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Quantitative risk assessment of *Mycobacterium avium* subsp. *paratuberculosis* survival in pasteurized milk in three dairy plants in Italy

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ABSTRACT

The objective of this study was to carry out a quantitative risk assessment (QRA) of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) survival in pasteurized milk produced by industrial dairy plants. Data were collected in three dairy plants (A, B and C) located in three different Italian regions and processing 38.75 (plant A), 89.29 (plant B) and 190.56 million litres (Plant C) of milk yearly. Plants A and plant C produce pasteurized milk, soft and hard industrial cheeses and yogurt; plant B produces only pasteurized milk. In-line milk filter (ILMF) samples and/or bulk milk samples were collected from all 569 herds delivering milk to the three dairy plants. Samples were analysed by quantitative real-time PCR (qPCR). The QRA considered the presence of MAP in ILMF and in bulk milk of all the dairy herds delivering milk to the three investigated dairy plants, estimating MAP concentration in raw milk on the basis of these data, the dilution effect due to mixing milk in collecting trucks and in plant silos, and the effect of pasteurization in reducing the MAP load. The expected fraction of litres of pasteurized milk with 0 MAP would be 99.02%, 99.45% and 99.12%, in plants A, B and C respectively, and an overall percentage 0.55% to 0.98% of pasteurized milk having a MAP contamination >0 colony forming units (CFU)/l and 0.04%–0.11% of pasteurized litres with a MAP contamination > 100 CFU/l was predicted. A daily variation was observed in the proportion of MAP-contaminated litres of milk. The study demonstrated that milk in the dairy plants investigated may be a source of MAP exposure for humans. The between-herd and within-herd MAP apparent prevalence in the investigated areas are likely comparable to those in other areas in Italy, Europe and North America, and the results are applicable to other geographical areas.

1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the aetiological agent of paratuberculosis, also known as Johne's disease (JD), a chronic and progressive granulomatous enteritis affecting ruminants and a variety of domestic and wildlife species (Beard et al., 2001; Chiodini, Van Kruiningen, & Merkal, 1984; Kennedy & Benedictus, 2001). MAP infection is widespread worldwide and its herd-level prevalence in dairy cattle has been reported to exceed 50% (Nielsen & Toft, 2009). MAP is thought to carry a zoonotic risk, on the basis of both clinical and gross lesion similarities between JD and human Crohn's disease (CD) and PCR evidence of MAP in the gut of CD patients (Chiodini, Chamberlin, Sarosiek, & McCallum, 2012). Human exposure to MAP most likely occurs via contaminated milk and milk products (Gill, Saucier, & Meadus, 2011); MAP may contaminate milk through direct excretion or by faecal contamination during milking (Grant, 2005). MAP has been detected in bulk tank raw milk (Hanifian, Khani, Barzegari, & Shayegh, 2013; Khol et al., 2013; Slana, Liapi, Moravkova, Kralova, & Pavlik, 2009; Stephan, Bohler, & Corti, 2002), and can survive low-temperature holding (63 °C for 30 min) and high temperature-short time (72 °C for 15 s) pasteurization (Grant, 2006; Grant, Ball, Neill, & Rowe, 1996; Millar et al., 1996; Sung & Collins, 1998; Van Brandt et al., 2011). Viable MAP has even been found in retail pasteurized milk (Ayele, Svastova, Roubal, Bartos, & Pavlik, 2005; Carvalho, Pietralonga,

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Schwarz, Faria, & Moreira, 2012; Ellingson, Koziczkowski, & Anderson, 2005; Grant, Ball, & Rowe, 2002; Shankar et al., 2010).

The number of decimal reductions of MAP in milk after industrial pasteurization remains a matter of some dispute. Industrial scale experiments reported a reduction of 4–7 log (Grant, Williams, Rowe, & Muir, 2005; Lynch, Jordan, Kelly, Freyne, & Murphy, 2007; McDonald, O'Riley, Schroen, & Condron, 2005; Pearce et al., 2001; Rademaker, Vissers, & te Giffel, 2007; Stabel & Lambertz, 2004). Given a thermal treatment of pasteurization, and the fact that the inactivation kinetics of unicellular organisms is a first order reaction so that the same proportion of cells is killed during each unit of time (Cerf, Griffiths, & Aziza, 2007), the concentration of survivors depends on the initial concentration of cells. Few data on the concentration of MAP in raw milk are currently available in the literature; in addition, the probability of MAP contamination of milk may vary in different geographical areas, depending on factors such as the herd-level prevalence, the within-herd prevalence, the presence of high shedders in the herds (Boulais, Wacker, Augustin, Cheikh, & Peladan, 2011; Khol et al., 2013; Sweeney, Whitlock, & Rosemberger, 1992) and milking hygiene (Jayarao et al., 2004).

To date, no attempts have been made to develop simulation models of MAP contamination of retail pasteurized milk based on quantitative data of raw milk contamination. The objective of this study was therefore to carry out a quantitative risk assessment (QRA) of MAP survival in pasteurized milk produced by three industrial dairy plants in Italy. The QRA considered the presence of MAP in in-line milk filter (ILMF) and in bulk milk (BTM) of all the dairy herds delivering milk to the three dairy plants, the dilution effect due to mixing milk in collecting trucks and in plant silos, and the effect of pasteurization in reducing the MAP load.

2. Material and methods

2.1. Nomenclature of distributions

Normal (μ, σ) stands for normal distribution with a mean of μ and a standard deviation of σ. Uniform (x, y) stands for the uniform distribution with a minimum of x and a maximum of y. Beta (a, b) stands for the beta distribution, with parameters a and b. Poisson (λ) stands for Poisson distribution with mean λ.

2.2. Exposure assessment

Data were collected in three dairy plants (A, B and C) located in three different Italian regions and processing 38.75 (plant A), 89.29 (plant B) and 190.56 million litres (Plant C) of milk a year. Plants A

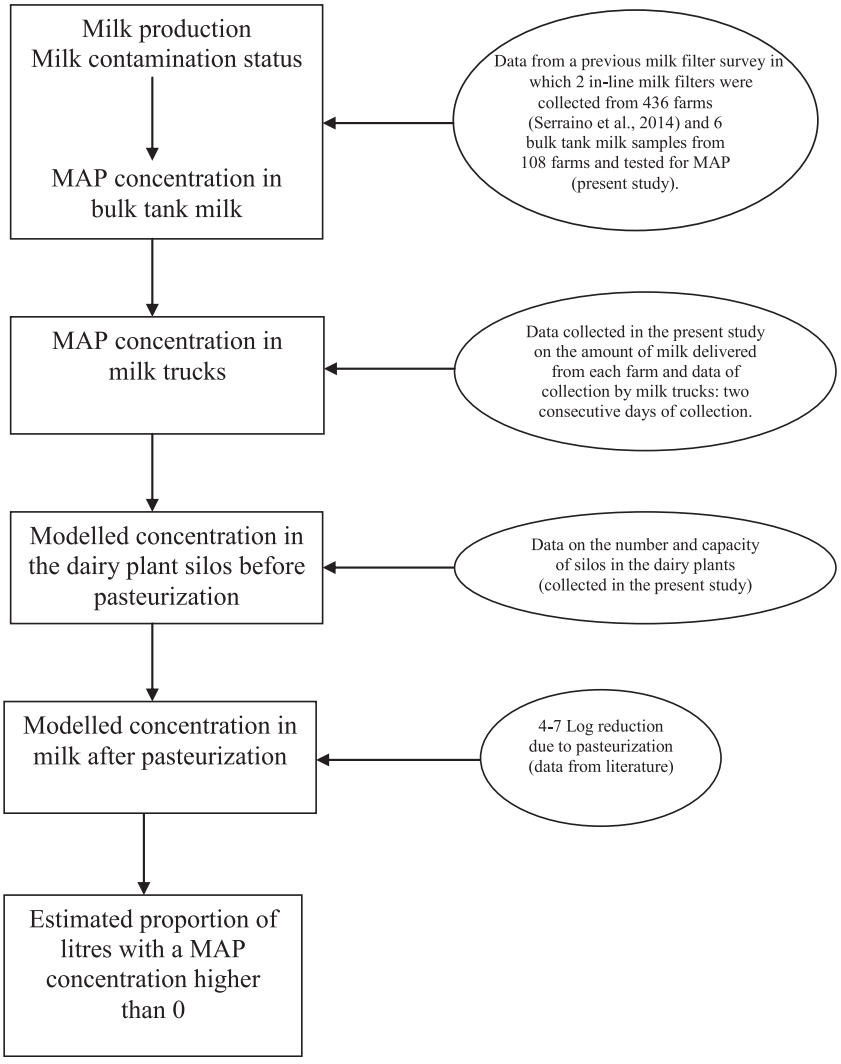

Fig. 1. Flow chart of the model used to estimate the proportion of milk litres with a *Mycobacterium avium* subsp. *paratuberculosis* (MAP) concentration higher than 0.

and plant C produce pasteurized milk, soft and hard industrial cheeses and yogurt whereas plant B produces only pasteurized milk. The risk assessment model developed is summarized in Fig. 1.

2.3. Collection of data on milk contamination

Data were collected from January 2010 to May 2013; all 569 dairy farms delivering milk to the three dairy plants were investigated in the study. Herds had from eight to 608 dairy cows (mean value: 123) and farming was the typical Italian system in which cows are housed in barns throughout the year; the main farmed breed was Holstein Friesian but some herds had a small proportion (about 10%) of Brown Swiss and Jersey cows.

Based on a previously described screening sampling plan (SSP) (Serraino et al., 2014), the dairy farms were classified as MAP positive or negative by performing quantitative real-time PCR (qPCR) examination of two ILMF samples and ELISA examination of two BTM samples (ID Screen® Paratuberculosis indirect, Confirmation test, IDVET, Montpellier, France; the S/*P* value, was considered positive when it was ≥0.15 for BTM samples). Briefly, BTM samples were obtained from all 569 herds included in the survey, whereas ILMF were collected from 436 herds as some small farms had no milking parlour: overall, the SSP proved able to detect 85.5% of herds in which at least one cow was positive by ELISA, performed on individual milk samples, (ID Screen® Paratuberculosis indirect, Confirmation test, IDVET, Montpellier, France; the S/*P* value was considered positive when it was ≥0.15 for individual milk samples), and 84.6–100% of farms with a within-herd apparent prevalence (AP) > 2.0%, tested by ELISA performed on individual milk samples. A total of 121/569 herds were MAP positive: 80 herds at ILMF examination and 78 herds at BTM examination. The limit of detection (LOD) of the analytical procedure performed on ILMF was estimated to be 10–20 (colony forming units) CFU/filter (Serraino et al., 2014).

2.4. IS900 q RT-PCR milk examination

Farmers of all the herds which resulted SSP MAP positive were requested to perform additional BTM samples: samples of raw milk were collected six times from each farm about one month apart by trained veterinary practitioners, frozen at −20 °C on the same day as sampling and shipped within 15 days after sampling for analysis by the accredited laboratory of the National Reference Centre for Paratuberculosis. Overall, the National Reference Centre for Paratuberculosis received the six milk samples from 108 MAP positive herds out of 121 (89.3%) and a total of 648 milk samples were analysed by IS900 q RT-PCR.

Fifty ml of milk were transferred into a Falcon tube and centrifuged at 2500 *g* for 15 min. The pellet obtained was suspended in 1 ml of PBS Tween 20, vortexed and transferred into a 1.5 ml tube. According to Foddai, Elliott, and Grant (2010), 10 μl of MyOne Tosylactivated Dynabeads (Life Technologies, Milan, Italy) coated with biotinylated aMp3 peptide (NYVIHDVPRHPA) and with biotinylated aMptD peptide (GKNHHHQHHRPQ) (Research Biochemicals, Cambridge, UK) (Stratmann, Dohmann, Heinzmann, & Gerlach, 2006) were added to the suspensions obtained from ILMF and mixed on a Stuart SRT6 rotator (Sigma–Aldrich, Milan, Italy) for 30 min at 30 rpm at room temperature. The samples were then subjected to magnetic separation for 10 min and washed twice with 1 ml PBS Tween 20. The magnetic beads were suspended in 300 μl of PBS Tween 20, 200 μl of sterile water, supplemented with 300 mg of SIGMA glass beads (150–212 μm diameter) and subjected to bead beating in Tissue Lyser for 10 min at 30 Hz. The DNA was then extracted adding 100 μl of the mixture to a 1.5 ml tube containing 20 μl of Proteinase K and 80 μl of Chelex resin (Biorad, Milan, Italy). The samples were incubated at 70 °C for 10 min followed by 15 min at 95 °C and then centrifuged at 12,000 *g* for 5 min. Four microlitres of supernatant were then used for qPCR.

The LOD was determined by processing ten replicates of 50 ml of BTM from a paratuberculosis-free herd (all animals had been paratuberculosis-negative in the previous ten years by Elisa testing and faecal cultures) spiked with MAP field isolate (IZSLER 76/2013) in a range from 10^0 to 10^3 CFU/ml. The LOD was 23 CFU/ml of milk and according to ISO 16140 statements the limit of quantification (LOQ) was fixed as 230 CFU/ml of milk. Real-time PCR was performed targeting sequence IS900 with primers and hydrolysis probe as already described (Donaghy, Totton, & Rowe, 2011; Ricchi et al., 2009). The amplification reactions were performed in duplicate for each sample, using a StepOne Plus System (Life Technologies, Milan, Italy) in 20 μl of master mix containing 300 nM of each primer, and 6 nM of the probe. In case of discrepant results, the analysis was repeated. An internal positive control (Life Technologies, Milan, Italy) was also added to avoid false negative results, and an internal extraction control, made by a spiked and frozen milk sample, was used to evaluate the reproducibility of the reaction. All PCR reactions were performed at the following conditions: 40 cycles of denaturation for 15 s at 95 °C and annealing/elongation for 60 s at 60 °C.

2.5. MAP concentration in raw milk

For all 121 MAP SSP positive dairy farms, data on qPCR analysis performed on two ILMF samples (80 farms) or six BTM samples (108 farms) were available and used for the estimation of the MAP concentration in raw milk that was calculated on the basis of qPCR analysis performed: in detail in 67 farms qPCR analysis was performed both on 2 ILMF samples and on six BTM samples; in 41 farms qPCR analysis was performed only on six BTM samples and in 13 farms qPCR analysis was performed only on ILMF samples.

Assuming that the true prevalence in a positive or negative herd is P, the number of positive filters S in a sample of N filters tested is binomial (N | P). If we assume a priori uniform [0,1] distribution for P (the probability of being positive) and find that S of N sampled herds have one or more positive filters, the posterior distribution of herd prevalence P is Beta distributed:

$$P^{+}_{\text{filter/milk}} = \text{Beta}\left(S_{\text{filter/milk}} + 1, N_{\text{filter/milk}} - S_{\text{filter/milk}} + 1\right) \quad (1)$$

For the aim of the model, ILMF examination was considered representative of a tenfold volume of bulk tank milk (500 ml) (Giacometti et al., 2012), assuming that 100% of bulk tank milk samples with MAP were detected and the similar LOD of qPCR in milk (23 CFU/ml) and in ILMF (10–20 CFU/filter); in fact, ILMF examination is three to ten times more sensitive in detecting pathogenic bacteria than milk examination (Latorre et al., 2009; Leone, Cremonesi, & Stella, 2010; Ruzante et al., 2010; Van Kessel, Karns, & Wolfgang, 2008; Warnick et al., 2003) and the presence of MAP in raw milk was tested using a qPCR after immunomagnetic concentration of 50 ml of raw milk.

Considering the sensitivity (85.5%) of the SSP (Serraino et al., 2014) in detecting herds in which at least 1 ELISA positive cow is present, and specificity of the tests used (Slana, Kralik, Kralova, Babak, & Pavlik, 2012; Van Weering et al., 2007), the real prevalence was adjusted as follows:

a) a test sensitivity of 85.5% was modelled using Beta distribution (85.5, 14.5)
b) a test specificity of 99% was modelled using Beta distribution (99, 1).

$$P^{+}_{\text{filter/milk}} = \text{Beta}\left(S_{\text{filter/milk}} + 1, N_{\text{filter/milk}} - S_{\text{filter/milk}} + 1\right) \Big/ \text{Beta distribution}\,(85.5,\ 14.5)^{*}\text{Beta distribution}\,(99,\ 1). \quad (1a)$$

Assuming a Poisson distribution of bacteria in milk, the probability that no MAP are present in a sample of ILMF or milk is a function of the mean concentration (C) of MAP in the volume tested:

$$C = -\ln\left(1 - P^{+}\right) \Big/ V \quad (2)$$

where P^{+} is the proportion of positive samples and V the volume of milk tested (50 ml for raw milk and 500 ml for ILMF).

Following these assumptions, we modelled the distribution of MAP in bulk tank milk for each farm, assuming the level of pathogens was lognormally distributed

$$C = \text{Normal}\,[\log(C),\ \sigma]. \quad (3)$$

Once the mean of the lognormal distribution was obtained, the standard deviation (σ) was calculated for each farm to match the fraction of positive samples actually observed in the survey.

2.6. MAP concentration in dairy plant milk silos before pasteurization

Milk is delivered to the dairy plants by milk trucks collecting milk from farms on the basis of logistics criteria; milk is collected daily from some farms and every two days from others and, in order to have a complete picture of milk consignments, the amount of milk collected (V_{tank}) from each farm was recorded for two consecutive days.

So the total amount of MAP, that originates by the contribution of the MAP amount of each farm, was modelled as:

$$\text{MAP}_{\text{farm}-ni} = 10^{\text{Normal}\,[\log(C),\ \sigma]}{}^{*}(V_{\text{tan}\,k}). \quad (4)$$

Obtaining these truck collecting milk data from each farm, for each milk truck the total amount of MAP collected is:

$$\text{MAP}_{\text{truck}} = \text{Sum}\left(\text{MAP}_{\text{farm1}},\ \text{MAP}_{\text{farm2}}, \cdots,\ \text{MAP}_{\text{farm}-n}\right) \quad (5)$$

The milk collected is spliced in the dairy plant silos on the basis of production needs, depending on their capacity. To simulate the milk dilution effect due to the mix of milk from different trucks, the number and capacity of the silos available in each dairy plant were recorded, and the amount of MAP modelled in (5) for each truck was randomly combined to model the silos concentration of MAP.

$$C_{\text{MAP silos}} = \text{Random}\,a, b, n- > \text{Sum}\,(\text{MAP}_{\text{truck}\,a}, \text{MAP}_{\text{truck}\,b}, \ldots, \text{MAP}_{\text{truck}\,n}) \times /\text{silos capacity}. \quad (6)$$

To calculate the pasteurization effects on decimal reduction of MAP, a uniform distribution of 4–7 log was chosen to model the pasteurization log reduction of MAP concentration on the basis of literature data (Cerf, Griffiths, & Aziza, 2007).

$$\text{pasteurization effect} = \text{Uniform}\,(4, 7) \quad (7)$$

The distribution of MAP after pasteurization per litre of milk produced was modelled as:

$$\text{Poisson}\left[10^{\log(6)-(7)}{}^{*}10^{3}\right]. \quad (8)$$

Per day of collection and per plant of milk pasteurization, a Monte Carlo simulation (with practical value of 100,000 iterations) was carried out using @Risk, version 4.5.2 (Palisade Corporation, Newfield, NY, USA). Six (three dairy plants for two consecutive days) simulations were run and the output of each simulation was the distribution of MAP per litre of milk produced after pasteurization.

3. Results

3.1. IS900 q RT-PCR milk examination

Six out of 648 bulk tank raw milk samples (0.9%) resulted positive at IS900 q RT-PCR (three from farms delivering milk to dairy plant A, two to plant B and one to plant C) but levels of contamination were under the LOQ in all positive samples.

3.2. Risk assessment

Table 1 reports the estimated concentration of MAP for each plant (A, B and C), on the basis of the different farm status (SSP positive or negative).

In dairy plant A, a daily average of 18 k L of milk were collected by milk trucks collected and in the pasteurization plant the collected milk was split between four silos with a mean capacity of 37 k L. The model output (concentration of MAP in each silo) was a Poisson distribution with a mean of 1.374 and 0.82774 CFU/litre using respectively the first or the second day of collection data stored. The model estimates zero MAP per litre of pasteurized milk in 99.02% of iterations (mean of the two days of collection) and 99.96% of simulated pasteurized milk litres on the two days of collection have less than 100 CFU of MAP.

In plant B, a daily average of 18 k L of milk were collected by milk trucks and in the pasteurization plant the collected milk was split between six silos with a capacity from 60 k L to 120 k L with a mean capacity of 100 k L. The model output (concentration of MAP in each silo) was a Poisson distribution with a mean of 0.210 and 0.061 CFU/litre using the first or the second day of collection data stored. The model estimates zero MAP per litre of pasteurized milk in 99.45% of iterations (mean of the two days of collection), and

Table 1
Estimated concentration (CFU/ml) of *Mycobacterium avium* subsp. *paratuberculosis* bulk tank raw milk from farms that resulted positive or negative to the screening sampling plan (SSP) based on milk filters and/or bulk tank milk examination by IS900 q RT-PCR and that deliver milk to three dairy plants.

Plant	SSP Farm status	Normal distribution of MAP concentration			
		Mean	5th Percentile	50th Percentile	95th Percentile
A	Positive	0.000442588	9.12011E-08	0.000446684	2.13796209
	Negative	1.68655E-05	1.41254E-09	1.69824E-05	0.204173794
B	Positive	0.000276058	6.30957E-08	0.000275423	1.202264435
	Negative	9.63829E-06	8.91251E-10	9.54993E-06	0.102329299
C	Positive	0.000427071	6.45654E-08	0.00042658	2.818382931
	Negative	8.57038E-06	1.07152E-09	8.51138E-06	0.067608298

99.94% of simulated pasteurized milk litres on the two days of collection have less than 100 CFU of MAP.

In dairy plant C, a daily average of 18 k L of milk were collected by milk trucks and in the pasteurization plant the milk collected was split between 11 silos each with a capacity of 100 k L. The model output (concentration of MAP in each silos) was a Poisson distribution with a mean 5.670 and 0.593 CFU/litre using the first or the second day of collection data stored. The model estimates zero MAP per litre in 99.12% of iterations on the two days of collection (mean of the two days of collection), and 99.89% of simulated pasteurized milk litres on the two days of collection have less than 100 CFU of MAP.

Histograms of the simulated values greater than zero of the pasteurized litre of milk produced in the three plants (mean of the two days of sampling) are shown in Fig. 2.

4. Discussion

To our knowledge, this is the first risk assessment model to estimate the proportion of retail pasteurized milk contaminated by MAP based on raw milk data.

The main pitfalls of the model are: i) the limited sensitivity (10–20 CFU/filter and 23 CFU/ml of milk) of the analytical method (IS900 q RT-PCR) used to assess the milk contamination that does not allow to estimate the concentration of MAP under the LOD; ii) the screening sampling plan we used to identify MAP-positive dairy herds was not able to detect a proportion of MAP-infected farms (14.5%); iii) the effect of pasteurization in the decimal reductions of MAP was estimated according to current literature and not measured in the three dairy plants.

The limited sensitivity of the analytical test reduced the proportion of MAP-positive samples detected (ILMF or BTM samples). However, the missing positive samples represent the proportion with the lowest level of contamination, whose contribution to the overall MAP contamination of retail milk is probably strongly reduced by pasteurization and thus the bias can be considered limited for the final estimation. In addition, the IS900 PCR methods are currently considered more sensitive than cultural methods for bulk tank examination (Okura, Tolf, & Nielsen, 2012) and therefore the risk assessment could not reach a higher level of accuracy.

The screening sampling plan used to identify MAP-infected dairy herds identified herds with a higher within-herd prevalence and a higher probability of milk contamination (Serraino et al., 2014), but the contribution of other undetected herds to the overall MAP contamination of retail milk, albeit limited, must be considered and for this reason an adjustment was added to the model.

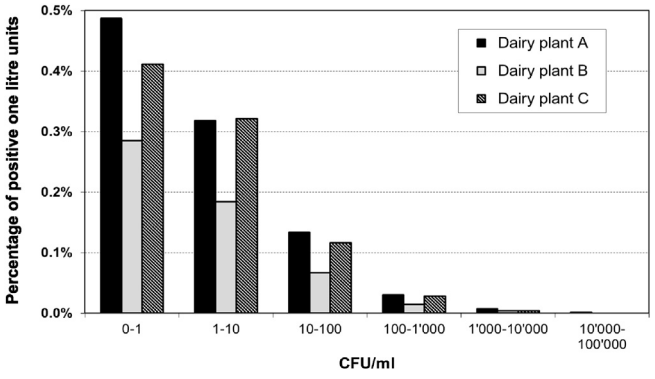

Fig. 2. Percentages of the simulated one litre units of pasteurized milk in which *Mycobacterium avium* subsp. *paratuberculosis* values greater than 0 were predicted and their predicted *Mycobacterium avium* subsp. *paratuberculosis* concentration (CFU/ml).

A very low proportion (0.9%) of MAP-contaminated milk samples was detected by IS900 RT-PCR in comparison with data reported in other surveys and reviewed by Okura et al. (2012) in which BTM MAP positivity was reported to be up to 80.0%. The reasons of the low level of contamination detected in the present study is not known: the investigated herds had no history of testing for paratuberculosis before the study and no actions designed to reduce paratuberculosis prevalence in the herds were taken during the study; in addition MAP AP (6.7%) detected in the farms delivering milk to the three dairy plants (Serraino et al., 2014) are similar to the prevalence reported in other studies performed in Italy, Europe and U.S.A. (Carter, 2012; Nielsen & Toft, 2009). However, all the investigated farms participated in an industry-driven scheme for milk quality improvement supported by the Italian Ministry of Agriculture, and this may have had an impact on the level of milking hygiene, one of the factors that may influence MAP milk contamination (Okura, Nielsen, & Tolf, 2013).

The model was developed considering two randomly chosen consecutive days for quantitative milk yield estimation of each dairy farm. MAP shedding by infected animals and pasteurized milk contamination may have seasonal variations (Cazer et al., 2013; Ellingson et al., 2005) related to a higher bacterial load or to seasonal calving practices (Cazer et al., 2013). However, the synchronization of calving is not common in Italy, because farmers are required to deliver the same quantity of milk throughout the year to meet consumers demand. Moreover, samples for the estimation of milk contamination were collected over a period of more than three years and this should have reduced any bias in our model due to seasonal variations. On the other hand, it cannot be ruled out that pasteurized milk could show a seasonal variation of MAP contamination, with a higher prevalence in some periods and a lower prevalence in others.

The Poisson distribution simulated a different mean CFU/litre using the first or second days of milk collection; this is particularly evident in plant C were the modelled MAP concentration on the first day of collection was tenfold the concentration modelled on the second day. This result is due exclusively to the different composition of the commingled milk in the trucks collecting milk from different farms on the first and second days of collection. On a yearly basis this finding may not affect the estimation of pasteurized milk MAP contamination, but a daily variation in the proportion of MAP-contaminated litres could be observed.

The model predicted a proportion of 0.55%–0.98% litres of pasteurized milk having a MAP contamination >0 CFU/l and 0.04–0.11% litres with a MAP contamination >100 CFU/l. No data are available in Italy on the prevalence of MAP-positive samples of pasteurized milk to compare our results, but researches in other countries with a MAP prevalence in dairy herds similar to that reported in Italy (Nielsen & Toft, 2009) showed percentages of positivity, by culture, in pasteurized milk samples ranging between 0.2% and 2.8% (Ayele et al., 2005; Carvalho et al., 2012; Ellingson et al., 2005; Grant et al., 2002; O'Reilly et al., 2004), in agreement with the occurrence estimated by our model.

Few attempts have been made to model human exposure to MAP via pasteurized milk consumption (Cerf, Griffiths, & Aziza, 2007; Nauta & van der Giessen, 1998); unlike both previously reported models were developed on the basis of theoretical estimations of raw milk contamination by MAP, our study is based on quantitative data of milk contamination and quantitative milk yield of each farm. Our results show a lower estimation than that reported by Nauta & Giessen that assumed a survivor concentration of 5.4 CFU/l in pasteurized milk, corresponding to a 25% probability of detecting MAP cells in a 50 ml samples as calculated by Cerf et al. (2007) which underline that this difference can be ascribed to the lower (2 log) reduction due to pasteurization

assumed in the Nauta & Giessen study. Our data are closer to those estimated by Cerf et al. (2007), which modelled a mean annual percentage detection of MAP in 0.12% of pasteurized milk samples of 50 ml volume in the most probable scenario, and a 97.5 percentile of 0.54% positive samples; however, several differences between these two studies make a comparison difficult. First of all, the pasteurization effect assumed in Cerf et al.'s study (up to 12 log reduction in some cases) is higher than that considered in this study: $a > 8$ log reduction of MAP during pasteurization has been reported (Stabel & Lambertz, 2004), but survivors were observed *n* some experiments, so in our study we adopted a conservative approach and modelled a maximum 7 log reduction. The second difference is that Cerf et al. calculated the proportion of contaminated pasteurized milk samples on 50 ml of sample volume assuming a sensitivity of the analytical technique able to detect one MAP cell in 50 ml of milk. By contrast, the analytical technique used in our study demonstrated a LOD of 23 CFU/ml and our study did not model the proportion of positive 50 ml milk samples but the proportion of contaminated 1 L retail pasteurized milk to assess consumer exposure to MAP.

The lack of a more accurate definition of the *D* value of MAP and more sensitive analytical techniques hamper a more precise estimation of the proportion of MAP-contaminated pasteurized milk litres.

For the purpose of the model, i.e. assessing human exposure due to milk consumption, all the pasteurized milk was considered sold as liquid milk. However, pasteurized milk accounts for only a small proportion of dairy products marketed in Italy, and for example, two of the three investigated dairy plants use a proportion of this milk for cheese making (soft, hard and stretched cheeses) and the production of yoghurt. The acidification that occurs during cheese-making and yoghurt production, and seasoning of cheeses may influence the survival of MAP (Donaghy et al., 2011; Van Brandt et al., 2011); in addition, during the production of "pasta filata" cheeses the stretching step with hot water (about 90 °C) may further reduce the MAP load as demonstrated for other pathogenic bacteria (Serraino et al., 2012; Serraino, Finazzi, et al., 2013; Serraino, Giacometti, et al., 2013). Hence the different use of milk must be taken into account in calculating the total human exposure to MAP due to dairy product consumption, and data on the evolution of the MAP population during different dairy production processes are needed.

The present study demonstrated that milk may be a source of MAP exposure for humans and quantified its exposure. Considering that our results are in agreement with experimental data on pasteurized milk contamination from studies performed in other geographical areas, and that the between-herd and within-herd AP in the investigated areas are comparable to those elsewhere in Italy, Europe and North America, our findings could be probably applicable to other geographical areas. Knowledge of the MAP contamination status of pasteurized milk will be useful to risk managers were the zoonotic potential of MAP confirmed.

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