

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

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**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*/L 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  $\text{€}16 \times 10^3$  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.

**dairy cattle / milk / paratuberculosis / stochastic simulation model / quality assurance programme**

## 1. 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<sup>1,2</sup> [5]. Milk is a possible

vehicle of transmission of *Map* to humans, because *Map* has been detected in raw milk [3, 10, 14, 28, 33, 36] and might not be effectively inactivated by pasteurisation [3, 9, 12–16, 24, 34]. 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.

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<sup>1</sup> EU, Possible links between Crohn's disease and Paratuberculosis, Report, Scientific Committee on Animal Health and Animal Welfare, European Commission, 76 pp. [on line] (2000) [http://europa.eu.int/comm/food/fs/sc/scah/out38\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/scah/out38_en.pdf) [consulted 21 December 2004].

<sup>2</sup> Herrewegh A.A.P.M., Roholl P.J.M., Overduin P., van der Giessen J.W.B., van Soelingen D., Is there

evidence for a link between Crohn's disease and exposure to *Mycobacterium avium* ssp. *paratuberculosis*? A review of current literature, RIVM report 230086001/2004, 81 pp. [on line] (2004) <http://www.rivm.nl/bibliotheek/rapporten/230086001.html> [consulted 3 July 2007].

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<sup>3</sup>, Australia, and the Netherlands [4, 20, 23]. 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 [4]. 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* [17, 37], 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.

## 2. MATERIALS AND METHODS

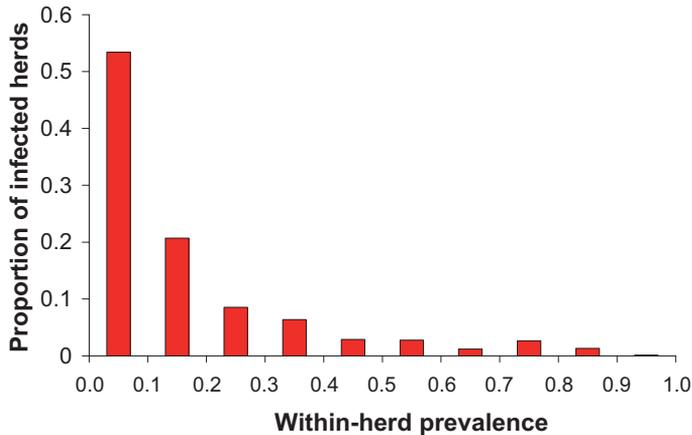
### 2.1. The JohneSSim model

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 [17, 37].

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 [18]. 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

<sup>3</sup> Buluga L.L., Collins, M.T., U.S. voluntary Johne’s disease herd status program for cattle, Proc. 6th Int. Coll. Paratuberculosis, Melbourne, 1999, pp. 39–47.



**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<sup>5</sup>.

*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,  $\geq$  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.

## 2.2. Assumptions in the 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 [17]. Both infected and non-infected herds are simulated. Therefore, in total 16 herd types were simulated separately (8 risk

profiles  $\times$  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 ( $\geq$  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 [26] using a Bayesian model [31] with assumptions on test characteristics based on a Dutch validation study<sup>4</sup> and is shown in Figure 1. Initial herd-level true prevalence was assumed to be 0.30, based on a recent study in the Netherlands<sup>5</sup>. Costs of participation in the quality assurance programme and costs of preventive management measures were updated for this study (Tab. I and II). Economic

<sup>4</sup> Van Maanen C., Koster C., van Veen B., Kalis C.H.J., Collins M.T., Validation of *Mycobacterium avium* subsp. *paratuberculosis* antibody detecting ELISA's, in: Juste R.A., Geijo M.V., Garrido J.M. (Eds.), Proc. 7th Int. Coll. Paratuberculosis, Bilbao, 2002, p. 182.

<sup>5</sup> Van Weering, Personal communication, 2004.

**Table I.** 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 <sup>1</sup>	Transport costs per submission <sup>1</sup>	Laboratory costs <sup>2</sup>	
			per submission	per test
Veterinarians' visit	22			
IFC <sup>3</sup>	2.75 per animal	10	7.80	30.00 per animal
PFC <sup>4</sup>	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

<sup>1</sup> Based on rates charged in 2004 by a convenience sample of veterinary practices.

<sup>2</sup> Based on rates charged in 2004 by the Animal Health Service.

<sup>3</sup> IFC = individual faecal culture.

<sup>4</sup> PFC = pooled faecal culture.

assumptions on losses by paratuberculosis and on the costs of culling of test-positive cattle were also updated (Tab. II). 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 III. Assumptions on effectiveness of additional preventive management measures, imposed on the 'background' management, were based on the opinions of an expert panel [17]. 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 available online in the Appendix at <http://www.vetres.org>.

### 2.3. 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 IV. These assumptions were based on the available quantitative data on direct shedding of *Map* in milk<sup>6,7</sup> [3, 10, 33, 36], faecal contamination of milk [32], shedding of *Map* in faeces<sup>8</sup> [6, 11, 27, 29, 35], and the concentration of *Map* in bulk milk [15]. 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 (Tab. IV) and is not restricted to a cow's own milk – meaning that variation in milk yield between cows can be ignored.

### 2.4. 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 pasteurisation. *Map* can survive high-temperature short-time (HTST) pasteurisation when the initial organism concentration is  $\geq 10^4$  cells per litre of milk [16, 34]. 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  $> 7.1 \log_{10}$  [30]. Therefore, in this study, we considered a concentration of *Map* organisms in milk less than  $10^3$  per litre acceptable.

### 2.5. 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

<sup>6</sup> McDonald W.L., O'Riley K.J., Schroen C.J., Condrón R.J., Heat inactivation of *Mycobacterium avium* subsp. *Paratuberculosis*, in: Juste R.A., Geijo M.V., Garrido J.M. (Eds.), Proc. 7th Int. Coll. Paratuberculosis, Bilbao, 2002, pp. 312–316.

<sup>7</sup> Rademaker, Personal communication, 2004.

<sup>8</sup> Stehman, Personal communication, 2004.

**Table II.** 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 [17] 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	Details / reference
<i>Losses caused by infection with Map</i>			
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 × €4/kg fat × 0.0435 kg fat/kg milk) [2]
Treatment	Treatment clinical case	€30	[2] <sup>b</sup>
Reduced slaughter value	Standard slaughter value (per cow): Reduction depends on infection state (lowly infectious 5%, highly infectious 10%, clinical 100%)	€448.75	
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 [19]
<i>Preventive management measures</i>			
Calving	Costs of cleaning per year Extra labour (hygiene, milking own dam) per calving	€100 per year Giving colostrum of own dam €9.11	[2]
Milk replacer <sup>a</sup>	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 L instead of waste milk = 6.83 238 L instead of bulk milk = -€9.11 Total = -€2.28	[2]

**Table II.** Continued.

Category	Parameter	Loss or costs	Details / reference
Hygiene barrier	Between adult stock and young stock	€726.71 per year (including labour)	Calculation includes: investment €1 850, depreciation of 15 years, 1% of maintenance costs, labour, materials [2]
Roughage	Better quality roughage, straw etc. during housing in summer season only.	€39.03 for calves 0–6 months	[2]
Housing	Separate housing of animals 0–70 days (initially 5 animals)	€487.5; per year; 5% increment per year	[2]
	Separate housing of animals 70–180 days (initially 7 animals)	€682.5 per year; 5% increment per year	[2]
	Separate housing of animals 180–360 days (initially 9 animals)	€877.5 per year; 5% increment per year	[2]

<sup>a</sup> 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).

<sup>b</sup> Lei, Farm Accountancy Data Network. [on line] (2004) <http://www.lei.wur.nl> [consulted 12 march 2004].

**Table III.** 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	1	0.997 <sup>4</sup>

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<sup>9</sup> 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 V, schemes A to D). Test schemes were based on herd examinations by serology (ELISA), individual faecal culture (IFC) [21] or pooled faecal culture (PFC) [22]. 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 (Tab. V, 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 exami-

nation by ELISA, the second to fifth examination by PFC). Surveillance is done by biennial herd examination 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.

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.

## 2.6. 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.

## 2.7. Sensitivity analyses

The influence of various input parameters on the study results was analysed, by changing one parameter at a time. These analyses were performed with test scheme D (Tab. V), with or without additional preventive management measures taken in all herds. The following parameter changes were made: (1) The default numbers of *Map* bacteria

<sup>9</sup> Van Weering, Personal communication, 2004.

**Table IV.** 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 <i>Map</i> in milk (organisms per litre)	<i>Map</i> in faeces (organisms per gram)	Total <i>Map</i> in milk (organisms per litre)
Latent infected		0	0	0
Lowly infectious	0.8	0	0	0
	0.2	0	10 <sup>2</sup>	4
Highly infectious	0.6	10 <sup>2</sup>	10 <sup>2</sup>	1.04 × 10 <sup>2</sup>
	0.24	10 <sup>2</sup>	10 <sup>4</sup>	5 × 10 <sup>2</sup>
	0.16	10 <sup>2</sup>	10 <sup>7</sup>	4.001 × 10 <sup>5</sup>
Clinical disease		10 <sup>4</sup>	10 <sup>9</sup>	4.001 × 10 <sup>7</sup>

**Table V.** 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 <sup>a</sup>		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 <sup>b</sup>	ELISA / PFC <sup>c</sup>	All, ≥ 3 yr / All, ≥ 2 yr	PFC	2 yr	All, ≥ 2 yr	IFC <sup>d</sup>	1 yr	All, ≥ 2 yr

<sup>a</sup> The initial assessment was done by a single herd examination (except in scheme E).

<sup>b</sup> Scheme E is the (current) test scheme for the ‘*Map*-free’ certification-and-surveillance programme for Dutch dairy herds.

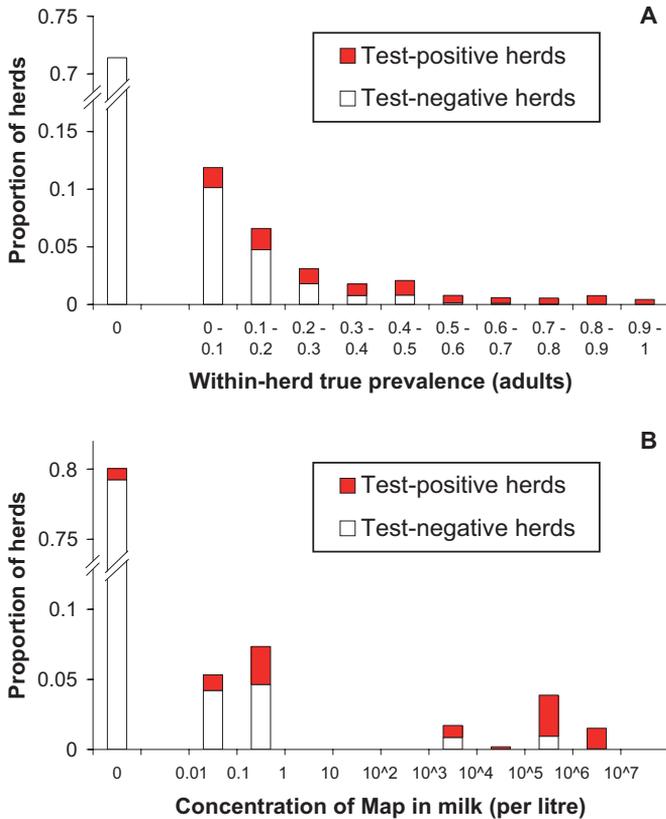
<sup>c</sup> The initial assessment of scheme E consists of five annual herd examinations (the first herd examination by ELISA followed by IFC of ELISA-positive animals; the 2nd through 5th examination by pooled faecal culture (PFC) followed by IFC of positive pools).

<sup>d</sup> 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’.

in milk (Tab. IV, last column) were multiplied by 10<sup>6</sup> – even though such high concentrations of *Map* in milk are probably not biologically plausible. For example, clinically diseased animals then shed 4 × 10<sup>13</sup> *Map*/L of milk (instead of the default value of 4 × 10<sup>7</sup> *Map*/L, Tab. IV). (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 (Tab. III)

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<sup>10</sup>. However, in a previous seroprevalence study the herd-level true prevalence was estimated at 0.31 to 0.71 [26].

<sup>10</sup> Van Weering, Personal communication.



**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  $\geq 3$  years of age (test schemes A to D).

Therefore, in this sensitivity analysis, the herd-level true prevalence was changed from 0.30 to 0.70.

## 2.8. Statistical analyses

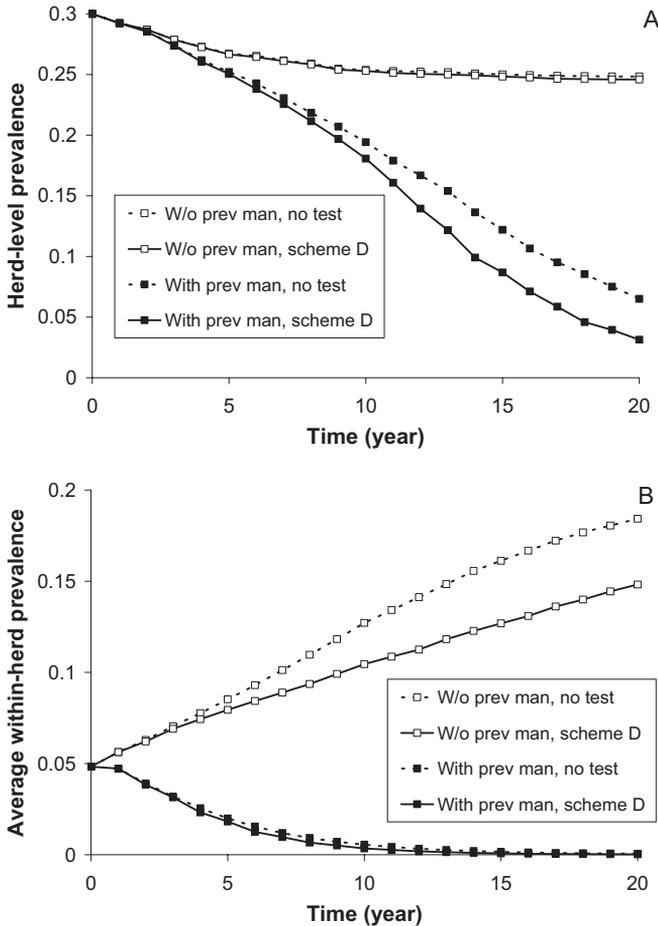
The proportions of dairy herds certified as 'green' (as a proportion of all dairy herds) by different test schemes were compared by Pearson  $\chi^2$ . If an overall  $\chi^2$  was significant then each alternative scheme was compared individually with scheme E (i.e. the Dutch certification-and-surveillance scheme for 'Map-free' herds, Tab. V) by Yate continuity corrected  $\chi^2_{cc}$ , using Bonferroni correction of  $p$  to adjust for multiple comparisons [1]. 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 correction of  $p$ . In all tests, significance was declared at  $p \leq 0.05$  (two-sided).

## 3. RESULTS

### 3.1. 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



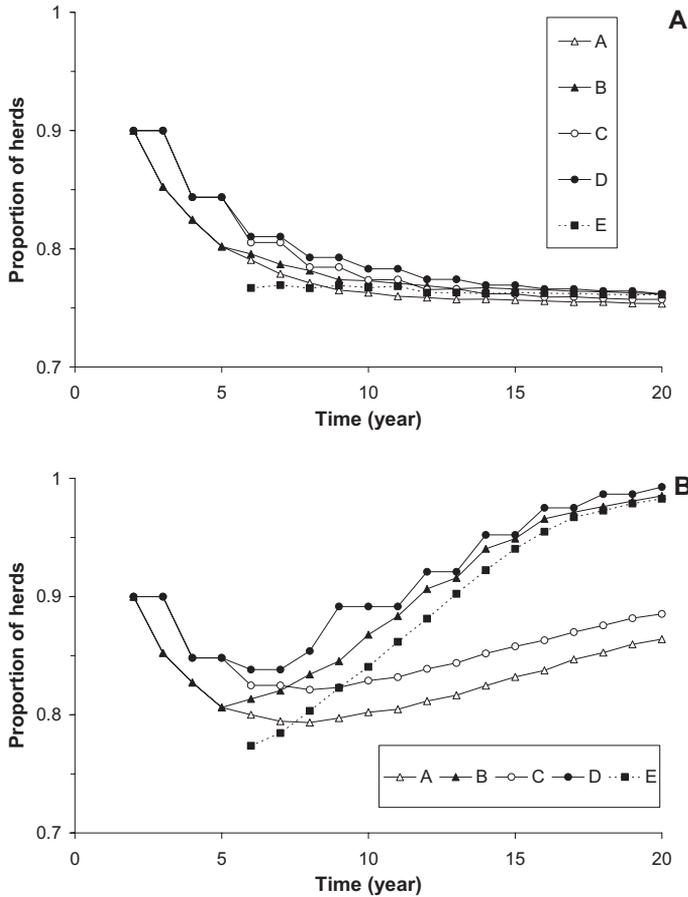
**Figure 3.** Estimated herd-level prevalence (A) and average within-herd prevalence (B) over time with or without (w/o) additional preventive management measures (prev man) and without testing (no test) or with test scheme D.

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<sup>3</sup>/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<sup>3</sup>/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<sup>3</sup> *Map*/L of milk, irrespective of whether or not additional preventive management measures were taken. Therefore, by default, ‘red’ herds were



**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 V.

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, Tab. V).

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 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,

**Table VI.** 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 V.

Year	Preventive management measures	Scheme	Proportion ‘green’ herds (%)		Overall $\chi^2$ (4 df)		Yates continuity-corrected $\chi^2_{cc}$ against scheme E (1 df)	
			Estimate	95% CI	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i> <sup>a</sup>
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			–	–

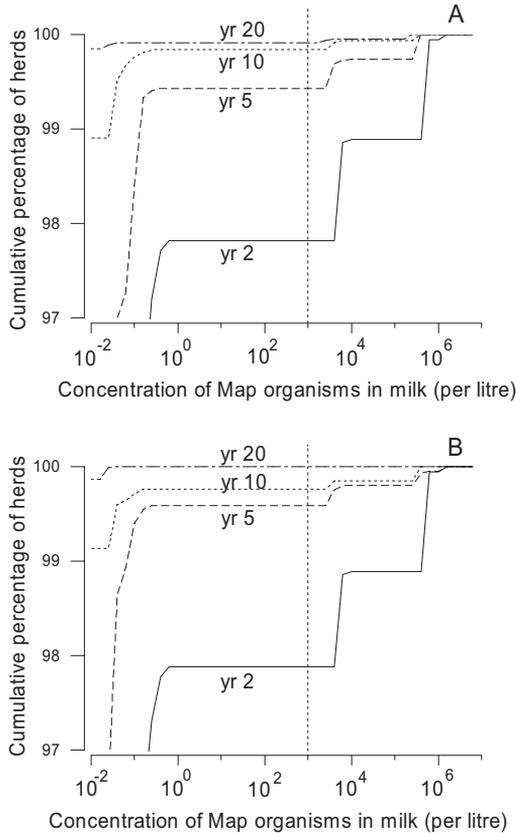
<sup>a</sup> Bonferroni-corrected *P*.

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 (Tab. VI).

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<sup>3</sup> 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<sup>3</sup>/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’ herds with < 10<sup>3</sup> *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<sup>3</sup> to €24 × 10<sup>3</sup> (Fig. 7A). For schemes with additional preventive measures these costs were much higher, ranging from €40 × 10<sup>3</sup> to €46 × 10<sup>3</sup>. 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 ×



**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.

$10^3$  to  $\text{€}7 \times 10^3$ . Additional preventive measures reduced these losses to on average  $\text{€}1 \times 10^3$  (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 programme plus losses due to paratuberculosis in Table VII. All alternative schemes were cheaper than scheme E (Tab. VII). 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 (Tab. VII).

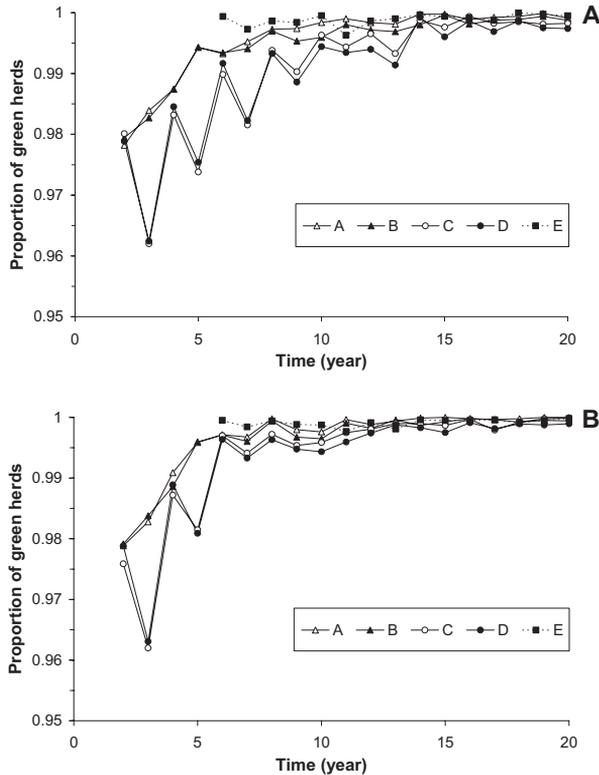
### 3.2. Sensitivity analyses

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*/L 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



**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 V.

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  $\geq 10^3$  *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  $\text{€}13 \times 10^3$  without additional preventive management measures, but only by  $\text{€}3 \times 10^3$  with additional preventive measures.

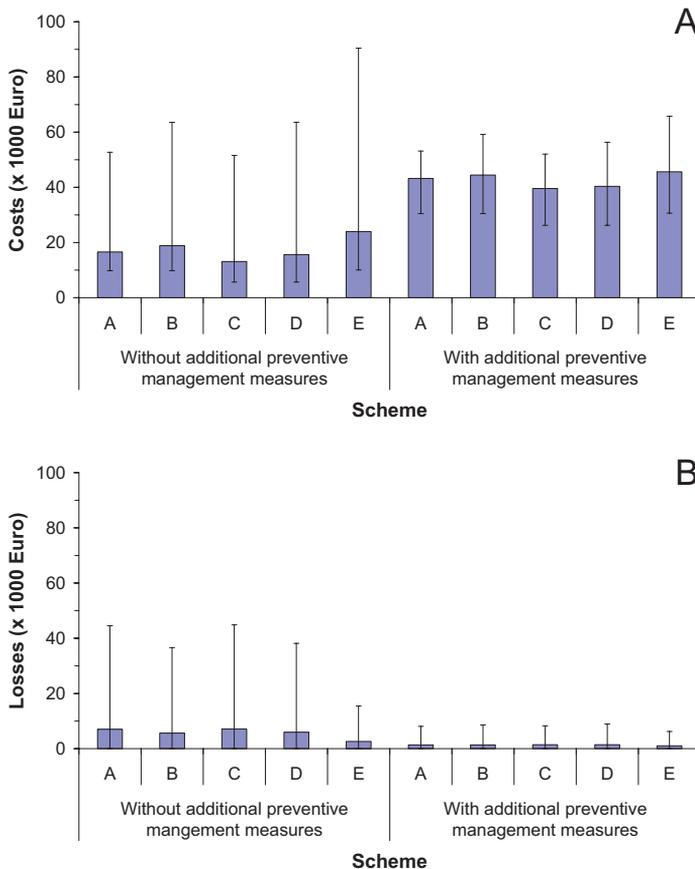
#### 4. DISCUSSION

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

**Table VII.** 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 V.

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 <sup>3</sup> )	P	W (× 10 <sup>6</sup> )		P <sup>a</sup>
				5%	50%							
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			—	—
20	Without	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			—	—
20	With	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			—	—
20	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			—	—

<sup>a</sup> Bonferroni-corrected P.



**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 V.

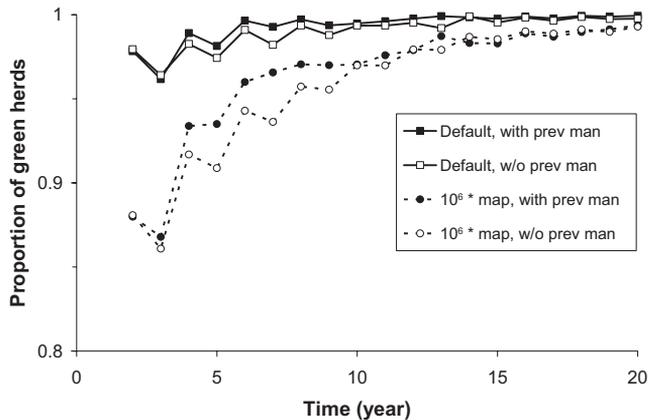
quality of ‘low-Map bulk milk’ herds, called ‘green’ herds in our simulations.

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 ten 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 Nether-

lands [4]. 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<sup>11</sup>.

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

<sup>11</sup> Velthuis A.G.J., Weber M.F., de Koeijer A.A., van Roermund H.J.W., Milk-quality-assurance program for Johne’s disease: decision analysis from a farmers perspective, in: Proc. 11th Int. Symposium on Vet. Epidemiology and Economics, Cairns, 2006, p. 313.



**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 IV. Alternatively, these concentrations were multiplied by  $10^6$  in the sensitivity analyses for this parameter.

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<sup>12</sup>. 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.

<sup>12</sup> Van Roermund H.J.W., Weber M.F., de Koeijer A.A., Velthuis A.G.J., de Jong M.C.M., Development of a milk quality assurance program for paratuberculosis: from within and between herd dynamics to economic decision analysis, in: Manning E.J.B., Nielsen S.S. (Eds.), Proc. 8th Int. Coll. Paratuberculosis, Copenhagen, 2005, pp. 51–59.

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 [25]) 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 the 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 [7, 14]. 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<sup>11,12</sup> 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 [4, 8, 37]. In the new pro-

gramme, farmers may choose freely between test schemes C and D (Tab. V). 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.

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

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