Surveillance of *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds

Maarten F. Weber

2009

Weber, M.F., 2009. **Surveillance of *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds**.

Keywords: Dairy cattle; Paratuberculosis; Milk; Surveillance; Quality assurance; Programme; Diagnostic test; Simulation; Modelling.

ISBN: 978-90-393-5238-0
Published by: *De* Gezondheidsdienst voor Dieren / GD Animal Health Service, Deventer, The Netherlands.

Layout and design: Marije Brouwer and Harry Otter, Division Multimedia, Faculty of Veterinary Medicine, Utrecht University.
Cover photograph: Otlis Sampimon.
Printed by: Ridderprint Offsetdrukkerij BV, Ridderkerk.

Surveillance of *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds

Surveillance van *Mycobacterium avium* subsp. *paratuberculosis* op melkveebedrijven

(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. J.C. Stoof,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
donderdag 10 december 2009 des middags te 3.45 uur

door

Maarten F. Weber

Promotor:	Prof.dr. J.A. Stegeman
Co-promotoren:	Dr. M. Nielen
	Dr. J. Verhoeff

Contents

Chapter 1

General introduction

1.1. Introduction

Paratuberculosis (Johne's disease) is a chronic inflammatory bowel disease, primarily affecting ruminants, and is caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). Clinical signs of paratuberculosis in cattle include reduced milk production, persistent diarrhoea, weight loss and progressive emaciation (National Research Council, 2003). Therefore, *Map* infection and paratuberculosis have negative effects on both animal welfare and the economics of dairy farming and the dairy processing industry. The economic effects on dairy farmers and the dairy processing industry include direct economic effects on infected farms and, potentially, indirect economic effects of concerns about a zoonotic potential of *Map*.

The direct economic effects on dairy farming depend on the herd-level prevalence and the economic impact on infected herds. The herd-level prevalence of *Map* infection amongst European dairy herds has been estimated to be over 50% (Nielsen and Toft, 2009). A similarly high herd-level prevalence was recently found in US dairy herds (USDA-APHIS, 2008). In the Netherlands, the herd-level true prevalence (95% CI) has been estimated at 0.31 to 0.71 (Muskens et al., 2000), at 0.54 (0.46, 0.63; van Schaik et al., 2003) and at 0.32 (0.21, 0.46; van Weering, personal communication, 2004). The direct economic impact on infected herds has been estimated at on average $35 per cow for a typical 100-head US dairy herd (increasing to $72 per cow after 20 years; Groenendaal and Galligan, 2003) and at £27 per cow in Scottish herds (Stott et al., 2005).

Economic effects due to a decreased demand of milk from infected herds can result from consumer concerns about the zoonotic potential of *Map* if no fully effective risk mitigation is available (Groenendaal and Zagmutt, 2008). The issue of a potential role of *Map* in the pathogenesis of Crohn's disease in humans has not yet been resolved (Scientific Committee on Animal Health and Animal Welfare, 2000; Feller et al., 2007; Uzoigwe et al., 2007; Waddell et al., 2008; Abubakar et al., 2008; Nacy and Buckley, 2008), and milk is a potential route of transmission of *Map* to humans (Nauta and van der Giessen, 1998; Grant, 2005).

1.2. Definition of surveillance, monitoring and control

There is only limited consistency in the literature about the definitions of surveillance, monitoring, and control of diseases (Christensen, 2001). In this thesis, the terms surveillance and monitoring are used synonymously and are defined as the efforts directed at assessing the infection status of a given population (e.g., a herd). This assessment may lead to control measures being taken if the data indicate a prevalence and/or incidence of *Map* infection and/or clinical paratuberculosis above a certain threshold. Control is defined as the whole spectrum of measures that are taken

to reduce the prevalence and/or incidence of *Map* infection and/or clinical paratuberculosis in a known infected population. Control is not necessarily directed at elimination of the infection from the population. Certification is defined as the process of obtaining an accredited status for populations (herds) in which (repeated) examinations have not indicated the presence of a *Map* infection; certified populations (herds) can subsequently be put under surveillance to determine whether their status can be maintained.

1.3. Surveillance of *Map*

Surveillance of *Map* in dairy herds can support decision makers (including dairy farmers) to take measures to reduce the negative economic effects of *Map* infection. Surveillance can alert farmers to an infection present in their herds, enabling them to take appropriate corrective actions to prevent future losses due to paratuberculosis (Collins et al., 2006). Surveillance can provide assurance to consumers about the safety of dairy products. Surveillance can reduce the between-herd transmission of *Map* by enabling low-risk trade of cattle. In addition, surveillance can enable farmers with herds enrolled in a certification-and-surveillance programme to increase revenues, for instance by selling livestock for higher prices (Kovich et al., 2006).

Certification-and-surveillance programmes in which *Map*-free dairy herds or dairy herds with a low likelihood of being infected are certified, have been initiated in several countries such as Australia, the USA, the UK, Italy and the Netherlands (Bulaga and Collins, 1999; Benedictus et al., 2000; Kennedy and Allworth, 2000; Wells et al., 2002; National Research Council, 2003; Kalis et al., 2004; Kennedy et al., 2005; Pozzato et al., 2007; Citer and Kennedy, 2009; Anon, 2009). Typically, these programmes aim to protect non-infected herds from *Map* infection and to reduce the between-herd spread of *Map* by enabling 'low-risk' trade of cattle between herds. However, the Australian National Bovine Johne's Disease Strategic Plan aims not only to protect non-infected herds and to reduce between-herd spread, but also to minimise the contamination of animal products with *Map* and human exposure to *Map* (Citer and Kennedy, 2009). The Italian programme was aimed at minimising the contamination of a single animal product: semen (Pozzato et al., 2007).

Certification-and-surveillance programmes generally start with an initial assessment of prevalence. Test-negative cattle populations are certified and advance to a surveillance procedure. Cattle populations that are test-positive in the initial assessment or surveillance procedure are either expelled from the programme or change to a control procedure, which aims to reduce the prevalence of *Map* infection to enable certification of the population eventually. However, the specific design of a certification-and-surveillance programme depends on its aims and the quality targets that should be met.

Quality targets for programmes aiming at 'low-risk' trade of cattle between herds are typically either a preset probability of herds at each level of certification to be truly *Map*-free (Bulaga and Collins, 1999; Collins, 1999) or an acceptably low between-herd transmission between certified '*Map*-free' herds (i.e. between-herd transmission ratio $R_h << 1$; van Roermund et al., 2002). The U.S. Voluntary Johne's Disease Herd Status Program for Cattle, VJDHSP was designed taking into account target probabilities of having a non-infected herd at the herd status 'level 1', 'level 2', 'level 3', and 'level 4' of 85%, 95%, 98%, and 99% respectively (USDA, 2006). The Dutch certification-and-surveillance programme for '*Map*-free' herds was designed to result in a between-herd transmission ratio amongst '*Map*-free' herds $R_h << 1$ (van Roermund et al., 2002). This latter approach does not only require insight about the progression of the infection-and-disease process in infected cattle (such as, the age at onset of shedding), the within-herd transmission of *Map*, the within-herd prevalence distribution and the diagnostic characteristics of tests used in the programme, but also insight about the rate and structure of contacts between herds (such as cattle transfers between herds) and the probability of infection given contact.

Quality targets for programmes that aim to minimise the contamination of food products with *Map* are not easily established, given the uncertainty about the zoonotic potential of *Map* and the lack of a dose-response curve. However, in the face of this uncertainty, targets may be chosen to ensure a high level of confidence that no viable *Map* are present in the final food product (such as pasteurised milk).

In most voluntary certification-and-surveillance programmes for *Map*, only a limited proportion of dairy herds in the target population participate. For example, only about 10% of the U.S. dairy herds are currently enrolled in the VJDHSP, which was initiated in 1998 (Bulaga and Collins, 1999; Carter et al., 2009). Similarly, a certification-and-surveillance programme for '*Map*-free' dairy herds was initiated in the Netherlands in 1998 (Benedictus et al., 2000). The uptake of this programme amongst farmers was low: by the end of 2002 only 1,231 of approximately 25,000 Dutch dairy herds were participating.

1.4. Objective of this thesis

The low uptake of certification-and-surveillance programmes for *Map* amongst dairy farmers may be related to the fact that participation in many certification-and-surveillance programmes is rather expensive while the direct benefits for most farmers to have a certified herd are small (Velthuis et al., 2006). Therefore, the overall objective of this thesis was to investigate the potential for improvements in surveillance of *Map* infection and paratuberculosis, leading to a reduction in surveillance costs whilst continuing to meet specific quality targets. In particular, differentiation of

surveillance strategies to accommodate the aims and needs of various groups of dairy farmers was studied.

Groups of dairy farmers to be distinguished are dairy farmers selling cattle to other dairy herds, dairy farmers purchasing cattle, dairy farmers selling milk to dairy processing industries, and all dairy farmers. These groups of dairy farmers are not mutually exclusive, but overlap each other. To accommodate the aims and needs of dairy farmers selling or purchasing cattle, improvements of the pre-existing Dutch certification-and-surveillance programme for '*Map*-free' herds were studied. To accommodate the aims and needs of dairy farmers selling milk to dairy processing industries, various alternative designs of a bulk milk quality assurance programme were studied. To accommodate the aims and needs of all dairy farmers, the preferred choice of test for surveillance of clinical paratuberculosis was studied.

1.5. Outline of this thesis

In this thesis, a pre-existing simulation model JohneSSim (Groenendaal et al., 2002; Groenendaal et al., 2003) was used to support improvement of the cost-effectiveness of the Dutch certification-and-surveillance programme for '*Map*-free' herds. To identify attractive alternative test schemes, various alternative test schemes for this programme were studied with the simulation model. The use of the model for this purpose was validated by comparing model results with results of a field study in 90 dairy herds (Chapter 2). Furthermore, the simulation model was used to support the development of a bulk milk quality assurance programme. Various alternative test schemes as well as the effect of preventive management measures were studied using the simulation model (Chapter 3).

In addition, field data were analysed to obtain information useful to the improvement of surveillance systems for *Map* infection and paratuberculosis in dairy herds. These field data were used to study three issues related to the surveillance of *Map* infection and clinical paratuberculosis. Firstly, detecting clinical paratuberculosis cases is an important component of surveillance for *Map* infection (Martin, 2008). However, the preferred choice of test for the confirmation of a presumptive diagnosis of clinical paratuberculosis was unknown. Therefore, two tests, a serum-ELISA and examination of Ziehl-Neelsen stained faecal smears, were evaluated for this purpose (Chapter 4). Secondly, trade of cattle is considered the main route of between-herd transmission of *Map* (Sweeney, 1996). However, cattle transfers between herds under paratuberculosis surveillance and their effect on the potential spread of *Map* had not been quantified. Therefore, these cattle transfers were studied (Chapter 5). Finally, the age at onset of shedding of *Map* may have an effect on the within-herd transmission of *Map*, and consequently on the between-herd transmission of *Map* in herds under

paratuberculosis surveillance. Therefore, the distribution of this age at onset of shedding of *Map* was studied (Chapter 6).

To conclude this thesis, general conclusions and prospects for future developments and improvements in the surveillance of *Map* infection and paratuberculosis in dairy herds are presented in Chapter 7.

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Chapter 2

Simulation of alternatives for the Dutch Johne's disease certification-and-monitoring programme

M.F.Weber [a], H. Groenendaal [b], H.J.W. van Roermund [c], M. Nielen [b]

Preventive Veterinary Medicine 62 (2004) 1-17

[a] GD Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands.

[b] Farm Management Group, Wageningen University, Hollandseweg 1, 6706 KN, Wageningen, The Netherlands

[c] Quantitative Veterinary Epidemiology, Institute for Animal Science and Health, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

2.1. Abstract

To identify optimal method(s) for certification and subsequent monitoring of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*)-unsuspected herds, certification-and-monitoring schemes were studied using a stochastic simulation model ("JohneSSim"). JohneSSim simulated the within-herd transmission and economic aspects of *Map* in closed Dutch dairy herds. The model was validated with field observations on *Map*-unsuspected herds. The current Dutch certification-and-monitoring schemes were compared with 11 alternative schemes in which individual and pooled faecal culture, ELISA, Johnin-intradermal test and γ-IFN ELISA were used, varying the test frequency, tested age group and number of tested animals.

On reaching the '*Map*-free' status with the standard certification scheme, 11% of the simulated herds were not truly *Map*-free. Therefore, the designation '*Map*-free' should be changed into, for instance, 'low-risk *Map*'. In the most-attractive alternative certification scheme, the '*Map*-free' status was reached after four herd examinations (at 2-year intervals) consisting of serial testing of all cattle ≥ 2 years of age with a pooled faecal culture and individual faecal culture of positive pools. This scheme resulted in lower total and annual discounted costs and a lower animal-level prevalence at reaching the '*Map*-free' status compared to the standard scheme, assuming that there was no new introduction of the infection.

Schemes to monitor the '*Map*-free' status were compared, assuming that this status was reached with the standard certification scheme. In comparison to the standard monitoring scheme, none of the alternative monitoring schemes resulted in both a lower animal-level prevalence of undetected pre-existing *Map* infections in closed herds, and lower median annual discounted costs.

Results of the model were very sensitive to the assumed sensitivity of the faecal culture test and to management measures that prevent within-herd transmission of *Map*. If these preventive measures were taken, the probability of undetected *Map* infections in closed '*Map*-free' herds was decreased substantially.

Keywords: Cattle; Paratuberculosis; Monte Carlo simulation; Certification; Monitoring; Control programme

2.2. Introduction

In a control programme for Johne's disease, certified *Mycobacterium avium* subsp. *paratuberculosis* (*Map*)-free cattle herds are important as a source of non-infected cattle. Certification programmes to identify *Map*-free (i.e. low-risk) herds have been developed in several countries (Kennedy et al., 2001). In the Netherlands, herds can obtain *'Map*-free*'* status following five annual herd examinations for which all results are negative (Benedictus et al., 1999). The first herd examination consists of serial testing of all cattle ≥ 3 years of age by serology (ELISA) and individual faecal culture of seropositive animals. The second to fifth herd examinations each consist of serial testing of all cattle ≥ 2 years of age with pooled faecal culture and individual-animal faecal culture of positive pools. The status of these certified '*Map*-free' herds is then monitored by annual herd faecal examinations, exactly as the second to fifth herd examination. For the pooled faecal culture, all animals ≥ 2 years of age are stratified by age. A pooled faecal sample is then obtained from each group of five animals and cultured as a single sample (Kalis et al., 2000). If a pooled sample is culture positive, the five animals are re-examined by individual faecal culture. If all individual faecal samples of a previously positive pool are negative, then this pool is regarded as culture negative. To reduce the risk of introduction of a *Map* infection in '*Map*-free' herds, cattle may be added to these herds only if the cattle originate from another '*Map*-free' herd. In addition, cattle may be added to herds that are in the process of '*Map*-free' certification only if they originate from a herd with an equal or higher number of negative annual herd examinations.

In September 2000, the first Dutch dairy herd obtained the '*Map*-free' status, and at the end of 2002 there were 233 '*Map*-free' certified herds in the Netherlands. However, because the current certification-and-monitoring scheme was felt to be too expensive (especially for closed herds) a study of alternatives was required. Alternative schemes had to fulfil three requirements to be considered for implementation: (1) the prevalence of undetected pre-existing *Map* infections in closed '*Map*-free' herds should not be higher than with the current scheme, (2) the costs of obtaining and monitoring a '*Map*-free' status had to be reduced, and (3) transmission of *Map* infections between '*Map*-free' herds had to be limited. (The transmission of *Map* infections between '*Map*-free' herds was studied in a separate study with a mathematical R_0 model by van Roermund et al., (2002b).) In the present study, alternative schemes were simulated to study their effects on the prevalence of pre-existing infections in closed dairy herds, and to study the associated costs.

2.3. Materials and methods

2.3.1. The JohneSSim model

The JohneSSim model is a stochastic and dynamic simulation model that simulates: (a) the herd dynamics, (b) the disease dynamics within the herd, (c) the control of Johne's disease and (d) the economic consequences at the herd-level. The model simulates a period of 20 years with, at the background, time steps of 6 months and generates output-data with time steps of 12 months. The time horizon of 20 years was chosen to support middle-to-long-term decisions. The herd dynamics of a typical Dutch dairy herd are simulated, including calves and replacement heifers. All animals in the herd have various attributes (such as parity, stage of infection, month in lactation, and milk production). The model contains many probability distributions for uncertain events (such as infection, progression of the stage of infection and culling). In the model, five infection routes are considered: (1) intra-uterine infections, (2) infections occurring around birth, (3) infections due to drinking colostrum, (4) infections due to drinking whole milk, and (5) infections due to environmental contamination with *Map*. Six stages in the infection-and-disease process are distinguished: (1) susceptible, (2) non-susceptible, (3) latent-infected, (4) lowly infectious, (5) highly infectious and (6) clinical disease. Both voluntary culling and involuntary culling are considered. The probability distributions for uncertain events are used for random sampling; repeated runs of the model provide insight into the variation in outcome at the farm level. Results at a higher aggregation level (e.g. national level) are obtained by simulating different types of dairy herds and aggregating the results according to their relative abundance. Preventive management and prevalence in the simulated herds was set to reflect the distribution of management practices and prevalence in the Dutch dairy industry (Muskens et al., 2000; Groenendaal et al., 2002). To represent the difference between preventive management on individual dairy farms, eight different herd risk-profiles were defined (van Roermund et al., 1999) and simulated separately. In total, the aggregation of all risk-profiles consisted of 7805 iterations. In the present study, relevant herd-specific model outcomes were the within-herd true prevalence and test prevalence over time, and costs spent over time on the certification-and-monitoring schemes. The JohneSSim model and assumptions made on parameters (such as herd size, yearly increase in herd size, herd prevalence and distribution of the within-herd seroprevalence at the start of simulations and probability distributions for uncertain events) were described in detail previously (Groenendaal et al., 2002). Resulting from these assumptions, the initial herd-level prevalence of the simulated dairy herd population was 79%, and the initial animal-level prevalence in the total simulated dairy population was 22%.

2.3.2. Assumptions in the JohneSSim model for present study

In the present study, all herds were assumed to be closed, and no new introduction of *Map* into any herd could occur during the simulations. Assumptions were made by an expert panel on the characteristics of tests (Table 1) and the costs of the programmes (Table 2). The estimated sensitivity of the ELISA depends on the stage of infection and the ELISA-kit used, and ranges from 12 - 24% in low-shedders to 68–79% in high-shedders and 87–88% in clinically diseased animals (Sweeney et al., 1995; Dargatz et al., 2001; Kalis et al., 2002). However, in the JohneSSim model, lowly infectious animals were defined as intermittent-shedders and highly infectious animals as continuous-shedders, and therefore sensitivities were estimated to be slightly lower than for low- and high-shedders, respectively. The sensitivity in latent-infected animals arbitrarily was set at 5%. The sensitivity of serial testing with the intradermal test (Johnin skin test) and the gamma interferon (γ-IFN) ELISA was calculated assuming independence of these tests. Combined specificity was based on field data (Kalis, personal communication, 2001). In the model, the minimal age at which infectious animals contribute to the transmission of *Map* was set to 2 years. Nevertheless, in the present study we assumed that faecal shedders between 1 and 2 years of age could be detected by faecal culture (Table 1). Discounted costs of the certification-and-monitoring programme were calculated assuming a real interest rate (approximated by interest rate minus inflation rate) of 5% per year.

2.3.3. Validation of the model

Results of a simulation of 7805 closed dairy herds were compared with the results of a field study of 90 dairy farms in the North of the Netherlands (Kalis et al., 2003a). In 100 herds entering the field study, herd management had been closed for ≥ 3 years, while clinical signs of paratuberculosis and positive laboratory results were absent for ≥ 5 years. Ten herds were withdrawn from the field study (for instance, because farmers ceased farming) and were excluded from the analyses here. In both the simulation and the field study, herds were selected in which a first herd examination of all cattle ≥ 2 years of age with the pooled faecal culture did not reveal any *Map* infections. This selection criterion was used to start the comparison with a set of herds that were *Map*-unsuspected in both the simulation and the field study. The number of selected herds in the simulation was 3995, and in the field study 77. Subsequently, the selected herds were examined a further eight times at half-year intervals by pooled faecal culture of all cattle ≥ 2 years of age. At each 1-year interval, the simulated proportion of remaining test-negative selected herds was compared with the observed proportion of remaining test-negative selected herds in the field study by Pearson's χ^2. Exact 95% confidence intervals were calculated for the number of remaining test-negative herds as a proportion of the number of herds test-negative at the first herd examination.

Table 1. Assumptions on sensitivity (Se) and specificity (Sp) of different tests in simulations of the certification-and-monitoring of '*Map*-free' herds (Johne's disease, the Netherlands).

	Age group (month of age)	Stage of infection	Individual faecal culture	Pooled faecal culture	ELISA	Intradermal test[a]	γ-IFN ELISA	Serial testing with intradermal test [a] and γ-IFN ELISA
Se (%)	12 – 36	Latent	0	0	–	60	60	36
		Lowly infectious	40	36	–	60	60	36
		Highly infectious	95	95	–	50	50	25
		Clinical disease [b]	90	90	–	30	30	9
	> 36	Latent	0	0	5	60	60	36
		Lowly infectious	40	36	10	60	60	36
		Highly infectious	95	95	60	50	50	25
		Clinical disease	90	90	80	30	30	9
Sp (%)	All	Not infected	100 [c]	100	99.7 [d]	88.8 [e]	96.0 [f]	98.6 [f]

[a] Intradermal test = Johnin skin test, at a cut-off value of 2 mm. [b] In JohneSSim simulations, animals do not become clinical diseased before 2 years of age. [c] Reinders (1963). [d] van Maanen et al., (1999). [e] Kalis et al. (2003b). [f] Kalis (personal communication, 2001).

Table 2. Variable costs (€) of participation in the '*Map*-free' certification-and-monitoring programme for Johne's disease in the Netherlands [a].

Test or action	Veterinarian	Transport	Laboratory (per submission)	Laboratory (per test)
Veterinarian's visit	18.15	–	–	–
Pooled faecal culture	2.72 per animal	7.26	6.81	34.49 per pool (maximum five animals)
Individual faecal culture	2.27 per animal	7.26	6.81	28.13 per animal
ELISA	2.27 per animal	7.26	6.81	5.67 per animal
Intradermal test [b]	3.18 per animal	–	–	–
γ-IFN ELISA	2.27 per animal	7.26	6.81	11.34 per animal

[a] Subscription costs were € 88.49 per year. Costs do not include value added tax (VAT). VAT for subscription and laboratory tests = 6%; VAT on other costs = 19%.
[b] Two veterinary visits are required for an intradermal test.

*2.3.4. Comparison of different 'Map-free' **certification** schemes*

The current (standard; St) and nine alternative schemes for certification of '*Map*-free' herds were simulated (Table 3). Herds with a positive individual faecal culture were expelled from the certification programme, and could not re-enter the programme. For each of the certification schemes, we determined: (1) the within-herd prevalence over time of pre-existing *Map* infections in the remaining test-negative closed dairy herds, (2) the animal-level prevalence over time in a dairy population consisting of all remaining test-negative iterations (i.e. herds) of the simulation, and (3) the costs from the start of the programme until reaching the '*Map*-free' status. Because the time from the start of the programme to reaching the '*Map*-free' status differed between the various certification schemes, both the total discounted costs and annual discounted costs (annuity costs) until the '*Map*-free' status were calculated. The animal-level prevalence (i.e. total number of infected animals/total number of animals in the population) at reaching the '*Map*-free' status and the total and annual discounted costs until reaching this status were compared for the different certification schemes.

*2.3.5. Comparison of different schemes to **monitor** 'Map-free' herds*

The current scheme to monitor the '*Map*-free' status and eight alternative monitoring schemes were simulated (Table 3). In all cases, simulations were started with herds that had reached the '*Map*-free' status in year 5 by the standard certification scheme (Table 3). Herds that were detected as infected were expelled from the certification-and-monitoring programme and could not re-enter the programme. For each monitoring scheme, we calculated: (1) the animal-level prevalence over time of undetected pre-existing *Map* infections in a dairy population consisting of all remaining test-negative herds and (2) the annual discounted costs for the remaining test-negative herds. To compare different monitoring schemes, the animal-level prevalence at 6 years after reaching the '*Map*-free' status and the annual discounted costs to that time were used. This time span was chosen to maximize discrimination between different test schemes with regard to the animal-level prevalence in remaining test-negative herds.

2.3.6. Sensitivity analysis

The influence of several parameters in the model was studied in a sensitivity analysis. The following parameters were changed one at the time:

(1) The default herd size at the start of the simulations was 50 adult cattle and 46 young stock. However, at the end of 2002, the mean number of adult cattle (± SD) in Dutch dairy herds was 65 (± 37). Therefore, to study the influence of herd size, an initial herd size of 100 adult cattle and 92 young stock was simulated with test schemes St, B and D.

Table 3. Simulated test schemes for the certification-and-monitoring of '*Map*-free' herds for Johne's disease in the Netherlands [a].

Scheme	Certification schemes	Monitoring schemes	First herd examination for certification		Second to fifth herd examination for certification and all herd examinations for monitoring			Year *'Map*-free' status achieved if all herd-tests negative
			Test	Animals and age (year)	Test	Frequency	Animals and age (year)	
St [b]	**x**	**x**	ELISA	All, ≥ 3	PF [c]	Once per year	All, ≥ 2	5
A [d]	**x**	**x**	ELISA	All, ≥ 3	IDT / γIFN	Once per year	All, 1 – 3	5
B [e]	**x**	**x**	ELISA	All, ≥ 3	PF / ELISA	Once per year	All, ≥ 2 / ≥ 3	8
C [f]	**x**		--	--	PF	Once per year	All, ≥ 2	4
D	**x**	**x**	ELISA	All, ≥ 3	PF	Once per 2 years	All, ≥ 2	8
E	**x**		ELISA	All, ≥ 3	PF	Twice per year	All, ≥ 2	3
F	**x**	**x**	ELISA	All, ≥ 3	PF	Once per year	30 youngest, ≥ 2	5
G		**x**	--	--	PF	Once per year	30 oldest, ≥ 2	--
H	**x**	**x**	ELISA	All, ≥ 3	PF	Once per year	All, ≥ 1	5
I		**x**	--	--	PF	Once per year	All, 1 - 3	--
CD [f]	**x**		--	--	PF	Once per 2 years	All, ≥ 2	7
DH	**x**	**x**	ELISA	All, ≥ 3	PF	Once per 2 years	All, ≥ 1	8

[a] Monitoring test schemes were simulated for herds that had reached the status '*Map*-free' by the standard certification scheme. A positive result in the ELISA or pooled faecal culture always was confirmed by individual faecal culture of the animals concerned. If an individual faecal culture was positive, the herd was expelled from the programme, and did not achieve the '*Map*-free status'. [b] Standard (current) scheme. [c] Pooled faecal culture (Kalis et al., 2000). [d] Scheme A includes testing of all cattle between 1 and 3 years of age with the intradermal test (Johnin skin test). Any animal tested positive with the intradermal test was tested with the γIFN – ELISA, and if positive, all cattle ≥ 2 years of age in the herd were tested with the pooled faecal culture. [e] Scheme B includes an annual herd examination with alternating a pooled faecal culture of all cattle ≥ 2 years of age and a serological examination (ELISA) of all cattle ≥ 3 years of age. The 'Map-free status' was obtained after eight herd examinations (four serological and four faecal examinations). [f] In schemes C and CD the 'Map-free' status can be obtained after only four herd examinations.

(2) The default sensitivity of the pooled faecal culture was 36% for lowly infectious cattle, 95% for highly infectious cattle and 90% for clinically diseased cattle (Table 1). Alternatively, test schemes St, B and D were simulated with a sensitivity of the pooled faecal culture equal to the default values multiplied by an arbitrary 0.75.

(3) In the current Dutch certification-and-monitoring programme, a confirmatory individual faecal culture of all animals in a faecal culture positive pool is allowed. Therefore, this was assumed by default in the present study. However, if all individual faecal samples of such a previously positive pool are negative, then the pool is regarded as culture negative – which means that an infected herd might not be detected. Therefore, as an alternative, test schemes St, B and D were simulated without confirmatory individual faecal culture of a culture positive pool.

(4) Because field data of the combined sensitivity of serial testing with the intradermal test and the γ-IFN ELISA were lacking, a combined sensitivity was calculated assuming independence of these tests. However, this is considered a worst-case scenario, because it is unlikely that these tests are independent. Therefore, the combined sensitivity was calculated alternatively assuming complete interdependence of the two tests (which means that these tests would be positive in the same infected individuals).

(5) By default, the results at the national level were calculated by aggregation of the results of the eight risk-profiles of herds. These risk-profiles reflected the wide variation in preventive measures taken by Dutch dairy farmers (Groenendaal et al., 2002). However, dairy farmers are stimulated to take preventive measures against the transmission of *Map*. Therefore, the results were calculated alternatively for the standard scheme and the two most-extreme risk-profiles:
   (a) Risk-profile A (rather good preventive management) in which (a) calves were fed colostrum of their own dams and milk replacer only, and (b) calves from 0 to 6 months of age were housed separately from adult cattle.
   (b) Risk-profile B (rather poor preventive management) in which (a) calves were fed mixed colostrum, whole milk and milk withdrawn from human consumption, and (b) calves from 0 to 6 months of age were housed together with the adult cattle.

2.3.7. Data analysis

Animal-level prevalences obtained by different test schemes were compared by Pearsons χ^2. If an overall χ^2 was significant, then each alternative scheme was compared individually with the standard scheme by Yate's continuity-corrected χ_{cc}^2, using Bonferroni's correction of *P* for adjusting for multiple comparisons (Altman, 1999). Costs of different test schemes were compared using the Kruskal-Wallis rank-sum test (adjusted for ties). If significant differences were found, then the alternative

test schemes were individually compared two-sided with the standard test scheme using the Mann-Whitney test (adjusted for ties) with Bonferroni's correction of *P*. In all tests, significance was declared at $P \leq 0.05$ (two-sided).

2.4. Results

2.4.1. Validation

Of 90 herds in the field study, 77 were pooled faecal culture negative at the first herd examination (Kalis et al., 2003a), Of these 77 herds, only 46% (35 herds) were still culture negative at the ninth herd examination (Fig. 1). No difference was found between the proportion of unsuspected herds in the field study and in the simulation after the third herd examination ($\chi^2 = 3.50$, df =1, $P = 0.06$), the fifth herd examination ($\chi^2 = 0.02$, df = 1, $P = 0.90$), the seventh herd examination ($\chi^2 = 0.69$, df = 1, $P = 0.41$) and the ninth herd examination ($\chi^2 = 0.30$, df = 1, $P = 0.58$). In retrospect, a true difference of >16% between the proportion of unsuspected herds after the ninth herd examination in the field study and in the simulation could have been detected with a power of 80%.

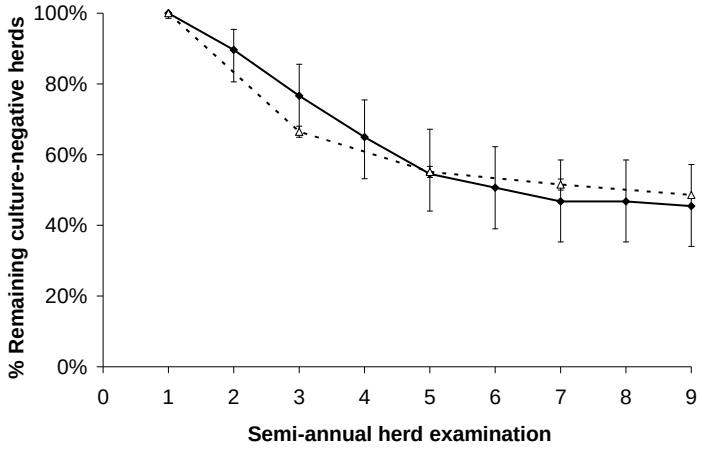

Fig. 1. Proportion of remaining test-negative dairy herds examined by semi-annual pooled faecal culture of all cattle > 2 year in a JohneSSim simulation of 3995 herds (Δ) and a field study on Johne's disease in 77 Dutch herds (♦) ± exact 95% confidence intervals.

2.4.2. Comparison of different 'Map-free' certification schemes

Using the standard certification scheme, test-negative herds obtained the '*Map*-free' status after 5 years. At that time, 23% of the simulated herds were truly free of *Map* infection, and 77% of the simulated herds were infected. Using the standard certification scheme, 74% of all simulated herds had a positive individual faecal culture in any of the first five annual herd examinations, and were expelled from the programme. Twenty-six percent of the herds remained test-negative (Fig. 2A), and therefore reached the '*Map*-free' status at a median cost of € 3412 (Table 4). Thus, with the standard certification scheme, an infection was present but not yet detected in 3% of the simulated herds (i.e., in 11% of the herds that remained test-negative). The initial animal-level prevalence over all simulated herds was 22%. After 5 years, this animal-level prevalence over all simulated herds had increased to 34%. By then, the animal-level prevalence over all remaining test-negative herds was only 0.56% (Fig. 2A). The distribution of the within-herd prevalence in herds that were positive in any herd examination and in remaining test-negative herds (i.e. certified '*Map*-free' herds) is shown in Fig. 2B.

With alternative certification schemes, 25–27% of the herds reached the '*Map*-free' status at median discounted total costs between € 1890 and € 4782 (Table 4). In comparison with the standard certification scheme, schemes C, E, F and CD resulted in lower median *total* discounted costs ($P < 0.001$; Table 4; Fig. 3A), and schemes B, D, F, CD and DH resulted in lower median *annual* discounted costs until the '*Map*-free' status was reached ($P < 0.005$; Fig. 3B). Schemes B, D, CD and DH resulted in a lower overall animal-level prevalence upon reaching the '*Map*-free' status (Table 4; Fig. 3D). In these four schemes, the pooled faecal culture was used only once every 2 years. Hence, the period until the '*Map*-free' status was reached was prolonged (Table 3). Only scheme CD resulted in a combination of both lower total and annual discounted costs until the '*Map*-free' status was reached, and a lower overall animal-level prevalence at that point.

2.4.3. Comparison of different schemes to ***monitor*** *'Map-free' herds*

After the '*Map*-free' status was reached in year 5 with the standard certification scheme, it took an additional 10 years to detect all infected '*Map*-free' herds with the standard monitoring scheme. With the alternative monitoring schemes A, B, D, F, H and DH this took 9–15 years. Therefore, with these schemes, the animal-level prevalence over all remaining '*Map*-free' herds decreased to zero in year 14–20 (Fig. 4). However, monitoring schemes G and I failed to detect all infected '*Map*-free' herds within the simulated 20-year period. If the standard monitoring scheme was used, the animal-level prevalence in remaining test-negative herds fell to 0.02% in year 11 (Fig. 4). The median annual discounted costs were by then € 708. None of the alternative monitoring schemes resulted in both lower median annual discounted costs up to year 11 and a lower animal-level prevalence in the remaining test-negative herds at the same

time. For instance, monitoring scheme DH resulted in a prevalence of 0.04% in year 11, although the median annual discounted costs to that point were only € 596.

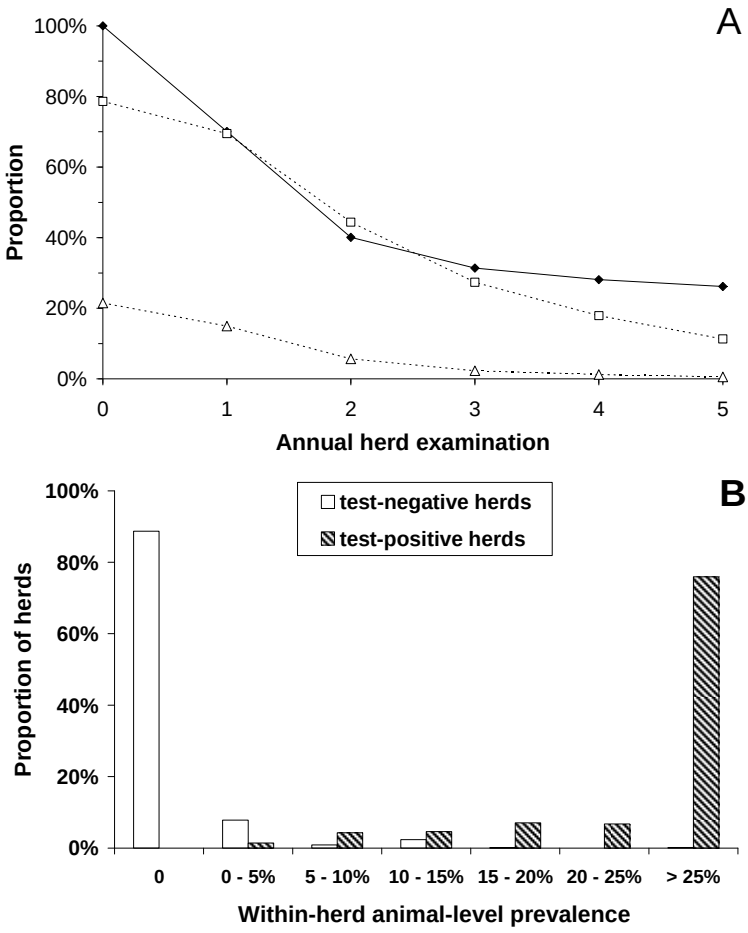

Fig. 2. Results of a simulation of the standard certification scheme for Johne's disease in the Netherlands. (A) Proportion of remaining test-negative herds (—◆—), proportion of infected herds in the group of remaining test-negative herds (- - -□- - -), and proportion of infected animals in the group of remaining test-negative herds (- - -△- - -), at each herd examination. (B) Distribution of within-herd animal-level prevalence after five herd examinations for herds that were test-positive in any of the herd examinations, and in herds that were test-negative in all herd examinations, and therefore reached the status '*Map*-free'.

Table 4. Estimated probability and total discounted costs (€) of reaching the '*Mycobacterium avium* subsp. *paratuberculosis*-free' ('*Map*-free') status, and animal-level prevalence upon reaching the '*Map*-free' status with various certification schemes [a].

Scheme	Probability of reaching '*Map* – free' status (%)	Costs (€)					Mann-Whitney against 'St'		Animal-level prevalence (%)	Yates continuity corrected χ^2_{cc} against 'St'	
		Min	Percentiles			Max					
			5%	50%	95%		W (*10^6)	P [b]		χ^2_{cc} (df = 1)	P [b]
St	26	2901	3019	3412	3732	3860			0.56		
A	26	1670	2380	3447	4603	5162	4.06	0.005	0.61	8.36	0.04
B	25	4040	4239	4782	5082	5685	2.09	< 0.001	0.24	418.33	< 0.001
C	27	2444	2593	2829	3288	3526	6.36	< 0.001	1.02	433.83	< 0.001
D	27	3090	3203	3563	3819	4174	3.08	< 0.001	0.44	47.01	< 0.001
E	27	2738	2906	3230	3649	3794	5.28	< 0.001	0.76	99.11	< 0.001
F	27	1816	1882	1890	2012	2316	6.45	< 0.001	1.44	1254.49	< 0.001
H	26	3667	3923	4387	4846	5029	2.09	< 0.001	0.58	1.99	> 0.5
CD	26	2667	2813	3106	3428	3570	5.71	< 0.001	0.48	17.61	< 0.001
DH	27	3929	4069	4596	4959	5275	2.09	< 0.001	0.43	60.74	< 0.001

[a] Overall, the costs for reaching the '*Map*-free' status were different (Kruskal Wallis test: H=17726.42, df=9, $P < 0.001$) and, the animal-level prevalences were different ($\chi^2 = 5539.29$, df = 9, $P < 0.001$).
[b] Bonferroni-corrected P.

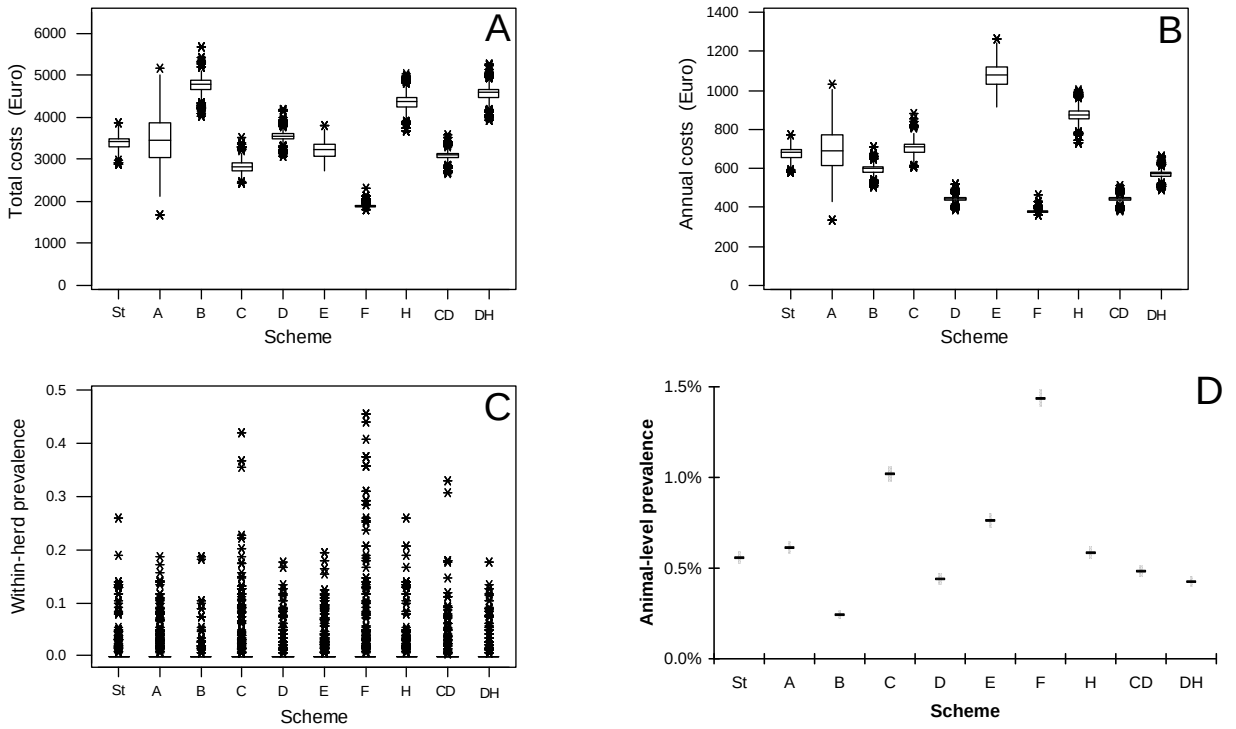

Fig. 3. Costs for reaching the '*Map*-free' status and prevalence at reaching this status for various simulated certification schemes for Johne's disease in the Netherlands. The certification schemes are defined in Table 3. (A) Boxplot of total discounted costs. (B) Boxplot of annual discounted costs. (C) Boxplot of within-herd animal-level prevalence. (D) Overall animal-level prevalence (i.e. number of infected animals/total number of animals in all herds reaching the '*Map*-free' status) with 95% confidence intervals. In the boxplots, the boxes indicate the first, second and third quartile. The whiskers extend from the top and bottom of the box to the lowest and highest observations that are within 1.5 times the inter-quartile range from the first and third quartile. Outliers outside this region are plotted with asterisks.

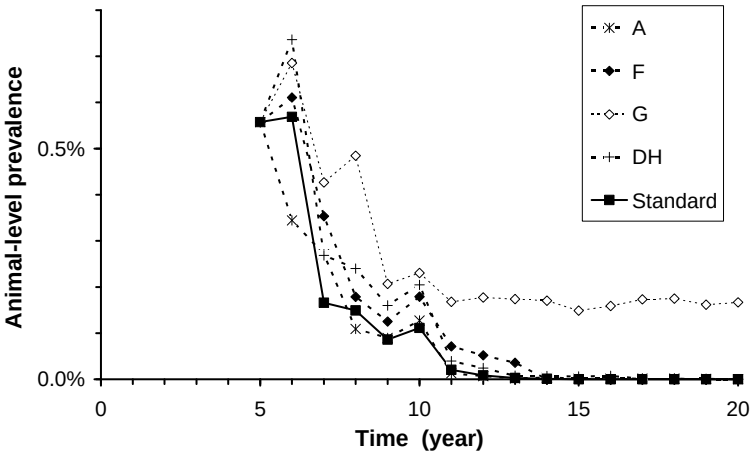

Fig. 4. Animal-level prevalence (i.e. total number of infected animals/total number of animals in all 'Map-free' herds) over time when different monitoring schemes are used after reaching the 'Map-free' status in year 5 by the standard certification scheme. The monitoring schemes are defined in Table 3.

2.4.4. Sensitivity analysis

The overall animal-level prevalence at reaching the '*Map*-free' status was estimated to be 0.1–0.3% lower in herds with 100 adult cattle than in herds with 50 adult cattle, depending on the test scheme used. The animal-level prevalence in '*Map*-free' herds at least doubled when the sensitivity of the pooled faecal culture was reduced to 0.75 of the default value (Fig. 5). However, if no confirmatory individual faecal culture of a culture positive pool was performed, then the animal-level prevalence upon reaching the '*Map*-free' status was reduced by a factor 0.3–0.6. Using alternative certification-scheme A, the animal-level prevalence upon reaching the '*Map*-free' status was 0.52% if the combined sensitivity of the intradermal test and the γ-IFN ELISA was calculated assuming complete interdependence of these tests, compared to 0.61% if independence of the tests was assumed. If the preventive management practices were rather good (risk-profile A), the prevalence in '*Map*-free' herds reached zero the year following the '*Map*-free' status. If the management practices were rather poor (risk-profile B), this took approximately 8 years (Fig. 6).

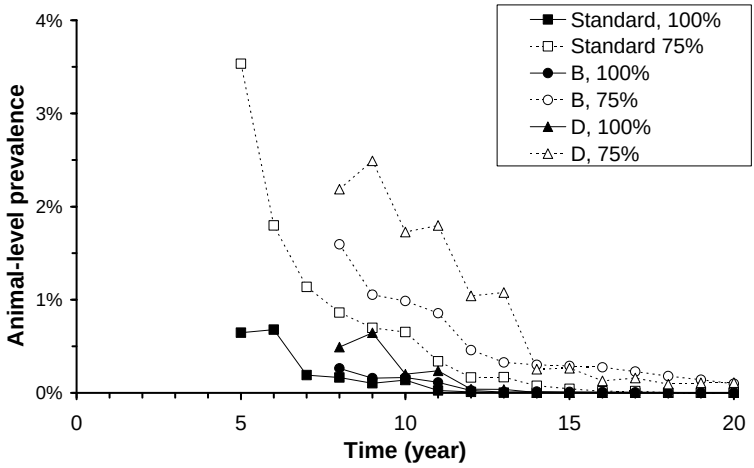

Fig. 5. Sensitivity analysis for sensitivity of the pooled faecal culture. Animal-level prevalence in '*Map*-free' herds over time at the default sensitivity ("100%") and 25% lower sensitivity for each stage of infection ("75%"). The different schemes are defined in Table 3.

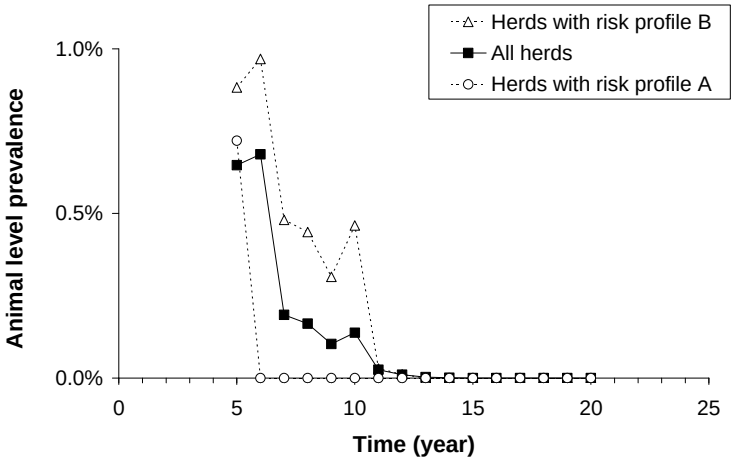

Fig. 6. Sensitivity analysis for preventive-management measures. Animal-level prevalence over time in all herds, herds with rather good calf management (risk-profile A) and herds with rather poor calf management (risk-profile B). (In all cases, the standard certification-and-monitoring scheme was used; Table 3).

2.5. Discussion

Simulations with the JohneSSim model were considered to be in general agreement with field observations on 77 closed dairy herds over a 4-year period. This does not necessarily mean that a similar agreement would be reached if field data over a longer time frame were available. For instance, the model could overestimate the proportion of remaining test-negative herds on the longer run. However, the results supported the validity of using the model for evaluation of alternative certification-and-monitoring schemes.

The JohneSSim model is a stochastic simulation model; therefore, the outcomes are probability distributions (as shown for within-herd prevalence in Fig. 2B). However, an individual farmer who buys cattle from a '*Map*-free' herd might be interested only in eliminating the risk of buying an infected animal. Because farmers lack information about the true *Map* infection status of the '*Map*-free' herd of origin, the only relevant parameter to purchasing farmers is the overall animal-level prevalence in the population of '*Map*-free' herds, which is used as a probability of the animal being infected. Therefore, in the present study, this overall animal-level prevalence of the population of '*Map*-free' herds was used to discriminate between alternative test schemes. To estimate this overall animal-level prevalence, the total animal population of '*Map*-free' herds was considered to consist of all iterations of a simulation. The resulting proportion (prevalence) is therefore a single point estimate and not a distribution. However, it is important to realize that infected cattle are clustered in a small proportion of '*Map*-free' herds – and that most herds truly are negative (Figs. 2 and 3C). The risk for the buyer is thus not spread evenly over all '*Map*-free' herds (in contrast to what might be suggested from our overall animal-level prevalences).

In the present study, comparisons between the standard and alternative schemes were supported by formal testing of the differences in animal-level prevalences and costs. However, the value of significance testing in a stochastic simulation is limited. With more iterations of the simulations, small and perhaps irrelevant differences between the standard and alternative schemes may become significant. Therefore, comparisons need to be focussed on the practical relevance of the differences between results obtained by different test schemes.

Our results predicted that an estimated 11% of the herds were not truly *Map*-free on reaching the '*Map*-free' status with the standard certification scheme. With the standard monitoring scheme, it took some 10 more years before all pre-existing infections were either extinct or detected. Therefore, the designation '*Map*-free' in the Dutch certification programme should be changed into, for instance, 'low-risk *Map*'.

The time from the start of the programme to reaching the '*Map*-free' status differed between the various *certification* schemes. Therefore, annual as well as total

discounted costs were estimated. Alternative certification schemes in which the interval between herd examinations is 2 years lengthened the certification process by 3 years (scheme B, D, CD and DH; see Table 3). However, these alternative certification schemes resulted in both lower estimated annual discounted costs and a lower estimated animal-level *Map* prevalence at reaching the '*Map*-free' status. This lower prevalence is probably because more individual-animals were tested for a *Map* infection over the longer period, and thus infected herds were more-likely to be detected. Only certification-scheme CD resulted in lower estimated annual and total discounted costs and a lower estimated *Map* prevalence at reaching the '*Map*-free' status, compared to the standard scheme. This might improve the acceptance of the programme by participants – although potential benefits of a '*Map*-free' status (such as trade and marketing advantages) are postponed by 3 years with this scheme. No data are available to estimate these benefits, but currently the financial benefits for herds that actually have achieved a '*Map*-free' status, compared to the benefits for herds that are half-way through the certification trajectory, appear to be limited**.** Thus, this scheme CD (in which the serologic herd examination was skipped and the '*Map*-free' status was reached after four pooled faecal cultures of all cattle ≥ 2 year of age at 2-year intervals) seemed to be the most-attractive alternative, assuming no new introduction of the infection.

Under the assumptions of the model, eventually all infected herds were detected by the standard *monitoring* scheme and the alternative monitoring schemes A, B, D, F, H and DH. However, the assumption that *Map* is not introduced into closed herds might not be realistic, especially if wildlife would be an important source of infection (Daniels et al., 2003). To our knowledge, field data of long-term (20-year) monitoring of *Map*-unsuspected herds are not available. However, a monitoring scheme for '*Map*-free' herds can be successful, even if introductions of *Map* into '*Map*-free' herds occur, as long as each infected herd is detected before the infection is transmitted to, on average, one other '*Map*-free' herd (van Roermund et al., 2002b).

In comparison to the standard scheme to monitor '*Map*-free' herds, none of the alternative *monitoring* schemes resulted in both a lower prevalence of undetected pre-existing *Map* infections in closed herds and lower median annual discounted costs. Monitoring scheme DH (faecal culture of all cattle ≥ 1 year of age at 2-year intervals) resulted in lower annual costs but a slightly higher prevalence of undetected *Map* infections in closed herds then the standard scheme. However, this scheme resulted in a sufficiently low between-herd transmission, if it was assumed that cattle could be traded between certified '*Map*-free' herds at a rate observed in 87 Dutch herds that were certified or in the process of certification as '*Map*-free' (van Roermund et al., 2002b). Therefore, we consider this scheme to be a suitable alternative for the standard monitoring scheme for maintaining a pool of '*Map*-free' herds.

The model was shown to be robust for initial herd size. This is important, because there is considerable variation in the herd size of Dutch dairy herds. If no

confirmatory individual faecal culture of positive pools in the pooled faecal culture was allowed, the prevalence in remaining test-negative herds was reduced markedly. Preclusion of the possibility of confirmatory individual faecal culture of positive pools might reduce the costs associated with testing of infected herds in a certification-and-monitoring programme and might simplify the programme. We assumed no changes in preventive management during the simulations. However, preventive measures against the transmission of *Map* infections resulted in a substantial lower probability of undetected *Map* infections in closed '*Map*-free' herds. Therefore, if a closed farming system is combined with preventive management, perhaps the certification-and-monitoring of such '*Map*-free' herds could be relaxed and carried out with considerable lower costs. Further studies in this field are needed.

We made important assumptions on the sensitivity of tests for the various stages of infection; published data are generally based on studies with high faecal shedders. The results of the JohneSSim model were very sensitive to the assumed sensitivity of the faecal culture. We assumed that young stock do not contribute to the transmission of *Map*. However, recently it has been suggested that calves contribute to the transmission of *Map* immediately after infection (van Roermund et al., 2002a). Furthermore, we assumed that faecal *Map*-shedders between 1 and 2 years of age could be detected by faecal culture. The efficacy of inclusion of this age group in herd examinations is expected to depend strongly on the sensitivity of faecal culture of this age group. In herds with clinical cases of Johne's disease, 2.1% of young stock between 1 and 2 years of age were culture positive (Kalis et al., 1999), but it is unknown whether this is similar in low-prevalence herds.

The present study was performed to assist decision-makers in selecting suitable alternatives for the Dutch certification-and-monitoring scheme for Johne's disease. A number of assumptions related specifically to Dutch dairy herds (such as the relative abundance of management risk-profiles, costs and initial (sero)prevalence). However, the mechanisms of transmission of the infection, disease and testing are comparable in other countries. Furthermore, the JohneSSim model has been adapted previously for use in Pennsylvanian dairy herds (Groenendaal et al., 2002) and Dutch beef herds, and thus provides a flexible tool for studying the within-herd transmission and detection of *Map* infections.

We conclude that the current Dutch certification-and-monitoring scheme for '*Map*-free' herds could be optimized by: (1) certification of '*Map*-free' herds after four herd examinations at 2-year intervals consisting of pooled faecal culture of all cattle ≥ 2 years of age, (2) monitoring of '*Map*-free' herds by pooled faecal culture of all cattle ≥ 1 year of age at 2-year intervals, and (3) vigorous execution of preventive management practices against the transmission of *Map* infections. In addition, the designation '*Map*-free' should be changed into, for instance, 'low-risk *Map*'.

2.6. Acknowledgements

The contributions of M.C.M. de Jong, and of the members of the expert-panel, C.H.J. Kalis, C. van Maanen, H.J. van Weering, and F.G. van Zijderveld, to this study were greatly appreciated. The authors thank A.R.W. Elbers and A.L.J. Gielkens for their comments on a previous version of the manuscript. This study was funded by the Dutch Ministry of Agriculture, Nature Management and Fisheries, and the Dutch Dairy Commodity Board.

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Chapter 3

Milk quality assurance for paratuberculosis: simulation of within-herd infection dynamics and economics

M.F. Weber [a], M. Nielen [b], A.G.J. Velthuis [c], H.J.W. van Roermund [d]

Veterinary Research (2008) 39:12

[a] GD Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands

[b] Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, The Netherlands,

[c] Business Economics, Wageningen University, Hollandseweg 1, 6706 KN, Wageningen, The Netherlands,

[d] Animal Sciences Group, Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

3.1. Abstract

A bulk milk quality assurance programme for *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) in dairy herds was simulated with a stochastic simulation model (JohneSSim). The aim of this study was to evaluate the epidemiological and economic effects of preventive management measures and various test schemes in a simulated population of closed Dutch dairy herds over a 20-year period. Herds were certified as 'low-*Map* bulk milk' if, with a certain probability, the concentration of *Map* in bulk milk did not exceed a maximum acceptable concentration of 10^3 *Map* organisms per litre (based on pasteurisation studies). The programme started with an initial assessment; test-negative herds entered a surveillance procedure and test-positive herds a control procedure. The simulations showed that herd examinations by ELISA for the initial assessment, surveillance and control procedures effectively ensure the quality of 'low-*Map* bulk milk': > 75% of simulated herds were certified and > 96% of certified herds produced bulk milk with < 10^3 *Map*/litre if the initial herd-level prevalence was 30%. Preventive management measures only had a minor effect on bulk milk quality of certified herds. Culling based on biennial faecal culture was more effective than culling based on annual ELISA. Average total discounted costs for 20-year participation in a programme consisting of initial assessment by ELISA, surveillance by biennial ELISA and control by biennial faecal culture were € 16×10^3 per herd. In conclusion, this study shows that a bulk milk quality assurance programme for closed Dutch dairy herds is feasible and provides information on the cost-effectiveness of different programmes. The concepts of this study equally apply to other countries because mechanisms of paratuberculosis infection, disease, and testing are comparable in other dairy cattle populations.

Keywords: Dairy cattle; Milk; Paratuberculosis; Stochastic simulation model; Quality assurance programme

3.2. Introduction

Mycobacterium avium subsp. *paratuberculosis* (*Map*) infections in cattle are of concern to the dairy industry due to the as-yet-unresolved issue of its potential role in Crohn's disease in humans (EU, 2000; Chacon et al., 2004; Herrewegh et al., 2004). Milk is a possible vehicle of transmission of *Map* to humans, because *Map* has been detected in raw milk (Sweeney et al., 1992b; Streeter et al., 1995; Giese and Ahrens, 2000; Grant et al., 2002b; O'Reilly et al., 2004; Ayele et al., 2005) and might not be effectively inactivated by pasteurisation (Grant et al., 1996; Sung and Collins, 1998; Grant et al., 1999; Gao et al., 2002; Grant et al., 2002a; Grant et al., 2002b; Ayele et al., 2005; Grant et al., 2005; McDonald et al., 2005). A milk quality assurance programme for paratuberculosis in dairy herds might reduce the potential risk of transmission of *Map* to humans through consumption of dairy products.

Certification-and-surveillance programmes for supposedly *Map*-free herds and control programmes for *Map*-infected herds have been developed in several countries, such as the USA, Australia and the Netherlands (Bulaga and Collins, 1999; Benedictus et al., 2000; Jubb and Galvin, 2000; Kennedy and Allworth, 2000). These certification-and-surveillance and control programmes generally aim at a low-risk trade of cattle and elimination of *Map*. Therefore, these programmes are inherently expensive and participation is often restricted to a minority of herds. For example, in the certification-and-surveillance programme that has been in place in the Netherlands since 1998, five negative annual herd examinations by different tests (including serology and faecal culture) are required to obtain '*Map*-free' status (Benedictus et al., 2000). By July 2005, only 473 of approximately 23,000 Dutch dairy herds had obtained this '*Map*-free' status. However, the goal of a milk quality assurance programme is to reduce the concentration of *Map* in bulk milk rather than eradication of *Map*. Herds in a milk quality assurance programme can be certified as 'low-*Map* bulk milk' if, with a certain probability, the concentration of *Map* in bulk milk does not exceed a pre-set maximum acceptable concentration. This does not necessarily mean that the herd is free of *Map*. Thus, such a milk quality assurance programme might possibly be run at considerable lower costs than a programme aiming at low-risk trade of cattle and elimination of *Map*.

A milk quality assurance programme would start with an initial assessment of each herd; test-negative herds enter a surveillance procedure and test-positive herds enter a control procedure. Herds subsequently testing positive in the surveillance procedure shift to the control procedure. The control procedure aims to decrease the concentration of *Map* in milk by suppression of the infection in the herds, such that the milk quality can be guaranteed and the herd can shift to the surveillance procedure. Test-negative herds in the surveillance procedure are assigned the 'low-*Map* bulk milk' status. However, various alternative test schemes for the initial assessment, surveillance and control procedures are available, and it was unknown which test

schemes would be most attractive. Furthermore, preventive management measures are important in programmes aiming at low-risk trade of cattle or elimination of *Map* (Groenendaal et al., 2002; Weber et al., 2004), but their cost-effectiveness in a milk quality assurance programme was unknown. Therefore, the aim of this study was to simulate a milk quality assurance programme in a population of closed Dutch dairy herds to evaluate the epidemiological effects and economic consequences of various alternative test schemes and preventive management measures.

3.3. Materials and methods

3.3.1. The JohneSSim model

The JohneSSim model is a stochastic and dynamic simulation model that simulates (a) herd dynamics, (b) disease dynamics within the herd, (c) the control of Johne's disease and (d) the economic consequences at the herd level. The model and its use to study certification-and-surveillance programmes have been described in detail (Groenendaal et al., 2002; Weber et al., 2004).

In short, the model simulates a period of 20 years with, at the background, time steps of 6 months and generates output-data with time steps of 12 months. The herd dynamics of a typical Dutch dairy herd are simulated, including calves and replacement heifers. All animals in the herd have various attributes (such as parity, stage of infection, month in lactation, and milk production). The model contains probability distributions for uncertain events (such as replacement, infection, progression of the stage of infection, testing). Both voluntary culling and involuntary culling are considered. The percentage of cows culled involuntarily is specified per lactation. Voluntary culling is based on the retention pay-off (RPO), i.e. the expected profit from trying to keep the animal until its optimal life-span is complete compared with immediate replacement, taking into account the risk of involuntary premature removal (Groenendaal et al., 2004). Cattle with the lowest RPO are culled until the number of adults is equal to the intended maximum number of adults in the herd.

In the model, five infection routes are considered: (1) intra-uterine infections, (2) infections at birth, (3) infections due to drinking colostrum, (4) infections due to drinking whole milk, and (5) infections due to environmental contamination with *Map*. Six stages are distinguished in the infection-and-disease process in individual cattle: (1) susceptible (i.e. uninfected, < 1 year of age), (2) non-susceptible (i.e. uninfected, ≥1 year of age), (3) latent infected, (4) lowly infectious, (5) highly infectious, and (6) clinical disease. The progress of the infection-and-disease process in infected cattle is influenced by the age at infection.

The probability distributions for uncertain events are used for random sampling. Repeated runs of the model provide insight into the variation in outcomes at the farm level. Results at a higher aggregation level (e.g. national level) are obtained by

simulating different types of dairy herds and aggregating the results according to their relative abundance.

All costs and losses are discounted, i.e. their net present values are calculated for a 10-year or 20-year period. Costs of culling an animal are estimated by its RPO.

3.3.2. Assumptions in JohneSSim model for present study

Preventive management in the simulated herds was set to reflect the distribution of management practices in the Dutch dairy industry ('background' management). To represent the differences between preventive management on individual dairy farms, eight different herd risk-profiles were defined and simulated separately (Groenendaal et al., 2002). Both infected and non-infected herds are simulated. Therefore, in total 16 herd types were simulated separately (8 risk profiles x 2 infection classes, infected and non-infected). The results of these herd types were aggregated according to their relative abundance in the Dutch dairy population to obtain results at the national level. In total, the aggregation of all herd types consisted of 10,500 iterations (including 200 to 1,944 iterations per herd type).

All herds were assumed to be closed (i.e. no purchase of animals and no new introductions of *Map*). Herd-size was assumed to be initially 65 adults (≥ 2 year), and to increase by 5% per annum. Eighty to 100% of heifer calves were raised in the herd, while a surplus of heifers was sold shortly before first calving. Mean annual milk production was 8,000 kg. The assumed distribution of the initial within-herd true prevalence in infected herds was based on analyses of Dutch seroprevalence data (Muskens et al., 2000) using a Bayesian model (van Schaik et al., 2003) with assumptions on test characteristics based on a Dutch validation study (van Maanen et al., 2002) and is shown in Fig. 1. Initial herd-level true prevalence was assumed to be 0.30, based on a recent study in the Netherlands (van Weering, personal communication, 2004). Costs of participation in the quality assurance programme and costs of preventive management measures were updated for this study (Tables 1 and 2). Economic assumptions on losses by paratuberculosis and on the costs of culling of test-positive cattle were also updated (Table 2). All costs were discounted at a real interest rate (approximated by interest rate minus inflation rate) of 5% per year. Assumptions on test characteristics are shown in Table 3. Assumptions on effectiveness of additional preventive management measures, imposed on the 'background' management, were based on the opinions of an expert panel (Groenendaal et al., 2002). By default, effective separation of young stock from adult cattle was assumed to reduce the number of effective cow-calf contacts through faecal contamination of the environment by 90%. Details of assumptions made for the present study are presented in Appendix I.

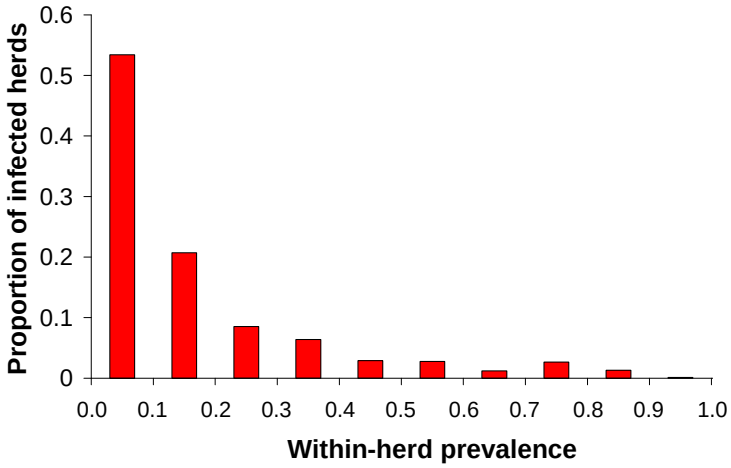

Figure 1. Assumed distribution of within-herd true prevalence of *Map* in infected Dutch dairy herds at the start of simulations. Thirty percent of herds were assumed to be infected, based on a recent study in the Netherlands (van Weering, personal communication).

Table 1. Variable costs (Euro) of participation in the bulk milk quality assurance programme. Additional annual subscription costs were € 90. Value added tax (VAT) was added to these costs (6% on costs of subscription and laboratory tests and 19% on other costs).

Test / action	Costs veterinarian [a]	Transport costs per submission [a]	Laboratory costs [b]	
			per submission	per test
Veterinarians' visit	22			
IFC [c]	2.75 per animal	10	7.80	30.00 per animal
PFC [d]	3.25 per animal	10	7.80	37.50 per pool (max. 5 animals)
ELISA	2.75 per animal	10	7.80	6.15 per animal

[a] based on rates charged in 2004 by a convenience sample of veterinary practices. [b] based on rates charged in 2004 by the GD Animal Health Service. [c] IFC = individual faecal culture; [d] PFC = pooled faecal culture.

Table 2. Assumed losses caused by infection with *Map* and costs of preventive management measures (including labour at € 18.21 per hour). Fifty percent of the costs of additional preventive management measures imposed on the 'background' management (Groenendaal et al., 2002) were attributed to the control of paratuberculosis. (The remaining 50% of costs were attributed to the control of other diseases.)

Category	Parameter	Loss or costs (Euro)	Details / reference
Losses caused by infection with Map			
Milk production	Reduction depends on infection state: 5% (lowly infectious) to 20% (clinical)	€ 0.08 / kg	Milk price (€ 0.33/kg) minus variable feed costs (€ 0.12/kg) minus costs of leasing milk if the loss occurs in the first 3/4 of the quota-year (3/4 x € 4 /kg fat x 0.0435 kg fat/kg milk; Animal Sciences Group, 2003)
Treatment	Treatment clinical case	€ 30	
Reduced slaughter value	Standard slaughter value (per cow): Reduction depends on infection state (lowly infectious 5%, highly infectious 10%, clinical 100%)	€ 448.75	(Animal Sciences Group, 2003; LEI, 2004)
Missed future income	Retention pay off, depending on parity, month in lactation, and production level assuming no alternative use of production factors	- € 111.63 to € 1431.23	Updated values, based on the methods described in Houben et al. (1994)

Table 2. Continued.

Category	Parameter	Loss or costs (Euro)	Details / reference
Preventive management measures			
Calving	Costs of cleaning per year	€ 100 per year	
	Extra labour (hygiene, milking own dam) per calving	Giving colostrum of own dam € 9.11	(Animal Sciences Group, 2003)
Milk replacer [a]	280 L of artificial milk, 8 L of milk replacer per kg milk powder, costs of milk powder € 1.30 per kg, value of bulk milk € 0.20 per litre.	42 litres instead of waste milk = € 6.83 238 litres instead of bulk milk = - € 9.11 Total = - € 2.28	(Animal Sciences Group, 2003)
Hygiene barrier	Between adult stock and young stock	€ 726.71 per year (including labour)	Calculation includes: Investment of € 1,850, depreciation of 15 years, 1% maintenance costs, labour, materials
Roughage	Better quality roughage, straw etc. during housing in summer season only.	€ 39.03 for calves 0 – 6 months	(Animal Sciences Group, 2003)
Housing	Separate housing of animals 0 – 70 days (initially 5 animals)	€ 487.5 per year; 5% increment per year	(Animal Sciences Group, 2003)
	Separate housing of animals 70 – 180 days (initially 7 animals)	€ 682.5 per year; 5% increment per year	(Animal Sciences Group, 2003)
	Separate housing of animals 180 – 360 days (initially 9 animals)	€ 877.5 per year; 5% increment per year	(Animal Sciences Group, 2003)

[a] Feeding milk replacer only and neither bulk milk nor waste milk (such as milk with a high somatic cell count or milk from cows within the withdrawal period after treatment).

Table 3. Assumptions on sensitivity (Se) and specificity (Sp) of individual faecal culture (IFC), pooled faecal culture (PFC) and ELISA.

	Stage of infection of animal	IFC	PFC	ELISA
Se	Latent infected	0	0	0.01
	Lowly infectious	0.40	0.36	0.10
	Highly infectious	0.95	0.95	0.60
	Clinical disease	0.90	0.90	0.80
Sp	Not infected	1.00	1.00	0.997 [a]

[a] (van Maanen et al., 2002)

3.3.3. Shedding of Map in milk

The assumptions made on shedding of *Map* in milk depending on the stage of infection of an animal are shown in Table 4. These assumptions were based on the available quantitative data on direct shedding of *Map* in milk (Sweeney et al., 1992b; Streeter et al., 1995; Giese and Ahrens, 2000; McDonald et al., 2002; Ayele et al., 2005; Rademaker, personal communication 2004), faecal contamination of milk (Stadhouders and Jørgensen, 1990), shedding of *Map* in faeces (Chiodini et al., 1984; van der Giessen et al., 1992; Sweeney et al., 1992a; Nauta and van der Giessen, 1998; Pearce et al., 2001; Stehman, personal communication, 2004) and the concentration of *Map* in bulk milk (Grant et al., 2002a). In the present study, the concentration of *Map* in bulk milk was approximated by the average of the concentration of *Map* in milk of each dairy cow in the herd. This approximation was justified because faecal contamination is the predominant source of *Map* in milk (Table 4) and is not restricted to a cow's own milk – meaning that variation in milk yield between cows can be ignored.

3.3.4. Acceptable concentration of Map-organisms in milk

The concentration of *Map* organisms in on-farm bulk milk that can be considered acceptable is unknown. No quantitative data on human exposure to *Map* (either alive or dead *Map* organisms) are available and the probability of human disease due to exposure is unknown. Therefore in the present study, we assumed that no viable *Map* organisms should be present after commercial pasteurization. *Map* can survive high-temperature short-time (HTST) pasteurisation when the initial organism concentration is $\geq 10^4$ cells per litre of milk (Sung and Collins, 1998; Grant et al., 2005). To our knowledge, no study indicated that *Map* could survive HTST pasteurisation when the initial organism concentration is $\leq 10^3$ cells per litre. Moreover, the inactivation of *Map* achieved by pasteurisation conditions used by the Dutch dairy industry has been estimated at > 4.2 $\log_{10}$ to > 7.1 $\log_{10}$ (Rademaker et al.,

2007). Therefore, in this study, we considered a concentration of *Map* organisms in milk less than 10^3 per litre acceptable.

3.3.5. Bulk milk quality assurance programmes

In our simulations, certified 'low-*Map* bulk milk' dairy herds were assigned a 'green' status, while other dairy herds were assigned a 'red' status. Thus, 'green' herds were herds with a high confidence that the concentration of *Map* in bulk milk was $< 10^3$/L. The initial assessment of herds was done two years after the start of the simulations, because it was anticipated that a bulk milk quality assurance programme would not be initiated within two years after the seroprevalence study (van Weering, personal communication, 2004) on which the assumptions on the herd-level prevalence were based. At the initial assessment, test-negative herds were assigned a 'green' status and test-positive herds a 'red' status. Thereafter, 'green' herds were regularly monitored in a surveillance procedure, with herds testing positive being moved to the pool of 'red' herds. A control procedure was applied to 'red' herds; test positive cattle and their last-born offspring were culled.

Various alternative test schemes for the initial assessment, surveillance procedure and control procedure were simulated (Table 5, schemes A to D). Test schemes were based on herd examinations by serology (ELISA), individual faecal culture (IFC; Kalis et al., 1999) or pooled faecal culture (PFC; Kalis et al., 2000). For each test scheme, the number of negative herd examinations required for a 'red' herd to move to the pool of 'green' herds was determined by the confidence that the concentration of *Map* in bulk milk was $< 10^3$/L. A test-negative 'red' herd became 'green' if this confidence was equivalent to the probability for a 'green' herd immediately after the initial assessment procedure to have $< 10^3$ *Map*/L. For each test scheme, the number of negative herd examinations required was calculated both with and without additional preventive management measures imposed by all herds on their 'background' management.

For comparison, the test scheme of the Dutch '*Map*-free' certification-and-surveillance programme was simulated as well (Table 5, scheme E). The aim of this programme is to enable low-risk trade of cattle between herds. The initial assessment of this programme consists of five annual herd examinations (the first herd examination by ELISA, the second to fifth examination by PFC). Surveillance is done by biennial herd examinations by PFC. ELISA-positive animals and culture-positive pools are re-tested by IFC. In the programme, various options are available for control of *Map* in test-positive herds. However, in this study, we simulated that these 'red' herds were tested annually by IFC. With scheme E, a 'red' herd only became 'green' after five negative annual herd examinations (by IFC or PFC), in line with the regulations of the Dutch '*Map*-free' certification-and-surveillance programme.

Table 4. Assumed concentration of *Map*-bacteria in milk of individual adult cattle for each stage of the infection-and-disease process (Total *Map* in milk = direct shedding + faecal contamination × *Map* in faeces. Faecal contamination was assumed to be 0.04 g/litre of milk). The concentration of *Map* in bulk milk was approximated by the average concentration of *Map* in milk of all individual cattle in the herd.

Stage	Proportion of animals within each stage	Direct shedding of *Map* in milk (organisms per litre)	*Map* in faeces (organisms per gram)	Total *Map* in milk (organisms per litre)
Latent infected		0	0	0
Lowly infectious	0.8	0	0	0
	0.2	0	10^2	4
Highly infectious	0.6	10^2	10^2	1.04×10^2
	0.24	10^2	10^4	5×10^2
	0.16	10^2	10^7	4.001×10^5
Clinical disease		10^4	10^9	4.001×10^7

Table 5. Simulated test schemes for initial assessment, surveillance (in 'green' herds) and control (in 'red' herds) procedures. In the initial assessment and surveillance procedure, a positive ELISA result was confirmed by individual faecal culture (IFC); IFC positive cattle and their lastborn calf were culled. In the control procedure, all ELISA or IFC positive cattle were culled.

Scheme	Initial assessment [a]		Surveillance (in 'green' herds)			Control (in 'red' herds)		
	Test	Animals	Test	Interval	Animals	Test	Interval	Animals
A	ELISA	All, ≥ 3 yr	ELISA	1 yr	All, ≥ 3 yr	ELISA	1 yr	All, ≥ 3 yr
B	ELISA	All, ≥ 3 yr	ELISA	1 yr	All, ≥ 3 yr	IFC	2 yr	All, ≥ 2 yr
C	ELISA	All, ≥ 3 yr	ELISA	2 yr	All, ≥ 3 yr	ELISA	1 yr	All, ≥ 3 yr
D	ELISA	All, ≥ 3 yr	ELISA	2 yr	All, ≥ 3 yr	IFC	2 yr	All, ≥ 2 yr
E [b]	ELISA / PFC [c]	All, ≥ 3 yr / All, ≥ 2 yr	PFC	2 yr	All, ≥ 2 yr	IFC [d]	1 yr	All, ≥ 2 yr

[a] The initial assessment was done by a single herd examination (except in scheme E).
[b] Scheme E is the (current) test scheme for the '*Map*-free' certification-and-surveillance programme for Dutch dairy herds.
[c] The initial assessment of scheme E consists of five annual herd examinations (the first herd examination by ELISA followed by IFC of ELISA-positive animals; the 2nd through 5th examination by pooled faecal culture (PFC) followed by IFC of positive pools).
[d] In case of a negative herd-examination by IFC in a 'red' herd, a further four negative annual herd examinations by PFC (including all animals ≥2 yr of age) are required before the herd is certified as '*Map*-free'.

All programmes were simulated with and without additional preventive management measures imposed by all participating herds (regardless of 'red' or 'green' status) on their 'background' management right from the start of the simulations. The following combined preventive measures were applied: improved hygiene around birth, colostrum from own dam only, feeding of artificial milk replacer only, and effective separation of young stock from adult cows for the first year after birth.

3.3.6. Model output

In the present study, relevant herd-specific predicted outcomes over time were the within-herd true- and test-prevalence, the concentration of *Map* in bulk milk, losses caused by paratuberculosis and costs spent on the milk quality assurance programme (including herd examinations, subscription costs, additional preventive management measures and culling of infected animals). Losses caused by paratuberculosis did not include a possible lower milk price due to potential consumer concerns. Relevant outcomes over time on the national herd level included the proportion of 'green' dairy herds (as a proportion of all dairy herds), the average concentration of *Map* in bulk milk from 'green' herds, the proportion of 'green' herds with $< 10^3$ *Map* organisms per litre of bulk milk (as a proportion of all 'green' herds), total national losses due to paratuberculosis and total national costs spent on the bulk milk quality assurance programme.

3.3.7. Sensitivity analyses

The influence of various input parameters on the study results was analysed, by changing one parameter at the time. These analyses were performed with test scheme D (Table 5), with or without additional preventive management measures taken in all herds. The following parameter changes were made: (1) The default numbers of *Map* bacteria in milk (Table 4, last column) were multiplied by 10^6 – even though such high concentrations of *Map* in milk are probably not biologically plausible. For example, clinically diseased animals then shed 4×10^{13} *Map*/L of milk (instead of the default value of 4×10^7 *Map*/L; Table 4). (2) The reduction of the effective cow-calf contacts through the environment by additional preventive management measures was changed from 90% (default value) to 50%. (3) The default sensitivities of both the ELISA and IFC for each stage of the infection (Table 3) were multiplied by 0.75. (4) The number of negative herd-examinations required for a 'red' herd to become 'green' (by default two negative herd examinations by IFC, see Results) was changed to only one negative herd-examination. (5) By default, the initial herd-level true prevalence was 0.30, based on a recent seroprevalence study (van Weering, personal communication). However, in a previous seroprevalence study the herd-level true prevalence was estimated at 0.31 to 0.71 (Muskens et al., 2000). Therefore, in this sensitivity analysis, the herd-level true prevalence was changed from 0.30 to 0.70.

3.3.8. Statistical analyses

The proportions of dairy herds certified as 'green' (as a proportion of all dairy herds) by different test schemes were compared by Pearson's χ^2. If an overall χ^2 was significant then each alternative scheme was compared individually with scheme E (i.e. the Dutch certification-and-surveillance scheme for '*Map*-free' herds; Table 5) by Yate's continuity corrected χ_{cc}^2, using Bonferroni's correction of p to adjust for multiple comparisons (Altman, 1999). Cumulative discounted costs of different test schemes plus cumulative discounted losses caused by paratuberculosis were compared using the Kruskal-Wallis rank sum test (adjusted for ties). If significant differences were found, then each alternative test scheme was individually compared with scheme E using the Mann-Whitney test (adjusted for ties), with Bonferroni's correction of p. In all tests, significance was declared at $p \leq 0.05$ (two-sided).

3.4. Results

3.4.1. Simulated bulk milk quality assurance programmes

At the initial assessment by ELISA (schemes A to D), 90% of all herds were test-negative and classified as 'green'. The remaining 10% of herds were test-positive and therefore classified as 'red'. The herd-level true prevalence decreased from 30% initially, to 29% at the time of the initial assessment in year 2, because the infection became extinct in some herds by random processes. Therefore, ~35% of the infected herds (and none of the non-infected herds) were classified as 'red' at the initial assessment. The within-herd prevalence of adult cattle in 'green' and 'red' herds at the initial assessment is shown in Figure 2A. The concomitant distribution of the concentration of *Map* in bulk milk is shown in Figure 2B. Immediately after the initial assessment by ELISA, 98% of 'green' herds had a concentration of *Map* in bulk milk $< 10^3$/L.

At the initial assessment in scheme E, 77% of all herds were test-negative at all five herd examinations (the first by ELISA and the second to fifth by PFC). Immediately after the end of the initial assessment (in year 6), > 99.9% of these test-negative herds had a concentration of *Map* in bulk milk $< 10^3$/L.

During control in 'red' herds with schemes A to D, two consecutive negative herd-examinations by IFC or six consecutive negative herd-examinations by ELISA were required to reach the same probability of 98% (see above) of having $< 10^3$ *Map*/L of milk, irrespective of whether or not additional preventive management measures were taken. Therefore, by default, 'red' herds were re-classified as 'green' only after two consecutive negative herd-examinations by IFC, or six consecutive negative herd-examinations by ELISA. However, this criterion did not apply to scheme E. In scheme E, 'red' herds were assigned 'green' status only after five consecutive negative annual herd examinations (by IFC or PFC; Table 5).

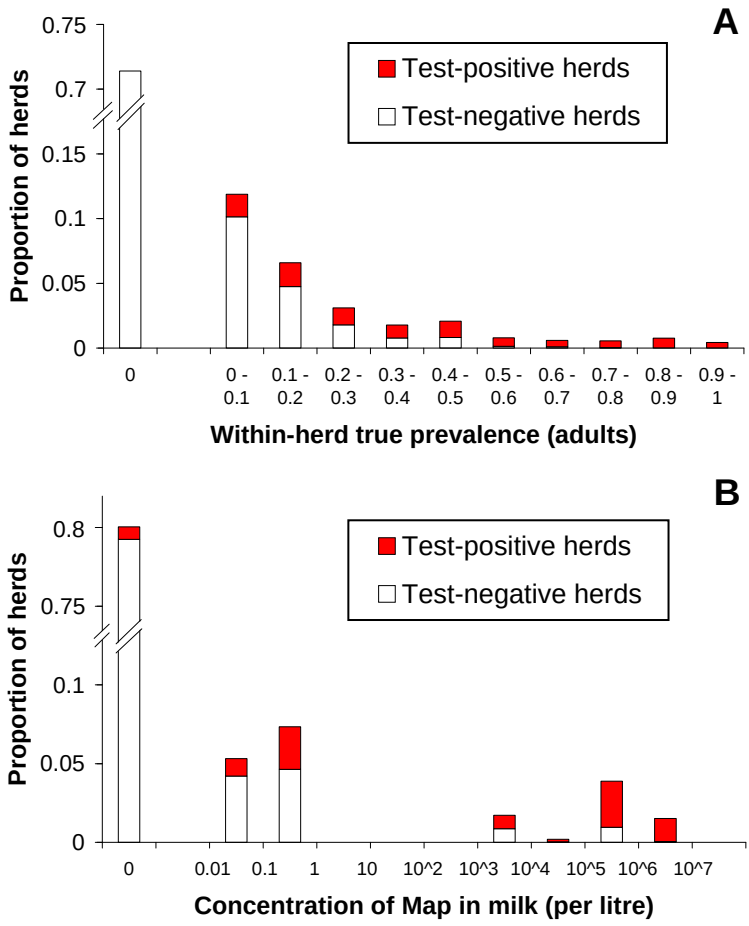

Figure 2. Estimated within-herd prevalence in adult cattle (A) and estimated concentration of *Map* organisms in bulk milk (B) immediately after initial assessment (and prior to culling of any test-positive cattle) in simulated herds that were test-positive ('red') and test-negative ('green') at the initial assessment by ELISA of all cattle ≥ 3 year of age (test schemes A to D).

Without additional preventive management measures, the herd-level prevalence decreased towards 24–25% in year 20 while the average within-herd prevalence increased towards 10–18%, depending on the test scheme. With additional preventive measures, the herd-level prevalence decreased towards 3–7% in year 20, while the average within-herd prevalence decreased to < 0.1% (Fig 3).

The proportion of herds classified as 'green' decreased continuously over time if no preventive measures were taken (Fig. 4A). However, if preventive management

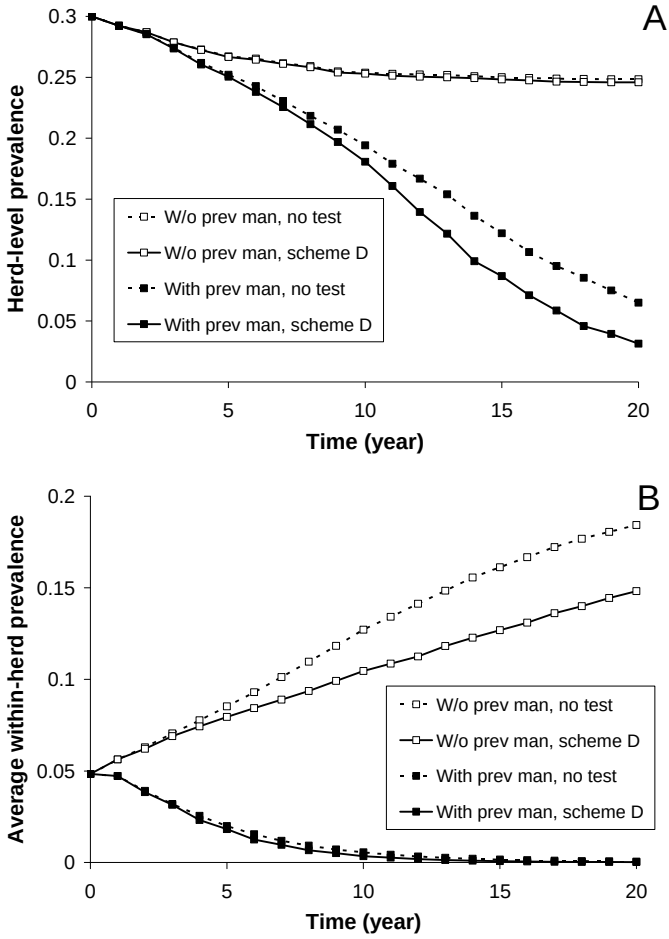

Figure 3. Estimated herd-level prevalence (A) and average within-herd prevalence (B) over time with or without (w/o) additional preventive management measures (prev man) and without testing (no test) or with test scheme D.

measures were taken by all participating herds, this proportion first decreased, but increased subsequently towards 86–99%, depending on the test scheme used (Fig. 4B). Preventive measures were pivotal for 'red' herds to become 'green'. Furthermore, these measures reduced the proportion of 'green' herds that lost their status. If preventive measures were taken, culling based on IFC (schemes B, D and E) was more effective than culling based on ELISA (schemes A and C): the proportion of 'green' herds in year 20 was approximately 10% higher with culling based on IFC (Table 6).

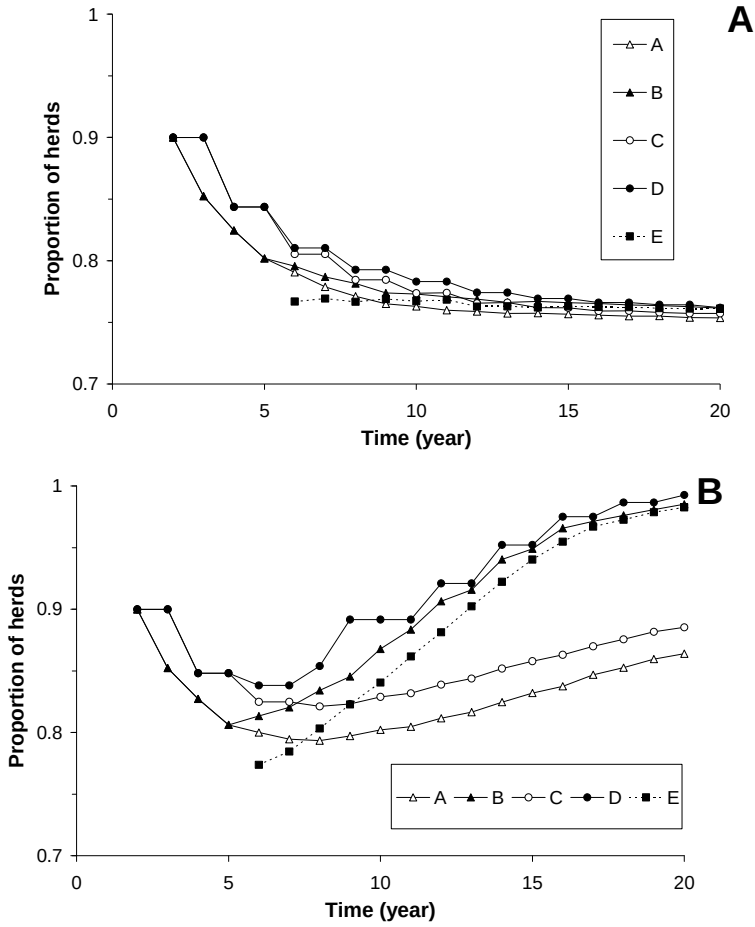

Figure 4. Proportion of herds that were classified as 'green' over time, assuming a population of closed herds with an initial herd-level true prevalence of 30% without additional preventive measures (A), and with additional preventive measures (B). Test schemes are defined in table 5.

With schemes A to D, the estimated average concentration of *Map* bacteria per litre of bulk milk in 'green' herds did not decrease below 10^3 before year 8 to 15, depending on the scheme used and whether or not additional preventive measures were taken. With scheme E the estimated average concentration of *Map* bacteria in 'green' herds was immediately and always < 10^3/L. However, with all schemes the distribution of the concentration of *Map* in milk was highly skewed, as noted before (Fig. 2). Furthermore, the proportion of 'green' herds with a high concentration of *Map* in milk decreased over time (Fig. 5). Therefore, with all test schemes, the proportion of 'green'

Table 6. Estimated proportion of herds with status 'green' in year 10 and 20, with various test schemes with or without additional preventive management measures taken in all herds. Test schemes are defined in Table 5.

Year	Preventive management measures	Scheme	Proportion 'green' herds (%)		Overall χ^2 (4 df)		Yates continuity-corrected χ_{cc}^2 against scheme E (1 df)	
			Estimate	95% CI	χ^2	*P*	χ^2	P^a
10	Without	A	76.3	75.5 ; 77.1	13.9	0.008	0.5	> 0.5
		B	77.3	76.5 ; 78.1			1.0	> 0.5
		C	77.4	76.5 ; 78.2			1.2	> 0.5
		D	78.3	77.5 ; 79.1			7.5	0.02
		E	76.7	75.9 ; 77.5			--	--
	With	A	80.2	79.4 ; 81.0	387.8	< 0.001	52.7	< 0.01
		B	86.8	86.1 ; 87.4			31.1	< 0.01
		C	82.9	82.2 ; 83.6			5.1	0.09
		D	89.2	88.6 ; 89.8			117.5	< 0.01
		E	84.1	83.3 ; 84.8			--	--
20	Without	A	75.4	74.5 ; 76.2	3.0	0.566	--	--
		B	76.2	75.3 ; 77.0			--	--
		C	75.7	74.9 ; 76.5			--	--
		D	76.2	75.4 ; 77.0			--	--
		E	76.1	75.3 ; 76.9			--	--
	With	A	86.4	85.7 ; 87.0	2957.8	< 0.001	1,043.8	< 0.01
		B	98.5	98.3 ; 98.7			2.0	> 0.5
		C	88.5	87.9 ; 89.1			805.3	< 0.01
		D	99.3	99.1 ; 99.4			42.2	< 0.01
		E	98.3	98.0 ; 98.5			--	--

[a] Bonferroni-corrected *P*.

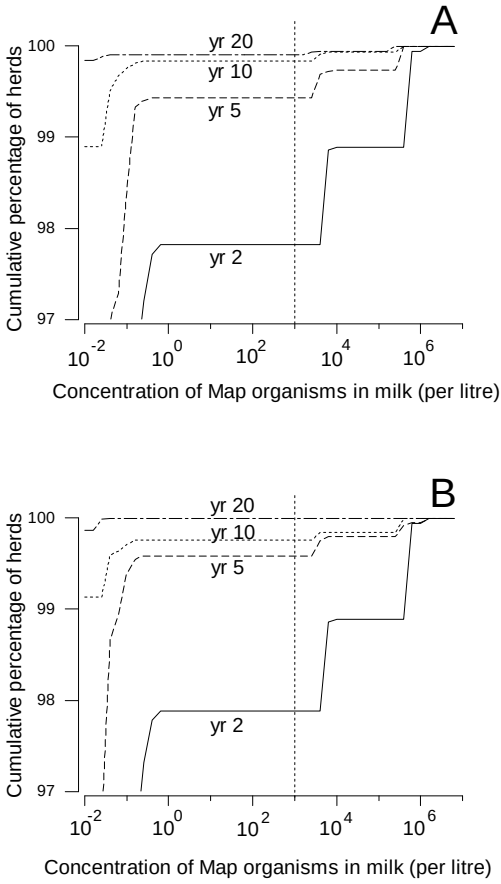

Figure 5. Cumulative distribution of the concentration of *Map* in bulk milk from herds certified as 'green' with test scheme A without additional preventive management measures (Fig. 5A) and with additional preventive management measures (Fig. 5B). Lines indicate the cumulative distribution in years 2, 5, 10 and 20. The dashed vertical line indicates the maximum acceptable concentration. For example, approximately 98% of 'green' herds in year 2 had a concentration of *Map* in bulk milk $< 10^3$/L.

herds with $< 10^3$ *Map*/L of bulk milk increased towards 100% in year 20. The differences in this proportion between the various test schemes with or without additional preventive management measures were small (Fig. 6).

The average cumulative discounted costs of 20-year participation in schemes A to D without additional preventive management measures ranged from € 13 × 10^3 to € 24 × 10^3 (Fig 7A). For schemes with additional preventive measures these costs were

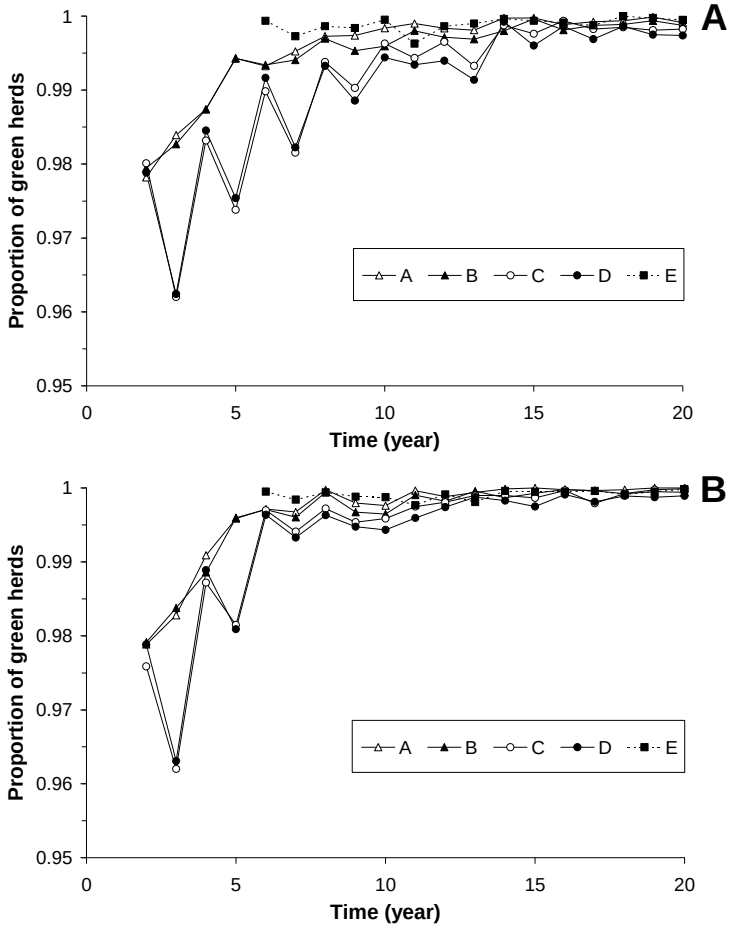

Figure 6. Proportion of 'green' herds with < 10^3 *Map* per litre of bulk milk without additional preventive measures (A), and with additional preventive measures (B). Test schemes are defined in Table 5.

much higher, ranging from € 40 × 10^3 to € 46 × 10^3. However, the 90% range of costs was much broader if no preventive measures were taken; therefore, for some schemes, the 95% percentile of costs were higher if no preventive measures were taken than if preventive measures were taken. The average cumulative discounted losses due to paratuberculosis up to year 20 with schemes A to D without additional preventive measures ranged from € 3 × 10^3 to € 7 × 10^3. Additional preventive measures reduced these losses to on average € 1 × 10^3 (Fig 7B). When comparing the costs of various programmes, this reduction in losses due to paratuberculosis should be taken into account. Therefore, we considered the sum of the costs of participation in the

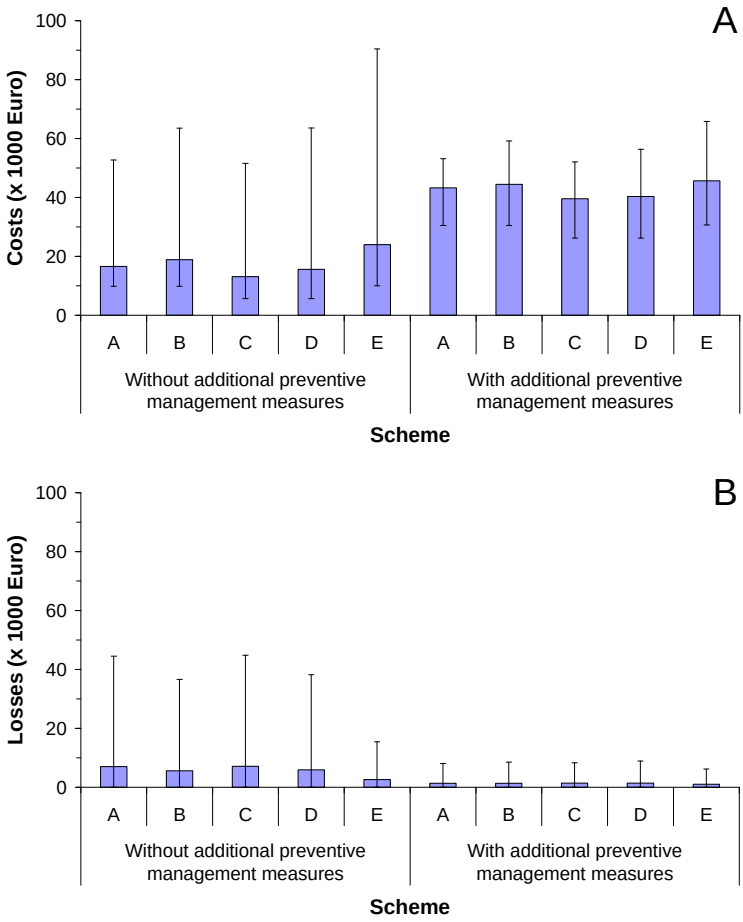

Figure 7. Mean cumulative discounted costs of participation in a bulk milk quality assurance programme (A) and losses due to paratuberculosis (B) per herd up to year 20 (averaged over all 'green' and 'red' herds). Bars indicate the 5% to 95% range. Test schemes are defined in Table 5.

programme plus losses due to paratuberculosis in Table 7. All alternative schemes were cheaper than scheme E (Table 7). For instance, over a 10-year period the total discounted costs of the programme plus losses due to paratuberculosis were on average 20% (with additional preventive measures) or 40% (without additional preventive measures) lower with scheme C compared to scheme E. However, over a 20-year period, the relative magnitude of the differences between schemes was smaller (Table 7).

3.4.2. Sensitivity analyses

If the default level of contamination of milk with *Map* was multiplied by 10^6, the proportion of 'green' herds with < 10^3 *Map*/L bulk milk was reduced from 98% to 88% in year 2. However, the effect decreased over time, and became very small beyond approximately year 10 (i.e. eight years after initial assessment, Fig. 8).

If additional preventive measures were taken with 50% reduction of effective cow-calf contacts through environmental transmission instead of 90%, the proportion of herds certified as 'green' after 20 years was reduced to 92% instead of 99%, compared to 76% of herds without additional preventive measures. However, the magnitude of the reduction in environmental transmission by preventive management measures had no effect on the proportion of 'green' herds with < 10^3 *Map* per litre bulk milk.

If the default sensitivities of both the ELISA and the IFC for each stage of the infection were multiplied by 0.75, the proportion of 'green' herds in year 4 increased to 89% (from 84% in the default analyses). However, beyond year 10, the differences became small. The proportion of 'green' herds with < 10^3 *Map*/L bulk milk was reduced by up to 2% without preventive management measures, and up to 1% with preventive measures. Again, beyond year 10, the differences became small.

By default, two negative herd examinations by IFC were required for a 'red' herd to be re-classified as 'green'. Alternatively, only one negative herd examination by IFC was required. This increased the proportion of herds classified as 'green' in year 8 to 92% (from 85% in the default analysis), if additional preventive management measures were taken. The reason was, of course, that 'red' herds moved to the pool of 'green' herds sooner. However, if no additional preventive measures were taken, there was only a minor increase of the proportion of 'green' herds (80% versus 78% in year 8). Moreover, beyond year 9 the differences decreased and were negligible in year 20 (< 0.5%). The bulk milk 'quality' of 'green' herds was slightly reduced: the percentage of 'green' herds with < 10^3 *Map*/L at any point in time was reduced by up to 1% if only one negative herd examination by IFC was required instead of two. This was due to the fact that slightly more infected herds with ≥ 10^3 *Map*/L shift to the population of 'green' herds if only one negative herd examination by IFC is required than if two negative herd examinations are required.

If the initial herd-level prevalence was 0.70 instead of 0.30, the proportion of 'green' herds in year 20 was markedly reduced to 45% without additional preventive management measures and to 76% with additional preventive measures. The proportion of 'green' herds with < 10^3 *Map* per litre of bulk milk during the first years of the simulations was decreased by up to 7%, but this decrease was small beyond year 10. The average cumulative discounted costs up to year 20 increased by € 13 × 10^3 without additional preventive management measures, but only by € 3 × 10^3 with additional preventive measures.

Table 7. Estimated total discounted costs of participation in the milk quality assurance programme plus total discounted losses due to paratuberculosis until year 10 and 20 for all participating herds (both 'green' and 'red' herds), with various test schemes with or without additional preventive management measures taken in all herds. Test schemes are defined in Table 5.

Year	Preventive management measures	Scheme	Costs of programme plus losses due to paratuberculosis (× € 1,000)						Kruskal-Wallis test (4 df)		Mann-Whitney test (against scheme E)	
			Min	Percentiles			Max	Mean	H	*P*	W (x 10^6)	P^a
				5%	50%	95%			(× 10^3)			
10	Without	A	4.6	4.8	5.0	34.9	87.9	9.5	16.4	< 0.001	77.5	< 0.001
		B	4.6	4.8	5.0	38.3	93.3	10.2			78.1	< 0.001
		C	2.9	2.9	3.0	34.7	87.9	7.8			75.6	< 0.001
		D	2.9	2.9	3.0	39.5	93.3	8.7			76.6	< 0.001
		E	5.5	5.7	5.9	47.5	94.8	12.5			--	--
	With	A	14.9	15.3	21.9	35.5	79.1	23.2	2.8	< 0.001	97.7	< 0.001
		B	14.9	15.3	22.1	39.4	85.6	23.9			99.9	< 0.001
		C	13.1	13.4	20.0	35.0	79.1	21.5			91.8	< 0.001
		D	13.1	13.4	20.1	39.5	85.6	22.2			93.6	< 0.001
		E	15.8	16.3	23.7	45.7	87.4	25.5			--	--

[a] Bonferroni-corrected *P*.

Table 7. Continued.

Year	Preventive management measures	Scheme	Costs of programme plus losses due to paratuberculosis (× € 1,000)						Kruskal-Wallis test (4 df)		Mann-Whitney test (against scheme E)	
			Min	Percentiles			Max	Mean	H (× 10^3)	*P*	W (x 10^6)	P^a
				5%	50%	95%						
20	Without	A	9.4	9.8	10.1	97.2	167.3	23.6	13.5	< 0.001	90.5	< 0.001
		B	9.4	9.8	10.1	100.3	164.8	24.5			90.7	< 0.001
		C	5.4	5.7	5.8	97.1	167.3	20.2			77.9	< 0.001
		D	5.4	5.7	5.8	101.9	164.8	21.6			78.4	< 0.001
		E	9.7	10.0	10.2	106.0	185.5	26.6			--	--
	With	A	29.8	30.5	44.3	59.5	107.1	44.6	2.2	< 0.001	103.6	< 0.001
		B	29.8	30.5	44.3	66.1	119.5	45.8			105.7	< 0.001
		C	25.9	26.3	40.0	59.0	107.1	41.0			95.4	< 0.001
		D	25.9	26.3	40.1	63.7	119.5	41.8			96.8	< 0.001
		E	30.1	30.6	44.5	71.5	127.0	46.7			--	--

[a] Bonferroni-corrected *P*.

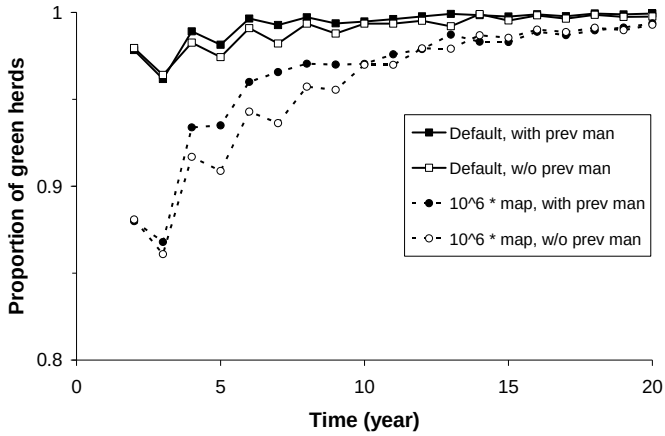

Figure 8. Proportion of 'green' herds with < 10^3 *Map* per litre, using test scheme D, with or without (w/o) preventive management measures (prev man), and with different assumptions on the concentrations of *Map* bacteria in milk. The default concentrations of *Map* in milk are given in Table 4. Alternatively these concentrations were multiplied by 10^6 in the sensitivity analyses for this parameter.

3.5. Discussion

To our knowledge, this is the first modelling study of a bulk milk quality assurance programme for paratuberculosis in dairy herds. The results indicate that the simulated programmes effectively guarantee the bulk milk quality of 'low-*Map* bulk milk' herds, called 'green' herds in our simulations.

The results of this study show that test schemes A to D (milk quality assurance), were considerably cheaper than scheme E (the '*Map*-free' certification-and-surveillance programme) during the first 10 years of participation – which is a time frame that is likely to strongly influence farmers' decisions to enter a programme. Moreover, with the simulated test schemes for a bulk milk quality assurance programme, a test-negative herd reaches the desired status in one herd-examination at the initial assessment, as opposed to five herd-examinations in the '*Map*-free' certification-and-surveillance programme in the Netherlands (Benedictus et al., 2000). These are attractive assets of the new bulk milk quality assurance programmes. However, incentives such as a milk price differentiation are still needed for farmers to justify the costs of participation (Velthuis et al., 2006).

Key elements in a successful bulk milk quality assurance programme are preventive measures to reduce the risk of introduction of *Map* in participating herds (including trade restrictions), preventive management measures to reduce within-herd spread of *Map*, and the initial assessment, surveillance, and control procedures. The

present study was restricted to closed herds. The effects of animal trade were analysed separately using a mathematical model (van Roermund et al., 2005). In the present study, additional preventive management measures to reduce within-herd spread of *Map* were found to have a major effect on the proportion of herds that can be certified as 'low-*Map* bulk milk' (i.e. 'green' in this study). These management measures were pivotal for test-positive ('red') herds to become certified as 'low-*Map* bulk milk' ('green'). This is, of course, related to the assumptions made on the effectiveness of management measures in reducing *Map* transmission. However, as expected, management measures only had a minor effect on the bulk milk quality of 'low-*Map* bulk milk' herds ('green') – since the majority of these herds were truly uninfected.

We simulated initial assessment-, surveillance-, and control-procedures based on tests at the animal-level (ELISA, faecal culture). Preferably, these procedures would be based on quantification of the concentration of *Map* organisms in bulk milk. Techniques to routinely quantify *Map* in large numbers of bulk milk samples might become available (for instance, see Metzger-Boddien et al., 2006) but have not yet been validated for use in the Dutch dairy population. However, our results showed that herd examinations by ELISA for the initial assessment and surveillance procedures effectively ensure the quality of 'low-*Map* bulk milk': > 96% of simulated certified herds (increasing to > 99% after 10 years) were below 10^3 *Map*/L. Surveillance by biennial herd examinations by ELISA was sufficient and resulted in the lowest costs to participants. However, control in 'red' herds by culling of test-positive animals and their last-born offspring based on biennial IFC was more effective than culling based on annual ELISA. Therefore, a programme including initial assessment by herd examination by ELISA, surveillance by biennial herd examinations by ELISA, and control in infected herds by biennial herd examinations by IFC (i.e. test scheme D) is the most attractive programme, according to the simulations.

In the face of uncertainty and lack of information, important assumptions were made in the present study. However, the assumptions considered to be most critical were studied in our sensitivity analyses. Due to deficiencies in the current methodology, it has so far been impossible to accurately quantify *Map* organisms in milk from a dairy herd with paratuberculosis (Dundee et al., 2001; Grant et al., 2002b). For instance, colony forming units can not simply be translated to concentrations of *Map* organisms, because of clumping of *Map* in specimens and insensitivity of culture. Our sensitivity analyses showed that a 10^6 fold increase in the assumed concentration of *Map* in milk from infected animals would initially decrease the number of certified 'low-*Map* bulk milk' ('green') herds with $<10^3$ *Map*/L by 10%. However, such high concentrations of *Map* in milk are probably not biologically plausible, even in so called 'super shedders'. Even so, the effects of such an increase in the concentration of *Map* in milk from infected animals on the bulk milk quality of 'green' herds were very small beyond year 10 (i.e. eight years after the initial assessment procedure). Due to the uncertainty and lack of quantitative information on the probability of human disease

due to exposure to *Map*, no target confidence for 'low-*Map* bulk milk' herds to be below 10^3 *Map*/L could be defined prior to this study. However, the results show that, over time, this confidence approximates to 100% with all simulated test schemes.

In this study, comparisons between simulated test schemes were supported by formal statistical testing. However, with increasing numbers of iterations in a stochastic simulation, small and irrelevant differences between schemes can become statistically significant. Therefore, comparisons between schemes need to be focussed on the practical relevance of differences, rather than on statistical significance alone.

Based on the results of this and other (van Roermund et al., 2005; Velthuis et al., 2006) studies, a new quality assurance programme for paratuberculosis in Dutch dairy herds has been initiated in January 2006. The new programme is run in addition to the pre-existing certification-and-surveillance programme for '*Map*-free' herds (Benedictus et al., 2000; Weber et al., 2004; Ezanno et al., 2005). In the new programme, farmers may choose freely between test schemes C and D (Table 5). Furthermore, farmers may choose to replace serological herd examinations with herd examinations by milk-ELISA, including all lactating cattle in the herd. The results of the new quality assurance programme will be studied when they become available over time.

This study was performed to assist decision-makers in selecting suitable alternatives for a bulk milk quality assurance programme. A number of assumptions related specifically to Dutch dairy herds (such as the relative abundance of management risk-profiles, costs and initial prevalence). However, the aim of the study, which is to develop a programme to improve bulk milk quality by reducing the contamination with *Map*, is of global interest. Furthermore, the mechanisms of paratuberculosis infection, disease and testing are comparable in other dairy cattle populations around the globe. Therefore, the concepts of this study equally apply to other countries.

It is concluded that a bulk milk quality assurance programme for paratuberculosis in closed dairy herds is feasible. Serology is sufficient for initial assessment and surveillance in the programme to warrant bulk milk quality. However, for control in test-positive herds, culling based on faecal culture is more effective than culling based on ELISA. Preventive management measures only had a minor effect on the bulk milk quality of 'low-*Map* bulk milk' herds, but may increase the probability of obtaining this status. The present study provided decision-makers with information on the cost-effectiveness of different programmes.

3.6. Acknowledgments

This study was funded by the Dutch Ministry of Agriculture, Nature and Food Quality and the Dutch Dairy Board. The authors thank the steering committee of the

Paratuberculosis Programme Netherlands for interesting discussions on the subject, and Peter Franken, Huybert Groenendaal, Gerdien van Schaik, Mart de Jong, and Koos Verhoeff for their advice and support during this study.

3.7. Declaration of interest

The principal author (MFW) is employed by the GD Animal Health Service, which is running a quality assurance programme for paratuberculosis, partially based on the results of this study.

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APPENDIX I.

Assumptions on input parameters of the JohneSSim model based on the literature and opinions of an expert panel (for details, see Groenendaal et al., 2002).

Table A. Assumptions on input parameters related to herd dynamics (part 1).

Parameter	Value
Initial herd size [a]	65 adult cattle and 103 young stock
Annual increase of maximum number of adult cattle	5% per annum
Age at first calving	2 years
Calving interval	1 year
Probability of calf born alive if dam is a heifer	0.88
Probability of calf born alive if dam is older	0.96
Proportion of live-born heifer calves raised in the herd [b]	0.8 – 1.0

[a] Based on median herd size of Dutch dairy herds in October 2003.
[b] Depending on the ratio of the number of adult cattle/maximum number of adult cattle in the previous time step. If the ratio was > 0.96, 80% of calves were retained; if the ratio was 0.92 to 0.96, 90% of calves were retained; and if ratio was < 0.92, 100% of calves were retained. A surplus of heifers was sold shortly before first calving.

Table B. Assumptions on input parameters related to herd dynamics (part 2).

Parity	Proportion of cattle at start of simulations (%) [a]	Probability of involuntary culling (%)
0 (calf)	20.4	10.0
0 (pregnant heifers)	16.5	7.5
1	16.5	13.6
2	13.6	14.9
3	11.7	17.9
4	8.7	19.8
5	4.9	22.7
6	3.9	24.5
7	1.9	25.9
8	1.9	27.3
9	0	29.0
10	0	31.0
11	0	32.6
12	0	34.5

[a] Based on age distribution of a random sample of 100 Dutch dairy herds with 65 adult cattle in October 2003.

Table C. Assumptions on probability of intra-uterine infection of a foetus, in relation to status of its dam when the dam is either highly infectious or clinically diseased.

	Number of months before becoming clinically diseased			Clinically diseased
	> 12 months	7–12 months	0–6 months	
Probability	0.035	0.07	0.22	0.5

Table D. Assumptions on infection probabilities at birth without additional preventive management measures.

Dam infection status	Herd infection status		
	No infectious cattle	≥ 1 lowly infectious cattle but no highly infectious cattle	≥ 1 highly infectious cattle
Not infectious	0.00	0.025	0.10
Lowly infectious, 2 calvings before highly infectious	N.A.	0.20	0.50
Lowly infectious, 1 calving before highly infectious	N.A.	0.50	0.50
Highly infectious or clinically diseased	N.A.	N.A.	0.95

Table E. Assumptions on infection through colostrum and milk.

Transmission route	Parameter	Value
Colostrum	Number of calves drinking colostrum from one cow if mixed colostrum is being fed (excluding own calf)	2
	Probability of infection by drinking colostrum from highly infectious or clinically diseased cow	1
	Probability of infection by drinking colostrum from lowly infectious cow	0.3
Waste milk	Number of calves drinking waste milk from one cow if waste milk is being fed (excluding own calf)	8
	Probability of infection by drinking waste milk from highly infectious or clinically diseased cow	1
	Probability of infection by drinking waste milk from lowly infectious cow	0.3
Bulk milk	Probability of one highly infectious or clinically diseased cow contaminating bulk milk	0.2
	Probability of infection by drinking contaminated bulk milk	0.95

Table F. Assumptions on infection probability of dairy calves due to environmental contamination with *Map*: infection probability = $1-(1-kS/N)^{I}$ (modified Reed Frost).

Parameter	Details
S	Susceptibility of calves to infection with *Map* $=100 \times \exp(-0.01 \times$ (age in days))
k	Total number of effective cow-calf contacts: 7 (0–6 months of age) and 63 (7–12 months of age)
N	Number of adult cattle, determined by the model
I	Number of infectious cattle in the last 6 months; lowly infectious cattle are assumed to only be infectious during the first two months postpartum

Table G. Assumptions on age (years) of cattle becoming highly infectious, modelled as triangular distribution. Infected cattle become lowly infectious two calvings before becoming highly infectious.

Age of infection	Age at which cattle become highly infectious		
	Minimum	Most-likely	Maximum
Congenital infection	1.5	2.5	20
At birth	2	3.5	20
0-6 Month	2	4	20
7-12 Month	4	6	20

Table H. Assumptions on interval between becoming highly infectious and culling in adult cattle.

Interval	Parameters of triangular distribution		
	Minimum	Most-likely	Maximum
Between becoming highly infectious and becoming clinically diseased (years)	0.5	1	2
Between becoming clinically ill and being culled (months)	0.5	1	3

Table I. Simulated risk-profiles used to represent the variation in the 'background' herd management.

Risk-profile	'Background' herd management				Proportion of Dutch dairy herds
	Cow-calf separation: immediately postpartum	Colostrum: own dam only or mixed	Milk: artificial milk replacer only	Proper separation of calves 0–6 months of age and adult cattle	
1	No	Own dam	Yes	Yes	0.08
2	No	Own dam	Yes	No	0.10
3	Yes	Own dam	No	Yes	0.08
4	Yes	Own dam	No	No	0.12
5	No	Own dam	No	Yes	0.18
6	No	Own dam	No	No	0.26
7	No	Mixed	No	Yes	0.06
8	No	Mixed	No	No	0.11

Table J.: Assumed default effects of additional preventive management measures.

Transmission route	Preventive measure	Effect on infection probability through this transmission route
Intrauterine infection	–	–
At birth	Improved hygiene at birth	90% reduction
Colostrum own dam	–	–
Mixed colostrum	Feeding colostrums from own dam only	100% reduction
Waste milk	Feeding milk replacer only	100%
Bulk milk	Feeding milk replacer only	100%
Environmental contamination	Effective separation of calves from adult cattle	90% reduction of the number of effective cow-calf contacts (k)

Chapter 4

Evaluation of Ziehl-Neelsen stained faecal smear and ELISA as tools for surveillance of clinical paratuberculosis in cattle in the Netherlands

M.F. Weber, J. Verhoeff, G. van Schaik, C. van Maanen

Preventive Veterinary Medicine 92 (2009) 256-266

GD Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands.

4.1. Abstract

Testing cattle suspected of clinical paratuberculosis is an important element of surveillance of paratuberculosis. The aim of this study was to evaluate the diagnostic-test characteristics of microscopic examination of Ziehl-Neelsen stained faecal smears for acid-fast Mycobacteria (ZN-test) and serum-ELISA in cattle suspected of clinical paratuberculosis in the Netherlands.

Results of all samples submitted for ZN-test and serum-ELISA between April 2003 and April 2006 to our laboratory were retrieved. Results from cattle for which both tests were performed were analysed using two Bayesian latent-class models for evaluation of diagnostic tests in two populations without a gold standard, assuming (a) conditional independence of tests, or (b) conditional dependence of tests in both infected and non-infected cattle. Sampled cattle were divided into two populations in different ways using four known risk factors for clinical paratuberculosis: region, soil type, clinical signs, and age.

For 892 cattle suspected of clinical paratuberculosis, both ZN-test and serum-ELISA results were retrieved: 250 ZN-positive and ELISA-positive, 12 ZN-positive and ELISA-negative, 260 ZN-negative and ELISA-positive, and 370 ZN-negative and ELISA-negative cattle.

With priors based on the available literature, the posterior estimates of sensitivity, specificity, and positive and negative predictive values of the ELISA were always higher than those of the ZN-test. Furthermore, lower limits of the 95% credibility intervals of the posterior positive predictive values of the ELISA were ≥ 99.7%, and of the negative predictive values of the ELISA ≥ 56.4%.

We conclude that the ELISA is preferred to the ZN-test to confirm the presumptive diagnosis of clinical paratuberculosis in the Netherlands. Little diagnostic information can be gained by performing the ZN-test in addition to the ELISA.

Keywords: Paratuberculosis; Cattle; Ziehl-Neelsen test; ELISA; Sensitivity; Specificity

4.2. Introduction

Paratuberculosis (Johne's disease) is a chronic inflammatory bowel disease, primarily affecting ruminants. The aetiological agent is *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). Clinical signs of paratuberculosis in cattle include loss of milk production, weight loss, and diarrhoea (National Research Council of the National Academies, 2003).

Testing cattle with clinical signs of paratuberculosis is an important element of surveillance for paratuberculosis (Collins et al., 2006; Martin, 2008). In many infected herds, control of *Map* is only initiated after detecting clinical paratuberculosis cases. Diagnostic tests used to confirm a clinical presumptive diagnosis and their characteristics are therefore important, not only in managing the individual patient, but also for paratuberculosis control on both herd and national levels.

The clinical presumptive diagnosis paratuberculosis can be confirmed by demonstrating presence of *Map* or presence of antibodies against *Map*. Methods to demonstrate the presence of *Map* include faecal culture, microscopic examination of Ziehl-Neelsen-stained faecal smears for the presence of clumps of acid-fast *Map* organisms (ZN-test), and polymerase chain reaction (PCR) assays[1] (National Research Council of the National Academies, 2003). Methods to demonstrate antibodies against *Map* include the complement fixation test and enzyme-linked immunosorbent assay (ELISA; Kalis et al., 2002; van Maanen et al., 2002).

To support culling decisions in cases suspected of paratuberculosis, a fast confirmation of the clinical presumptive diagnosis is preferred. Faecal culture is often regarded as a gold standard but takes at least several weeks before a test result is obtained, depending on the culture method used. Therefore, cattle suspected of paratuberculosis are often tested by quicker methods such as ZN-test on faecal samples and serum-ELISA. The sensitivity of the ZN-test has been estimated at 49% (Zimmer et al., 1999) in clinically affected cattle. The specificity of the ZN-test was estimated at 83% (Ris et al., 1988) in faecal culture-negative cattle from paratuberculosis-free herds. However, only small numbers of cattle were included in these studies. The sensitivity of the serum-ELISA used at the GD Animal Health Service[2] laboratory (ELISA Paratuberculosis Antibody Screening, Institut Pourquier, Montpellier, France) has been estimated at 28.0–40.8% in faecal culture-positive cattle (van Maanen et al., 2002; Collins et al., 2005). However, to our knowledge, this ELISA has not been evaluated in clinical paratuberculosis cases. Therefore, and because of the rather small

[1] In November 2008, after submission of the present paper, a PCR assay for direct testing of faecal samples has also become available at the GD Animal Health Service laboratory for routine diagnostic submissions.

[2] GD Animal Health Service is a private organisation providing laboratory services, animal health programmes, monitoring and surveillance of animal health, consultancy and training to livestock farmers, veterinarians, industries and government bodies.

sample sizes in the studies on the ZN-test, it is difficult to give a clear advice on the preferred choice of test and interpretation of test-results in cases suspected of clinical paratuberculosis. For instance, in the Netherlands, in case of suspected paratuberculosis in cattle that have recently been purchased, there is often debate on whether a positive ELISA result is sufficient to reclaim the loss of the animal from its seller. This would be the case if the positive predictive value of the ELISA is equal to or higher than the corresponding value of the ZN-test, because the ZN-test is generally accepted as sufficient to support such claim. Therefore, the aim of the present study was to evaluate the diagnostic-test characteristics of microscopic examination of Ziehl-Neelsen stained faecal smears and a serum-ELISA in cattle suspected of clinical paratuberculosis.

4.3. Materials and methods

4.3.1. Samples

All test results of faecal samples from cattle in the Netherlands submitted between April 1st, 2003 and April 1st, 2006 for the ZN-test were retrieved from the laboratory information system of the GD Animal Health Service. In addition, all ELISA results of serum samples submitted in the same period were retrieved. Diagnostic samples from the Dutch cattle population to be tested for paratuberculosis are predominantly submitted to the GD Animal Health Service laboratory. Therefore the retrieved laboratory results were assumed to be representative of Dutch cattle tested with either test. Faecal samples submitted for ZN-test and serum samples submitted for ELISA were considered to be collected simultaneously from the same animal only if (a) collected from cattle in the same herd and (b) the animal identification provided with both samples was exactly the same or both samples could be matched to the same unique cattle identification number in the national cattle identification & registration (I&R) database (Nielen et al., 1996), and (c) the difference between the submission dates of both samples was <8 days. The choice of test (ZN-test, ELISA or both) was at the discretion of the attending veterinarian in consultation with the farmer, who was charged for the costs of testing.

4.3.2. ZN-test

For each sample, 5 g of faeces were suspended in 25 ml tap water. The mixture was filtrated through a tea-strainer. The filtrate was mixed with 25 ml of a 3% v/v sodiumhypochlorite solution, kept overnight at 15-25°C, and centrifuged for 10 min at 1500 x g after which the supernatant was poured off. A smear was made of the top layer of the sediment, fixated in hot air and stained for 10 min in a warm carbolfuchsine solution (i.e. an aqueous solution of approximately 0.04 g/ml phenol and 8.7% v/v of a saturated solution of carbolfuchsine in 96% ethanol; Boom, Meppel, the Netherlands). After rinsing with tap water, the smear was decolorized for 1 min in

an aqueous solution of 5 ml 37% v/v HCl and 95 ml ethanol 96% v/v. The smear was then rinsed with tap water and stained in a methylene-blue solution (i.e. a 10% v/v aqueous solution of a saturated solution of methylene-blue in 96% ethanol; Boom) for 3 min, again rinsed with tap water and dried on air. Each sample was examined microscopically for 10 min using a 10x oculair and 100x oil immersion objective. Samples were considered positive if at least 2 groups of at least 3 acid-fast Mycobacteria were detected. With each batch of samples, a positive control sample was processed in which the presence of *Map* had been confirmed by culture or PCR. In total, five laboratory technicians were involved in microscopic examination of the samples. To improve reproducibility of the test, each positive or doubtful smear was re-examined microscopically by a second technician before a final decision on the test result was made.

4.3.3. ELISA

Serum samples were tested with the ELISA Paratuberculosis Antibody Screening (Institut Pourquier, Montpellier, France) according to the manufacturers' instruction. A sample to positive control (S/P) ratio was calculated based on the optical density of the sample and controls. To increase the diagnostic-test specificity, higher S/P cut-off values were used than recommended by the manufacturers. The results of samples with an $S/P \leq 0.90$ were considered negative, results of samples with an $S/P \geq 1.10$ were considered positive, and results of samples with $0.90 < S/P < 1.10$ were considered inconclusive.

4.3.4. Herd-level and animal-level data

For each sample, the unique herd identification number, the animal identification and recorded clinical signs were retrieved, as provided at the submission of the sample. In our analyses, inconclusive test results were assumed to be negative. Samples for which the test result was missing (6 faecal samples and 3 serum samples of cattle for which a faecal sample was submitted as well), were excluded from our analyses. Each individual was assumed to have been tested only once by the combination of ZN-test and ELISA.

For each herd from which samples were submitted, the region of the country and its 4-digit postal code were retrieved. The postal code could only be retrieved if the herd still existed. For each 4-digit postal code area, the percentage of surface area covered by water or low-moor bog, high-moor bog, loess, sand, sandy loam or clay was retrieved from a database of Alterra, Wageningen University and Research Centre. Soil type at a surface point was defined as the predominant soil type in the upper 80 cm-layer of soil. From the percentages of surface area of each postal code covered by the various soil types, the soil type that covered the largest past of the terrestrial surface area (water area excluded) was considered the soil type of the herd.

Clinical signs as recorded by the veterinarian on the submission form accompanying the samples were grouped as (a) diarrhoea, (b) loss of bodyweight, (c) loss of milk production and (d) other clinical signs. The date of birth of cattle to which a sample could be uniquely matched was retrieved from the I&R database.

4.3.5. Frequentist analyses

The association between the choice of tests (ZN-test alone or the combination of ZN-test and ELISA) and ZN-test result was tested by Chi-square test. Because of the low average number of samples per herd (on average 1.3 faecal samples and 1.2 serum samples per herd), it was considered appropriate to ignore nesting of samples within herd in this analysis. Significance was declared at $p < 0.05$.

4.3.6. Bayesian analyses

4.3.6.1. Models

To estimate the diagnostic test characteristics (sensitivity, specificity, positive and negative predictive values) of both ZN-test and ELISA, Bayesian latent-class models for evaluation of two tests (with or without conditional independence) in two populations described by Branscum et al. (2005) were adapted. Their models are available on-line at http://www.epi.ucdavis.edu/diagnostictests/. Two models were used. In model 1, we assumed conditional independence of the ZN-test and ELISA. In model 2 we assumed conditional dependence of the ZN-test and ELISA. The reason for this second model was that conditional dependence of the tests can not be precluded, even though the tests measure different aspects of the infection-and-disease process. In this second model, conditional dependence was modelled using four parameters γ_D, λ_D, γ_{Dc} and λ_{Dc} that were defined as the probabilities of the ELISA being positive or negative given disease status and the result of the ZN-test: γ_D = P(ELISA-positive| diseased, ZN-negative), λ_D = P(ELISA-positive|diseased, ZN-positive), γ_{Dc} = P(ELISA-negative|non-diseased, ZN-positive), and λ_{Dc} = P(ELISA-negative|non-diseased, ZN-negative). Thus, when $\gamma_D = \lambda_D$ and $\gamma_{Dc} = \lambda_{Dc}$ the ZN-test and ELISA are conditionally independent. The models are given in appendix I. The models were run with the freeware program WinBUGS version 1.4.1 (Lunn et al., 2000); available online at http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml).

For each of the two models, the population of cattle tested with both tests was divided into two subpopulations with a presumed different prevalence of infection. Known risk factors for clinical paratuberculosis were used to create subpopulations. The incidence of the various differential diagnoses of clinical paratuberculosis was assumed not to be influenced by these risk factors, and to be equally distributed across the subpopulations. Therefore, the proportion of true paratuberculosis cases within the

population of cattle showing clinical signs resembling paratuberculosis was expected to be different for the subpopulation exposed to these risk factors and the subpopulation not exposed to these factors.

Four risk factors were used to create subpopulations: region of the Netherlands, soil type, clinical signs, and age at testing. Firstly, the incidence of clinical paratuberculosis is generally thought to be higher in the North of the Netherlands than in other parts of the country, even though there is little published evidence to support this statement. Huitema (1962) described an annual figure of confirmed clinical diagnoses of 786 in the North, 188 in the East, 240 in the West, and 117 in the South of the Netherlands. More recently, the herd-level seroprevalence was found to be higher in the North compared to the East and the West of the Netherlands (Muskens et al., 2000). Therefore, we divided our dataset in a subpopulation of cattle from the North of the Netherlands (i.e. the provinces of Fryslân, Groningen, and Drenthe) and a subpopulation of cattle from the rest of the Netherlands. Secondly, soil type has been associated with paratuberculosis prevalence (Reviriego et al., 2000; Ward and Perez, 2004). Paratuberculosis seroprevalence is positively associated with acidic soil and high iron content (Johnson-Ifearulundu and Kaneene, 1997, 1999). Favourable conditions for survival of *Map* in soil are a low pH, low temperature, and wet soil (Schroen et al., 2002). In the Netherlands, the general opinion is that the incidence of clinical paratuberculosis is higher if the soil type is low-moor bog (i.e. low pH, wet), although, to our knowledge, this is not substantiated by published evidence. Therefore, we divided our dataset in a subpopulation of samples from postal code areas with predominantly low-moor bog, and samples from other postal code areas. Thirdly, cattle with diarrhoea as well as loss of body weight, as recorded at sampling, were compared to all other cattle, because we assumed that the prevalence of clinical disease was higher in cattle with this combination of clinical signs in comparison to cattle with none or only one of these signs. Fourthly, cattle ≥4 years of age have a 2.7 fold higher incidence of clinical paratuberculosis compared to cattle between 2 and 4 years of age (Reinders, 1963). Similarly, Benedictus et al. (1987) found that 86 (76%) of 113 cattle with clinical paratuberculosis were ≥ 4 years of age. Therefore, we divided our dataset in subpopulations of cattle <4 year of age and cattle ≥4 years of age. Finally, cattle ≥4 years of age with diarrhoea as well as loss of body weight were compared to all other cattle. Each of the five sets of two subpopulations was analysed separately with the two models described above.

Each subpopulation was assumed to include ≥1 clinical case of paratuberculosis. Because of the low average number of samples per herd (1.2), it was considered appropriate to ignore nesting of samples within herd in our analyses. Furthermore, the size of the population of cattle with clinical signs that may be caused by paratuberculosis is very large, and therefore sampling of cattle was assumed to have been done without replacement.

4.3.6.2. Priors

Uninformative prior distributions ($\beta(1, 1)$, i.e. Uniform distributions from 0 to 1) were used for the proportion of paratuberculosis cases within each subpopulation. Prior $\beta(s+1, n-s+1)$ distributions for sensitivity (specificity) of tests were created based on the number s of test-positive (test-negative) and total number n of infected (non-infected) cattle obtained in various studies. Based on the study by Zimmer et al. (1999) a $\beta(38, 39)$ distribution was used as a prior for the sensitivity of the ZN-test (Table 1). Based on the study by Ris et al. (1988) a $\beta(16, 4)$ distribution was used as prior for the specificity of the ZN-test. To our knowledge, the sensitivity of the ELISA used in this study has not been evaluated in clinical paratuberculosis cases. However, the sensitivity of other ELISA kits in cattle with clinical paratuberculosis has been estimated at 50% (Bech-Nielsen et al., 1992), 77% (Egan et al., 1999), and 87% (Sweeney et al., 1995). Therefore, a $\beta(44, 14)$ distribution was used as a prior for the sensitivity of the ELISA, based on the study by Egan et al. (1999). The specificity of the Pourquier ELISA in uninfected cattle has been estimated at 100% (Collins et al., 2005) and 99.7% (van Maanen et al., 2002). In the latter study, of 2135 non-infected cattle, 3 were ELISA-positive, and 5 ELISA-inconclusive. In the present study, inconclusive test results were considered to be negative. Therefore, a $\beta(2133, 4)$ distribution was used as prior for the specificity of the ELISA. All default prior distributions on the sensitivity and specificity of tests are reported in Table 1.

Table 1. Default prior distributions for the sensitivity (Se) and specificity (Sp) of microscopic examination of Ziehl-Neelsen stained faecal smears (ZN) and ELISA.

Test	Parameter	Prior distribution	Mode	Percentile of prior distribution		
				2.5%	50%	97.5%
ZN	Se	$\beta(38, 39)$	0.493	0.383	0.493	0.604
	Sp	$\beta(16, 4)$	0.833	0.604	0.810	0.939
ELISA	Se	$\beta(44, 14)$	0.768	0.642	0.762	0.859
	Sp	$\beta(2133, 4)$	0.999	0.996	0.998	0.999

4.3.6.3. Sensitivity analyses

Based on the available literature, important assumptions were made in the present study on the prior distributions of the sensitivity and specificity of the ZN-test and ELISA. Firstly, the effect of these assumptions was evaluated by running both model 1 and model 2 with uninformative prior $\beta(1, 1)$ distributions (i.e. a Uniform distribution from 0 to 1) for all model parameters at the same time. Secondly, the effect of these assumptions was evaluated by replacing, one-at-a-time, the prior sensitivity and specificity of both tests by an uninformative prior. These analyses were done with model 2 and subpopulations based on age. Model 2 was chosen because this model contained the largest number of model parameters, and was therefore likely to be less robust to alterations in prior distributions. Thirdly, the effects of lower and higher prior sensitivities of the ELISA were studied. Arbitrarily, the default prior sensitivity of the ELISA had been based on the results of Egan et al. (1999). The effect of a lower prior sensitivity was studied using a $\beta(21, 21)$ distribution based on the results of Bech-Nielsen et al. (1992), i.e. a distribution with a mode and median of 0.500, and 2.5% and 97.5% percentiles of 0.351 and 0.649, respectively. The effect of a higher prior sensitivity was studied using a $\beta(55, 9)$ distribution based on the results of Sweeney et al. (1995), i.e. a distribution with a mode of 0.871 and 2.5%, 50% and 97.5% percentiles of 0.765, 0.863 and 0.933 respectively. Fourthly, the effect of a down-weighted prior specificity of the ELISA was studied, using a $\beta(427.4, 1.6)$ distribution with the same mode of 0.999 as the default prior distribution, and 2.5%, 50% and 97.5% percentiles of 0.989, 0.997 and 1.000 respectively. Finally, the effect of a higher specificity of the ZN-test was studied, using a prior $\beta(18, 2)$ distribution, i.e. a distribution with a mode of 0.944, and 2.5%, 50% and 97.5% percentiles of 0.740, 0.913 and 0.987 respectively.

4.3.6.4. Sampling

Each model was run for 1×10^6 iterations, after a 5000 iterations burn in. Because preliminary analyses indicated some autocorrelation, only every 5^{th} iteration was selected to contribute to the statistics being calculated. However, because preliminary analyses indicated that the level of autocorrelation increased with uninformative priors, each sensitivity analysis was run for 4×10^6 iterations, after a 5000 iterations burn in and only every 20^{th} iteration was selected to contribute to the statistics being calculated. For each analysis, history plots, quantile plots and autocorrelation plots were checked for indications of a lack of model convergence. With all models, the Gelman-Rubin convergence statistic was monitored in a separate run using two initial strains with initial values for all parameters of 0.001 and 0.999 respectively. Model fit was evaluated with the Deviance Information Criterion (DIC; Spiegelhalter et al., 2002). An important assumption in the models is that the estimated proportion of true paratuberculosis cases of the two subpopulations is substantially different. This assumption was assumed to be violated if the 95% credibility interval

(95% CI) of the posterior difference between the proportions of true paratuberculosis cases in the two subpopulations included zero. In that case, no further interpretation of the results of the analysis was made.

4.4. Results

4.4.1. Descriptive statistics

ZN-test results of 1968 samples were retrieved. In total, 538 (27%) of the 1968 samples tested positive (ZN-positive). For 892 of the 1968 faecal samples from 729 herds, an ELISA result of a simultaneous serum sample of the same individual was available as well. Of these 892 faecal samples, 262 (29%) were ZN-positive. Of the remaining 1076 faecal samples, for which no simultaneous ELISA result of the same individual was available, 276 (26%) were ZN-positive. There was a non-significant indication that cattle tested with the combination of ZN-test and ELISA were more often ZN-positive than cattle tested by ZN-test alone (χ^2=3.401, df=1, p=0.065).

Table 2. Results of microscopic examination of Ziehl-Neelsen stained faecal smears and ELISA of 892 cattle suspected of clinical paratuberculosis per region of the Netherlands, per age group, and in relation to clinical signs recorded at sampling (diarrhoea as well as loss of body weight, versus otherwise).

Region	Age	Diarrhoea and weight loss	Test results			
			ZN-pos ELISA-pos	ZN-pos ELISA-neg	ZN-neg ELISA-pos	ZN-neg ELISA-neg
North	< 4 yrs	Yes	8		8	16
		No	12	2	27	45
	≥ 4 yrs	Yes	27		23	17
		No	44	3	45	28
	Unknown	Yes	12		13	11
		No	12	3	14	19
Other regions	< 4 yrs	Yes	11		8	11
		No	11		18	53
	≥ 4 yrs	Yes	28	1	29	22
		No	44	2	37	85
	Unknown	Yes	15		13	18
		No	26	1	25	45
Total			250	12	260	370

The 892 cattle for which both faecal and serum samples had been submitted originated from 729 herds (619 herds with one case, 168 herds with two cases, 57 herds with three cases, four herds with four cases, one herd with six cases, one herd with seven cases and one herd with 19 cases). Region could be retrieved for all of these herds and cases (Table 2). Soil type could be retrieved for 701 of the 729 herds, with 863 of the 892 cases. In 787 of the 892 cases for which both faecal and serum samples were submitted, one or more clinical signs had been indicated on the submission form: diarrhoea (738 cases), loss of body weight (332 cases), loss of milk production (63 cases) and other clinical signs (30 cases). In 18 cases the presence of clinical signs had been indicated without a specification of these signs. No clinical signs had been indicated on the submission form in the remaining 87 cases. However, because faecal samples are submitted for the ZN-test almost exclusively in case of clinical signs of paratuberculosis, these cases were not excluded from our analyses. The cattle identification provided at submission could be matched to a unique animal identification in the national I&R database for 665 of the 892 samples. The age distribution at testing of these 665 cattle is shown in Fig. 1.

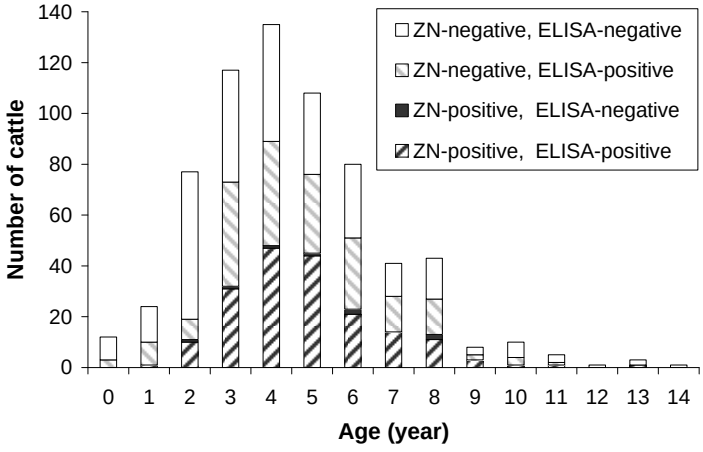

Fig. 1. Age at microscopic examination of ZN-stained faecal smears and ELISA on serum samples of 665 cattle suspected of clinical paratuberculosis from which faecal samples were submitted to the GD Animal Health Service (Deventer, the Netherlands) between 1 April 2003 and 1 April 2006.

4.4.2. Bayesian analysis

In none of the default analyses, inspection of history plots, quantile plots, autocorrelation plots, and plots of the Gelman-Rubin convergence statistic indicated a lack of convergence. The 95% CI's of the differences between the posterior proportions of true paratuberculosis cases in the subpopulations based on soil type included zero. Therefore, no further interpretation was made of the results of analyses with subpopulations based on soil type. However, the posterior distributions of the proportions of true paratuberculosis cases in subpopulations based on region, clinical signs, age, or the combination of age and clinical signs were substantially different, and therefore the results of these analyses were interpreted. In line with our hypotheses, the posterior proportion of true paratuberculosis cases was highest in the North of the Netherlands compared to other regions, in cattle with diarrhoea and weight loss compared to other cattle, in cattle ≥4 yrs of age compared to younger cattle, and in cattle ≥4 yrs of age with diarrhoea and weight loss compared to other cattle (data not shown).

The DIC values of model 1 (Table 3) were only slightly higher than those of model 2 (Table 4); indicating that the fit of both models to the data was comparable.

Point estimates of the overall proportion of true paratuberculosis cases ranged from 60% to 68%. Although model 2 resulted in higher point estimates of this proportion compared to model 1, the 95% CI's of the estimates obtained with both models overlapped. Moreover, the effect of the choice of parameter used to create subpopulations (region, age, clinical signs, or the combination of age and clinical signs) on the estimated overall proportion of true paratuberculosis cases was negligible.

The posterior estimates of the overall diagnostic sensitivity, specificity and positive and negative predictive values of the ELISA were always higher than those of the ZN-test. Point estimates of the overall negative predictive value of ZN-test and ELISA ranged from 0.457 to 0.555 and 0.740 to 0.904, respectively (Tables 3 and 4). Point estimates of the overall positive predictive value of the ZN-test ranged from 0.972 to 0.979. In contrast, the point estimates of the overall positive predictive values of the ELISA were always 0.999 (Tables 3 and 4).

The estimates of the specificities and positive predictive values of the tests were almost identical across models 1 and 2 (Tables 3 and 4). This was related to the identical probabilities with model 2 of a positive ELISA result in ZN-positive and ZN-negative non-diseased cattle i.e. the tests were conditionally independent in non-diseased cattle (Table 4). However, point estimates of the sensitivity and negative predictive values of the ELISA were somewhat higher with model 1 compared to model 2. This also applied to the ZN-test, but for this test the 95% CI's of the estimates obtained with model 1 and model 2 always overlapped (Tables 3 and 4).

Table 3. Results of a Bayesian latent class model assuming conditional independence of the microscopic examination of Ziehl-Neelsen stained faecal smears (ZN) and ELISA (model 1). Deviance Information Criterion (DIC), median (95% CI) of the estimated overall proportion of true paratuberculosis cases and median (95% CI) of the estimated diagnostic-test characteristics of the ZN and ELISA.

Parameter	Test	Subpopulations based on			
		Region (n=892)	Signs (n=892)	Age (n=665)	Signs x age (n=665)
DIC		51.28	52.70	51.04	47.67
Proportion of true paratuberculosis cases		0.604 (0.569, 0.640)	0.602 (0.567, 0.638)	0.605 (0.565, 0.647)	0.604 (0.563, 0.646)
Sensitivity	ZN	0.482 (0.442, 0.523)	0.482 (0.441, 0.523)	0.477 (0.431, 0.524)	0.477 (0.431, 0.523)
	ELISA	0.927 (0.893, 0.954)	0.930 (0.895, 0.957)	0.920 (0.880, 0.951)	0.921 (0.881, 0.952)
	Difference	-0.444 (-0.491, -0.396)	-0.447 (-0.494, -0.399)	-0.442 (-0.494, -0.387)	-0.443 (-0.496, -0.389)
Specificity	ZN	0.983 (0.962, 0.995)	0.981 (0.959, 0.994)	0.980 (0.956, 0.994)	0.979 (0.954, 0.993)
	ELISA	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)
	Difference	-0.015 (-0.036, -0.003)	-0.018 (-0.039, -0.004)	-0.018 (-0.042, -0.004)	-0.019 (-0.044, -0.005)
Negative predictive value	ZN	0.554 (0.510, 0.596)	0.555 (0.511, 0.598)	0.550 (0.498, 0.598)	0.551 (0.500, 0.599)
	ELISA	0.900 (0.849, 0.939)	0.904 (0.854, 0.943)	0.891 (0.832, 0.935)	0.893 (0.834, 0.937)
	Difference	-0.344 (-0.386, -0.303)	-0.347 (-0.389, -0.306)	-0.339 (-0.385, -0.293)	-0.340 (-0.387, -0.294)
Positive predictive value	ZN	0.977 (0.950, 0.993)	0.974 (0.944, 0.992)	0.974 (0.941, 0.992)	0.972 (0.938, 0.991)
	ELISA	0.999 (0.997, 1.000)	0.999 (0.997, 1.000)	0.999 (0.997, 1.000)	0.999 (0.997, 1.000)
	Difference	-0.021 (-0.049, -0.006)	-0.025 (-0.054, -0.007)	-0.025 (-0.058, -0.007)	-0.027 (-0.060, -0.007)

Table 4. Results of a Bayesian latent class model assuming conditional dependence of the microscopic examination of Ziehl-Neelsen stained faecal smears (ZN) and ELISA (model 2). Deviance Information Criterion (DIC), median (95% CI) of the estimated overall proportion of true paratuberculosis cases, median (95% CI) of the estimates of diagnostic-test characteristics of microscopic examination of Ziehl-Neelsen stained faecal smears (ZN) and ELISA, and median (95% CI) probabilities of a positive and negative ELISA result given disease status and result of the ZN-test (i.e. model parameters related to conditional dependence of tests).

Parameter	Test	Subpopulations based on			
		Region (n=892)	Signs (n=892)	Age (n=665)	Signs x age (n=665)
DIC		51.19	52.16	50.89	47.34
Proportion of true paratuberculosis cases		0.674 (0.616, 0.754)	0.676 (0.616, 0.759)	0.673 (0.611, 0.757)	0.673 (0.610, 0.752)
Sensitivity	ZN	0.437 (0.385, 0.487)	0.435 (0.381, 0.485)	0.436 (0.380, 0.489)	0.435 (0.381, 0.489)
	ELISA	0.832 (0.754, 0.890)	0.831 (0.749, 0.890)	0.829 (0.750, 0.888)	0.829 (0.753, 0.888)
	Difference	-0.393 (-0.442, -0.342)	-0.394 (-0.444, -0.343)	-0.391 (-0.445, -0.336)	-0.392 (-0.446, -0.338)
Specificity	ZN	0.980 (0.954, 0.994)	0.976 (0.947, 0.992)	0.976 (0.946, 0.993)	0.975 (0.943, 0.992)
	ELISA	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)
	Difference	-0.018 (-0.044, -0.004)	-0.022 (-0.051, -0.006)	-0.022 (-0.052, -0.005)	-0.023 (-0.055, -0.006)
Negative predictive value	ZN	0.457 (0.346, 0.535)	0.454 (0.336, 0.533)	0.457 (0.341, 0.538)	0.457 (0.346, 0.539)
	ELISA	0.743 (0.576, 0.845)	0.740 (0.564, 0.845)	0.740 (0.570, 0.843)	0.740 (0.580, 0.844)
	Difference	-0.283 (-0.331, -0.222)	-0.284 (-0.333, -0.220)	-0.280 (-0.331, -0.219)	-0.281 (-0.332, -0.223)
Positive predictive value	ZN	0.979 (0.952, 0.993)	0.974 (0.946, 0.992)	0.975 (0.944, 0.992)	0.973 (0.940, 0.992)
	ELISA	0.999 (0.998, 1.000)	0.999 (0.998, 1.000)	0.999 (0.998, 1.000)	0.999 (0.998, 1.000)
	Difference	-0.020 (-0.047, -0.006)	-0.025 (-0.053, -0.007)	-0.024 (-0.055, -0.007)	-0.026 (-0.059, -0.007)

Table 4. Continued.

Parameter	Subpopulations based on			
	Region (n=892)	Signs (n=892)	Age (n=665)	Signs x age (n=665)
P(ELISA-positive | diseased, ZN-negative)	0.759 (0.641, 0.855)	0.754 (0.633, 0.853)	0.759 (0.641, 0.856)	0.758 (0.644, 0.855)
P(ELISA-positive | diseased, ZN-positive)	0.927 (0.894, 0.954)	0.931 (0.897, 0.958)	0.921 (0.881, 0.951)	0.922 (0.883, 0.953)
P(ELISA-negative | non-diseased, ZN-positive)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)
P(ELISA-negative | non-diseased, ZN-negative)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)

4.4.3. Sensitivity analyses

With uniform priors for all model parameters, both model 1 and 2 became unstable, with the final result depending on the initial values of the parameters. Therefore, the results of these analyses could not be interpreted.

In none of the sensitivity analyses in which default priors were replaced one-at-a-time, inspection of history plots, quantile plots, autocorrelation plots and plots of the Gelman-Rubin convergence statistic indicated a lack of model convergence. In each analysis, the posterior proportion of true paratuberculosis cases in the two subpopulations was substantially different (results not shown). The posterior estimates of the sensitivity, specificity, and negative and positive predictive values of the ELISA remained to be higher than those of the ZN-test with uninformative priors for the sensitivity of the ZN-test or ELISA (Table 5). The same applied to higher and lower priors for the sensitivity of the ELISA. However, with an uninformative prior for the specificity of the ZN-test, the posterior estimates of the specificity and positive predictive values of the ZN-test and ELISA were no longer different (as the 95% CI of the estimated difference between the tests of these parameters included zero; Table 5). Similarly, with a higher prior specificity of the ZN-test, the posterior estimates of the specificities of both tests were no longer different. Moreover, the results were sensitive to the prior specificity of the ELISA: with an uninformative prior specificity the posterior estimate of the specificity of the ZN-test was higher than the posterior specificity of the ELISA. However, there was no difference between the posterior positive predictive values of both tests, and both the posterior sensitivity and negative predictive value of the ELISA remained higher than those of the ZN-test. Moreover, with a down-weighted, rather than uninformative, prior specificity of the ELISA, also the posterior specificity and the posterior positive predictive value of the ELISA were higher than those of the ZN-test.

4.5. Discussion

The results of this study show that, with priors based on the available literature, the posterior estimates of the sensitivity, specificity, and positive and negative predictive values of the ELISA were significantly higher than those of the ZN-test, in cows suspected of clinical paratuberculosis (Tables 3 and 4). Therefore the ELISA is superior to the ZN-test to confirm the presumptive diagnosis of clinical paratuberculosis. The positive predictive value of the ELISA was estimated at 0.999 (0.997, 1.000). This means that very little diagnostic information can be gained with the ZN-test if the ELISA has a positive result. Also, if the ELISA has a negative result, the likelihood of gaining diagnostic information with the ZN-test is very small, because this test was positive in only 3% of ELISA-negative cases (Table 2). Furthermore, the ZN-test is very laborious, can not be fully automated and is expensive in comparison to

the ELISA. Current (2008) rates (excl. value added tax) charged by our laboratory are € 64.47 and € 7.30 for the ZN-test and ELISA respectively, whereas the results of both tests are available, on average, within a week. The results of this study indicate that the ELISA alone is a cost-effective test to confirm the presumptive diagnosis of clinical paratuberculosis.

In the Netherlands, the buyer of cattle that develop clinical paratuberculosis within 6 months of the date of purchase may reclaim their value from the seller. To avoid false claims it is customary that a refund is made only if the presence of *Map* bacteria has been demonstrated, either by ZN-test or faecal culture. However, because the estimated positive predictive value of the ELISA (lower limit of its 95% CI $\geq$ 0.997) was higher than the positive predictive value of the ZN-test (upper limit of its 95% CI $\leq$ 0.993), it is justifiable to accept a positive ELISA result of cattle with clinical signs of paratuberculosis as sufficient evidence to support a claim from the purchaser. This particularly applies to the ELISA kit used in this study in our study population (i.e. Dutch cattle with clinical signs of paratuberculosis). However, it may also apply to other ELISA kits provided they have an equal or higher positive predictive value in the population to which they are applied.

The posterior estimates of the sensitivity of the ZN-test were comparable to previously published estimates of 49% in clinically affected cattle (Zimmer et al., 1999) and 56% in faecal culture-positive cattle (Ris et al., 1988). This is, of course, partially explained by our prior for the sensitivity that was based on these studies. However, the posterior estimates of specificity (>95%) in this study were higher than the estimate of 0.83 (95% CI: 0.59, 0.96) of Ris et al. (1988). This may be related to the strict criterion used to declare a sample positive in the ZN-test in the present study: samples were only considered positive if at least 2 groups of at least 3 acid-fast Mycobacteria were detected at microscopic examination of smears. Other factors that may have contributed to the high specificity were a conscientious examination of smears by experienced laboratory technicians in this study, cross-checking of positive smears and, possibly, a lower background population of Mycobacteria compared to the study by Ris et al. (1988).

The posterior estimate of the sensitivity of the ELISA in this study depended on the choice of its prior distribution, but was always higher than the estimate of 50% by Bech-Nielsen et al. (1992). The posterior estimate of the specificity of the ELISA used in this study was in line with previously published estimates (van Maanen et al., 2002; Collins et al., 2005) which is related to fact that a highly informative prior was used, based on the study of van Maanen et al. (2002).

In our study, two models were used, with and without the assumption of conditional independence of the tests. Conditional dependence of tests resulted in somewhat lower point estimates of the sensitivities and positive predictive values. However, the effect of this assumption on the posterior distributions of the specificity

Table 5. Estimated sensitivity (Se), specificity (Sp), negative predictive value (PVN) and positive predictive value (PVP) of microscopic examination of ZN-stained faecal smears (ZN) and ELISA across subpopulations by age with model 2 and various changes (one-at-a-time) of prior distributions of model parameters.

Test characteristic	Test	Uninformative prior for			
		Se ZN	Sp ZN	Se ELISA	Sp ELISA
Se	ZN	0.417 (0.349, 0.479)	0.441 (0.387, 0.493)	0.439 (0.350, 0.511)	0.498 (0.413, 0.590)
	ELISA	0.812 (0.713, 0.881)	0.834 (0.761, 0.889)	0.873 (0.684, 0.986)	0.820 (0.751, 0.875)
	difference	-0.392 (-0.449, -0.335)	-0.391 (-0.445, -0.337)	-0.430 (-0.516, -0.321)	-0.319 (-0.407, -0.227)
Sp	ZN	0.975 (0.942, 0.992)	0.995 (0.973, 1.000)	0.962 (0.919, 0.986)	0.822 (0.750, 0.921)
	ELISA	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.998 (0.996, 0.999)	0.577 (0.486, 0.737)
	difference	-0.023 (-0.056, -0.006)	-0.004 (-0.025, 0.002)	-0.036 (-0.079, -0.012)	0.243 (0.151, 0.305)
PVN	ZN	0.432 (0.280, 0.527)	0.469 (0.364, 0.546)	0.478 (0.220, 0.595)	0.756 (0.585, 0.872)
	ELISA	0.708 (0.478, 0.832)	0.749 (0.597, 0.845)	0.807 (0.382, 0.982)	0.860 (0.741, 0.926)
	difference	-0.273 (-0.328, -0.193)	-0.278 (-0.327, -0.222)	-0.327 (-0.416, -0.159)	-0.100 (-0.196, -0.035)
PVP	ZN	0.974 (0.942, 0.992)	0.994 (0.970, 1.000)	0.958 (0.920, 0.986)	0.599 (0.341, 0.867)
	ELISA	0.999 (0.998, 1.000)	0.999 (0.998, 1.000)	0.999 (0.997, 1.000)	0.507 (0.283, 0.777)
	difference	-0.025 (-0.057, -0.007)	-0.005 (-0.029, 0.001)	-0.041 (-0.079, -0.014)	0.087 (-0.003, 0.181)

Table 5. Continued.

Test characteristic	Test	Lower prior for Se ELISA	Higher prior for Se ELISA	Higher prior for Sp ZN	Down-weighted Sp ELISA
Se	ZN	0.365 (0.318, 0.426)	0.461 (0.411, 0.511)	0.439 (0.385, 0.492)	0.436 (0.381, 0.490)
	ELISA	0.661 (0.597, 0.752)	0.898 (0.839, 0.940)	0.832 (0.758, 0.889)	0.829 (0.750, 0.888)
	difference	-0.297 (-0.354, -0.248)	-0.435 (-0.487, -0.383)	-0.391 (-0.445, -0.337)	-0.391 (-0.445, -0.336)
Sp	ZN	0.965 (0.909, 0.990)	0.976 (0.948, 0.992)	0.989 (0.965, 0.998)	0.976 (0.946, 0.993)
	ELISA	0.998 (0.996, 0.999)	0.998 (0.996, 1.000)	0.998 (0.996, 1.000)	0.997 (0.989, 1.000)
	difference	-0.034 (-0.089, -0.008)	-0.022 (-0.050, -0.006)	-0.009 (-0.033, 0.000)	-0.020 (-0.050, -0.002)
PVN	ZN	0.234 (0.121, 0.393)	0.519 (0.440, 0.580)	0.465 (0.357, 0.544)	0.458 (0.343, 0.540)
	ELISA	0.372 (0.200, 0.606)	0.854 (0.748, 0.918)	0.746 (0.590, 0.845)	0.740 (0.572, 0.843)
	difference	-0.138 (-0.219, -0.076)	-0.333 (-0.378, -0.286)	-0.279 (-0.329, -0.221)	-0.279 (-0.330, -0.219)
PVP	ZN	0.981 (0.955, 0.994)	0.970 (0.936, 0.990)	0.988 (0.962, 0.998)	0.975 (0.943, 0.992)
	ELISA	1.000 (0.999, 1.000)	0.999 (0.997, 1.000)	0.999 (0.998, 1.000)	0.998 (0.993, 1.000)
	difference	-0.019 (-0.044, -0.005)	-0.028 (-0.063, -0.008)	-0.011 (-0.037, -0.001)	-0.023 (-0.054, -0.005)

and negative predictive values was negligible. Moreover, the assumption of conditional dependence of tests had no practical consequences for the preferred choice of test, because with both models, the test characteristics of the ELISA were more attractive than those of the ZN-test. The results of model 2 indicate conditional dependence of tests in infected cattle but independence of tests in uninfected cattle. This result seems to be biologically plausible: an association between the level of shedding of *Map* in faeces (which is related to the probability of a positive ZN result) and the seroresponse in cattle with clinical paratuberculosis is biologically conceivable. Of course, the conditional independence of the tests in cattle without clinical paratuberculosis is related to the high prior and posterior estimates of the specificity of the ELISA (Toft et al., 2007).

Constant test accuracy across the subpopulations within an analysis was assumed in the present study. At first sight, this assumption may seem in conflict with observed associations between diagnostic sensitivity of tests for paratuberculosis and age (Jubb et al., 2004; Nielsen and Toft, 2006). However, these observed associations are likely to be the result of an association between age and the stage of the infection-and-disease process on the one hand and an association between stage of the infection-and-disease process and sensitivity on the other hand. In the present study, only the final stage of the infection-and-disease process, i.e. clinical disease, was studied. Therefore, the assumption of constant test accuracy across subpopulations, including subpopulations based on age and/or clinical signs, was considered not to be violated.

It is generally accepted that tests should be evaluated in their target populations because conditions differ between populations. Preferably, the prior distributions for the sensitivity and specificity of the tests in this study would have been based on studies with the same tests in the same target population (i.e. Dutch cattle suspected of clinical paratuberculosis) using the same target condition (i.e. clinical paratuberculosis in this study). However, such data were not available. Therefore, assumptions on the prior distributions of the sensitivity and specificity of both tests were based on the available literature. The effects of these assumptions were studied in the sensitivity analyses.

The sensitivity analyses indicated that without any informative priors (i.e. Uniform priors for all model parameters) both model 1 and 2 were unstable, reflecting the importance of prior information in our analyses. However, the opportunity to estimate test characteristics based on the combination of data obtained in a field study and prior information is generally considered a major advantage of Bayesian analyses. Moreover, in the present study the sample size (892 cattle) was large compared to the prior weights meaning that the posterior estimates can shift from the prior values. An exception to this was the specificity of the ELISA for which a highly informative prior was used. An uninformative prior for the specificity of the ELISA resulted in a posterior estimate of the specificity of the ZN-test being higher than the corresponding estimate of the ELISA. However, an uninformative prior for the specificity of the

ELISA is very unrealistic, because this ELISA is widely used and its specificity was found to be high in two independent large studies (van Maanen et al., 2002; Collins et al., 2005). Furthermore, none of the other sensitivity analyses (including an analysis with a down-weighted prior specificity of the ELISA) resulted in posterior estimates of the sensitivity, specificity, negative predictive value or positive predictive value of the ZN-test being higher than those of the ELISA.

There was a non-significant indication that the ZN-test was more often positive in cattle from which both serum and faecal samples were submitted compared to cattle from which only faecal samples were submitted. This suggests that the sample of cattle from which both serum and faecal samples were submitted came from a different study population than the sample of cattle from which faecal samples were submitted alone. Therefore, there is a potential selection bias, and caution is required in extrapolating the results obtained in the cattle from which both samples were submitted to other cattle suspected of paratuberculosis. The direction of this selection bias is not easily determined. However, selection biases are inherent to any observational study based on routine laboratory submissions. In this retrospective study, the choice of test was at the discretion of the attending veterinarian, in consultation with the farmer. Possibly, veterinarians are more inclined to submit samples for both tests simultaneously from cases with high odds of paratuberculosis, or in herds where paratuberculosis is perceived as an economical problem. Such inclination could possible explain the observation that the ZN-test was more often positive in cattle from which both faecal and serum samples were submitted. Theoretically, the preferred study design would be based on random sampling from the population of cattle suspected of clinical paratuberculosis. However, since paratuberculosis is not notifiable in the Netherlands, no central registration of suspected paratuberculosis cases was available.

The results of the present study are specific to Dutch cattle suspected of clinical paratuberculosis. However, the mechanisms of paratuberculosis infection, disease and testing are comparable in other cattle populations around the globe. Therefore, the concepts of this study equally apply to other countries.

The present study focussed on the ZN-test and a serum-ELISA for paratuberculosis. PCR assays for direct detection of *Map* in faeces may offer an alternative for the confirmation of a clinical presumptive diagnosis, providing rapid results and a diagnostic sensitivity comparable to faecal culture (Collins et al., 2006; Alinovi et al., 2009). However, the costs of a PCR assay are often considerably higher than those of a serum-ELISA. Moreover, access to a validated PCR assay for routine diagnostic testing of faecal samples is not yet available in all countries where *Map* is endemic. In those cases, the serum-ELISA offers a cheap and valid alternative for the confirmation of clinical paratuberculosis.

We conclude that the sensitivity, specificity, and positive and negative predictive value of the ELISA in cattle suspected of clinical paratuberculosis are higher than those of the ZN-test. Therefore, to confirm the presumptive diagnosis of paratuberculosis, the

ELISA is preferred above the ZN-test. Positive ELISA results can be considered sufficient to support a claim if recently purchased cattle develop signs of clinical paratuberculosis. If the ELISA is used to confirm this presumptive diagnosis, little diagnostic information can be gained by performing the ZN-test as well.

4.6. Conflict of interest statement

The authors are employed by the GD Animal Health Service, which is providing a laboratory service including the ZN-test and ELISA described in this study.

4.7. Acknowledgements

The authors thank I. Gardner for his advice on the Bayesian models prior to the study and comments on the manuscript, S.S. Nielsen for his comments on the manuscript, F. de Vries for providing the data on soil types, W. Swart for advice on statistics, and A. Luppen and H. Assink for assistance in data collection.

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Appendix I, Bayesian models

Model 1

```
Model;
{
y[1:Q, 1:Q] ~ dmulti(p1[1:Q, 1:Q], n1)
z[1:Q, 1:Q] ~ dmulti(p2[1:Q, 1:Q], n2)

#subpopulation1
p1[1,1] <- pi1*SeZN*SeE + (1-pi1)*(1-SpZN)*(1-SpE)
p1[1,2] <- pi1*SeZN*(1-SeE) + (1-pi1)*(1-SpZN)*SpE
p1[2,1] <- pi1*(1-SeZN)*SeE + (1-pi1)*SpZN*(1-SpE)
p1[2,2] <- pi1*(1-SeZN)*(1-SeE) + (1-pi1)*SpZN*SpE

#subpopulation2
p2[1,1] <- pi2*SeZN*SeE + (1-pi2)*(1-SpZN)*(1-SpE)
p2[1,2] <- pi2*SeZN*(1-SeE) + (1-pi2)*(1-SpZN)*SpE
p2[2,1] <- pi2*(1-SeZN)*SeE + (1-pi2)*SpZN*(1-SpE)
p2[2,2] <- pi2*(1-SeZN)*(1-SeE) + (1-pi2)*SpZN*SpE

#positive and negative predictive values subpopulation1
PVP_E_1 <- (pi1*SeE)/(pi1*SeE+(1-pi1)*(1-SpE))
PVN_E_1 <- (SpE*(1-pi1))/(SpE*(1-pi1)+(1-SeE)*pi1)
PVP_ZN_1 <- (pi1*SeZN)/(pi1*SeZN+(1-pi1)*(1-SpZN))
PVN_ZN_1 <- (SpZN*(1-pi1))/(SpZN*(1-pi1)+(1-SeZN)*pi1)

#positive and negative predictive values subpopulation2
PVP_E_2<- (pi2*SeE)/(pi2*SeE+(1-pi2)*(1-SpE))
PVN_E_2 <- (SpE*(1-pi2))/(SpE*(1-pi2)+(1-SeE)*pi2)
PVP_ZN_2 <- (pi2*SeZN)/(pi2*SeZN+(1-pi2)*(1-SpZN))
PVN_ZN_2 <- (SpZN*(1-pi2))/(SpZN*(1-pi2)+(1-SeZN)*pi2)

#overall positive and negative predictive values
pi<- (pi1*n1+pi2*n2)/(n1+n2)
PVP_E<- (pi*SeE)/(pi*SeE+(1-pi)*(1-SpE))
PVN_E <- (SpE*(1-pi))/(SpE*(1-pi)+(1-SeE)*pi)
PVP_ZN<- (pi*SeZN)/(pi*SeZN+(1-pi)*(1-SpZN))
PVN_ZN<- (SpZN*(1-pi))/(SpZN*(1-pi)+(1-SeZN)*pi)

# differences between populations and test
dpi<- (pi1-pi2)
dSe<-SeZN-SeE
dSp<-SpZN-SpE
dPVN<-PVN_ZN-PVN_E
dPVP<-PVP_ZN-PVP_E

#priors
```

```
SeZN ~ dbeta(38, 39)
SpZN ~ dbeta(16, 4)
SeE ~ dbeta(44, 14)
SpE ~ dbeta(2133, 4)
pi1~ dbeta(1, 1)
pi2 ~ dbeta(1, 1)
}

# data
# sequence c(ZN-positive and ELISA-positive, ZN-positive and ELISA-negative, ZN-negative and
ELISA-positive, ZN-negative and ELISA-negative)

list(n1=389, n2=503, y=structure(.Data=c(115,8,130,136),.Dim=c(2,2)),
z=structure(.Data=c(135,4,130,234),.Dim=c(2,2)), Q=2)

# region: list(n1=389, n2=503, y=structure(.Data=c(115,8,130,136),.Dim=c(2,2)),
z=structure(.Data=c(135,4,130,234),.Dim=c(2,2)), Q=2)
# soil: list(n1=159, n2=704, y=structure(.Data=c(58,1,39,61),.Dim=c(2,2)),
z=structure(.Data=c(185,11,210,298),.Dim=c(2,2)), Q=2)
# age: list(n1=230, n2=435, y=structure(.Data=c(42,2,61,125),.Dim=c(2,2)),
z=structure(.Data=c(143,6,134,152),.Dim=c(2,2)), Q=2)
# signs group n1: signs (both [diarrhoea] and [loss of body weight / loss of body condition / wasting]:
# signs: list(n1=291, n2=601, y=structure(.Data=c(101, 1, 94, 95),.Dim=c(2,2)),
z=structure(.Data=c(149, 11, 166, 275),.Dim=c(2,2)), Q=2)
# signs x age group n1: signs (both [diarrhoea] and [loss of body weight / loss of body condition /
wasting] and >=4 yr:
# signs x age: list(n1=147, n2=518, y=structure(.Data=c(55, 1, 52, 39),.Dim=c(2,2)),
z=structure(.Data=c(130, 7, 143, 238),.Dim=c(2,2)), Q=2)

#initial values
list(pi1=0.5, pi2=0.5, SeZN=0.56, SpZN=0.83, SeE=0.38, SpE=0.99)
```

Model 2

```
model;
{
y1[1:Q, 1:Q] ~ dmulti(p1[1:Q, 1:Q], n1)
y2[1:Q, 1:Q] ~ dmulti(p2[1:Q, 1:Q], n2)

#subpopulation1
p1[1,1] <- pi1*eta11 + (1-pi1)*theta11
p1[1,2] <- pi1*eta12 + (1-pi1)*theta12
p1[2,1] <- pi1*eta21 + (1-pi1)*theta21
p1[2,2] <- pi1*eta22 + (1-pi1)*theta22

#subpopulation2
p2[1,1] <- pi2*eta11 + (1-pi2)*theta11
p2[1,2] <- pi2*eta12 + (1-pi2)*theta12
p2[2,1] <- pi2*eta21 + (1-pi2)*theta21
p2[2,2] <- pi2*eta22 + (1-pi2)*theta22
```

```
# conditional dependence
eta11 <- lambdaD*SeZN
eta12 <- SeZN - eta11
eta21 <- gammaD*(1-SeZN)
eta22 <- 1 - eta11 - eta12 - eta21
theta11 <- 1 - theta12 - theta21 - theta22
theta12 <- gammaDc*(1-SpZN)
theta21 <- SpZN- theta22
theta22 <- lambdaDc* SpZN
SeE <- eta11 + eta21
SpE<- theta22 + theta12
rhoD <- (eta11 - SeZN*SeE) / sqrt(SeZN*(1-SeZN)*SeE*(1-SeE))
rhoDc <- (theta22 - SpZN*SpE) / sqrt(SpZN*(1-SpZN)*SpE*(1-SpE))

#positive and negative predictive values subpopulation1
PVP_E_1 <- (pi1*SeE)/(pi1*SeE+(1-pi1)*(1-SpE))
PVN_E_1 <- (SpE*(1-pi1))/(SpE*(1-pi1)+(1-SeE)*pi1)
PVP_ZN_1 <- (pi1*SeZN)/(pi1*SeZN+(1-pi1)*(1-SpZN))
PVN_ZN_1 <- (SpZN*(1-pi1))/(SpZN*(1-pi1)+(1-SeZN)*pi1)

#positive and negative predictive values subpopulation2
PVP_E_2<- (pi2*SeE)/(pi2*SeE+(1-pi2)*(1-SpE))
PVN_E_2 <- (SpE*(1-pi2))/(SpE*(1-pi2)+(1-SeE)*pi2)
PVP_ZN_2 <- (pi2*SeZN)/(pi2*SeZN+(1-pi2)*(1-SpZN))
PVN_ZN_2 <- (SpZN*(1-pi2))/(SpZN*(1-pi2)+(1-SeZN)*pi2)

#overall positive and negative predictive values
pi<- (pi1*n1+pi2*n2)/(n1+n2)
PVP_E<- (pi*SeE)/(pi*SeE+(1-pi)*(1-SpE))
PVN_E <- (SpE*(1-pi))/(SpE*(1-pi)+(1-SeE)*pi)
PVP_ZN<- (pi*SeZN)/(pi*SeZN+(1-pi)*(1-SpZN))
PVN_ZN<- (SpZN*(1-pi))/(SpZN*(1-pi)+(1-SeZN)*pi)

# differences between populations and test
dpi<- (pi1-pi2)
dSe<-SeZN-SeE
dSp<-SpZN-SpE
dPVN<-PVN_ZN-PVN_E
dPVP<-PVP_ZN-PVP_E

#priors
pi1 ~ dbeta(1, 1)
pi2 ~ dbeta(1, 1)
SeZN ~ dbeta(38, 39)
SpZN ~ dbeta(16, 4)
lambdaD ~ dbeta(44, 14) ## based on prior for SeE
gammaD ~ dbeta(44, 14)  ## based on prior for SeE
lambdaDc ~ dbeta(2133, 4) ## based on prior for SpE
gammaDc ~ dbeta(2133, 4) ## based on prior for SpE
}
```

```
#data
# sequence c(ZN-positive and ELISA-positive, ZN-positive and ELISA-negative, ZN-negative and
ELISA-positive, ZN-negative and ELISA-negative)

list(n1=389, n2=503, Q=2, y1=structure(.Data=c(115,8,130,136),.Dim=c(2,2)),
y2=structure(.Data=c(135,4,130,234),.Dim=c(2,2)))

# region: list(n1=389, n2=503, Q=2, y1=structure(.Data=c(115,8,130,136),.Dim=c(2,2)),
y2=structure(.Data=c(135,4,130,234),.Dim=c(2,2)))
# soil: list(n1=159, n2=704, Q=2, y1=structure(.Data=c(58,1,39,61),.Dim=c(2,2)),
y2=structure(.Data=c(185,11,210,298),.Dim=c(2,2)))
# age: list(n1=230, n2=435, Q=2, y1=structure(.Data=c(42,2,61,125),.Dim=c(2,2)),
y2=structure(.Data=c(143,6,134,152),.Dim=c(2,2)))
# signs group n1: signs (both [diarrhoea] and [loss of body weight / loss of body condition / wasting]:
# signs: list(n1=291, n2=601, Q=2, y1=structure(.Data=c(101, 1, 94, 95),.Dim=c(2,2)),
y2=structure(.Data=c(149, 11, 166, 275),.Dim=c(2,2)))
# signs x age group n1: signs (both [diarrhoea] and [loss of body weight / loss of body condition /
wasting] and >=4 yr:
# signs x age: list(n1=147, n2=518, Q=2, y1=structure(.Data=c(55, 1, 52, 39),.Dim=c(2,2)),
y2=structure(.Data=c(130, 7, 143, 238),.Dim=c(2,2)))

#initial values
list(pi1=0.5, pi2=0.5, SeZN=0.56, SpZN=0.83, lambdaD=0.38, lambdaDc=0.99, gammaD=0.38,
gammaDc=0.99)
```

Chapter 5

Cattle transfers between herds under paratuberculosis surveillance in the Netherlands are not random

M.F. Weber [a], H.J.W. van Roermund [b], J.C.M. Vernooij [c], C.H.J. Kalis [a], J. A. Stegeman [c]

Preventive Veterinary Medicine 76 (2006) 222-236

[a] GD Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands.

[b] Quantitative Veterinary Epidemiology, Animal Sciences Group, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

[c] Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, The Netherlands

5.1. Abstract

The rate and structure of cattle transfers between 206 Dutch cattle herds with a '*Mycobacterium avium* subsp. *paratuberculosis* (*Map*)-free' status by November 2002, were analyzed over a 3-year period (November 1999 – November 2002). Of the 206 '*Map*-free' herds, 184 were closed herds during the period studied. In total, 280 cattle had been introduced into 22 herds at an average rate of 0.33 animals per year per 100 cattle present in the 206 herds. Assuming a random herd-contact structure, the observed rate of cattle transfers between certified '*Map*-free' herds was sufficiently low to relax the surveillance scheme to biennial herd examinations by pooled faecal culture of all cattle ≥ 2 years of age.

The cattle transfers were not randomly distributed over the herds. Forty-four of the 280 cattle originated from 12 other '*Map*-free' herds. The other 236 cattle did not originate from a '*Map*-free' herd and were introduced into a herd before it obtained the '*Map*-free' status. No cattle were introduced into any of the '*Map*-free' herds from which cattle were transferred to other '*Map*-free' herds. Thus, continued propagation of the infection by cattle transfers was impossible in the group of herds studied during the study period. Therefore the surveillance scheme may be further relaxed, and may be differentiated regarding the risk herds pose to other herds.

Keywords: Cattle; Paratuberculosis; Surveillance programme; Herd-contact rate; Herd-contact structure; Cattle transfers; Animal trade

5.2. Introduction

Paratuberculosis (Johne's disease) is a chronic inflammatory bowel disease, primarily affecting ruminants. The etiologic agent is *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). Certified *Map*-free cattle herds are important in a control programme for paratuberculosis because they provide a source of non-infected cattle. In the Netherlands, herds can obtain '*Map*-free' status following five annual herd examinations, if all test results are negative (Benedictus et al., 2000). The first herd examination consists of serial testing of all cattle ≥ 3 year of age by serology (ELISA) and individual faecal culture of seropositive cattle. The second to fifth herd-examinations each consist of pooled faecal culture (Kalis et al., 2000) and individual-animal faecal culture of positive pools. Until recently, the status of these herds was subsequently surveyed by annual herd faecal examinations, exactly as the second to fifth herd examination. Recently, the surveillance scheme was adapted to biennial herd faecal examinations, based on the results of both the present study and a modelling study (van Roermund et al., 2002).

Trade of cattle is the main route of between-herd transmission of *Map* (Sweeney, 1996). However, trade of cattle may be necessary to achieve goals of farmers, such as optimizing production. To reduce the risk of introduction of a *Map* infection, the Dutch paratuberculosis certification-and-surveillance programme requires that cattle that are added to a '*Map*-free' herd originate from another '*Map*-free' herd. In addition, cattle may be added to herds that are in the process of '*Map*-free' certification only if the cattle originate from a herd with an equal or higher number of negative annual herd examinations. However, cattle that have been raised in a '*Map*-free' herd are allowed to return to this herd after a period of shared grazing in a herd with a lower status, because it is assumed that the risk of introducing *Map* through such re-introductions is small.

At present, there are approximately 24,000 dairy herds in the Netherlands. In May 2000, the first Dutch dairy herd obtained the '*Map*-free' status and, at 1 January 2005 there were 457 '*Map*-free' herds in the Netherlands. However, the surveillance of '*Map*-free' herds by annual herd-examinations was considered expensive, in comparison to the incentives for farmers to participate in the programme. Therefore, an effective but cheaper surveillance programme was desired. Surveillance is necessary to detect infected herds in time. Certified '*Map*-free' herds might in reality be infected, either because a pre-existing infection remains undetected or because of a new introduction of *Map*. To maintain a group of herds free of *Map* long-term, an infected herd should be detected and removed from the group before the infection has spread to, on average, one other herd (i.e. the between-herd reproduction ratio R_h, defined as the average number of herds infected by a single infected herd in a large population of susceptible herds, is <1). Then, only small outbreaks might be expected (e.g. de Jong and Diekmann 1992; de Jong 1995; Graat et al. 2001).

Transmission between herds is determined by the probability of infection given contact, and the rate and structure of contacts between herds. The herd-contact rate is the number of cattle transferred divided by the number of cattle-years at risk of being transferred between herds. The herd-contact structure defines the pattern of herds which are in contact with other herds.

To study alternative test schemes in a surveillance programme, a deterministic model for within- and between-herd transmission of *Map* has been developed (van Roermund et al., 2002). The model described transmission following introduction of *Map* into a population of (previously) *Map*-free herds. A similar approach for bovine herpesvirus-1 infections in cattle was developed by Graat et al. (2001). The model consisted of three parts: (a) an S-I model of within-herd dynamics of the infection following introduction of *Map* into a herd, (b) the detection probability of an infected herd at any point in time, and (c) between-herd dynamics of the infection, based on cattle transfers between herds. The model was age-structured because of the long latency period of *Map* infections: susceptibility, infectiousness, diagnostic-test sensitivity as well as culling depended on age. The probability of detecting an infected herd depended on the frequency of herd examinations, the number of animals tested at a herd examination, and the diagnostic-test sensitivity. Between-herd transmission was assumed to depend on transfer of infected cattle between herds. In the model, cattle transfers between herds were assumed to be random. The output of the model was R_h. The model results showed that R_h was linearly dependent on the herd-contact rate if a random herd-contact structure was assumed. Then, if the herd-contact rate in the target population is low, a cheaper surveillance scheme (less frequent herd examinations, fewer animals tested at a herd examination or a cheaper but less-sensitive test) could also result in an $R_h < 1$. Furthermore, aberrations of a random herd-contact structure might influence between-herd transmission, and the required surveillance scheme. Therefore, in the present study, the rate and structure of cattle transfers between Dutch '*Map*-free' herds were characterized to support the design of a cost-effective surveillance programme for these herds. However, characterization of the herd-contact rate and structure is equally important for other infectious diseases and other countries and therefore the approach followed in this study can also be useful in the design of other surveillance programmes

5.3. Materials and methods

5.3.1. Study population and data collection

All 206 Dutch cattle herds with a '*Map*-free' status on 1 November 2002 were selected from the national cattle identification-and-registration (I&R) system (Nielen et al., 1996). These 206 herds are indicated as '*Map*-free' herds throughout this paper, irrespective of the time at which they actually obtained this status. For each '*Map*-free'

herd, data on all cattle present in the herd, introduced into the herd, transferred to another herd, exported to other countries or that had died or were culled between 1 November 1999 and 1 November 2002 were retrieved from the I&R system. Furthermore, the herd type (dairy, beef or other) and the date at which the '*Map*-free' status was obtained were retrieved for each herd. For each individual animal, the date of birth was retrieved. For each animal introduced in a '*Map*-free' herd or transferred from a '*Map*-free' herd to another herd, the time of introduction or transfer and the identification of the herd of origin (at the time of transfer) or herd of destination was retrieved. Furthermore, for each herd of origin or destination, the paratuberculosis status (unknown or infected, in the process of certification, or '*Map*-free') at 1 November 2002 was retrieved.

The data sets were extensively checked for inconsistencies. Furthermore, consistency of the data in relation to the regulations of the paratuberculosis certification-and-surveillance programme was also checked (i.e. whether recorded cattle transfers were in line with the regulations).

5.3.2. Data analysis

All cattle transfers were included in a matrix of the herds of origin and destination and the year of study in which the animal was transferred (year 1 was defined as 1 November 1999 to 1 November 2000, year 2 as 1 November 2000 to 1 November 2001 and year 3 as 1 November 2001 to 1 November 2002). Fixed effects (and their interactions) of year of study (1, 2 or 3) and time of obtaining '*Map*-free' status (before or after 1 May 2001) on the annual probability for a '*Map*-free' herd to introduce cattle (≥1) or not to introduce cattle were evaluated in a logistic regression. In this logistic regression, nesting of data within herd was incorporated while the logarithm of the annual average number of cattle present in the herd year was used as an offset to correct for herd size (i.e. to calculate the odds per animal in the herd). The final model was selected using the smallest Akaike Information Criterion (Dohoo et al., 2003). Model fit was checked by studying the deviance residuals against the predicted probabilities of introducing cattle. Similarly, effects on the annual probability for a '*Map*-free' herd to transfer or not to transfer cattle (≥1) to other '*Map*-free' herds were evaluated.

For each '*Map*-free' herd, the number of cattle-years present in each year (1, 2 and 3) of study was approximated by the mean of the numbers of cattle present at the beginning and end of the year (including young stock). For each '*Map*-free' herd with herd number h, the annual rate of cattle introductions (I) into the herd in year y was expressed in relation to the number of cattle-years present in the herd in that year ($I_{h,y}$):

$$I_{h,y} = \frac{number\ of\ cattle\ introduced_{(h,y)}}{number\ of\ cattle{-}years_{(h,y)}}$$

Similarly, the annual rate of cattle transfers ($M_{h,y}$) from each of the selected herds to another herd was expressed as

$$M_{h,y} = \frac{number\ of\ cattle\ transferred\ to\ another\ herd_{(h,y)}}{number\ of\ cattle{-}years_{(h,y)}}$$

To determine whether averaging the annual rates of introduction of a herd in years 1 – 3 was appropriate, equality of the median rate of cattle introductions into the selected '*Map*-free' herds in years 1, 2 and 3 was tested with the Friedman test by year blocked for herd. Similarly, equality of the median rate of cattle transfers from the selected '*Map*-free' herds to other herds in years 1, 2 and 3 was tested.

The average annual rates of cattle introductions ($\bar{\bar{I}}$) and transfers ($\bar{\bar{M}}$) from the 206 selected herds during the 3 years of study were calculated as

$$\bar{\bar{I}} = \frac{1}{206}\frac{1}{3}\left(\sum_{h=1}^{h=206}\sum_{y=1}^{y=3} I_{h,y}\right) yr^{-1} \quad \text{and} \quad \bar{\bar{M}} = \frac{1}{206}\frac{1}{3}\left(\sum_{h=1}^{h=206}\sum_{y=1}^{y=3} M_{h,y}\right) yr^{-1}$$

respectively. Confidence intervals of these point estimates $\bar{\bar{I}}$ and $\bar{\bar{M}}$ were omitted because the whole target population of Dutch '*Map*-free' herds at the time of the study was included.

The proportion of herds in which cattle were introduced and the rate of cattle introductions were compared between the '*Map*-free' herds and all 26,790 Dutch dairy herds in which young stock were raised. Data of the latter group were obtained from a study of all cattle movements in the Netherlands between 1 September 2001 and 1 September 2002 (Velthuis, 2004; access to these data was kindly permitted by the Dutch Ministry of Agriculture, Nature and Food Quality). For this comparison, Dutch dairy herds in which young stock were raised were selected, because this group of herds most closely resembled the group of herds in the present study. The '*Map*-free' dairy herds in the present study were also included in the total population of 26,790 herds; however their contribution in this total population of herds was considered negligible because of their relative small number. The proportions of herds that introduced cattle were compared by χ^2-test. Annual rates of cattle introductions in the subsets of herds that introduced cattle were compared by Mann-Whitney test (adjusted for ties).

Friedman, Mann-Whitney and χ^2-tests were performed using Minitab release 12.1 statistical software (Minitab Ltd., Coventry, U.K.). Logistic regression was performed using a non-normal repeated-measurements models package (Lindsey,

2004) for the R statistical computing environment (R Development Core Team, 2004). In all analyses, statistical significance was declared when $p \leq 0.05$.

5.3.3. Simulation of a random herd-contact structure

To characterize the structure of cattle transfers observed in our field study, random cattle transfers were simulated using an add-in for risk analysis (@Risk, version 4.5, Palisade corporation, Newfield, USA) in a spreadsheet program (Excel '97, Microsoft, Redmond, USA). Output of the simulation was the distribution of number of herds of origin and destination of transferred cattle. The numbers of herds and cattle used as input in the simulation were based on observations made in our field study. Within a simulated group of 206 '*Map*-free' herds, 44 cattle were randomly transferred between herds. For each transferred animal, a herd of origin was selected randomly from the 206 '*Map*-free' herds. Then, a herd of destination was randomly drawn from the remaining 205 '*Map*-free' herds. A further 236 cattle, originating from non-'*Map*-free' herds were introduced into the group of '*Map*-free' herds. For each of these 236 cattle, a herd of destination was randomly selected from the 206 '*Map*-free' herds. Herds were selected randomly by Latin hypercube sampling (in which stratified samples are taken from the input probability distribution) from a discrete uniform distribution. Latin hypercube sampling was chosen because it tends to force convergence in fewer iterations, as opposed to Monte Carlo sampling. The simulation consisted of 20,000 iterations. For each iteration, the number of '*Map*-free' herds of origin and/or destination was determined. Convergence of output was declared if the mean, the standard deviation, as well as percentiles (0% to 100% in 5% increments) of the number of herds of origin and/or destination changed less then 1% with an additional 500 iterations.

5.4. Results

Of the 206 selected herds, 194 herds were dairy herds, 8 herds were mixed dairy and beef herds, and 4 herds were beef or other herds. These herds are indicated as '*Map*-free' herds throughout this paper, although they had actually obtained this status at a varying time between early May 2000 and late October 2002 (Fig. 1). The location of these herds in the Netherlands is shown in Fig. 2. The mean herd size (±SD) of the 206 herds was 114 (±52) cattle, including 66 (±30) adult cattle and 48 (±23) young stock (Fig. 3). In comparison, the mean herd size of all 26,790 Dutch dairy herds in which young stock were raised was 102 (±56) cattle, including young stock.

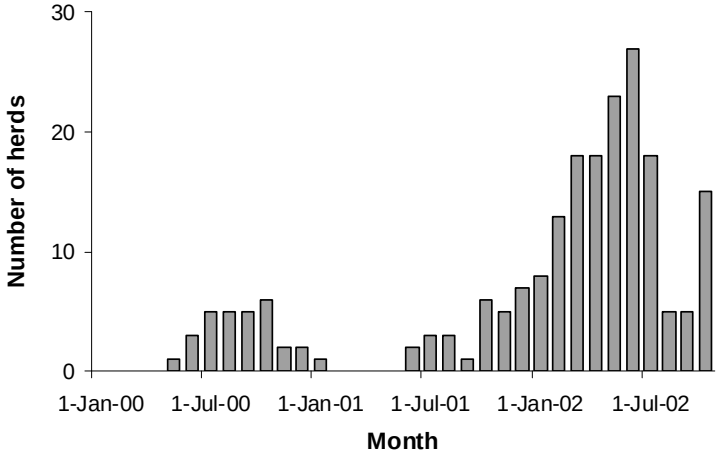


Fig. 1. Time at which the 206 herds obtained their '*Map*-free' status in the paratuberculosis certification-and-surveillance programme in the Netherlands.

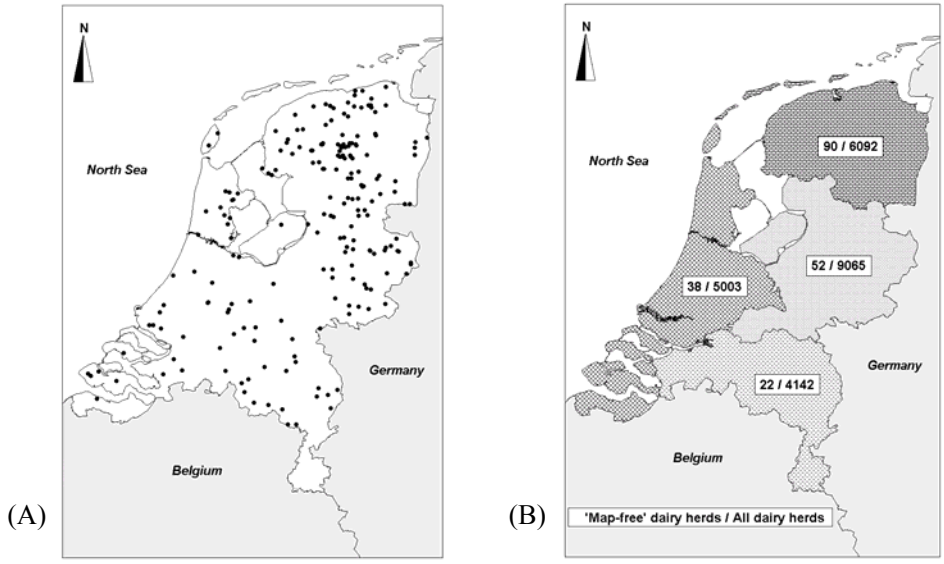


Fig. 2. (A) Observed location of all 206 herds with a '*Map*-free' status in the paratuberculosis certification-and-surveillance programme in the Netherlands on 1 November 2002. (B) Observed number of '*Map*-free' dairy herds as a proportion of all dairy herds per region in the Netherlands.

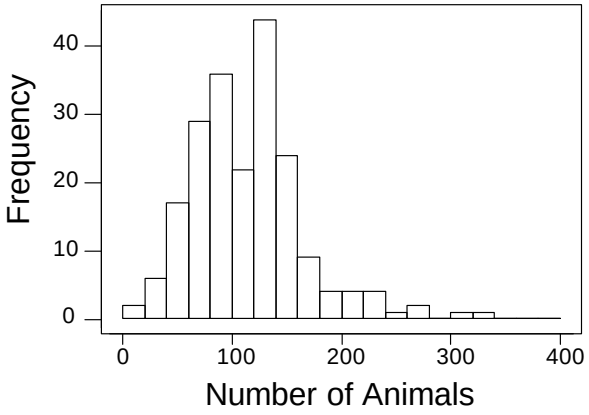


Fig. 3. Distribution of herd size, including young stock, of 206 herds with a '*Map*-free' status in the paratuberculosis certification-and-surveillance programme in the Netherlands on 1 November 2002.

5.4.1. Introduction of cattle into 'Map-free' herds

During the 3 years of study, cattle were introduced into 22 (11%) of the 206 '*Map*-free' herds (Fig. 4). Cattle were not transferred from any of these 22 herds to another '*Map*-free' herd during the 3 years of study. Thus, transferred cattle had only one transfer event during this study. In the remaining 184 herds (89%), no cattle at all were introduced from another herd. Neither year of study (1, 2 and 3), nor time at which a herd obtained the '*Map*-free' status (before or after 1 May 2005), nor their interaction had a significant effect on the annual probability for a herd to introduce or not to introduce cattle (≥1 versus 0). Also, a plot of deviance residuals did not indicate a lack of fit of the model.

In all, 280 cattle were introduced into the '*Map*-free' herds. Most (223, i.e. 80%) of these 280 cattle were introduced into only four (2%) of the herds (Fig. 5A). Therefore, the distribution of the annual rate of introduction over this 3-year period was skewed (Fig. 5B). There was no significant difference in the median rate of introduction between the 3 years of study ($p = 0.956$). The overall average rate of introduction $\bar{\bar{I}}$ in the total population of 206 herds was 3.3×10^{-3} per year**.**

Forty-four of the 280 introduced cattle originated from another herd with a '*Map*-free' status on 1 November 2002 (Fig 4). The remaining 236 introduced cattle originated from a herd without a '*Map*-free' status by that time. These 236 cattle were introduced before the herd of destination had obtained '*Map*-free' status. These cattle included 84 cattle originating from a herd that was in the process of certification at the

time of introduction, but had not yet obtained the '*Map*-free' status at 1 November 2002. Another 146 cattle were raised until at least 6 months old in a '*Map*-free' herd and returned to this same herd after a period of shared grazing elsewhere. The remaining six cattle were introduced from herds with an unknown paratuberculosis status to '*Map*-free' herds, in conflict with the regulations of the paratuberculosis certification-and-surveillance programme. Consequentially, these herds lost their '*Map*-free' status, but regained this status after subsequent removal of these six cattle.

5.4.2. Comparison of introductions in the general Dutch dairy population and in 'Map-free' herds

In a 1-year study (September 2001 – September 2002) of cattle movements in the whole Dutch cattle population, cattle were introduced in 12,067 of the 26,790 dairy herds in which young stock were raised. The proportion (20 / 194) of '*Map*-free' pure dairy herds in which cattle were introduced during the 3 years of the present study was much lower ($\chi^2 = 94.0$, df = 1, $p < 0.001$; no cattle were introduced in the eight '*Map*-free' mixed dairy and beef herds). However, no difference was found between the median rate of introductions in the 12,067 herds and the annual rates of introductions in the '*Map*-free' dairy herds in which cattle were introduced in years 1, 2 and 3, possibly because of the low numbers of herds and the low statistical power of the Mann-Whitney test (Table 1).

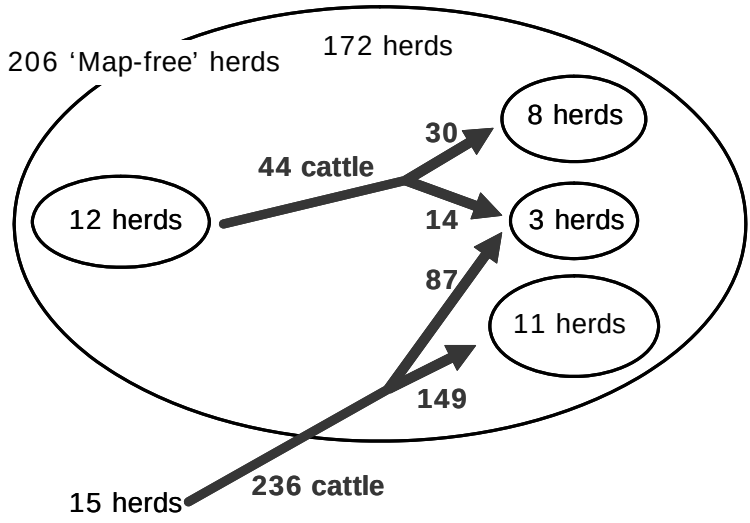
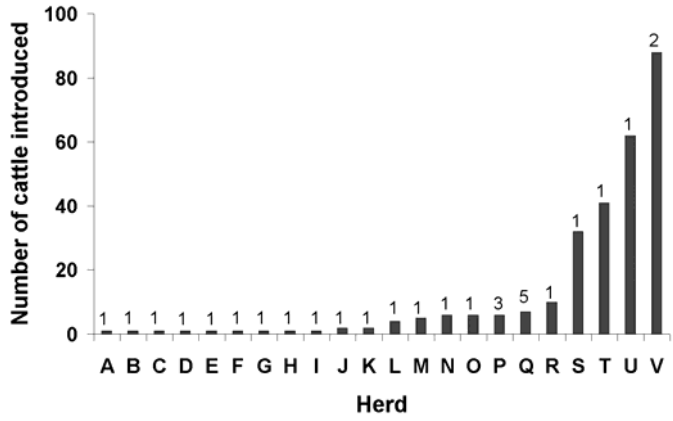
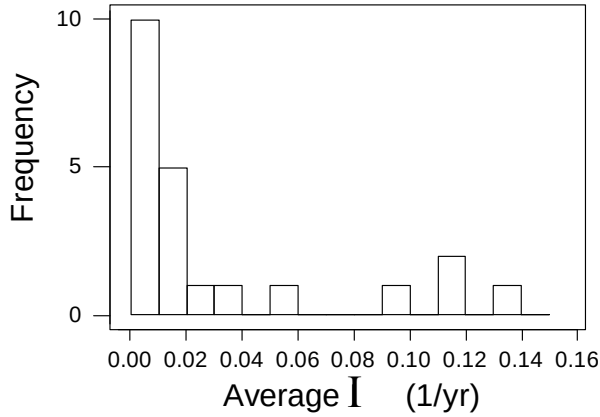


Fig. 4. Cattle introductions observed during a 3-year period in 206 '*Map*-free' herds in the Netherlands.



(A)



(B)

Fig. 5. (A) Observed absolute **number of cattle introduced** per herd over a 3-year period for the 22 '*Map*-free' herds into which cattle were introduced, ranked by number of cattle introduced. Numbers above each bar indicate the number of herds of origin. All herds were dairy herds with the exception of herds E and K. (B) Observed average **rate of introduction** (number of cattle introduced per year / average number of cattle present in the herd) over a 3-year period for the 22 '*Map*-free' herds into which cattle were introduced.

Table 1. Comparison of cattle introductions in 194 '*Map*-free' pure dairy herds[a] in the paratuberculosis certification-and-surveillance programme in the Netherlands with cattle introductions in 26,790 Dutch dairy herds in which young stock were raised.

	Period	Cattle introductions		Mann-Whitney *p* (vs. $0.064 \cdot yr^{-1}$)
		Number of herds [b]	Rate [c] (yr^{-1})	
Dutch dairy herds	1 Sept 2001 – 1 Sept 2002	12067	0.046	-
'*Map*-free' pure dairy herds (present study)	Year 1 (1 Nov 1999 – 1 Nov 2000)	11	0.013	0.07
	Year 2 (1 Nov 2000 – 1 Nov 2001)	8	0.031	0.30
	Year 3 (1 Nov 2001 – 1 Nov 2002)	7	0.013	0.22

[a] No cattle were introduced in the eight '*Map*-free' mixed dairy and beef herds in the present study, although three animals were introduced in year 2 in two out of four '*Map*-free' beef herds at a rate of 0.097 per year.

[b] Number of herds in which cattle were introduced during a one-year period.

[c] Median rate of cattle introductions in herds in which cattle were introduced (i.e. number of cattle introduced per year / number of cattle-years present in the herd).

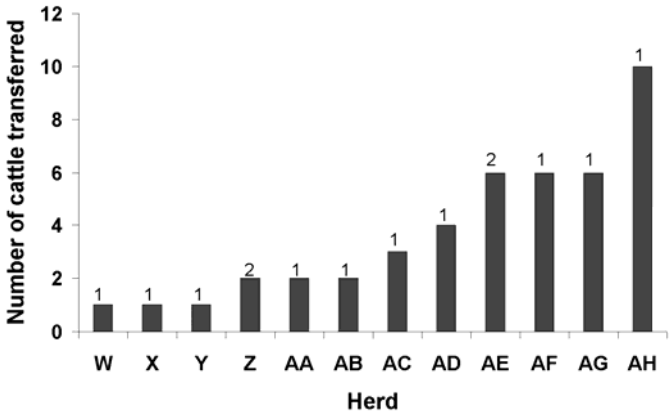
5.4.3. Cattle transferred from 'Map-free' herds

During the 3 years, 24,526 cattle (including calves for veal production) were transferred from the 206 '*Map*-free' herds to other herds. Of these, 44 cattle from 12 herds were transferred to 11 other '*Map*-free' herds (Fig. 4). No cattle were introduced into any of these 12 '*Map*-free' herds of origin during the period of study. The distribution of the average annual rate of cattle transferred from these 12 '*Map*-free' herds to other '*Map*-free' herds was positively skewed (Fig. 6).

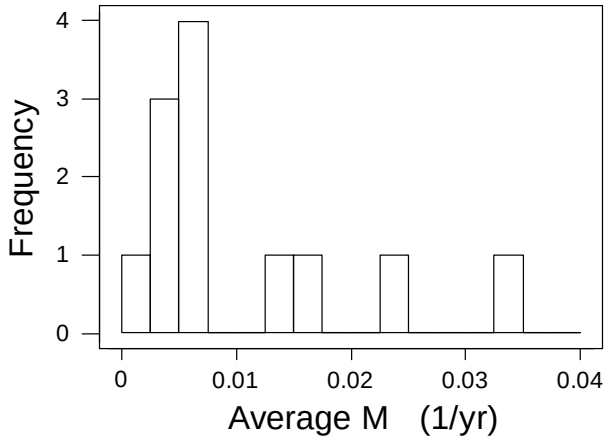
Both year of study and time at which the '*Map*-free' status was obtained, but not their interaction, were included in the final logistic model on the probability for a '*Map*-free' herd to transfer or not to transfer cattle to other '*Map*-free' herds (Table 2). Herds that obtained the *Map*-free status before 1 May 2001 were much more likely to transfer cattle to other '*Map*-free' herds than herds that obtained the status after that date. However, effects of year of study were not significant. A plot of deviance residuals did not indicate a lack of fit of the model.

The median rates of cattle transferred from '*Map*-free' herds *to other 'Map-free' herds* in years 1, 2 and 3 were not significantly different ($S = 0.42$, $df = 2$, $p = 0.809$). For all 206 '*Map*-free' herds, the average rate $\overline{\overline{M}}$ of cattle transferred to other '*Map*-free' herds during the 3 years of study equalled 0.6×10^{-3} per year. Similarly, the

median annual rates of cattle transferred from '*Map*-free' herds *to herds that where in the process of obtaining a 'Map-free' status* on 1 November 2002 were not significantly different (S = 2.67, df = 2, p = 0.263). For all 206 '*Map*-free' herds, the average rate $\overline{\overline{M}}$ of cattle transferred to herds still in the process of obtaining this status on 1 November 2002 equalled 3.1×10^{-3} per year.



(A)



(B)

Fig. 6. (A) Observed absolute **number of cattle transferred** to other '*Map*-free' herds over a 3-year period for 12 '*Map*-free' herds of origin, ranked by number of cattle transferred. Numbers above each bar indicate the number of '*Map*-free' herds of destination. All 12 herds were dairy herds. (B) Observed average **rate of transfer** of cattle to '*Map*-free' herds (number of cattle transferred per year / average number of cattle present in the herd) over a 3-year period for the 12 '*Map*-free' herds of origin.

Table 2. Final model of logistic regression on the annual probability of 206 '*Map*-free' herds in the paratuberculosis certification-and-surveillance programme in the Netherlands to transfer cattle to another '*Map*-free' herd.

Factor	Level	OR		*p*
		Estimate	95% CI	
Period[a]	Year 1	1	–	–
	Year 2	3.53	0.78; 15.9	0.10
	Year 3	0.93	0.17; 5.08	0.93
Time of certification [b]	Early	13.5	3.42; 52.9	<0.01
	Late	1	–	–

[a] Year 1 = 1 November 1999 – 1 November 2000; Year 2 = 1 November 2000 – 1 November 2001; Year 3 = 1 November 2001 – 1 November 2002
[b] Time at which the herd has obtained the '*Map*-free' status. Early = before 1 May 2001; late = after 1 May 2001.

5.4.4. Simulation of a random herd-contact structure

Convergence was reached for all output-parameters of the simulation. The mean number of simulated '*Map*-free' herds from which cattle were transferred to other '*Map*-free' herds was 40 (i.e. 19% of herds; Table 3). The mean number of '*Map*-free' herds in which cattle were introduced was 153 (74% of herds). On average, 29 '*Map*-free' herds (14% of herds) were both a source and destination of cattle.

To support the credibility of the model results, a single herd can be considered. If 44 cattle are randomly transferred between 206 herds, the number of cattle M transferred from this single herd follows a binomial distribution $Bin(206^{-1}, 44)$ and the probability to transfer ≥1 animal from this herd equals $P(M \geq 1) \approx 19\%$. Therefore, given random transfers, the expected proportion of herds from which animals are transferred to other herds is 19%. Similarly, if 280 cattle are randomly introduced into 206 herds, the number of cattle I introduced into a single herd follows $Bin(206^{-1}, 280)$, and the probability $P(I \geq 1) \approx 74\%$.

The numbers of '*Map*-free' herds in our field study in which cattle were introduced or from which cattle were transferred to other '*Map*-free' herds were well below the first percentiles of the numbers of herds in the simulation (Table 3). This means that the number of herds potentially becoming infected by cattle transfers in case any '*Map*-free' herds are not truly free of infection is much lower in the observed situation than in case of random cattle transfers.

Table 3. Comparison of field observations of 206 '*Map*-free' herds with results of a simulation of random cattle transfers to and between '*Map*-free' herds.

	Simulation			Field observation
	1% percentile	Mean	99% percentile	
Number of '*Map*-free' herds from which cattle were **transferred to** other '*Map*-free' herds	35	40	43	12
Number of '*Map*-free' herds in which cattle **from other 'Map-free'** herds were **introduced**	35	40	43	11
Number of '*Map*-free' herds in which cattle **from non-'Map-free' herds** were **introduced**	130	141	151	14
Total number of '*Map*-free' herds in which cattle were **introduced**	143	153	164	22
Number of '*Map*-free' herds in which cattle were **introduced and** from which cattle were **transferred to** other '*Map*-free' herds	23	29	36	0

5.5. Discussion

In the present study, the *rate* and *structure* of cattle transfers between Dutch '*Map*-free' herds were characterized to enable the design of a cost-effective alternative to the Dutch surveillance programme for '*Map*-free' herds. Until recently, '*Map*-free' herds were monitored by annual herd faecal examination consisting of a pooled faecal culture of all cattle ≥ 2 years of age (Kalis et al., 2000). However, at the *rate* of cattle transfers observed in the present study (0.06 to 0.3 animal per year per 100 animals present), biennial herd examinations are expected to result in a sufficiently low between-herd reproduction ratio R_h, if a random herd-contact structure is assumed (van Roermund et al., 2002). Therefore, a surveillance scheme was recently adopted in which '*Map*-free' herds are monitored by biennial herd faecal examinations by pooled faecal culture of all cattle ≥ 2 years.

The observed *structure* of cattle transfers warrants even further relaxation of the surveillance scheme for '*Map*-free' herds. The numbers of '*Map*-free' herds into which cattle were introduced and from which cattle were transferred to other '*Map*-free' herds observed in the field were well below the first percentiles of the simulation, in which a random structure of transfers was assumed. Thus, we conclude that the herd-contact structure is not random − and that the assumption of a random herd-contact structure made by van Roermund et al. (2002) was invalid. Most (89%) of '*Map*-free' herds were closed and 80% of introduced cattle were introduced in only 2% of the '*Map*-free' herds. No cattle were transferred to '*Map*-free' herds that were themselves a source of

cattle for other '*Map*-free' herds. This can be considered a special case of an under-dispersed herd-contact structure in which continued propagation of the infection between '*Map*-free' herds by cattle transfers was impossible. In case of an under-dispersed herd-contact structure, animals from an infected herd are transferred to a smaller number of different herds than in case of a random herd-contact structure. Thus, an under-dispersed contact structure results in a lower number of secondary infected herds (R_h is smaller) if infected cattle entering a susceptible herd act independently in spreading the infection in that herd. Therefore, with the observed under-dispersed contact structure, the surveillance scheme for '*Map*-free' herds may be even further relaxed in comparison to the optimal scheme for a random herd-contact structure. Moreover, an effective surveillance scheme might then be differentiated with respect to the threat individual '*Map*-free' herds pose to other '*Map*-free' herds (and to herds with a lower status) by transfer of cattle.

The assumption of a random herd-contact structure is generally considered convenient in modelling transmission of infectious diseases as part of the paradigm of "mass action" (i.e. equal infection probabilities for all susceptibles; de Jong, 1995; Heesterbeek, 2005; Keeling, 2005). Therefore, this assumption is made in many models for between-herd transmission by animal transfers within or between groups of herds (Jalvingh et al., 1999; Ferguson et al., 2001; Graat et al., 2001; Mangen et al., 2002; Tomassen et al., 2002; Vonk Noordegraaf et al., 2002). However, aberrations of the real herd-contact structure from the assumed randomness might also have a major effect on other infectious diseases. Therefore, models in which a non-random (under-dispersed or over-dispersed) herd-contact structure can be incorporated are required for the development of cost-effective surveillance programmes.

The present study demonstrates the importance of using situation-specific data. The proportion of herds introducing cattle was lower in the population of '*Map*-free' herds (11% in a 3-year period) than in the general dairy population (45% in a 1-year period). Thus, the specific rate of transfer of cattle in the target population needs to be taken into account in modelling studies.

All cattle transfers to and from herds with a '*Map*-free' status on 1 November 2002 during the previous 3 years were included in the present study, even if the herd had not yet obtained the '*Map*-free' status by the time of the transfer (Fig. 1). Obtaining '*Map*-free' status during the study influenced the herd-contact structure of a herd: herds that obtained the '*Map*-free' status before 1 May 2001 were a more likely source of cattle for other '*Map*-free' herds than herds that obtained the status at a later date. However, no effect of year of study on cattle introductions in '*Map*-free' herds and cattle transfers between '*Map*-free' herds was found. Therefore, the data from these 3 years were pooled, irrespective of the time at which a herd had obtained '*Map*-free' status. However, over a longer period, the herd-contact structure might alter. For instance, '*Map*-free' herds in which cattle have been introduced in the past, might possibly become a source of cattle for other '*Map*-free' herds in the future. Therefore,

we recommend continuous monitoring of the herd-contact rate and structure of herds participating in any surveillance programme for an infectious disease.

Most cattle introduced into herds with a '*Map*-free' status on 1 November 2002 originated from a herd without this status by that time. Most of these cattle were introduced before the herds had actually obtained the '*Map*-free' status, and were raised in a herd with, at the time of transfer, an equal or higher number of negative annual herd examinations than the herd of destination. Therefore, these introductions were not in conflict with the regulations of the Dutch paratuberculosis programme.

Almost without exception, introduction of paratuberculosis into a herd is through addition of infected farm animals (Sweeney, 1996). Therefore, the present study focussed on cattle transfers only. However, it has been suggested that herds also become infected through other routes, such as application of manure from infected herds or wildlife (Sweeney, 1996; Daniels et al., 2003). Such other introductions potentially result in transfer of infected cattle to other herds (with or without '*Map*-free' status). A breakdown in any '*Map*-free' herd caused by such introduction would reduce the average net return to farmers of participation in the '*Map*-free' certification-and-surveillance programme. However, in the present study, contacts between '*Map*-free' herds, other than by transfer of cattle, are presumably uncommon because of the geographical distribution of these herds (Fig. 2). Thus, given the structure of cattle transfers observed in this study, introductions of *Map* into '*Map*-free' herds through routes other than cattle transfers would not result in a continuous propagation of the infection between '*Map*-free' herds.

The concepts of the present study equally apply to other infectious diseases and other countries.

5.6. Conclusions

In conclusion, the majority of Dutch '*Map*-free' herds were closed. The observed rate of cattle transfers between certified '*Map*-free' herds was sufficiently low to relax the surveillance scheme to biennial herd examinations by pooled faecal culture of all cattle ≥ 2 years of age. The observed structure of cattle transfers was not random but under-dispersed. Continued propagation of the infection between herds was impossible during the 3 years of study, and therefore the surveillance scheme may be even further relaxed and differentiated regarding the risk herds pose to other herds.

5.7. Declaration of interest

The first and third author (MFW and CHJK) are employed by the Dutch Animal Health Service, which is running a certification-and-surveillance programme for '*Map*-free' herds.

5.8. Acknowledgments

We thank A. Velthuis and the Dutch Ministry of Agriculture, Nature and Food Quality (E.G.M. van Klink, W. Pelgrim) for providing data on cattle transfers in the general Dutch dairy population. The assistance of H.B.J. Assink in data collection, the contributions of J.A.P. Heesterbeek and D. Klinkenberg to the study, and the comments from J. Verhoeff on the manuscript are gratefully acknowledged.

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Chapter 6

Age at which dairy cattle become *Mycobacterium avium* subsp. *paratuberculosis* faecal-culture positive

M.F. Weber [a], J. Kogut [b], J. de Bree [b], G. van Schaik [a], M. Nielen [c]

Submitted for publication

[a] GD Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands.

[b] Central Veterinary Institute of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

[c] Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, The Netherlands.

6.1. Abstract

Age at which cattle become faecal culture positive for *Mycobacterium avium subsp. paratuberculosis (Map)* can be used as a proxy parameter for age at becoming infectious, which is an important parameter in the control of *Map* in cattle herds. To investigate the age at becoming faecal culture positive, survival analysis methods were applied. The analyses were carried out on asynchronous interval censored data of faecal culture results of samples collected from 37,151 female cattle of dairy breeds in 373 Dutch herds between 1996 and 2002. A Weibull proportional hazards model was employed to study risk factors, such as apparent prevalence (AP) within the herd, breed, herd size and season of birth.

The analyses showed that the hazard of becoming faecal culture positive (i.e., the instantaneous rate of becoming culture positive at a certain age, given that the animal was not positive up to that age) increased with the age of cattle. At a higher within-herd apparent prevalence, cattle became faecal culture positive at younger age. In high prevalence herds (apparent prevalence ≥ 0.10), an estimated 5% to 14% of cattle became culture positive before 2 years of age, depending on breed and herd size.
Our findings indicate that a considerable proportion of young stock was shedding *Map*. Therefore, infectious young stock should be a major concern in the control of paratuberculosis, especially in high prevalence herds.

Keywords: Cattle; Paratuberculosis; Faecal culture; Age; Survival analysis

6.2. Introduction

Paratuberculosis (or Johne's disease) in cattle is an infectious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). The disease is widespread world-wide and causes significant economic losses. Control programmes for paratuberculosis are based on improvement of animal husbandry and on early culling of infected cattle to reduce transmission of *Map* within a herd.

In developing control programmes, it has frequently been assumed that young stock do not become infectious. Provided that adult cattle are highly resistant to infection, this assumption means that effective separation of young stock from adult cattle largely prevents postnatal infection. However, this assumption is in contrast with observed faecal shedding of *Map* in experimentally infected young stock (Rankin, 1959; Rankin, 1961; McDonald et al., 1999; Waters et al., 2003), young stock exposed to experimentally infected cattle (Rankin, 1961; van Roermund et al., 2007) and naturally infected young stock (Kalis et al., 1999; McDonald et al., 1999; Waters et al., 2003; Antognoli et al., 2007). Moreover, calf-calf transmission has experimentally been demonstrated (van Roermund et al., 2007).

The age at onset of faecal shedding depends on the progression of the infection within an infected individual. Progression of the infection depends on the age at infection and the infectious dose (Begg and Whittington, 2008), and these parameters are related to the within-herd prevalence. Also, genetic variation exists in dairy cattle for susceptibility to (progression of) infection with *Map* and faecal shedding of *Map* (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006). This genetic variation may result in differences in susceptibility between breeds. Therefore, the age at onset of faecal shedding might depend on within-herd prevalence and breed. However, the aforementioned studies demonstrating faecal shedding of *Map* in young stock (Rankin, 1959; Rankin, 1961; Kalis et al., 1999; McDonald et al., 1999; Waters et al., 2003; Antognoli et al., 2007; van Roermund et al., 2007) included only small numbers of cattle and herds, and quantitative data on the age at onset of faecal shedding in relation to breed and within-herd prevalence were lacking. Therefore, the aim of the present study was to quantify the distribution of age at onset of faecal shedding in dairy cattle of various breeds in infected herds. The age at onset of faecal shedding was approximated by the age at which cattle become faecal culture positive.

6.3. Materials and methods

6.3.1. Data

A database was set up containing test results of all bovine faecal samples submitted between 1st January 1996 and 31st December 2002 to the GD Animal Health Service's laboratory for individual faecal culture (IFC), or pooled faecal culture (PFC)

for *Map*. To check for completeness, the annual numbers of results retrieved for each of the test methods were compared with numbers presented in financial reports of the laboratory. For all year and test combinations, results were retrieved for > 95% of samples tested according to the financial reports.

From this database, a subset of IFC results was selected according to the following criteria: (1) a positive or negative culture result (i.e. missing results, such as a sample regarded contaminated, were excluded from the subset), (2) at least 20 adult (i.e. ≥ 2 years of age) cattle in the herd in the quarter of the year of sampling, (3) at least one herd examination by IFC, (4) at least one positive IFC or PFC result in the herd during 1996 - 2002. The number of adult cattle present in the herd in the quarter of the year in which the sample was submitted was retrieved from census data of the Dutch agricultural statutory bodies. A herd examination was defined as a submission of samples within a 30-day period, of which the number of samples was ≥ 80% of the recorded number of adults in the same quarter. Because herds with at least one positive faecal culture were selected, cattle in these herds were considered at risk of being infected.

All IFC samples were assumed to be collected 2 days before the date of registration at the AHS laboratory. Using the combination of (1) the unique herd number, (2) the animal identification as provided at submission, and (3) the assumed date of sampling, each sample was matched to a unique animal identification as recorded in the national cattle identification and registration system (Nielen et al., 1996). For uniquely identified animals, the date of birth, sex and breed were retrieved from the pedigree records of the Dutch National Cattle Syndicate (NRS).

6.3.2. Laboratory tests

IFC was performed on Löwenstein Jensen (L.J.) medium using the modified Jørgensen method (Kalis et al., 1999). In short, for each sample, four culture tubes were inoculated and inspected for *Map* colonies and contamination at four, eight, 12, 16 and 26 weeks of incubation. Suspect colonies were examined by acid fast staining; acid fast colonies were confirmed to be *Map* by IS900 PCR. A sample was regarded positive if one or more *Map* colonies were detected. A sample was regarded contaminated if three or more tubes were contaminated at 16 weeks of incubation or earlier. A sample was regarded negative if it was neither regarded positive nor regarded contaminated. During the six years of study (1996-2002), the culture method was slightly adapted. From January 2001 onwards, inspection at 26 weeks of incubation was ceased, because this inspection only marginally improved sensitivity. From March 2001 onwards, natamycine (1.18 g/l) was added to the LJ-culture media to reduce the risk of fungal contamination. From January 2002 onwards, a sample was regarded contaminated only if three or more tubes were contaminated at 8 weeks of incubation or earlier; samples found to be contaminated at >8 weeks were considered negative. From September 2002 onwards, acid fast staining on suspect colonies was no longer

performed; suspect colonies were directly tested by IS900 PCR. However, because the effect of these adaptations on diagnostic sensitivity and specificity was considered of minor importance, the IFC results were analysed as if performed by a single culture method.

PFC, in which samples of up to 5 individuals were pooled, was performed as previously described (Kalis et al., 2000), with the same adaptations of the culture protocol during the years of study as described above.

6.3.3. Definition of variables

Test results as provided in the database were classified as positive or negative. Age at sampling was calculated as the number of days between birth and sampling.

The within-herd prevalence of infectious cattle at birth of an individual was considered a potential risk factor for the age at onset of faecal shedding in this individual. This within-herd prevalence of infectious cattle was assumed to be constant over time. Apparent prevalence (AP) in the herd was used as a proxy parameter for this within-herd prevalence of infectious cattle. AP was defined as the total number of positive IFC results in a herd during 1996-2002, divided by the total number of IFC results in that period. Thus, each herd was assigned a single value of AP throughout the study period. AP was categorized as $AP < 0.05$, $0.05 \leq AP < 0.1$ or $AP \geq 0.1$.

Herd size was defined as the average of the number of adult cattle in the quarters of years of sampling (weighed according to the number of cattle sampled at each sampling). Herd size was categorised as 'small' (number of adult cattle < 50), 'medium' (50 ≤ number of adult cattle < 80) or 'large' (number of adult cattle ≥ 80). Thus, each herd was assigned to a single category of herd size throughout the study period.

Season of birth was defined as quarter 1 (January – March), quarter 2 (April – June), quarter 3 (July – September) or quarter 4 (October – December). Alternatively, season was defined as 'summer' (April – September) and 'winter' (October – March), because it was hypothesized that calf rearing management would differ between summer and winter period.

Breed of cattle was categorised into three groups: pure Holstein-Frisian cattle ('pure HF'), Holstein-Frisian crossbred cattle ('HF-cross'), other dairy breeds ('other dairy'). Data on beef breeds (n= 609) and cattle for which breed was unknown (n=29) were discarded. Sex of cattle was either female or male; all data on male cattle (n= 63) were discarded.

6.3.4. Data analysis

A survival analysis was performed to determine the distribution of age at which cattle start detectable shedding of *Map*, taking into account the asynchronous interval censored nature of the data. Let A_{ij} be the unknown age at which individual i in a well defined group j started to shed *Map*. We assumed that an animal was consistently IFC-

negative up to age A_{ij} and remained IFC-positive beyond this age. So, in our survival analysis, the "event" was detectable shedding of *Map* and "time to event" was equal to age A_{ij}. Two datasets were created: one dataset including all available faecal culture results, and one dataset including only samples of young stock (≤ 2 yrs of age). The dataset for each analysis was structured according to the description for asynchronous interval censoring by Radke (2003). In short, two variables L_i and U_i were defined as lower and upper bounds of the age interval for A_i. For an interval censored observation (i.e. an animal with a negative sample followed by a positive sample) U_i was set to the age at sampling of the first positive sample of the animal, and L_i to the age at sampling of the last preceding negative sample. For a right censored observation (i.e. an animal without any positive test result) L_i was set to the age at sampling of the last negative sample and U_i was set to missing. For a left censored observation (i.e. the first known test result of an animal is positive) L_i was set to missing and U_i was set to the age at sampling of the positive test.

Survival analysis for asynchronous interval censored data was performed using the PROC LIFEREG command of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A Weibull proportional hazards model was chosen. The general form of the Weibull survival curve $S_j(t)$ in a group of animals j is defined as (Cox and Oakes, 1994):

$$S_j(t) = Prob(A_{ij} \geq t) = e^{-\left(\frac{t}{scale_j}\right)^{shape}} \qquad \text{(eq. 1)}$$

i.e. the probability that an animal will not shed *Map* before age t depends on the scale parameter of group j and all groups in an analysis have a common shape parameter. Differences between groups are modelled by:

$$scale_j = e^{\beta_0 + \sum_{k \varepsilon \, group\, j} \beta_k X_k} \qquad \text{(eq. 2)}$$

where β_0 is the intercept and X_k are factors or covariates to define group j. Then the failure function $F_j(t)$ is the probability of shedding by an individual in group j before age t:

$$F_j(t) = Prob(A_{ij} < t) = 1 - S_j(t) \qquad \text{(eq. 3)}$$

The probability density of this failure function $F_j(t)$ is:

$$f_j(t) = \frac{d}{dt}\left[1 - S_j(t)\right] = \frac{shape}{scale_j}\left(\frac{t}{scale_j}\right)^{shape-1} e^{-\left(\frac{t}{scale_j}\right)^{shape}} \quad \text{(eq. 4)}$$

while the hazard $h_j(t)$ is finally modelled as:

$$h_j(t) = \lim_{\Delta t \to 0+} \frac{P\left(t \le A_{ij} < t + \Delta t \mid A_{ij} \ge t\right)}{\Delta t} = \frac{f_j(t)}{S_j(t)} =$$

$$= \frac{shape}{scale_j}\left(\frac{t}{scale_j}\right)^{shape-1} \quad \text{(eq. 5)}$$

i.e. the instantaneous rate of *Map* shedding at age *t*, given that the animal was not shedding up to age *t*.

Putative risk factors were included in this Weibull proportional hazards model to improve the model fit. Putative risk factors studied were AP, herd size, season of birth and breed group. Risk factors were considered significant at *p*-values ≤ 0.05 generated by two-sided Wald tests. A final model was selected using the likelihood ratio test. Most likely values and 95% confidence intervals of the hazard ratio's were generated by simulation.

6.3.5. Sensitivity analysis on simulated data

A sensitivity analysis was performed to evaluate the robustness of the LIFEREG procedure for our asynchronous interval censored data with a high proportion of missing values (i.e. left and right censored observations). A total of 100 simulated datasets of 5000 cattle each were analysed. To create these datasets, the shape and scale parameter of the Weibull distribution for the true survival of the simulated infected cattle were set to 1.739 and 9,475 respectively (similar to our results of field data for pure HF cattle in large herds with a low apparent prevalence). Age A_i, at which detectable shedding of *Map* started, was randomly drawn from this survival distribution for each individual. Furthermore, two sampling dates were generated for each individual, using a gamma distribution for the age at first sampling:

$$f(x) = \frac{\beta^{-\alpha} x^{\alpha-1} e^{\left(-x/\beta\right)}}{\Gamma(\alpha)}, x \ge 0 \quad \text{(eq. 6)}$$

for which $\alpha = 4.13$ and $\beta = 316.5$ and $\Gamma(\alpha)$ is the gamma function:

$$\Gamma(\alpha) = \int_0^\infty t^{\alpha-1} e^{-t} dt \qquad \text{(eq. 7)}$$

A second gamma distribution was used for the interval between first and second sampling for which α = 3.53 and β = 100.5. The parameters of these gamma distributions were chosen to approximate the distributions observed in the group of pure HF cattle in large herds with a low apparent prevalence. Then, for each individual, values of the two variables L_i and U_i were determined by comparison of the age at samplings with the age at which detectable shedding of *Map* started for each individual (A_{ij}). If the age at first sampling was ≥ A_{ij}, L_i was set to missing and U_i was set to the age at first sampling (left censored observation). If the age at second sampling was <A_{ij}, L_i was set to the age at second sampling and U_i was set to missing (right censored observation). Otherwise, L_i was set to the age at first sampling and U_i was set to age at the second sampling (interval censored observation). The 100 datasets consisted of, on average, 178 (range 159 – 200) left censored observations, 4744 (4714-4764) right censored observations, and 78 (59 – 98) interval censored observations. Each simulated dataset was analysed using the LIFEREG procedure of SAS as described above.

6.4. Results

6.4.1. Herds and cattle

Observations were obtained for 37,151 female cattle of dairy breeds (including all age groups) from 373 herds (Table 1), for which at least one IFC result was available, a unique animal identification could be matched, and information on date of birth was available. From these 37,151 cattle, a total number of 59,575 individual faecal samples were cultured (Fig. 1). Of these 59,575 samples, 6,158 samples were collected before 2 years of age, from 4,661 cattle in 174 herds (Table 1). The number of herds and cattle in the various classes of herd size and AP are shown in Table 2.

Table 1. Categorisation of cattle included in the analyses.

Breed group	Description	All age groups		Young stock (<2 year)	
		Number of cattle	Number of herds	Number of cattle	Number of herds
Pure HF	Pure Holstein-Frisian	18,979	353	3,182	149
HF cross	Holstein-Frisian crossbred	17,345	371	1,426	126
Other dairy	Other dairy breeds[1]	827	91	53	11
Total		37,151	373	4,661	174

[1] Including: Frisian-Holland (FH), Maas-Rijn-IJssel (MRY), Brown Swiss, Dutch Belted, Dutch Glassed, Jersey, Montbeliarde and Swedish Red and White

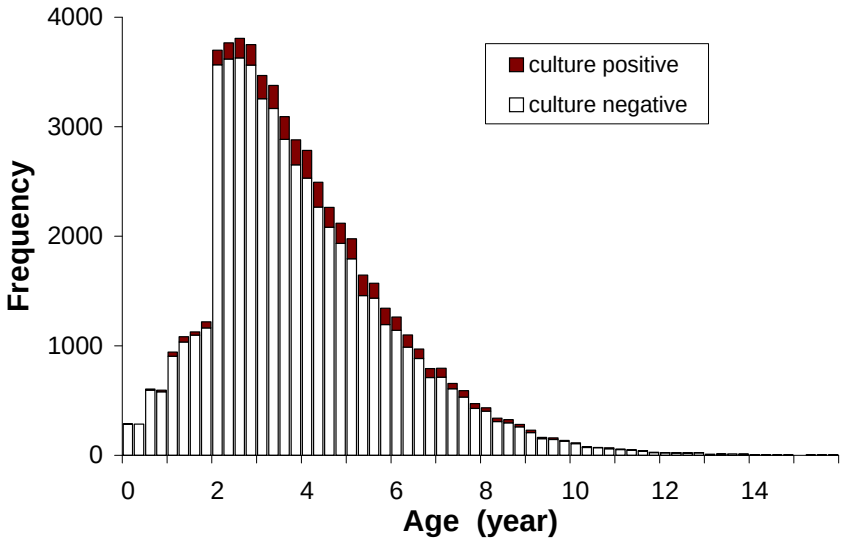

Fig. 1. Distribution of age at sampling and result of individual culture for *M. a. paratuberculosis* on 59,575 faecal samples collected from 37,151 female cattle of dairy breeds submitted between 1996 and 2002 to the GD Animal Health Service.

Table 2. Number of herds (number of cattle) included in the analyses in each herd size class by apparent prevalence (AP) class.

Herds	Age group	AP class	Herd size class (i.e. number of adult cattle)			
			<50	50 - 80	>80	Total
All herds	All age groups	AP< 0.05	26 (1,409)	75 (5,739)	59 (9,081)	160 (16,166)
		0.05 ≤ AP < 0.1	14 (711)	45 (4,167)	51 (7,552)	110 (12,430)
		AP ≥ 0.1	18 (843)	37 (2,291)	48 (5,421)	103 (8,555)
		Total	58 (2,963)	157 (12,197)	158 (21,991)	373 (37,151)
Herds with young stock (< 2 yr) sampled	All age groups	AP< 0.05	12 (1,009)	29 (2,669)	36 (6,457)	77 (10,135)
		0.05 ≤ AP < 0.1	6 (360)	22 (2,535)	27 (4,924)	55 (7,819)
		AP ≥ 0.1	7 (414)	17 (1,333)	18 (2,675)	42 (4,422)
		Total	25 (1,783)	68 (6,537)	81 (14,056)	174 (22,376)
	Young stock (< 2 yr) only	AP< 0.05	12 (299)	29 (603)	36 (1,609)	77 (2,511)
		0.05 ≤ AP < 0.1	6 (26)	22 (412)	27 (908)	55 (1,346)
		AP ≥ 0.1	7 (59)	17 (232)	18 (513)	42 (804)
		Total	25 (384)	68 (1,247)	81 (3,030)	174 (4,661)

6.4.2. Survival analysis of faecal culture results of all age groups

Left censored (first observation positive), interval censored (negative observation followed by positive observation) and right censored (all observations negative) observations were made for 2,538, 1,083 and 33,530 of the 37,151 female cattle respectively. Thus, 34,613 observations of L_i and 3,621 observations of U_i were made (Figs. 2A and B).

The shape parameter of the Weibull model was found to be significantly different for the three classes of AP (shape ± SE = 1.739 ± 0.074, 1.336 ± 0.044, 1.061 ± 0.041 for the low, intermediate and high AP class, respectively; Tables 3, 4 and 5). Because the shape parameter is required to be equal amongst the various groups in a single analysis, no overall analysis was possible. Further subdivisions of each of the three AP classes did not result in significant differences of the shape parameter. Thus, only separate analyses were performed for the three AP classes. Within each AP class, shape parameters were not significantly different for the breed groups and herd size classes apart. Therefore, all breed groups and herd size classes could be included in a single analysis within each AP class.

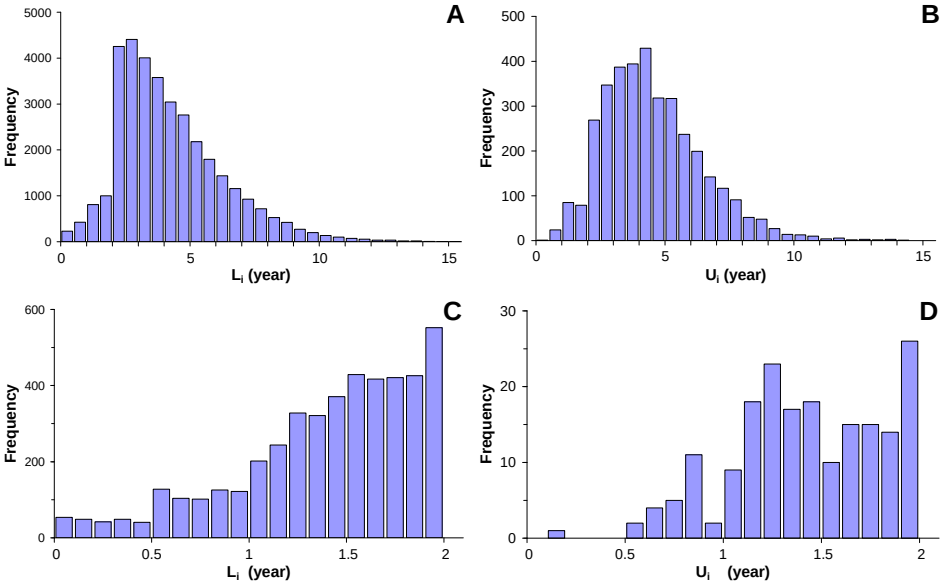

Fig. 2. Age distribution for lower (L_i) and upper (U_i) limits for age A_i at which individual *i* start detectable shedding of *M. a. paratuberculosis*. (A) distribution of L_i for analysis of data from all age groups (n=34,613); (B) distribution of U_i for analysis of data from all age groups (n=3,621), (C) distribution of L_i for analysis from young stock only (n=4,528); (D) distribution of U_i for analysis from young stock only (n=190).

Table 3. Final survival model of age (in days) at onset of detectable shedding for all age groups based on individual faecal culture results of 16,166 cattle in 160 herds with an apparent prevalence < 0.05 (low).

Parameter	(β_i)	Estimate	Standard error	Pr > Chi-square	Overall Pr > Chi-square	Hazard ratio		
						Lower limit 95% CI	Most Likely	Upper Limit 95% CI
Intercept (β_0)		9.5786	0.1801	<0.0001				
Breed	HF pure	-0.4222	0.1649	0.0105	0.0004	1.191	2.001	3.704
	HF cross	-0.2600	0.1639	0.1127		0.899	1.313	2.775
	Other dairy	0.0000	0.0000			1.000	1.000	1.000
Herd size	Small	-0.2033	0.0747	0.0065	<0.0001	1.105	1.403	1.844
	Medium	0.1411	0.0534	0.0082		0.653	0.800	0.939
	Large	0.0000	0.0000			1.000	1.000	1.000
Shape		1.7392	0.0744					

Table 4. Final survival model of age (in days) at onset of detectable shedding for all age groups based on individual faecal culture results of 12,430 cattle in 110 herds with 0.05 ≤ apparent prevalence < 0.10 (medium).

Parameter	(β_i)	Estimate	Standard error	Pr > Chi-square	Overall Pr > Chi-square	Hazard ratio		
						Lower limit 95% CI	Most Likely	Upper Limit 95% CI
Intercept (β_0)		9.035	0.1382	<0.0001				
Breed	HF pure	-0.0812	0.1338	0.5441	0.0049	0.784	1.127	1.583
	HF cross	0.0535	0.1334	0.6880		0.656	0.900	1.324
	Other dairy	0.0000	0.0000			1.000	1.000	1.000
Shape		1.3362	0.0441					

Table 5. Final survival model of age (in days) at onset of detectable shedding for all age groups based on faecal culture results of 8,555 cattle in 103 herds with an apparent prevalence ≥ 0.1 (high).

Parameter	(β_i)	Estimate	Standard error	Pr > Chi-square	Overall Pr > Chi-square	Hazard ratio		
						Lower limit 95% CI	Most Likely	Upper Limit 95% CI
Intercept (β_0)		9.318	0.1944	<0.0001				
Breed	HF pure	-0.5038	0.1829	0.0059	<0.0001	1.165	1.545	2.500
	HF cross	-0.3233	0.1825	0.0764		0.964	1.483	2.059
	Other dairy	0.0000	0.0000			1.000	1.000	1.000
Herd size	Small	-0.4056	0.0746	<0.0001	<0.0001	1.314	1.547	1.807
	Medium	-0.1415	0.0538	0.0085		1.039	1.159	1.300
	Large	0.0000	0.0000			1.000	1.000	1.000
Shape		1.0608	0.0410					

For ease of interpretation of the results, survival curves $S_j(t)$, failure density curves $f_j(t)$ as well as hazard curves $h_j(t)$ were plotted for herds with a low AP and herds with a high AP (Fig. 3), even though the three curves are mathematically determined by the parameters in the corresponding Tables 3 and 5. Note that the failure density curve $f_j(t)$ is the first derivative of the failure curve, which is the complement of the survival curve (eqs. 3 and 4). Therefore, this failure density curve represents the incidence rate at which cattle start shedding *Map* at a certain age within the total population of cattle studied. In contrast, the hazard function is the ratio of the failure density curve and the survival curve. The hazard function therefore represents the incidence rate at which cattle start shedding *Map* at a certain age within the subpopulation of cattle that did not shed *Map* prior to that age.

The hazard of becoming faecal culture positive increased with age in all three AP-classes. With an increasing AP, the shape parameter of the Weibull curve decreased towards one (Tables 3 - 5). This reflects that the weight of the hazard curve $h_j(t)$ shifted to a lower age with increasing AP class (Figs. 3C and F). Consequently, the survival curve $S_j(t)$ decreased at a higher rate with increasing AP (Figs. 3A and D). In low-AP herds, an estimated 0.4% to 1.6% of cattle became faecal culture positive before 2 years of age, depending on breed and herd size. These proportions are represented by the areas under the failure density curves $f_j(t)$ in Figs. 3B and E. In intermediate-AP herds, 3.7% to 4.4% of cattle became faecal culture positive before two years of age, depending on breed (but independent of herd size). Finally, in high-AP herds, an estimated 5.4% to 14% of cattle became faecal culture positive before two years of age, again depending on breed and herd size. An estimated 2.6% to 6.8% of the cattle in these high-AP herds even became culture positive before one year of age.

In the low-AP class, both breed and herd size effects were significant in the final model, but not their interaction (Table 3). Pair wise comparisons revealed a higher hazard in ‘pure HF’ compared to ‘other dairy breeds’. Differences between ‘pure HF’ and ‘HF cross’ and between ‘HF cross’ and ‘other dairy breeds’ were not significant. In small herds the hazard was higher than in large herds, while the hazard was lowest in medium sized herds.

In the intermediate-AP class the effect of breed was significant, even though pair wise comparisons did not reveal any significant difference between the three breed groups (Table 4).

In our final model for the high-AP class, both breed and herd size were significant, but not their interaction (Table 5). Similar to the low-AP class, pair wise comparisons revealed a higher hazard in ‘pure HF’ compared to ‘other dairy breeds’. The hazard was lower in large herds than in both small and medium sized herds.

Neither season of birth (modelled as quarter of year, or modelled as summer versus winter), nor two-way interactions between season and the other explanatory variables, were significant in any of the analyses.

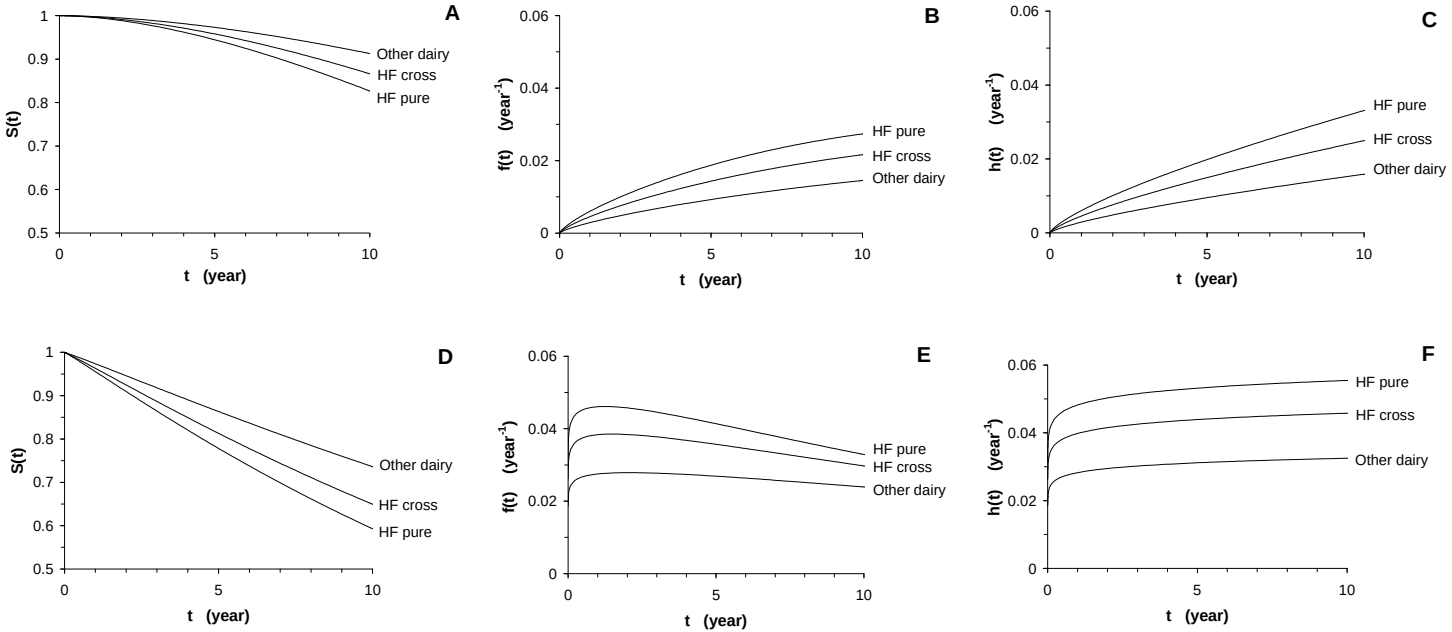

Fig. 3. Fitted survival $S_j(t)$, failure density $f_j(t)$ and hazard $h_j(t)$ curves in herds (≥ 80 adult cattle) with a low apparent prevalence (< 0.05; A, B, C) and a high apparent prevalence (≥ 0.10; D, E, F). For illustrative purposes, the dimension of age is years.

6.4.3. Survival analysis of faecal culture results of young stock

Left censored, interval censored and right censored observations were made for 133, 57 and 4,471 of the 4,661 female young stock respectively. Thus, observations of L_i were obtained for 4,528 cattle and observations of U_i were obtained for 190 cattle (Figs. 2C and D).

The shape parameter of the Weibull model was not found to be different for the three classes of AP. However, because this shape parameter was significantly different for the three AP classes in the analyses of faecal culture results of all age groups, the results of a similar subgroup analysis are presented here.

None of the effects of the explanatory variables (breed, herd size, season of birth or two-way interactions) were found to be significant in any of the AP classes. Within each AP class, the failure density curve for young stock did not differ from the failure density curve for all age groups (Table 6). When the data on faecal culture results of young stock were analysed over all AP classes, then AP class but none of the other variables was found to have a significant effect (higher hazard in herds with higher AP; results not shown).

In analyses without explanatory variables, the estimated proportion of cattle that started to shed *Map* before two years of age did not substantially depend on whether the analysis was based on data from all herds or on data from herds in which young stock had been tested. Furthermore, if the analysis was based on data from herds in which young stock had been tested, the estimated proportion did not substantially depend on whether the analysis was based on data from all age groups, or data of young stock only (Table 7).

6.4.4. Sensitivity analysis

In the sensitivity analysis, 100 simulated datasets were analysed. In 99 of the 100 datasets, the 95% CI of the estimate of the shape parameter included the true value that was used to create the datasets. In 98 of the 100 simulated datasets, the 95% CI of the estimate of the scale parameter included its true value. In each of these 98 datasets, also the 95% CI of the estimate of the shape parameter included its true value. These results indicate robustness of the LIFEREG procedure for our asynchronous interval censored data.

Table 6. Final survival models of age (in days) at onset of detectable shedding for young stock (≤ 2 years of age) in the various apparent prevalence (AP) classes.

AP class	Number of cattle (herds)	Parameter	df	Estimate	Standard error	Chi-square	Pr > Chi-square
< 0.05	2511 (77)	Intercept (β_0)	1	8.2556	0.4009	424.16	<0.0001
		Shape	1	2.1240	0.4436		
0.05 ≤ AP <0.10	1346 (55)	Intercept (β_0)	1	8.7228	0.4847	323.95	<0.0001
		Shape	1	1.1961	0.2379		
AP ≥ 0.10	804 (42)	Intercept (β_0)	1	7.7510	0.2563	914.31	<0.001
		Shape	1	1.4737	0.2522		

Table 7. Estimated proportion (95% CI) of cattle with onset of detectable shedding before 2 years of age, in analyses based on data of cattle of all ages in all herds, all ages in herds in which young stock were sampled, or only young stock in herds in which young stock were sampled. For numbers of herds and cattle included in each analysis, see Table 2.

Herds included in analysis	AP class[1]	Age group included in analysis	
		All age groups	Young stock only
All herds	AP < 0.05	0.009 (0.006; 0.016)	
	0.05 ≤ AP < 0.10	0.038 (0.031; 0.050)	
	AP ≥ 0.10	0.090 (0.075; 0.112)	
Herds with young stock sampled	AP < 0.05	0.012 (0.007; 0.020)	0.006 (0.002; 0.192)
	0.05 ≤ AP < 0.10	0.041 (0.032; 0.055)	0.045 (0.015; 0.262)
	AP ≥ 0.10	0.099 (0.080; 0.127)	0.130 (0.063; 0.344)

[1] AP: apparent prevalence.

6.5. Discussion

In this study, the age distribution at onset of infectiousness of dairy cattle in infected herds was approximated by the distribution of age at onset of detectable faecal shedding of *Map*. The results show that this age distribution strongly depends on the within-herd prevalence. With increasing within-herd prevalence, cattle start to shed *Map* at, on average, younger age. A considerable proportion of young stock become faecal culture positive, especially in high-prevalence herds (5.4 to 14% before 2 years of age in herds with an AP ≥ 0.10).

Several putative risk factors were included in our analyses: breed, AP, and herd size. 'Pure-HF' cattle started to shed *Map* at, on average, younger age than 'other dairy' breeds. Possibly, pure-HF cattle are more susceptible to infection or progression of the infection. Alternatively, other factors such as high milk production or herd management might be confounders for the association between breed and time to detectable shedding. Infected cattle have been described to produce more milk than uninfected cows before they began shedding, possibly caused by a genetic component to susceptibility to *Map* with a positive genetic correlation to greater milk production (Smith et al., 2009).

Cattle in herds with a higher AP started to shed *Map* at, on average, younger age. There are two potential explanations for this observation. Firstly, in herds with a low AP, more cattle are uninfected. Uninfected cattle will remain non-shedders throughout their lifetime in the herd. Consequently, in herds with a low AP, relatively fewer animals will start to shed *Map* at any age. Thus, cattle in herds with a high AP start to

shed at younger age on average. Secondly, infected cattle in herds with a high AP may have a different course of the infection-and-disease process compared to infected cattle in herds with a low AP. Susceptible calves in herds with a high prevalence of infection are likely to be exposed at a younger age to a higher infectious dose than susceptible calves in herds with a low prevalence. Possibly, this results in onset of shedding in infected cattle at a younger age. However, experimental studies are needed to elucidate to what extent either of these two potential explanations contributes to the observed difference between herds with a low and herds with a high AP.

Herd size was included in our analyses because it was hypothesized that simultaneous presence of young susceptible calves and heavy shedders is more likely in large herds. Therefore calves might be more likely to become infected at young age, and consequently more likely to become infectious at younger age. However, in both low-prevalence and high-prevalence classes, age at onset of detectable faecal shedding was lower in small herds compared to large herds whereas herd size had no significant effect in the intermediate-prevalence class. Possibly, the effect of herd size is confounded with the effect of herd management. Unfortunately, data on herd management were not available in the present study.

Our observation of onset of faecal shedding before adulthood is in line with the results of previous studies on shedding in young stock (Rankin, 1959; Rankin, 1961; McDonald et al., 1999; Waters et al., 2003; Antognoli et al., 2007; van Roermund et al., 2007). Nevertheless, our results are in contrast with a survival analysis by Nielsen and Ersbøll (2006) in Danish dairy cows in which cattle only became faecal culture positive after two years of age. However, faecal culture results of young stock were included in our study, but not in the study by Nielsen and Ersbøll (2006). Moreover, in the present study asynchronous interval censoring of the data was taken into account. Radke (2003) demonstrated that by taking asynchronous interval censoring into account, the parameter estimates are less biased compared to the 'upper bound approximation method' used by Nielsen and Ersbøll (2006). Consequently, our survival curve probably provides a less biased estimation of the age at onset of faecal culture positivity.

The sensitivity analysis on simulated datasets demonstrated the robustness of the LIFEREG procedure for our asynchronous interval censored data with a large proportion of missing values (in case of left and right censored observations). A Weibull hazard function was chosen in our analyses because it is a flexible function for which suitable statistical procedures are available to deal with asynchronous interval censored data. More flexible hazard functions were not used because, to our knowledge, no appropriate measures exist to compare the fit of these functions on asynchronous interval censored data with a large proportion of missing values. A limitation of the Weibull model is that it enforces a monotonically increasing or decreasing hazard function $h(t)$. Therefore, in the analyses of faecal culture results from all age groups, the data on adult cattle possibly influenced the estimated survival of

young stock. However, the survival curve was not substantially different in the separate analyses of data from young stock only (Table 7). Therefore, it was considered legitimate to interpret the results from the overall analyses of data from all age groups for young stock as well.

Several sources of bias might have influenced our results. Firstly, onset of faecal culture positivity was used as proxy parameter for the event of interest: onset of infectiousness. Because shedding might be intermittent and faecal culture has an imperfect sensitivity, true onset of shedding is most likely at younger age than estimated in our study. However, sensitivity of faecal culture is relatively high in high shedders, which probably contribute most to the transmission of *Map*. Therefore, it was considered reasonable to use onset of faecal culture positivity as a proxy parameter for onset of infectiousness. Secondly, the true within-herd prevalence of infectious cattle at the time of infection of each individual was unknown, in part because a large proportion of individuals in the study was already infected at the time their herd entered the study. Therefore, AP was used as a proxy parameter of the true within-herd prevalence, assuming a constant prevalence over time. Thirdly, effects of AP and breed might be confounded by herd. The LIFEREG procedure does not allow inclusion of a random herd effect, which would cover all unmeasured confounding at the clustered herd level. For the current analysis, our interest was more biological, where AP and breed were seen as causal variables, while a random herd effect would preclude any biological interpretation. Additionally, the inclusion of a random herd effect in our current models would most likely only change the standard errors of parameter estimates of explanatory variables on the animal-level (meaning that their effect might become insignificant) and not alter their point estimates. This disadvantage of the LIFEREG procedure was therefore acceptable for the current analysis. Finally, participating herds were not a random sample of the Dutch dairy population, but were predominantly participating in a control programme for paratuberculosis or participating in research projects. However, the variation in the herd management and AP in the 373 study herds is probably not much different from those of the general Dutch dairy population.

Our finding that a considerable proportion of young stock started to shed *Map* before adulthood, especially in herds with a high prevalence, has important implications for the control of paratuberculosis. Shedding of *Map* in young stock can result in transmission of the infection amongst young stock. Calf-calf transmission has been shown experimentally (van Roermund et al., 2007), and in a field study of *Map* transmission in 21 herds (van Roermund et al., 2002), the best fitting model included calf-calf transmission. In the presence of infectious young stock, separation of young stock from adult cattle might be insufficient to prevent postnatal infections, although it will remain to be important to reduce the numbers of initially infected young stock. However, additional preventive measures to reduce infectious contacts between young stock might be important, such as a strict separation between various age groups of

young stock. Studies to evaluate the effectiveness of such additional preventive measures are required.

In translating the results of the present study to input parameters for future modelling studies, it is important to realise that these results apply to cattle at risk of being infected and provide an underestimation of the hazard in infected cattle.

In conclusion, the hazard of becoming infectious increased with age. Within-herd prevalence had a major influence on the distribution of the age at which cattle became infectious. In high prevalence herds, a considerable proportion of cattle started shedding *Map* before adulthood. This might result in transmission of *Map* amongst young stock. Therefore, potential transmission of *Map* amongst young stock should be a major concern in the control of *Map*, especially in high prevalence herds.

6.6. Acknowledgements

This study was financially supported by the Dutch Ministry of Agriculture, Nature and Food Quality, and the Dutch Dairy Production Board. The authors would like to thank G. de Jong, A.P.W. de Roos and R. van Hoorne (NRS) for providing the pedigree records, A. Luppen and H.B.M. Assink for providing access to the laboratory results, and H. Brouwer-Middelesch for advice on SAS. Comments of H. Groenendaal, T.J.G.M. Lam, H.J.W. van Roermund, and H.J. van Weering on the manuscript are gratefully acknowledged.

6.7. References

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Chapter 7

General discussion

7.1. Introduction

The overall objective of this thesis was to investigate the potential for improvements in surveillance of *Mycobacterium avium* subsp. *paratuberculosis* (*Map)* infection and paratuberculosis, leading to a reduction in surveillance costs whilst continuing to meet specific quality targets. In particular, the research focussed on the differentiation of surveillance strategies to accommodate the aims and needs of various groups of farmers (such as dairy farmers selling cattle to other dairy herds, dairy farmers purchasing cattle, dairy farmers selling milk to dairy industries, and all dairy farmers). To achieve the objective of this thesis, a simulation model JohneSSim (Groenendaal et al., 2002) was used to study various alternatives for the Dutch certification-and-surveillance programme for '*Map*-free' herds and for a Bulk Milk Quality Assurance Programme (BMQAP) for dairy herds. In addition, data from field studies were analysed to provide further insights into specific aspects of surveillance of *Map* infection and paratuberculosis.

7.2. Major findings

Simulations of the Dutch certification-and-surveillance scheme for '*Map*-free' herds indicated that an initial assessment consisting of four biennial herd examinations with pooled faecal culture of all cattle ≥ 2 years was most attractive (Chapter 2). In closed herds, this scheme resulted in lower total and annual discounted costs and a lower animal-level prevalence at reaching the '*Map*-free' status compared to the standard scheme (i.e. five herd examinations at one-year intervals, the first by ELISA and the 2^{nd} to 5^{th} by pooled faecal culture). In the surveillance procedure, annual herd examinations consisting of pooled faecal culture of all cattle ≥ 2 years were most attractive; none of the simulated alternatives resulted in both a lower animal-level prevalence of undetected pre-existing *Map* infections in closed herds, and lower median annual discounted costs. Other simulated schemes resulted in higher annual discounted costs and/or a higher animal-level prevalence of undetected *Map* infections. However, at the rate of cattle transfers between herds observed in Chapter 5, the between-herd transmission was acceptably low (i.e. between-herd reproduction ratio R_h << 1) with a surveillance procedure consisting of biennial herd examinations (van Roermund et al., 2002). Therefore, the higher animal-level prevalence of undetected *Map*-infections with biennial herd examinations compared to annual herd examinations (as described in Chapter 2) was considered acceptable.

The results of simulations of a BMQAP for Dutch dairy herds (Chapter 3) showed that herd examinations by ELISA for the initial assessment, surveillance and control procedures effectively ensure the quality of 'low-*Map* bulk milk': > 75% of simulated herds were certified and > 96% of certified herds produced bulk milk with a

concentration of *Map* below the assumed maximum acceptable concentration if the initial herd-level prevalence was 30%. In test-positive herds, culling based on biennial faecal culture was more effective than culling based on annual ELISA.

The results of the simulations in Chapters 2 and 3 indicate that the probability of reaching and retaining a certified status was substantially higher if preventive management measures were taken in a herd, given the assumptions made on the effectiveness of these measures on the within-herd transmission of *Map*. Furthermore, the probability of undetected *Map* infections in certified closed herds decreased substantially if preventive management measures were taken. However, the results of the simulations also indicate that preventive management measures only had a minor effect on bulk milk quality of herds that were certified in a BMQAP. This means that information on the preventive herd management with respect to spread of *Map* within the herd is not required to assign a herd a certified status in a BMQAP.

Detecting clinical paratuberculosis cases is an important component of surveillance for *Map* infection. To confirm the presumptive diagnosis of clinical paratuberculosis, a serum-ELISA was found to be preferable to examination of Ziehl-Neelsen stained faecal smears (ZN test; Chapter 4). Little diagnostic information could be gained by performing the ZN-test in addition to the ELISA.

In Chapter 5, the observed structure of cattle transfers was found not to be random but under-dispersed. It can be mathematically shown that an under-dispersed contact structure between herds is likely to result in a lower between-herd transmission than a random contact structure. The results described in Chapter 5 stress the importance of using situation specific data with regard to cattle transfers between herds in the target population.

In Chapter 6, a considerable proportion of young stock in infected herds was shown to start shedding *Map*, especially in herds with a high within-herd prevalence. This result does not agree with the assumption made in the simulation studies in Chapters 2 and 3 that infected cattle do not become infectious before two years of age. Consequently, the transmission of *Map* in the presence of an effective separation between young stock and adult cattle will be higher than expected based on the simulation studies in Chapters 2 and 3. However, recent simulations with an adapted version of the JohneSSim model indicated that in the presence of transmission among young stock, an effective separation between young stock and adult cattle remained the most beneficial (Weber and Groenendaal, 2009). Moreover, these simulations showed that in a population of closed herds this increased transmission of *Map* results in earlier detection of infected herds in a BMQAP. The proportion of certified herds with a high concentration of *Map* in bulk milk was not increased. Therefore, it was concluded that the bulk milk ‘quality’ of certified herds in the BMQAP is hardly affected by infectious young stock (Weber and Groenendaal, 2009).

7.3. Practical applications

The results of the studies in this thesis provided guidance to decision-makers in improving the surveillance of *Map* infection and paratuberculosis, by differentiating surveillance strategies to accommodate the aims and needs of various groups of farmers.

In 1998, the Dutch certification-and-surveillance programme for '*Map* free' herds was initiated (Benedictus et al., 2000). In 2003, the surveillance procedure of this programme was relaxed from annual herd examinations to biennial herd examinations. This relaxation of the surveillance scheme resulted in a considerable reduction of the costs. Therefore it is likely that this relaxation increased the number of farmers for which the benefits of having a certified '*Map*-free' herd weigh up to the costs of participation in the programme. The decision to relax the surveillance procedure was based on the results of simulations presented in Chapter 2, the observed rate of cattle transfers between certified '*Map*-free' herds (Chapter 5) and the results of a mathematical model of within- and between-herd transmission (van Roermund et al., 2002). At the observed rate of cattle transfers between herds, the results of the model indicated that the population of '*Map*-free' herds was likely to be sustainable with this relaxed surveillance scheme ($R_h << 1$). This was also confirmed in another modelling study that took into account both introduction of infected cattle and environmental infections as a way of introducing *Map* into herds (Ezanno et al., 2005).

A voluntary bulk milk quality assurance programme (BMQAP) for paratuberculosis in Dutch dairy herds was initiated in January 2006. The design of the programme was based in part on the results of simulations presented in Chapter 3, the results of a mathematical model describing within- and between-herd transmission of *Map* (van Roermund et al., 2005), a decision analysis from a farmers' perspective (Velthuis et al., 2006) and a validation of an ELISA on milk samples (van Weering et al., 2007). Following the initiation of this BMQAP, the total number of Dutch herds participating in a paratuberculosis certification-, surveillance- and control-programme increased from 1,071 in December 2005 to 1,837 in December 2006 (Weber et al., 2007). This indicated a beneficial effect of differentiating surveillance of *Map* infection and paratuberculosis towards the needs of various groups of farmers on the uptake of these programmes. While the pre-existing certification-and-surveillance programme for '*Map* free' herds focussed on the needs of dairy farmers selling or purchasing cattle only (providing farmers with a high confidence of absence of *Map* in cattle transferred between herds), the new BMQAP focussed on the needs of all dairy farmers delivering milk to the dairy processing industry (providing farmers with information on their herd infection status and guarantees on milk quality at relatively low cost).

To confirm the clinical presumptive diagnosis of paratuberculosis, testing serum samples by ELISA was shown to be suitable (Chapter 3). Prior to this study, it was customary to confirm the presumptive diagnosis of paratuberculosis in Dutch dairy

cattle by microscopical examination of Ziehl-Neelsen stained smears (ZN test) or faecal culture. In the USA, testing by ELISA has been considered appropriate provided its use is restricted to herds with prior confirmed cases of paratuberculosis (Collins et al., 2006). Based on the results of Chapter 3, there is no need to restrict the use of the serum ELISA for this purpose to herds with prior paratuberculosis cases. The use of the ELISA reduces the costs of testing cattle with suspected clinical paratuberculosis. For example, costs of a direct PCR assay and ZN-test are approximately five-fold and ten-fold higher than the costs of an ELISA, respectively. Such a reduction of costs of testing can be beneficial to the control of paratuberculosis, provided it does increase the proportion of cattle suspected of clinical paratuberculosis that are tested.

7.4. Interests of consumers, dairy processing industry and dairy farmers

The interests of consumers, the dairy processing industry as well as dairy farmers are best served if exposure of humans to *Map* through the consumption of dairy products is reduced. Alongside pasteurisation, control and prevention of paratuberculosis in the national dairy herd are considered to be an effective way of reducing a potential human health risk of exposure to *Map* through consumption of dairy products (O'Reilly et al., 2004). If a causal link between *Map* and Crohn's disease were established, an effective risk-mitigation strategy can limit the milk demand reduction caused by consumer concerns (Groenendaal and Zagmutt, 2008). Therefore, the decision of individual dairy farmers to join a BMQAP reduces potential health risks to the public and in addition reduced demand and economic risks to dairy farmers and the dairy processing industry.

Recognition of similarities as well as differences between the interests of the dairy processing industry, the collective of dairy farmers and individual dairy farmers is pivotal in the design of surveillance programmes for *Map* infection and paratuberculosis.

The interests of the dairy processing industry are served by a reduction in the concentration of *Map* in milk delivered to the milk factories and certification of herds with a low probability of a high concentration of *Map* in milk. The concentration of *Map* in milk is related to the prevalence of cattle shedding *Map*, which may be reduced by preventive management measures directed at a reduction in both within- and between-herd transmission of *Map* (van Roermund et al., 2005). In many infected herds, preventive measures to reduce the transmission of *Map* are only considered and started after the detection of clinical paratuberculosis cases. Therefore surveillance for clinical paratuberculosis in individual dairy herds is likely to serve the interests of the dairy processing industry. The between-herd transmission of *Map* may be reduced if sufficient numbers of cattle with a low-risk of *Map* infection are available for trade from certified '*Map*-free' herds. Between September 2001 and September 2002, cattle

were introduced into 45% of the Dutch dairy herds (Weber et al., 2006). Thus, it is in the interest of the dairy processing industry that a sufficient number of individual dairy farmers participate in a surveillance programme for '*Map-free'* herds. Furthermore, a short term reduction of the concentration of *Map* in milk may be achieved by culling test-positive cattle in infected herds participating in a BMQAP (van Roermund et al., 2005). Finally, participation of individual dairy herds that have a low probability of a high concentration of *Map* in bulk milk in a BMQAP is in support of the dairy processing industry which seeks to safeguard future access to the international dairy market. In summary, the interests of the dairy processing industry are served by a high uptake of the certification-and-surveillance programme for '*Map*-free' herds, the BMQAP and surveillance for clinical paratuberculosis amongst dairy farmers.

Given the dependence of dairy farmers on the dairy processing industry for marketing their products, the interests of the collective of dairy farmers with respect to controlling *Map* infection and paratuberculosis are similar to those of the dairy processing industry, as described above.

The interests of individual dairy farmers are not totally aligned with those of the dairy industry and the collective of dairy farmers. A common interest is the need for a continued demand for milk and dairy products. However, individual farmers will only have an incentive to participate in a surveillance programme for '*Map*-free' herds or a BMQAP if this is perceived to provide economic benefits. Insufficient perceived benefits to participating farmers are probably the main reason why paratuberculosis programmes often fail to attract a substantial number of participants. In fact, even a 'perfect' programme is useless if it fails to attract participants. Therefore, in the development and marketing of voluntary paratuberculosis programmes, it is important to focus on the interests of individual participants – even if their interests are different from those of the decision makers initiating the programme. Surveillance for *Map* can be economically justified by individual farmers for various reasons. Firstly, dairy farmers may benefit from surveillance for (clinical) paratuberculosis in their herds – because detecting the disease may alert them to take appropriate corrective actions to prevent future losses due to paratuberculosis (Collins et al., 2006). Secondly, in the future, certified dairy farmers in a BMQAP may receive a market advantage, depending on the situation on the (international) dairy market. A cost-benefit analysis from the farmers' perspective showed that a milk price differentiation between accredited and non-accredited herds of € 0.005 per litre milk is already sufficient to economically justify participation in the initial assessment of the BMQAP (Velthuis et al., 2006). Velthuis et al. also found that without incentives such as a milk price differentiation, the preferred economic option for farmers should be not to participate in such programmes. Thirdly, pedigree dairy farmers selling cattle to other herds or breeding organisations might benefit from evidence of freedom of *Map* infection if this herd status results in a higher price of cattle sold. Anecdotal evidence indicates that Dutch dairy farmers purchasing cattle are increasingly willing to pay a premium for

cattle from '*Map*-free' herds, given that they wish to prevent introduction of *Map* when purchasing cattle is necessary to increase their production. In the United States, approximately half of the producers certified at 'level 3' or 'level 4' that sold cattle to other herds were able to do so at a premium (Kovich et al., 2006). The demand for cattle from '*Map*-free' herds and other certified herds (such as 'green' herds in a BMQAP) could be further increased through regulation in all certification-and-surveillance programmes, allowing the introduction of cattle into a herd only from herds of equivalent or higher status (i.e. a status with an equal or higher probability of freedom of *Map*). In the Netherlands, this is currently only regulated in the certification-and-surveillance programme for '*Map*-free' herds.

The importance of (the prospects of) economic incentives to participate in a paratuberculosis certification-, surveillance-, and control programme, in combination with a reduction of costs to enter the programme, is demonstrated by recent developments in the Netherlands. In early 2008, the Dutch dairy processing industry announced a requirement for Dutch dairy farmers delivering milk to the milk factories to participate in a paratuberculosis programme by 2010. Also, by 2011 these farmers are required to have their herds assigned status 'A' (i.e. test-negative herd, addressed as 'green' in our modelling studies) or 'B' (i.e. test-positive herd in which all test-positive cattle have been removed from the herd). Depending on the consequences for farmers not fulfilling these requirements (such as, their milk not being collected, or being collected at a lower milk price), these requirements can be a strong economic incentive. Also, laboratory costs were temporarily subsidised in 2008 by the dairy industry. These efforts resulted in a major increase in the uptake of the BMQAP (Fig. 1). By September 2009, approximately 95% of the 19,600 Dutch dairy herds participated in either the BMQAP or the certification-and-surveillance programme for '*Map*-free' herds. When designing incentives for farmers to participate in a programme, it may be relevant that quality penalties have been described to be more effective in motivating farmers than quality premiums (Valeeva et al., 2007).

An important characteristic of surveillance programmes for *Map* is that they do promote risk-adverse behaviour of participating farmers. Risk-taking behaviour can result in the introduction or spread of *Map* in the herd which increases the risk of losing the benefits of an accredited status. Education of participants about the magnitude of risks associated with certain farming practices is a prerequisite for rational risk-adverse behaviour. For instance, purchasing cattle may result in future outbreaks of *Map* infections and loss of the herd status. Therefore, education of participants regarding the risks of *Map* being introduced through the purchase of cattle is a key issue. For education of farmers in the Netherlands, a checklist with risk factors for paratuberculosis has been developed (van Weering et al., 2005).

Initiation of a BMQAP raises the question who will actually benefit. To determine potential benefits to producers and consumers of disease control and risk mitigation measures, the equilibrium between supply and demand functions can be

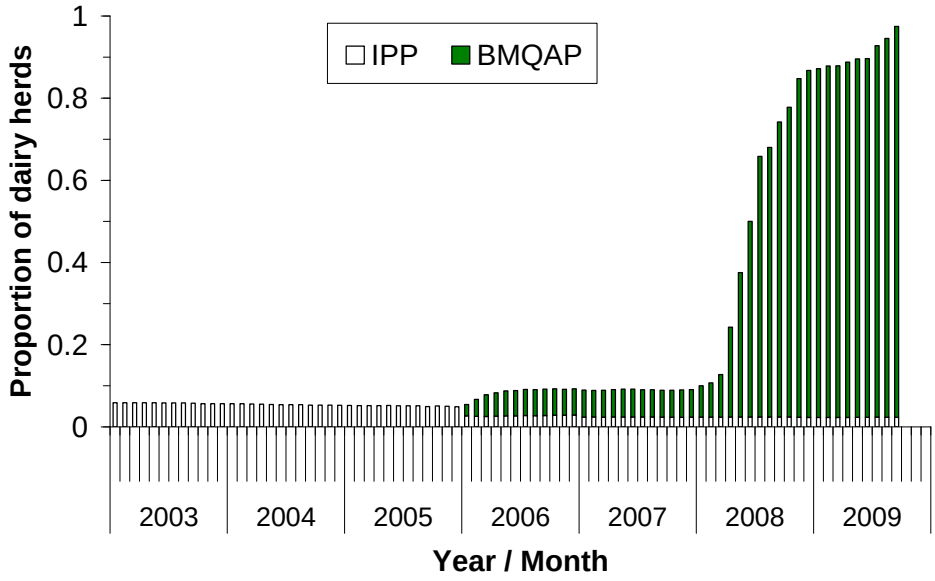

Fig. 1. Proportion of Dutch dairy herds participating in the Bulk Milk Quality Assurance Programme (BMQAP) and the Intensive Paratuberculosis Programme (IPP, i.e. the certification-, surveillance-, and control- programme in which Dutch cattle herds can be certified as '*Map*-free').

studied (Groenendaal and Zagmutt, 2008; Weldegebriel et al., 2009). From a shift in these functions as a result of a disease control programme, a change in producer surplus and consumer surplus can be derived. The producer surplus is defined as the amount by which producers gain by selling their product at a market price that is higher than the minimum price they would consider acceptable. The consumer surplus is defined as the amount by which consumers gain by being able to purchase a product for a price that is less than they are willing to pay. The changes in producer surplus and consumer surplus, together with the change in government costs, result in the total economic effect (Boardman et al., 2006).

To illustrate the economic effects of initiation of a BMQAP, four situations can be considered: (a) absence of consumer concerns about the safety of dairy products and absence of a causal link between *Map* and human disease such as Crohn's disease, (b) absence of consumer concerns but presence of a causal link between *Map* and human disease, (c) presence of consumer concerns but absence of causal link between *Map* and human disease, and (d) presence of consumer concerns about the safety of dairy products and presence of a causal link between *Map* and human disease. To examine those four situations, no government costs, a global implementation of BMQAP's, linear supply and demand functions, and perfect competition on the dairy market were

assumed. No government costs and the assumption of perfect competition can be viewed in light of the planned abolition of the system of milk quotas by the year 2014/2015 as part of the Common Agricultural Policy reform in the EU.

(a) In both the absence of consumer concerns about the safety of dairy products and of a causal link between *Map* and human disease, the initiation of a BMQAP increases the costs of production in both infected and uninfected herds, whereas the on-farm losses due to (sub)clinical paratuberculosis in infected herds are reduced. For the total population of dairy herds (infected and uninfected herds), the costs of participation in the BMQAP exceed the benefit of a reduction of on-farm losses due to (sub)clinical paratuberculosis (Velthuis et al., 2006). Therefore, introduction of the BMQAP increases the costs of production. This is likely to result in an upward shift of the supply function, meaning that producers will only produce a certain quantity of milk at a higher price, without a change in the demand function. This upward shift of the supply function results in a decrease of both the consumer and producer surplus, i.e. an economic loss to society. However, this is presumably a minor loss, given that a milk price differentiation between certified and non-certified herds of € 0.005 per litre of bulk milk is already sufficient for a farmer to justify entering the BMQAP (Velthuis et al., 2006). This is approximately 1% of the total costs (including labour) of production of milk on a Dutch dairy farm (€ 0.53 per kg milk; Anon, 2008).

(b) In the absence of consumer concerns about the safety of dairy products but the presence of a causal link between *Map* and human disease, the initiation of a BMQAP is expected to result in a similar decrease of both the consumer surplus and producer surplus. However, the resulting economic loss to society in this scenario can be balanced against the potential health and economic benefits due to a reduction of human disease by a reduction of exposure to *Map*. The magnitude of these benefits can currently not be estimated, given the uncertainty about a potential role of *Map* in the pathogenesis of Crohn's disease (Scientific Committee on Animal Health and Animal Welfare, 2000; Feller et al., 2007; Uzoigwe et al., 2007; Waddell et al., 2008; Abubakar et al., 2008; Nacy and Buckley, 2008).

(c) In the presence of consumer concerns about the safety of dairy products but the absence of a causal link between *Map* and human disease, the initiation of a BMQAP might provide reassurance to consumers about the safety of dairy products. Consumer concerns about the safety of dairy products are expected to result in a shift of the demand curve such that at a certain price of dairy products, a lower quantity is consumed. Therefore, these concerns are expected to decrease both the consumer surplus and the producer surplus (Groenendaal and Zagmutt, 2008). This loss to society is expected irrespective of whether the consumer concerns are based on a real hazard or a falsely believed hazard posed by *Map* to

human health. This loss to society might be reduced by reassurance of consumers about the safety of dairy products through the initiation of a BMQAP. At the same time, initiation of the BMQAP will result in a shift of the supply function, which increases the loss to society. However, as argued above, this increase is likely to be small. Thus, provided consumers are reassured by the presence of a BMQAP, the total economic loss to society caused by the initiation of a BMQAP plus the (reduced) consumer concerns in presence of a BMQAP is likely to be lower than the economic loss due to consumer concerns in absence of a BMQAP.

(d) In both the presence of consumer concerns about the safety of dairy products and of a causal link between *Map* and human disease, the initiation of a BMQAP might result in a similar reduction of the losses to society due to consumer concerns. In addition, the BMQAP might reduce any potential future losses to society due to human disease caused by exposure to *Map* through consumption of dairy products.

At present, there appear to be no major consumer concerns about the safety of dairy products, whereas the issue of a potential role of *Map* in the pathogenesis of Crohn's disease in humans has not yet been resolved (Scientific Committee on Animal Health and Animal Welfare, 2000; Feller et al., 2007; Uzoigwe et al., 2007; Waddell et al., 2008; Abubakar et al., 2008; Nacy and Buckley, 2008). In this situation, the presence of a BMQAP might prevent or reduce negative economic effects to society due to potential future consumer concerns about the safety of dairy products as well as reduce any potential future losses to society due to human disease caused by exposure to *Map* through consumption of dairy products. The presence of a BMQAP can therefore be regarded as an insurance policy against the economic effects to society of *Map* infection in cattle and consumer concerns about the safety of dairy products.

In the situations examined above, global implementation of BMQAP's was assumed. If a BMQAP is only implemented in one or a few countries in the absence of consumer concerns, the increased costs of production in these countries will not result in a higher world milk price, meaning that, if import and export markets were efficient, all costs are likely to be transferred to owners of production factors (such as dairy farmers). However, should consumer concerns arise in the future, dairy farmers and dairy processing industries in the countries with a BMQAP might have a strategic advantage in avoiding barriers in international trade and in serving high quality niche markets (such as the baby food industry), resulting in potential higher revenues for their milk and dairy products.

Some may argue that the costs of a programme should be allocated according to the 'beneficiary pays' principle (Weldegebriel et al., 2009). Given that a BMQAP might reduce potential future losses due to human disease caused by exposure to *Map* through consumption of dairy products and is likely to reduce a decrease of the

consumer surplus should concerns about the safety of dairy products arise, it could be argued that consumers are beneficiaries. This would mean that part of the costs of a BMQAP should be allocated to consumers or governments. However, the benefits to consumers and governments are currently debatable. Moreover, it may be argued against the 'beneficiary pays' principle that in competitive industries, costs of improvement of the quality of products are typically paid by producers and not by the government (Groenendaal, personal communication). In addition to this, as described above, the dairy industry may also be an important beneficiary of a BMQAP in the future by providing it a strategic advantage. More research into this field is needed.

7.5. Future developments and research

Future developments in, and research on, surveillance of *Map* infection and paratuberculosis are likely to be directed at monitoring the results of surveillance programmes in relation to their objectives and quality targets. In addition, developments and research may be directed at a further reduction of costs and simplification of current procedures.

The aim of the BMQAP for paratuberculosis is to reduce the concentration of *Map* in bulk milk delivered to the milk factories. The quality target of the BMQAP is a high proportion (e.g. > 96%) of certified herds, addressed as 'green' herds in Chapter 3, having less than 10^3 *Map* organisms per litre of bulk milk. Preferably, a herd status in a BMQAP would be assigned on a quantification of the concentration of *Map* in bulk milk. Several studies on PCR assays to detect and quantify *Map* in bulk milk have been performed (Stratmann et al., 2002; Jayarao et al., 2004; Bosshard et al., 2006; Metzger-Boddien et al., 2006; Gao et al., 2007; Herthnek et al., 2008; Donaghy et al., 2008). Also, phage-based methods to detect and quantify *Map* in milk have been described (Botsaris et al., 2009a; Botsaris et al., 2009b). However, these methods have not yet been validated for use in Dutch dairy herds. Therefore, the design of the BMQAP was based on use of individual animal tests. However, to validate the design of the BMQAP and evaluate whether the quality target is met over the course of time, quantification of *Map* in bulk milk from herds participating in the BMQAP would be very valuable.

The cost-effectiveness of surveillance programmes for *Map* infection and paratuberculosis depends in part on the rate of state transitions of the participating herds. If the rate at which certified herds lose their status is high, the average pay-back time in which a farmer can recover the investments in achieving the certified status by increased revenues will be low. This reduces the cost-effectiveness of participating in the programme. The results of the modelling studies in Chapter 2 and 3 indicate that a proportion of herds participating in the programmes will have a change of status over time. It is important to compare 'field' observations on these state transitions of herds

participating in the programmes with these model predictions, and to study hypotheses on any relevant differences between these observations and model predictions. Furthermore, a study of risk factors for the various state transitions may provide insight in factors (such as introduction of cattle from other herds) that determine why prevention and control of *Map* infections are successful in some herds whilst not in others.

The performance of certification-and-surveillance programmes against their objectives does not only depend on the design of the programme but also on the behaviour of participating farmers. It is generally assumed that trade of cattle between herds is the dominant route of between-herd transmission of *Map* (Sweeney, 1996). Model predictions on *Map* prevalence and the concentration of *Map* in bulkmilk of the herds under surveillance (van Roermund et al., 2002; van Roermund et al., 2005), were sensitive to assumptions on transfers of cattle between herds. Therefore, to ensure the quality of the programmes, it is important to compare the model assumptions with the true rate and structure of cattle transfers. In Chapter 5, cattle transfers into and between '*Map*-free' herds were studied in a three-year period (1999-2002). An underdispersed contact structure was observed with a low rate of cattle introductions in '*Map*-free' herds and a complete segregation between herds from which cattle were transferred and herds in which cattle were introduced. However, a recent study showed that the annual rate of introductions of cattle into '*Map*-free' herds between 2002 and 2007 was approximately 10-fold higher than between 1999 and 2002 (Weber, unpublished observations). Although the contact structure was still underdispersed, there was no longer a complete segregation between herds from which cattle were transferred and herds in which cattle were introduced. This means that propagation of *Map* infections between '*Map*-free' herds can not be fully excluded. It is important to keep track of such changes in contact rate and structure for herds in the certification-and surveillance programme for '*Map*-free' herds as well as herds in the BMQAP. Recently, the uptake of these programmes increased to approximately 95% of Dutch dairy herds, and farmers face the prospects of incentives from the dairy industry for a favourable herd status in the coming years. This may increase the awareness of farmers about the risks of introduction of *Map* infection with purchased cattle. Also, to increase such awareness of farmers, adult cattle introduced from non-certified herds ('red' in Chapter 3) into certified herds ('green' in Chapter 3) have to be tested serologically. Possibly, this increased awareness of farmers will influence the structure of cattle transfers between herds, which may have a beneficial effect on the between-herd transmission of *Map*. Depending on the observed rate and structure of cattle transfers between herds under surveillance, implementation of additional regulations to reduce the between-herd transmission of *Map* should be considered.

Prospects for future developments include a further reduction of costs and simplification of current procedures through the use of herd-level tests, such as testing bulk milk samples or environmental samples and risk based surveillance.

Bulk milk samples are a readily available specimen. Provided tests for bulk milk samples have acceptable diagnostic test characteristics, testing bulk milk samples may be an attractive alternative to testing individual animals in the surveillance of certified herds. In addition to tests for quantification of *Map* in bulk milk, antibody detection tests may be considered. Testing bulk milk samples for antibodies against *Map* by ELISA has been evaluated in several studies (Nielsen et al., 2000; Beyerbach et al., 2004; van Weering et al., 2007; Geue et al., 2007). So far, these studies indicated a lack of sensitivity at cut-off values in a range required for an acceptable specificity. However, bulk milk tests were developed for the surveillance of various other bacterial infections such as leptospirosis, brucellosis and salmonellosis, and the diagnostic performance of the bulk milk ELISA's for paratuberculosis warrants further consideration (van Weering et al., 2007).

Culture of environmental samples has been evaluated as a tool to detect infected dairy herds in several studies in the USA (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006). In these studies, 76% to 94% of dairy herds with concurrent individual animal tests were identified as infected by environmental culture. Based on these results, culture of environmental samples has been recommended as the first choice of test for classification of dairy herds as infected or uninfected (Collins et al., 2006) and is allowed as a method of entry-level testing in the test-negative component of the United States Department of Agriculture's Voluntary Bovine Johne's Disease Control Program (USDA, 2006). Recently, the relative herd-level sensitivity of culture of environmental samples in comparison to the concurrent initial assessment of the BMQAP was evaluated in Dutch dairy herds housed in free stalls. The relative sensitivity (95% CI) of culture of a pooled sample from the slurry pit in comparison to the initial assessment was estimated at 92.1% (87.7%, 95.3%) (Weber et al., 2009). However, before implementation of culture of environmental samples in the BMQAP can be considered, the relative sensitivity in comparison to the current surveillance scheme of the BMQAP, the relative specificity and the cost-effectiveness of culture of environmental samples should be evaluated. Furthermore, also PCR methods may be suitable for testing environmental samples to classify herds as infected or uninfected (Cook and Britt, 2007). PCR assays take considerably less time than culture and may be more suitable for high throughput applications. Validation of PCR assays for suitability of testing environmental samples of Dutch dairy herds under paratuberculosis surveillance is therefore warranted.

Risk based surveillance (Stark et al., 2006) can increase the cost-effectiveness of surveillance for *Map* infection and paratuberculosis, in BMQAP's as well as in surveillance programmes for '*Map*-free' herds. In a BMQAP, the surveillance strategy can be differentiated towards the risk of delivering milk to the milk factory with a concentration of *Map* above the maximum acceptable concentration. This risk can be estimated based on variables related to the likelihood of the herd being infected or becoming infected (such as, region, the time since the herd was certified, the number of

cattle introduced into the herd). In a certification-and-surveillance programme for '*Map*-free' herds, the surveillance strategy can be differentiated towards the risk the herd poses to other herds. This risk can be estimated based on variables related to the likelihood of the herd being infected or becoming infected and on variables related to the likelihood that an infection present in the herd will spread to other herds (such as, the number of cattle sold to other herds).

A desirable future development would be the implementation of a standard of the World Organisation for Animal Health (OIE) for evidence of absence of *Map* infection in cattle that are exported. Currently, there is no such standard, and therefore importing countries design their own standards. Often these standards require absence of any indication of *Map* infection in the herd of origin, including absence of positive test results. However, this effectively penalises infected herds participating in a paratuberculosis control programme as opposed to infected herds not participating in such programme. Therefore, an OIE standard that appropriately deals with this issue is urgently needed.

Results of research projects generally raise new questions, and the results of studies in this thesis are no exception in that respect. In addition to the issues discussed above, key topics for further studies related to the subject of this thesis include:

(a) Development of models for the between-herd transmission of *Map* in which the effect of non-random contact structures can be studied. The assumption of a random herd-contact structure is generally considered convenient in modelling transmission of infectious diseases (de Jong, 1995; Heesterbeek, 2005; Keeling, 2005). Traditionally, this assumption has been made in many models for between-herd transmission by animal transfers within or between groups of herds (Jalvingh et al., 1999; Ferguson et al., 2001; Graat et al., 2001; Mangen et al., 2002; Tomassen et al., 2002; Vonk Noordegraaf et al., 2002). However, aberrations of the real herd-contact structure from the assumed randomness might have a major effect on the transmission of *Map* as well as other infections. More recently, models with a non-random contact structure between herds have been developed (e.g., Bates et al., 2003; Gilbert et al., 2005; Mariner et al., 2006; Xiao et al., 2007; Robinson et al., 2007; Sharkey et al., 2008). The application of such models to the transmission and surveillance of *Map* may improve estimates of the cost-effectiveness of surveillance programmes.

(b) Risk factors for *Map* infection in dairy herds, in particular factors explaining regional differences. Risk factors for *Map* infection or clinical paratuberculosis in dairy herds have been studied extensively (Collins et al., 1994; Goodger et al., 1996; Obasanjo et al., 1997; Johnson-Ifearulundu and Kaneene, 1998; Johnson-Ifearulundu and Kaneene, 1999; Wells and Wagner, 2000; Chi et al., 2002; Daniels et al., 2002; Muskens et al., 2003; Hacker et al., 2004; Hirst et al., 2004; Ward and Perez, 2004; van Weering et al., 2005; Berghaus et al., 2005; Scott et al., 2006; Kobayashi et al., 2007; Nielsen and Toft, 2007; Tiwari et al., 2008;

Tavornpanich et al., 2008; Dieguez et al., 2008; Nielsen et al., 2008). However, most of these studies have been performed in limited numbers of herds and therefore lack power to detect region-specific risk factors. Moreover, it is unknown whether the risk factors found can explain the regional differences in *Map* prevalence observed in dairy herds in the Netherlands (Muskens et al., 2000). However, the large uptake (approximately 95%) of paratuberculosis programmes in Dutch dairy herds offers the opportunity to study regional differences in prevalence and risk factors associated with these differences.

7.6. Generalisation of results of this thesis

The studies in this thesis focussed on *Map* infection and paratuberculosis in Dutch dairy herds. Assumptions in the modelling studies were made specifically for Dutch dairy herds. Consequently, the results can not be translated directly to dairy populations in other countries. For instance, herd- and animal-level prevalence each influence the focus of paratuberculosis certification-, surveillance-, and control-programmes. In a metapopulation with a low herd-level prevalence of *Map* infections, successful prevention of infection being introduced into test-negative herds is the prime determinant of the average concentration of *Map* in milk produced in this metapopulation. In contrast, the average concentration of *Map* in milk produced in a metapopulation with a high herd-level and animal-level prevalence will be determined primarily by the effectiveness of control of *Map* in test-positive herds. Also the benefits to farmers of having a certified *Map*-free herd are likely to be considerably higher in a metapopulation with small proportion of herds being '*Map*-free' than in a metapopulation with a large proportion of herds being '*Map*-free'. Such differences mean that paratuberculosis programmes can not be simply copied from one country to another. However, the concepts and approach followed in this thesis may equally apply to other countries.

7.7. Concluding remarks

In this thesis the potential for improvements in surveillance of *Map* infection and paratuberculosis was investigated. Knowledge gained during these studies assisted Dutch decision-makers as they sought to design surveillance strategies suited to the needs of various groups of dairy farmers.

To accommodate the needs of dairy farmers selling cattle to other herds or purchasing cattle from other herds, ways to improve the certification-and-surveillance programme for '*Map*-free' herds were indicated. An initial assessment consisting of four biennial herd examinations and surveillance procedure consisting of biennial herd

examinations resulted in lower annual discounted costs than alternative test schemes. This test scheme was expected to result in an acceptably low between-herd transmission of *Map* given the rate and structure of cattle transfers between '*Map*-free' herds observed in this thesis.

To accommodate the needs of farmers selling milk to dairy industries, a bulk milk quality assurance programme was developed. In this programme, test-negative herds were certified. Certified and non-certified herds were tested by biennial and annual herd examinations by ELISA, respectively. This effectively ensured that >96% of certified herds produced bulk milk with a concentration of *Map* below a maximum acceptable concentration (10^3 *Map*/litre, based on pasteurisation studies). In non-certified herds, culling based on biennial herd examinations by faecal culture was more effective than culling based on annual herd examinations by ELISA. Information on the preventive herd management with respect to the within-herd transmission of *Map* was not required to assign a certified status to a herd.

Finally, to accommodate the needs of other dairy farmers, improved methods of surveillance for clinical paratuberculosis were needed. To confirm a presumptive diagnosis of clinical paratuberculosis, a serum-ELISA is preferred to the examination of Ziehl-Neelsen stained faecal smears. Little diagnostic information can be gained by performing the ZN-test in addition to the ELISA.

The results of this thesis show that recognition of similarities as well as differences between the interests of the consumers, dairy processing industry, the collective of dairy farmers and individual dairy farmers is pivotal in the design of surveillance programmes for *Map* infection and paratuberculosis. The decision of the Dutch dairy processing industry to provide incentives for farmers to participate in certification-, surveillance-and-control programmes for *Map* can be considered the single most important development in controlling *Map* in the Dutch dairy population during the last decades. Participation of dairy herds in these programmes can be regarded as an insurance policy against the economic effects to society of *Map* infection in dairy cattle.

7.8. References

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Chapter 8

Summary / Samenvatting

8.1. Summary

The overall objective of this thesis was to investigate the potential for improvements in surveillance of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection and paratuberculosis in dairy herds, leading to a reduction in surveillance costs whilst continuing to meet specific quality targets. In particular, differentiation of surveillance strategies to accommodate the aims and needs of various groups of dairy farmers was studied.

To accommodate the aims and needs of dairy farmers selling or purchasing cattle, improvements of the Dutch certification-and-surveillance programme for '*Map*-free' herds were studied. Herds participating in the programme were simulated with a simulation model (JohneSSim). To identify cost-effective test schemes for the programme, various alternative test schemes were simulated (Chapter 2). Furthermore, field data on cattle transfers between certified '*Map*-free' herds were studied to enable quantification of their effect on the potential spread of undetected *Map* infections (Chapter 5). The results of the simulations indicated that an initial assessment consisting of four biennial herd examinations by pooled faecal culture of all cattle ≥ 2 years was most attractive. In the surveillance of certified '*Map*-free' herds, annual herd examinations consisting of pooled faecal culture of all cattle ≥ 2 years were most attractive. Other simulated schemes resulted in higher annual discounted costs and/or a higher animal-level prevalence of undetected *Map* infections. However, analysis of our field data showed a rate of cattle transfers between '*Map*-free' herds at which a surveillance scheme consisting of biennial herd examinations by pooled faecal culture of all cattle ≥ 2 years was expected to result in a sustainable population of certified '*Map*-free' herds. Moreover, there was a clear separation between '*Map*-free' herds in which cattle were introduced, and '*Map*-free' herds from which cattle were transferred to other '*Map*-free' herds. Therefore, continued propagation of *Map* infections between '*Map*-free' herds was impossible. This increased the 'quality' of the '*Map*-free' status considerably. Based on these results, the surveillance scheme for '*Map*-free' herds was relaxed from annual to biennial herd examinations.

To accommodate the aims and needs of dairy farmers selling milk to dairy processing industries, various alternative designs of a bulk milk quality assurance programme (BMQAP) were simulated with the JohneSSim model (Chapter 3). The results indicate that herd examinations by ELISA for the initial assessment-, surveillance- and control-procedures effectively ensure the quality of certified (i.e. 'green', or 'status A') herds in a BMQAP: > 75% of simulated herds were certified and > 96% of certified herds produced bulk milk with a concentration of *Map* below the assumed maximum acceptable concentration. The maximum acceptable concentration was based on pasteurisation studies. The probability of reaching and retaining the certified status was substantially higher if preventive management measures were taken in a herd (given the assumptions made on the effectiveness of these measures).

However, preventive management measures only had a minor effect on bulk milk quality of certified herds, because the majority of certified herds were uninfected. This means that information on the preventive herd management is not required to certify a herd in a BMQAP. In 2006, a BMQAP was initiated in the Netherlands, based in part on the results of this thesis.

To accommodate the aims and needs of all dairy farmers, the preferred choice of test for surveillance of clinical paratuberculosis was studied (Chapter 4). Test results of cattle suspected of clinical paratuberculosis were analysed using a Bayesian latent class model. The results showed that to confirm the presumptive diagnosis of clinical paratuberculosis, the Pourquier serum-ELISA is preferred to the examination of Ziehl-Neelsen stained faecal smears (ZN test). Little diagnostic information can be gained by performing the ZN test in addition to the ELISA.

The age at onset of shedding of *Map* may have an effect on the within- and between-herd transmission of *Map* in herds under paratuberculosis surveillance. Therefore, the distribution of this age at onset of shedding of *Map* was studied using field data (Chapter 6). A considerable proportion of cattle in infected herds started to shed *Map* before 2 years of age, especially in herds with a high within-herd prevalence. This may result in transmission of *Map* amongst young stock. However, recent simulations with an adapted version of the JohneSSim model indicate that an effective separation between young stock and adult cattle remains the most beneficial, to avoid initial infection of young stock. Moreover, these simulations showed that transmission of *Map* amongst young stock results in earlier detection of infected herds under *Map* surveillance, meaning that the bulk milk quality of certified herds in a BMQAP is hardly affected by infectious young stock.

Recognition of similarities as well as differences between the interests of the dairy processing industry, the collective of dairy farmers and individual dairy farmers is pivotal in the design of surveillance programmes for *Map* infection and paratuberculosis (Chapter 7). To increase the uptake of the programmes it is essential to focus on the interests of individual participants – even if their interests differ from those of the decision makers initiating the programme. Recognising this, the Dutch dairy industries have announced incentives for dairy farmers to participate in a paratuberculosis programme by 2010 and to have their herds assigned at least status ‘A’ (i.e. test-negative herd, addressed as ‘green’ in our modelling studies) or ‘B’ (i.e. test-positive herd from which all test-positive cattle have subsequently been culled) by 2011. Also, laboratory costs were temporarily subsidised in 2008 by the dairy industry. These efforts resulted in a major increase in the uptake of the paratuberculosis programmes from 8% of the Dutch dairy herds in January 2008 to 95% in September 2009. This is probably the single most important development in the control of *Map* in the Dutch dairy population during the last decades. Participation of dairy herds in paratuberculosis programmes can be regarded as an insurance policy against the economic effects to society of *Map* infection in dairy cattle (Chapter 7).

Prospects for future developments in, and research on, surveillance of *Map* infection and paratuberculosis include monitoring of the results of surveillance programmes in relation to their objectives and quality targets. In addition, developments and research may be directed at a further reduction of costs of surveillance (such as risk based surveillance and the use of herd-level tests) and international standardisation of surveillance procedures (Chapter 7).

8.2. Samenvatting

Toezicht op *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infecties en paratuberculose op melkveebedrijven is een belangrijk hulpmiddel voor veehouders en beleidsmakers om de economische schade door *Map* infecties te kunnen beperken. In de diergeneeskunde wordt dit toezicht vaak aangeduid met de term 'surveillance'. Surveillance is in dit proefschrift gedefinieerd als de inspanningen die gericht zijn op het beoordelen van de infectiestatus van een populatie runderen (bijvoorbeeld alle runderen op één melkveebedrijf). Deze beoordeling kan leiden tot het nemen van beheers- of bestrijdingsmaatregelen indien blijkt dat de prevalentie en/of incidentie van *Map* infecties en/of klinische paratuberculose een bepaalde drempelwaarde overschrijden. Door surveillance kunnen veehouders zich bewust worden van een *Map* infectie op hun bedrijf en maatregelen nemen om de schade hiervan te beperken. Surveillance geeft inzicht in de infectiestatus van bedrijven en hiervan kan in de dierhandel gebruik worden gemaakt om het risico op insleep van *Map* bij de aankoop van rundvee te beperken. Tot slot kan surveillance het vertrouwen van consumenten in de veiligheid van zuivelproducten versterken.

Het doel van het in dit proefschrift beschreven onderzoek was mogelijkheden te onderzoeken voor verbetering van de surveillance van *Map* infecties en paratuberculose op melkveebedrijven. In het bijzonder werd differentiatie van surveillancestrategieën onderzocht in relatie tot de doelstellingen van verschillende groepen melkveehouders.

Om de doelstellingen van melkveehouders die runderen afvoeren naar andere melkveebedrijven of aanvoeren van andere bedrijven beter te kunnen ondersteunen werden mogelijkheden voor verbetering van het Nederlandse certificeringsprogramma voor 'paratuberculose-vrije' ('Status 10') bedrijven onderzocht. Bedrijven die aan het programma deelnemen werden gesimuleerd (nagebootst) met het computersimulatie-model JohneSSim. Om kosten-effectieve testschema's voor het programma te vinden werden verschillende alternatieve testschema's gesimuleerd (Hoofdstuk 2). Tevens werden verplaatsingen van runderen tussen gecertificeerd 'paratuberculose-vrije' bedrijven geanalyseerd op basis van gegevens uit het Identificatie & Registratie (I&R) systeem voor runderen. Het doel hiervan was de bijdrage van dierverplaatsingen aan de potentiële verspreiding van niet-gedetecteerde *Map* infecties te kwantificeren

(Hoofdstuk 5). De resultaten van de simulaties lieten zien dat het meest aantrekkelijke testschema voor het behalen van de 'paratuberculose-vrij' status bestond uit vier koppelonderzoeken met een interval van twee jaar, bestaande uit gepoolde faeceskweken van alle runderen ≥ 2 jaar. Voor het bewaken van de status van 'paratuberculose-vrije' bedrijven waren jaarlijkse koppelonderzoeken bestaande uit gepoolde faeceskweken van alle runderen ≥ 2 jaar het meest aantrekkelijk. Andere testschema's resulteerden in hogere jaarlijkse verdisconteerde kosten en/of een hogere prevalentie op dierniveau van niet-gedetecteerde *Map*-infecties. Uit de I&R gegevens bleek echter dat slechts zo weinig runderen werden verplaatst tussen 'paratuberculose-vrije' bedrijven dat verwacht mocht worden dat tweejaarlijkse bewakingsonderzoeken resulteren in een duurzame groep van gecertificeerd 'paratuberculose-vrije' bedrijven. Bovendien bleek er een volledige scheiding te zijn tussen 'paratuberculose-vrije' bedrijven waarop runderen werden aangevoerd, en 'paratuberculose-vrije' bedrijven waarvandaan dieren naar andere 'paratuberculose-vrije' bedrijven werden afgevoerd. Dit betekende dat een voortgaande verspreiding van niet-gedetecteerde *Map* infecties door runderverplaatsingen tussen 'paratuberculose-vrije' bedrijven onwaarschijnlijk was. Dit draagt aanzienlijk bij aan de 'kwaliteit' van de status 'paratuberculose-vrij'. De hogere prevalentie van niet-gedetecteerde *Map* infecties bij tweejaarlijkse koppelonderzoeken, in vergelijking met jaarlijkse koppelonderzoeken, werd daarbij als acceptabel beoordeeld. Op basis van deze resultaten werd het bewakingsschema voor 'paratuberculose-vrije' bedrijven verlicht van jaarlijkse naar tweejaarlijkse koppelonderzoeken.

Om de doelstellingen van melkveehouders die melk aan een melkfabriek afleveren te ondersteunen werden verschillende ontwerpen voor een programma voor melkkwaliteit met betrekking tot paratuberculose bestudeerd. Met het JohneSSim model werden Nederlandse melkveebedrijven die aan een dergelijk programma deelnemen gesimuleerd (Hoofdstuk 3). De resultaten van deze simulaties lieten zien dat met ELISA koppelonderzoeken voldoende garanties kunnen worden gegeven over de kwaliteit van tankmelk van gecertificeerde bedrijven (zoals bedrijven met Status A in het Paratuberculose Programma Nederland): > 75% van de gesimuleerde bedrijven werd gecertificeerd en > 96% van de gecertificeerde bedrijven had een concentratie van *Map* in tankmelk beneden de aangenomen maximaal acceptabele concentratie. De maximaal acceptabele concentratie werd gebaseerd op onderzoek naar de effectiviteit van pasteurisatie. De kans op het behalen en behouden van de gecertificeerde status (Status A) was substantieel hoger wanneer preventieve maatregelen in de bedrijfsvoering werden genomen, gegeven de aannames die over het effect van deze maatregelen werden gemaakt. Preventieve maatregelen bleken echter slechts een klein effect te hebben op de kwaliteit van tankmelk van gecertificeerde bedrijven, omdat de meerderheid van gecertificeerde bedrijven niet geïnfecteerd is. Dit betekent dat voor het certificeren van bedrijven geen informatie nodig is over de bedrijfsvoering. In 2006

werd het Paratuberculose Programma Nederland gestart, wat onder meer is gebaseerd op de resultaten die beschreven zijn in dit proefschrift.

Om de doelstellingen van alle melkveehouders te ondersteunen werd onderzocht welke test, onderzoek van serum met een ELISA of onderzoek van mest met de Ziehl-Neelsen test (ZN test), het meest geschikt is voor de surveillance van klinische paratuberculose (Hoofdstuk 4). De testresultaten van runderen die verdacht werden van klinische paratuberculose werden geanalyseerd. Hieruit bleek dat de gebruikte ELISA beter geschikt is dan de ZN test voor het bevestigen van de waarschijnlijkheids-diagnose paratuberculose. Wanneer serum wordt onderzocht met deze ELISA, kan slechts weinig extra informatie worden verkregen door tevens mest van het rund te onderzoeken met de ZN test.

De leeftijd waarop geïnfecteerde runderen *Map* beginnen uit te scheiden kan effect hebben op de verspreiding van de infectie binnen en tussen bedrijven die deelnemen aan een paratuberculoseprogramma. Daarom werd de verdeling van deze leeftijd onderzocht (Hoofdstuk 6). Een substantieel deel van het jongvee op geïnfecteerde bedrijven bleek *Map* uit te scheiden vóór de leeftijd van 2 jaar, in het bijzonder op zwaar besmette bedrijven. Dit kan leiden tot verspreiding van de infectie onder jongvee. Recente simulaties met een aangepaste versie van JohneSSim laten zien dat een effectieve scheiding tussen jongvee en volwassen vee belangrijk blijft, om te voorkomen dat jongvee *Map* gaat uitscheiden. Bovendien laten deze simulaties zien dat verspreiding van *Map* tussen jongvee leidt tot eerdere detectie van geïnfecteerde bedrijven. Dat betekent dat de melkkwaliteit van gecertificeerde bedrijven in een melkkwaliteitsprogramma voor paratuberculose nauwelijks beïnvloed wordt door uitscheiding van *Map* bij jongvee.

Erkenning van overeenkomsten èn verschillen tussen de belangen van drie groepen, te weten de zuivelverwerkende industrie, het collectief van melkveehouders en individuele melkveehouders, is essentieel bij het ontwerpen van surveillance-programma's voor *Map* infecties en paratuberculose (Hoofdstuk 7). Aandacht voor de belangen van individuele veehouders is belangrijk om de deelname aan deze programma's te vergroten – zelfs als de belangen van deze veehouders ogenschijnlijk verschillen van de belangen van de beleidsmakers die het programma opzetten. De Nederlandse zuivelverwerkende industrie heeft daarom stimulansen aangekondigd voor melkveehouders om deel te nemen aan een paratuberculoseprogramma in 2010 en tenminste status 'A' (test-negatief bedrijf) of status 'B' (test-positief bedrijf, waar na het betreffende koppelonderzoek alle test-positieve dieren zijn afgevoerd) te behalen in 2011. Daarnaast werden in 2008 tijdelijk de laboratoriumkosten voor deelnemers aan een paratuberculoseprogramma gesubsidieerd. Deze inspanningen van de zuivelindustrie hebben geleid tot een grote stijging van de deelname aan paratuberculoseprogramma's van 8% van de Nederlandse melkveebedrijven in januari 2008 tot 95% in september 2009. Dit is waarschijnlijk de belangrijkste ontwikkeling in de bestrijding van *Map* in de Nederlandse melkveehouderij gedurende de laatste

decennia. Deelname aan een paratuberculoseprogramma kan worden beschouwd als een verzekering tegen de economische effecten voor de maatschappij van *Map* infecties onder melkvee (Hoofdstuk 7).

Vooruitzichten voor toekomstige ontwikkelingen in, en onderzoek naar, de surveillance van *Map* infecties en paratuberculose omvatten onder meer de analyse en bewaking van de resultaten van surveillanceprogramma's, in relatie tot de doelstellingen van deze programma's. Bovendien kunnen ontwikkelingen en onderzoek gericht zijn op een verdere reductie van de kosten van surveillance en internationale standaardisatie van surveillanceprocedures.

Chapter 9

Curriculum vitae and publications

9.1. Curriculum vitae

The author of this thesis obtained his veterinary degree at the Faculty of Veterinary Medicine of Utrecht University and is currently employed by GD Animal Health Service, Deventer, the Netherlands. He has been a Diplomate of the European College of Bovine Health Management since 2004 and a Diplomate of the European College of Veterinary Public Health (subspecialty Population Medicine) since 2005. The focus of his work is on the epidemiology and control of bovine infectious diseases, especially paratuberculosis and salmonellosis.

9.2. List of publications

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