STOCHASTIC SIMULATION OF A MILK QUALITY ASSURANCE PROGRAMME FOR PARATUBERCULOSIS: WITHIN-HERD INFECTION DYNAMICS AND ECONOMICS

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SUMMARY

A milk quality assurance programme for *Mycobacterium avium* subsp. *paratuberculosis* (Map) in dairy herds was simulated with a stochastic simulation model. 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 (MAC; 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 aim of this study was to evaluate the epidemiological and economic effects of various test schemes and preventive management measures in a simulated population of closed dairy herds.

The simulations showed that herd examinations by ELISA effectively ensure the quality of 'low-Map bulk milk': >96% of certified herds were below the MAC. Preventive management measures considerably increased the number of 'low-Map bulk milk' 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 €$6 \cdot 10^3$ per herd. On average, additional preventive measures increased these costs to €$40 \cdot 10^3$ per herd.

This study showed that a bulk milk quality assurance programme for closed dairy herds is feasible and provided information on the cost-effectiveness of different programmes.

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 (Anon. 2000, Chacon et al. 2004, Herrewegh et al 2004). If Map is implicated, then milk is a possible vehicle of transmission of the organism to humans, because Map has been detected in raw milk and may not be effectively inactivated by pasteurisation (Sweeney et al., 1992b, Streeter et al. 1995, Grant et al. 1996, Millar 1996, Sung and Collins 1998, Grant et al. 1999, Giese and Ahrens 2000, Corti and Stephan 2002, Gao et al. 2002, Grant et al. 2002a & b, MacDonald et al. 2005, Pillai and Jayaro 2002, Sevilla et al. 2002). A milk quality assurance programme for paratuberculosis in dairy herds may reduce the potential risk of transmission of Map to humans through consumption of milk and milk products.

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Certification-and-surveillance programmes for Map-free herds have been developed in several countries (Kennedy et al. 2001). These programmes generally aim at a low-risk trade of cattle. In the Netherlands, a certification-and-surveillance programme has been developed in which herds can obtain 'Map-free' status following five negative annual herd examinations (the first herd examination by ELISA and faecal culture of ELISA-positive animals, the 2nd through 5th examination by pooled faecal culture; Benedictus et al., 1999). Control programmes for Map infected herds generally aim at elimination of Map in these herds. Because of their aims, these certification, surveillance and control programmes are inherently expensive and participation is often restricted to a minority of herds. By July 1st, 2005, only 473 of approximately 23.000 Dutch dairy herds had obtained '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 infection. Thus, such a milk quality assurance programme might possibly be run at considerable lower costs than the current Dutch certification-, surveillance- and control programme. Therefore, the aim of this study was to simulate different milk quality assurance programmes to evaluate their epidemiological effects and economic consequences for a population of closed Dutch dairy herds.

A milk quality assurance programme starts with an initial assessment; test-negative herds enter a surveillance procedure and test-positive herds enter a control procedure. Test-positive herds in the surveillance procedure shift to the control procedure. The control procedure aims to suppress the infection in the herds, such that the milk quality can be guaranteed and the herd can shift to the surveillance procedure. Different milk quality assurance programmes were simulated with a stochastic model JohneSSim (Groenendaal et al. 2002). Various alternative test schemes based on herd examinations by serology (ELISA) or individual faecal culture (IFC) were simulated. All programmes were simulated with and without preventive management measures taken by all participating herds. All simulated herds were closed.

MATERIALS AND METHODS

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 herd dynamics of a typical Dutch dairy herd and the infection-and-disease process in a 20-year period are simulated. The model and its use to study certification-and-surveillance programmes have been described in detail (Groenendaal et al. 2002, Weber et al. 2004). 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. Both infected and non-infected herds are simulated.

Assumptions in JohneSSim model for present study.

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 yr.), 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 1st calving. Mean annual milk production was 8000 kg. Initial herd-level true prevalence was assumed to be 0.30, based on a recent study in the Netherlands (van Weering, personal communication, 2004). The assumed distribution of the initial within-herd true prevalence in infected herds is shown in Fig. 1. Economic assumptions on losses caused by infection with Map, costs of participation in the quality assurance programme and costs of preventive management measures were updated (Tables 1-3). 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 4. Preventive management in the simulated herds was set to reflect the distribution of management practices in the Dutch dairy industry ('background' management; Groenendaal et al. 2002). Assumptions on effectiveness of additional preventive management measures, imposed on the 'background' management, have been described in detail previously (Groenendaal et al. 2002). By default, effective separation of young stock from adult cattle was assumed to reduce infections through faecal contamination of the environment by 90%.

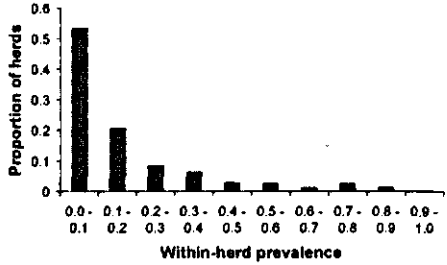

Fig. 1. Assumed distribution of within-herd true prevalence in infected herds at the start of simulations.

Table 1. Assumptions on losses caused by infection with Map. Losses did not include effects of a potential reduction in milk consumption due to consumer concerns.

	CATEGORY	COSTS (EURO)
Milk production	Reduction depends on infection state: 5% (lowly infectious) to 20% (clinical)	0.08 / kg
Treatment	Treatment clinical case	30
Reduced slaughter value	Standard slaughter value (per cow):	448.75
	Reduction depends on infection state (lowly infectious 5%, highly infectious 10%, clinical 100%)	
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

Table 2. Variable costs (Euro) of participation in the bulk milk quality assurance programme. Subscription costs were 90 Euro per year. Costs do not include Value Added Tax (VAT for subscription and laboratory tests = 6%; VAT on other costs 19%).

TEST / ACTION	COSTS VETERINARIAN	TRANSPORT COSTS	LABORATORY COSTS	
			PER SUBMISSION	PER TEST
Veterinarians' visit	22			
IFC	2.75 per animal	10	7.80	30.00 per animal
ELISA	2.75 per animal	10	7.80	6.15 per animal

Table 3. Assumed costs (Euro) of preventive management measures (including labour at 18.21 Euro 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.

CATEGORY		LOSS OR COSTS
Calving	Costs of cleaning per year	€ 100 per year
	Extra labour (hygiene, milking own dam) per calving	Giving colostrum of own dam € 9.11
Milk replacer	280 litres of artificial milk, 8 litre of milk replacer per kg milkpowder, costs of milkpowder € 1.30 per kg, value of bulkmilk €0.20 per litre.	42 litre instead of rest milk = € 6.83 238 litre instead of bulkmilk = - € 9.11 Total = - € 2.28
Hygiene barrier	Between adult stock and young stock	€ 726.71 per year (including labour)
Roughage	Better quality roughage, straw et cetera during housing in summer season only.	€ 39.03 for calves 0 – 6 months
Housing	Separate housing of animals 0 – 70 days (initially 5 animals)	€ 487.5 per year; 5% increment per year
	Separate housing of animals 70 – 180 days (initially 7 animals)	€ 682.5 per year; 5% increment per year
Calving	Separate housing of animals 180 – 360 days (initially 9 animals)	€ 877.5 per year; 5% increment per year

Table 4. Assumptions on sensitivity (Se) and specificity (Sp) of individual faecal culture (IFC) and ELISA.

	STAGE OF INFECTION	IFC	ELISA
Se	Latent infected	0	0.01
	Lowly infectious	0.40	0.10
	Highly infectious	0.95	0.60
Sp	Not infected	1	0.997 (a)

(a) van Maanen et al. (2002).

Shedding of Map in milk.

The assumptions made on shedding of Map in milk depending on the stage of infection are shown in Table 5. These assumptions were based on the available quantitative data on direct shedding of Map in milk, faecal contamination of milk and shedding of Map in faeces (Chiodini et al 1984, Stadhouders and Jørgensen 1990, van der Giessen et al. 1992, Sweeney et al. 1992a

& b, Streeter et al. 1995, Millar 1996, Rossiter and Burhans 1996, Nauta and van der Giessen 1998, Giese and Ahrens 2000, Pearce et al. 2001, Corti and Stephan 2002, MacDonald et al. 2005, Grant et al 2002a,b, Pillai and Jayaro 2002, Sevilla et al 2002, Rademaker, personal communication 2004, Stehman, personal communication, 2004).

Table 5. Assumed concentration of Map-bacteria in milk for each stage of the infection-and-disease process in adult cattle. (Total Map in milk = direct shedding + faecal contamination • Map in faeces. Faecal contamination was assumed to be 0.04 gram/litre milk.)

STAGE	PROPORTION OF ANIMALS	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	10^2
	0.24	10^2	10^4	$5 \cdot 10^2$
	0.16	10^2	10^7	$4 \cdot 10^5$
Clinical disease		10^4	10^8	$4 \cdot 10^5$

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 exposure to Map (either alive or dead organisms) and the probability of human disease are available. Therefore in the present study, we assumed that no viable Map organisms should be present after commercial pasteurization. Sung and Collins (1998) concluded that Map may survive HTST pasteurisation when the initial organism concentration is greater than 10^4 cells per litre. To our knowledge, no studies indicated that Map may survive HTST pasteurisation when the initial organism concentration is less than 10^4 cells per litre. Therefore, in this study, we considered a concentration of Map organisms in milk less than 10^3 per litre acceptable (allowing some safety margin).

Bulk milk quality assurance programmes

In our simulations, certified 'low-Map bulk milk' dairy herds were assigned a status 'green', while other dairy herds were assigned a status 'red'. Thus, 'green' herds were herds with a high probability that the concentration of Map in bulk milk was $<10^3$ per litre. The initial assessment of herds was done two years after the start of the simulations. Initial assignment of a status to a herd was based on the results of this assessment: test negative herds were classified as 'green' and test-positive herds as 'red'. Thereafter, 'green' herds were regularly monitored in a surveillance scheme; test-positive herds moved to the pool of 'red' herds. A control scheme was applied to 'red' herds. Test positive cattle and their last-born offspring were culled.

Various alternative test schemes for the initial assessment (i), surveillance (s) and control (c) were simulated (Table 6). The number of negative herd examinations required for a 'red' herd to move to the pool of 'green' herds was determined by the probability that the concentration of Map in bulk milk was $<10^3$ per litre. A test-negative 'red' herd became 'green' if this probability

was equal to, or higher than, the probability for a 'green' herd immediately after the intake procedure to have $<10^3$ Map per litre.

All programmes were simulated with and without additional preventive management measures imposed by all participating herds on their 'background' management. The following 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 from birth to the end of the first year.

Table 6. Simulated test schemes for initial assessment (i), surveillance (s) and control (c). In the initial assessment and the 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		SURVEILLANCE			CONTROL			
	TEST (ONCE)	ANIMALS	TEST	INTERVAL	ANIMALS	TEST	INTERVAL	ANIMALS	TEST
i1-s1-c1	ELISA	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA
i1-s1-c7	ELISA	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	IFC	2 yr	All, ≥2 yr	ELISA
i1-s2-c1	ELISA	All, ≥3 yr	ELISA	2 yr	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA
i1-s2-c7	ELISA	All, ≥3 yr	ELISA	2 yr	All, ≥3 yr	IFC	2 yr	All, ≥2 yr	ELISA

Model output.

In the present study, relevant herd-specific outcomes over time were the within-herd true prevalence, test prevalence, concentration of Map in bulk milk, and costs spent on the quality assurance programme. Relevant outcomes over time on the aggregate level included the proportion of 'green' 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 and costs spent on the bulk milk quality assurance programme (including herd examinations, subscription costs, preventive measures and cull of infected animals).

Sensitivity analyses.

The influence of various input parameters on the study results was analysed, by changing one parameter at the time. These sensitivity analyses were performed with test scheme i1_s1_c1, with or without additional preventive management measures taken in the herds. These sensitivity analyses were: (1) The default numbers of Map bacteria in milk (Table 5, last column) were multiplied by 10^6 to study the effect of this insecure parameter. (2) By default, preventive management measures reduced the probability of infection through the environment by 90%. Alternatively, this reduction was only 50%. (3) By default, the initial herd-level true prevalence was 0.30. Alternatively, a prevalence of 0.56 was simulated. (4) By default, a number of negative herd-examinations were required for a 'red' herd to become 'green' (based on the probability for such herd to have $<10^3$ Map per litre of bulk milk). Alternatively, only one negative herd-examination was required to become 'green'.

The proportion of herds classified as 'green' decreased over time if no preventive management measures were taken (Fig 3A). However, if preventive measures were taken, this proportion first decreased, but, thereafter, increased towards 86% - 99%, depending on the test scheme used (Fig 3B). 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 biennial IFC was more effective than culling based on annual ELISA.

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. The proportion of 'green' herds with $<10^3$ Map per litre of bulk milk increased towards 100% in year 20 (Fig. 4).

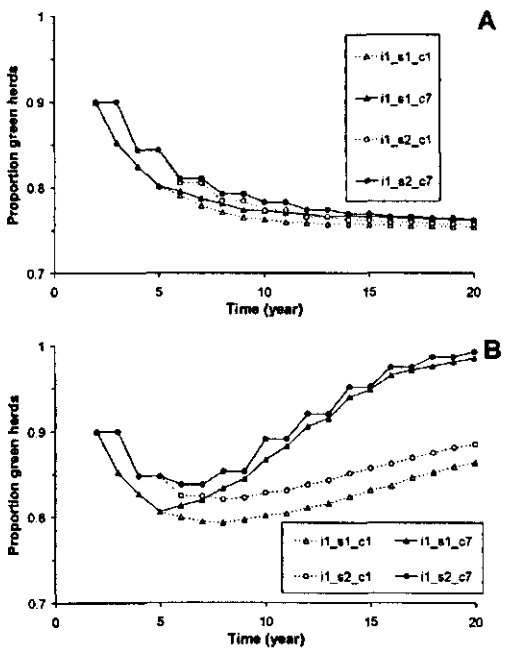

Fig. 3. Proportion of herds that are classified as 'green' over time, assuming a population of closed herds with an initial herd-level true prevalence of 30%. (A) Without additional preventive management measures, (B) with additional preventive measures. Test schemes are defined in table 6.

RESULTS

Simulated bulk milk quality assurance programmes.

At the initial assessment (scheme i1: ELISA, all cattle ≥3 yr), 90% of all herds were test-negative and classified as 'green'. The remaining 10% of herds were test-positive (i.e. ~35% of the infected herds at that time, and none of the non-infected herds) and therefore classified as 'red'. 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 (with scheme i1), 98% of 'green' herds had a concentration of Map in bulk milk $<10^3$ per litre. During control in 'red' herds, two consecutive negative herd-examinations by IFC or six consecutive negative herd-examinations by ELISA were required to reach the same probability of having $<10^3$ Map per litre milk. 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.

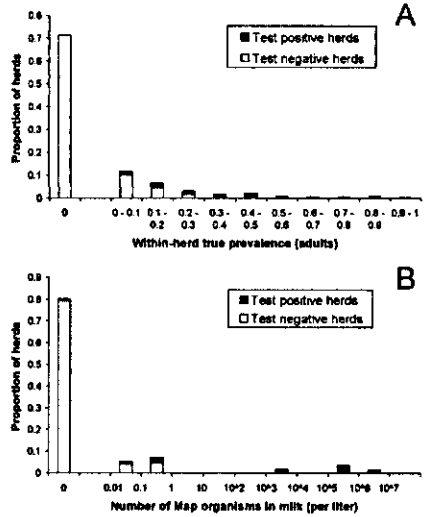

Fig. 2. Estimated within-herd prevalence in adult cattle (A) and estimated number of Map organisms per litre of bulk milk (B) immediately after the initial assessment in simulated herds that were test-positive ('red') and test-negative ('green') at the initial assessment, using scheme i1 (ELISA, all cattle ≥3 yr).

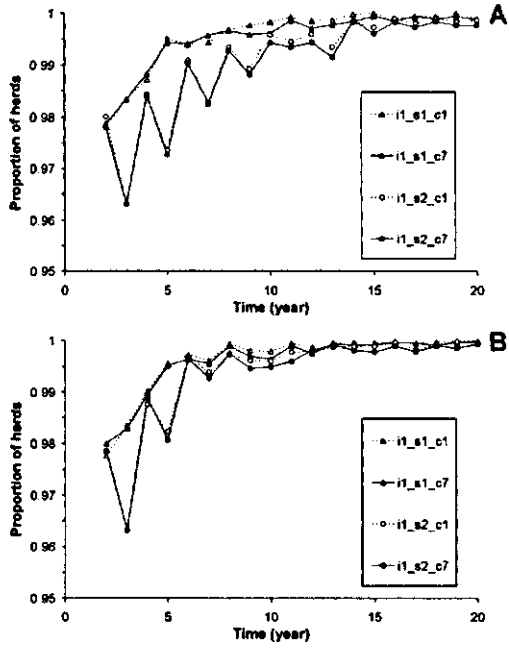

Fig. 4. Proportion of 'green' herds with $<10^3$ Map per litre of bulk milk (A) without additional preventive measures. (B) with additional preventive measures. Test schemes are defined in Table 6.

The median cumulative discounted costs during the 20 simulated years for schemes without additional preventive measures ranged from $6 \cdot 10^3$ to $10 \cdot 10^3$ Euro (Fig. 5). For schemes with additional preventive measures these costs were higher, ranging from $40 \cdot 10^3$ to $44 \cdot 10^3$ Euro. 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.

Sensitivity analyses

If additional preventive measures were taken but their effect was assumed to be 50% effective in reducing transmission through the environment instead of the default value of 90%, the proportion of herds certified as 'green' after 20 years was 81% instead of 86%. However, not taking any additional preventive measures resulted in only 75% of herds being certified as

'green'. Moreover, decreasing the reduction in transmission through the environment had no effect on the proportion of 'green' herds with $<10^3$ Map per litre bulk milk.

If the default level of contamination of milk with Map was multiplied by 10^6, the proportion of 'green' herds with $<10^3$ Map per litre bulk milk was reduced by up to 10% during the first years after intake. However, beyond approximately year 10 (i.e. 8 years after the initial assessment), the effect was very small (Fig. 6).

If a higher initial herd-level prevalence of 0.56 instead of 0.30 was assumed, the proportion of 'green' herds in year 20 was reduced by 21% (54% instead of 75% with additional preventive management measures; 75% instead of 86% without 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 2%, but this decrease was small beyond year 10. Effects on the cumulative discounted costs up to year 20 were negligible.

By default, six negative herd examinations by ELISA were required for a 'red' herd to be re-classified as 'green'. Alternatively, only one negative herd examination by ELISA was required. Then, over 99% of herds were classified as 'green' in year 20 (instead of 86%), if additional preventive management measures were taken. The reason is, of course, that 'red' herds move to the pool of 'green' herds sooner. If no additional preventive measures were taken, there was only a minor effect on the proportion of 'green' herds. However, the bulk milk 'quality' of 'green' herds was lower: the proportion of 'green' herds with $<10^3$ Map/l was reduced by up to 2% if only one negative herd examination by ELISA was required instead of six.

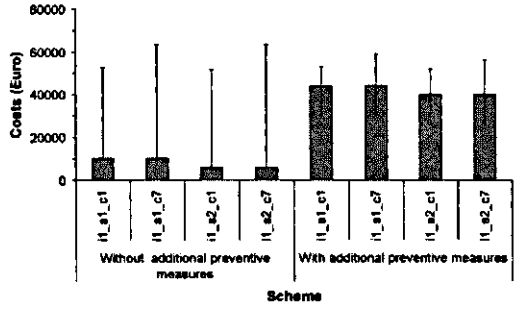

Fig. 5. Median cumulative discounted costs per herd up to year 20 (averaged over all 'green' and 'red' herds). Error bars indicate the 5% to 95% range. Test schemes are defined in Table 6.

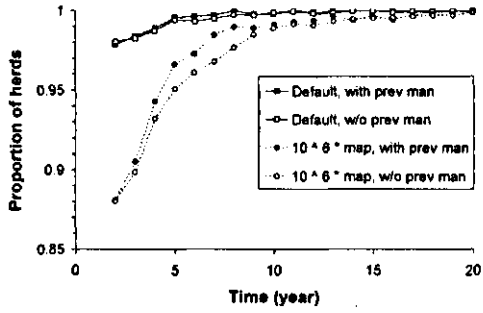

Fig. 6. Proportion of 'green' herds with $<10^3$ Map per litre of bulk milk in sensitivity analysis for the effect of contamination of milk with Map, using scheme i1_s1_c1, with or without (w/o) preventive management measures (prev man). Default concentrations of Map bacteria in milk are given in Table 5. Alternatively, these concentrations were multiplied by 10^6.

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DISCUSSION

To our knowledge, this is the first modelling study into a bulk milk quality assurance programme for paratuberculosis in dairy herds. By aiming at suppression of Map to guarantee milk quality, such a milk quality assurance programme can be run at considerable lower costs than certification-, surveillance- and control programmes aiming at a low-risk trade of cattle and elimination of Map at the herd-level.

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 the risk of within-herd spread of Map, and the intake-, surveillance-, and control procedures. The present study was restricted to closed herds. 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'). However, these measures only had a minor effect on the bulk milk quality of 'low-Map bulk Milk' herds ('green'). The intake, surveillance and control procedures would preferably be based on quantification of the concentration of Map organisms in bulk milk. However, to our knowledge, techniques to routinely quantify Map in large numbers of bulk milk samples are not yet available. Therefore, we simulated procedures for initial assessment surveillance and control based on tests at the animal-level (ELISA, faecal culture). The results showed that herd examinations by ELISA for intake and surveillance effectively ensure the quality of 'low-Map bulk milk': >96% of simulated certified herds (increasing to >99% after 10 years) were below the 10^3 Map/l. However, culling of test-positive animals and their last-born offspring based on biennial faecal culture was more effective than culling based on annual ELISA.

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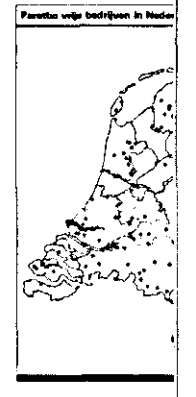

Fig. 2. Distribution over The Netherlands of all 206 Dutch herds with a 'Map-free' status on¶

In the face of uncertainty and lack of information, important assumptions were made in the present study. However, 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, 2002a). 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 indeed $<10^3$ Map per litre by 10%. However, such high concentrations of Map in milk are probably not biologically plausible (for example, a clinical animal would then shed $4 \cdot 10^{12}$ Map/litre of milk). Even though, the effects of such an increase in Map in milk from infected animals on the bulk milk quality of 'green' herds were very small beyond year 10 (i.e. 8 years after the intake procedure).

It is concluded that a bulk milk quality assurance programme for paratuberculosis in closed dairy herds is feasible. Preventive management measures should be advised to participants, because they considerably increase the probability of obtaining and keeping a 'low-Map bulk milk' status in the long term. Serology is sufficient for initial assessment and surveillance in the programme. However, for control in test-positive herds, culling based on faecal culture results is more effective than culling based on ELISA results. The present study provided decision-makers with information on the cost-effectiveness of different programmes.

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