. Johan, wij kenden elkaar al veel langer. Ik vind het geweldig dat je mijn keuze hebt ondersteund door actief betrokken te blijven bij dit project! Henri, jij was het die me attendeerde op de vacature. Bedankt hiervoor! En natuurlijk ook omdat je me altijd, op elk moment van de dag, bij wilde staan met raad en daad! Ad, wat bewonder ik jou om je gedetailleerde kennis en brede interesse. Samen hebben we een onderzoeksopzet 'geit' bedacht, geiten gemolken, in het lab gestaan maar ook het opzetten van het epidemiologisch model was zonder jou niet gelukt!

… mijn paracollega's. Suus, hoogtepunt samen was voor mij onze trip naar New York. Slapen doen we thuis wel! Sorry Wiebren, bij pathologie ben ik helaas niet op mijn best! Jeroen, zonder jouw 'labtraining' had ik het niet gered! Marina, maandag, dinsdag en donderdag om half zeven 's ochtends bij de McDonald's in Veenendaal. Heel vroeg, maar altijd heel gezellig! Victor Rutten, Lindert Benedictus, Maarten Weber, Karianne Lievaart-Peterson en Geert Benedictus, de parameeting eens per twee maanden was altijd leuk en informatief. Bedankt voor jullie bijdrage hieraan.

… CRV. Bedankt Gerben, Mathijs, Herwin, Lydia, Marianne, Jolanda en Pedro van de Animal Evaluation Unit voor het verschaffen van een fijne werkplek, data en deskundigheid! Bedankt Ghyslaine, Chris en Erwin van Research voor de fijne samenwerking waarmee de GWAS paper tot stand is gekomen.

… Bennie en Heleen Aarts en het personeel van de geitenfarm. Zonder jullie en jullie mooie bedrijf was ons geitenonderzoek niet tot stand gekomen.

… ELDA ICT & Services B.V. Met name dank aan Lia en Floor voor wie niets te veel was om het geitenonderzoek te faciliteren.

… Judith, Marieke, en Dieuwertje, AIO ben je in voor- en tegenspoed!

… Marjolein, we hebben wat kilometers hardgelopen maar aan jouw doorzettingsvermogen kan ik helaas niet tippen.

… Saskia, samen hadden we een waar 'koffietafelproject' met als resultaat een paper in JDS! Bedankt voor de geweldige samenwerking!

... Don, omdat je het idee over een genetisch-epidemiologisch model zag zitten! Jouw technische skills waren onmisbaar maar ook hoe je je hebt ingezet om het verhaal rond te hebben voor de deadline (mijn deadline!) vind ik geweldig! Nogmaals bedankt!

… mijn andere collega's op het departement landbouwhuisdieren. Dank voor de leuke, inspirerende, relativerende en ontspannende koffiepauzes! En natuurlijk de dames van het secretariaat. In het bijzonder Annet, gelukkig had ik jou om op terug te vallen als ik mijn persoonlijke administratie niet op orde had, Fros, jij krijgt altijd alles geregeld, en Sakoen, bedankt voor je gezelligheid!

… Barbara, Marieke en Angelien, voor de kans die jullie me bieden om mijn mogelijkheden binnen de humane genetica te onderzoeken. Dank aan alle nieuwe collega's, en in het bijzonder Alejandro, Janita, Marina, Saskia, Remco, Johanne, Mascha, Marlies en Janneke voor de fijne werksfeer waarin ik me gelijk thuis heb gevoeld!

… Auk. Ondanks onze verschillen in achtergrond doorlopen wij hetzelfde traject op alle vlakken in het leven. Dat wij tegelijkertijd AIO waren was dan ook geen verrassing. Het hoogtepunt (of dieptepunt?) waren de zondagochtenden samen naar Mick's kantoor om allebei aan de discussie te werken! Bedankt dat je er altijd voor me bent!

… mijn paranimfen. Judith, wij begonnen tegelijk als AIO op uiteenlopende onderwerpen maar kwamen wel op dezelfde kamer terecht. Jij was voor mij onmisbaar met je advies over presentaties, brainstormsessies over methoden en het lezen van mijn discussies. Simone, ooit mijn buurvrouw maar toen dat eenmaal voorbij was is ons 'lijntje' nooit verbroken. Bedankt voor je eeuwige interesse in waar ik mee bezig ben! En natuurlijk de etentjes die ik kan verdienen als er een paper geaccepteerd is. Judith en Simone, wat een eer dat ik jullie mijn paranimfen mag noemen!

… mijn (schoon-) familie. Jullie belangstelling en ondersteuning heeft het leven een stuk fijner gemaakt! Piet en Truce, bedankt hiervoor! Oma, uw foto's van vroeger laten me zien waar mijn liefde voor de koeien vandaan komt. Pa en ma, Rick, en Rutger en Anne, altijd laten jullie merken hoe trots jullie zijn. Bedankt dat jullie er altijd voor me zijn!

… Mick. Dertien jaar kennen we elkaar. Die tijd staat voor verandering. Ik verheug me erop binnenkort een nieuw hoofdstuk aan ons leven samen te kunnen toevoegen! En Puck, mijn grootste trots. Jullie zijn mijn 'thuis' en dat maakt me gelukkig!

Curriculum Vitae

Kimm van Hulzen was born on January 30, 1981 in Arnhem (the Netherlands). She attended the Stedelijk Gymnasium Nijmegen and ROC Nijmegen from 1993 to 2001 and studied Animal Sciences with a masters degree in Animal Breeding and Genetics at Wageningen University from 2001 to 2006. After her studies, she started working on the Animal Breeding and Genomics Centre (ABGC) at Wageningen University as a coordinator of the European Master in Animal Breeding and Genetics. In July 2008, she started her PhD studies on genetic variation in paratuberculosis in dairy populations at the Farm Animal Health department of the faculty of Veterinary Medicine at Utrecht University. In August 2012 she started working as a genetic-epidemiologist within the group Multifactorial Diseases at the department of Human Genetics of the University Medical Center Nijmegen.

List of publications

PEER REVIEWED PUBLICATIONS

van Hulzen, K.J. , R.C. Sprong, R. van der Meer and J.A. van Arendonk. 2009. Genetic and nongenetic variation in concentration of selenium, calcium, potassium, zinc, magnesium, and phosphorus in milk of Dutch Holstein-Friesian cows. J. Dairy Sci. 92:5754-5759.

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Poster presentations

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