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Can the Grana Padano and Parmigiano Reggiano production process guarantee a reduction in pathogenic microorganisms equivalent to the pasteurization process?

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Abstract

Italian hard cheeses made in the Pianura Padana area, such as Grana Padano and Parmigiano Reggiano, are traditionally produced from raw milk and undergo extended ripening periods. These processes generate multiple microbiological hurdles that can inactivate pathogens. However, current European regulations do not formally recognize the impact of these hurdles as equivalent to pasteurization, limiting trade opportunities. This extensive literature review evaluated experimental studies published between 2000 and 2025 assessing pathogen reduction during Grana-type cheese production. Seven studies met the inclusion criteria and examined *Escherichia coli*, O157:H7 *Listeria monocytogenes, Salmonella Typhimurium, Staphylococcus aureus, Mycobacterium bovis, M. avium* subsp. *paratuberculosis* (MAP), and avian influenza viruses (H1N1, H5N1). Across trials, high inoculum levels declined by >4 $\log_{10}$ within 24-48 hours, primarily during curd cooking and acidification. MAP and *M. bovis* were eliminated during ripening, within 90 days, while both influenza viruses were inactivated within 30 days. Calculated F-values were used as comparative indicators of equivalence, allowing the overall reduction achieved through the production process to be compared with the standard pasteurization benchmark (72°C for 15 seconds). F-values exceeded this high-temperature short-time reference, confirming the substantial lethality of the process. Overall, evidence indicates that traditional Grana-type cheese production ensures microbiological safety at least comparable to pasteurization through the synergistic action of multiple hurdles. While European regulations currently treat ripening as an additional measure rather than an equivalent to pasteurization, our findings support reconsideration of this approach and provide a scientific basis for future regulatory evaluation.

Key words: raw-milk cheese, Grana Padano, Parmigiano Reggiano, cheese production, pasteurization process.

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Introduction

According to Regulation (EC) 853/2004, raw milk is defined as "milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment having equivalent effect" (European Commission, 2004a). In the absence of thermal processing, raw milk contains a complex microbial community, encompassing both spoilage organisms and potentially pathogenic microorganisms, which may have implications for human health (EFSA, 2015).

Cheese is one of the oldest forms of processed food, originally developed as a means to concentrate and preserve milk (Nájera *et al.,* 2021). Raw-milk cheeses are increasingly appreciated by consumers for their more intense and complex flavor profiles compared with pasteurized-milk varieties (Yoon *et al.,* 2016). Among these, Grana Padano (GP) and Parmigiano Reggiano (PR) stand out as Italy's most prominent Protected Designation of Origin (PDO) Italian hard cheeses. These extra-hard, cooked-curd cheeses are made exclusively from raw cow's milk and undergo extended maturation periods, ranging from a minimum of 9 months for GP and 12 months for PR, to 20-24 months or beyond 30 months for gold-certified PR (www.granapadano.it; www.parmigianoreggiano.com). In 2024, production reached 219,259 tonnes for GP and 163,495 tonnes for PR, accounting for approximately 74% of total PDO cheese production in Italy and generating an average annual export revenue of €734 per tonne (CLAL, 2024).

Although GP and PR differ in terms of geographical origin, regulatory standards, and certain technological practices, their core production process is fundamentally similar (Consorzio del Formaggio Grana Padano, 2025; Consorzio del Formaggio Parmigiano Reggiano, 2025) (*Supplementary Figure 1*) (Neviani *et al*., 1995; Pellegrino *et al*., 1997; Giraffa *et al*., 1998). Both cheeses are produced from raw cow's milk obtained by blending partially skimmed evening milk, naturally separated overnight, with whole milk from the following morning's milking, typically in a 1:1 ratio. The milk is transferred into traditional copper vats and heated to 32-34°C before inoculation with a natural whey starter, rich in thermophilic lactic acid bacteria. Calf rennet is then

added to initiate coagulation. Once the curd reaches the desired firmness, it is cut into small granules using a traditional tool called the "*spino*" and cooked at 53-56°C for 5 to 15 minutes under constant agitation. During this phase, the curd gradually settles at the bottom of the vat, remaining immersed in hot whey. The core temperature of the curd is maintained for several hours, facilitating further syneresis, texture firming, and microbial stress. Concurrently, pH decreases, typically reaching values between 6.0 and 5.3, depending on the microbial activity and fermentation dynamics of the whey starter (Coppola *et al.,* 2000; Malacarne *et al.,* 2006). The curd is then extracted, divided, and placed into molds for approximately 2 days, followed by immersion in a brine bath for 18-25 days. Finally, the cheeses undergo long-term maturation in controlled environments, which may last from several months to multiple years.

The combination of thermal treatment, acidification, brining, and prolonged maturation represents a paradigmatic example of the "hurdle technology" concept, in which multiple mild stress factors act synergistically to inhibit microbial growth while preserving the product's quality (Leistner and Gorris, 1995). Hurdle technology is widely applied in dairy and meat products, where preservation is achieved through combinations of mild heat, reduced water activity (Aw), acidification, natural antimicrobial compounds, and controlled atmospheres (Aaliya *et al.,* 2021). The strength of this approach lies in imposing simultaneous sublethal stresses on microorganisms, which collectively lead to inactivation through metabolic exhaustion (Leistner, 2000). In GP and PR production, this approach allows effective microbial control without resorting to intensive processing methods or synthetic additives, thus maintaining both safety and the traditional characteristics of the cheeses. From a public health perspective, the principle of equivalence of effect plays a central role in food safety legislation. This principle holds that different interventions may be deemed acceptable if they ensure the same level of protection, particularly against microbiological hazards (WTO, 1995; CAC, 1999). The concept emphasizes outcomes rather than the specific method employed and allows for alternative treatments to be adopted when they demonstrate comparable efficacy. Internationally, the Terrestrial Animal Health Code of the World Organisation for Animal Health (WOAH, 2023) recognizes that substantially different sanitary systems may provide equivalent protection, provided they are supported by scientific risk analysis. Within the European framework, Regulation (EC) 852/2004 defines an "equivalent measure" as one "capable of achieving the same objectives" (European Commission, 2004b), allowing recognition of alternative treatments if they ensure comparable safety levels. A practical example is found in Delegated Regulation (EU) 2020/687, which permits milk pasteurization *via* alternative methods as long as the effect is equivalent to the standard high-temperature short-time (HTST) treatment of 72°C for 15 seconds (*Supplementary Table 1*; European Commission, 2020). Scientific evaluation of equivalence often relies on quantitative parameters such as the F-value, which quantifies the lethal effect of thermal treatments on microbial populations and provides a framework for objective comparisons across different processes (Holdsworth and Simpson, 2015). Despite the protective effects of hurdle technology and prolonged maturation, European legislation does not formally recognize the Grana-type cheese production process as equivalent to pasteurization. This regulatory position, while precautionary, may limit export opportunities for raw-milk hard cheeses, even when scientific evidence demonstrates effective pathogen inactivation. Given this background, the present extensive literature review aims to synthesize experimental evidence published between 2000 and 2025 on the impact of the production process on the microbiological safety of GP and PR. The review focuses on quantitative reductions in microbial populations and evaluates whether the manufacturing process can achieve pathogen reduction comparable to pasteurization, based on F-value calculations and other thermal lethality metrics. By providing a comprehensive overview of both the technological and microbiological aspects of Grana-type cheese production, this study seeks to inform both regulatory considerations and risk-based assessments of traditional raw-milk hard cheeses.

Methods

Search strategy and study selection

A literature review was conducted to evaluate the microbiological effects of Grana-type cheese production. The research question was formulated as: "Can the production process of Italian hard cheeses be considered equivalent to pasteurization at 72°C for 15 seconds?".

The literature search targeted manuscripts published between 2000 and 2025 across five electronic databases: PubMed, Scopus, ScienceDirect, Web of Science, and Google Scholar. In addition, reference lists of selected articles were screened to capture additional relevant studies. The keywords and corresponding search string [("Grana Padano" OR "Parmigiano Reggiano" OR "Grana cheese" OR "Parmigiano cheese" OR "hard cheese" OR "hard cheeses")] were adapted according to the specific electronic database used. Given the focus on pathogen inactivation in raw-milk hard cheeses with extended ripening (≥9 months), similar varieties produced under comparable conditions, such as Emmental, Gruyère, and Sbrinz, were included in the search strategy. The search was submitted on July 20, 2025.

Eligibility criteria

Study selection followed a two-step process: initial screening of titles, abstracts, and keywords, followed by full-text review. Studies were included if they met all of the following criteria: i) published in English or Italian between 2000 and 2025, ii) reported detailed experimental data, including initial and final microbial loads of the inoculated target pathogens, and iii) focused on Grana-type cheeses (GP and PR) or comparable raw-milk hard cheeses. Studies without quantitative microbial data or unavailable in full text were excluded.

Data extraction and management

For each included study, relevant data were extracted independently by two reviewers (GM and AR) and cross-verified by a third reviewer (PB) to ensure consistency. Extracted information included: i) author(s) and year of publication, ii) cheese type, iii) microorganisms investigated, iv) initial inoculated load ($\log_{10}$ CFU/mL), and v) stage of production at which the microorganism was no longer detected. Data were recorded in a standardized Microsoft Excel spreadsheet to facilitate comparison across studies. Discrepancies were resolved through consensus discussions.

Quantitative assessment of pathogen inactivation

To assess the thermal equivalence of Grana-type cheese production to pasteurization, the F-value was calculated using the following relationship [Eq. 1]:

$$F\ value = D \cdot log_{10}(\frac{N_0}{N}) \quad \text{[Eq. 1]}$$

where D is the decimal reduction time at the reference temperature, N_0 is the initial microbial load in the challenge tests considered, and N is the final microbial load observed at a given stage of the production process.

The D-values at 72°C for each pathogen were obtained directly from the literature sources. When a D-value was not explicitly reported, it was derived from data available in those same studies, following the standard first-order kinetic model [Eq. 2]:

$$log_{10} D_T = log_{10} D_{ref} - \frac{(T - T_{ref})}{z} \quad \text{[Eq. 2]}$$

where D_T is the decimal reduction time at temperature T, D_{ref} is the decimal reduction time at the reference temperature T_{ref}, and z is the thermal resistance constant expressed in °C. This approach follows the Bigelow model, and it is widely used in thermal processing studies (Dhotre, 2019).

The F-values obtained from the selected studies for each microorganism were compared with the reference F-value corresponding to HTST pasteurization. When the F-value derived from the cheese production process exceeded the HTST benchmark (15 seconds at 72°C), the process was considered to provide a higher level of microbial inactivation. In other words, achieving the same reduction observed in challenge tests through thermal treatment at 72°C would require at least 15 seconds or more.

Synthesis of results

Due to substantial heterogeneity in experimental design, inoculum levels, and environmental conditions across studies, meta-analysis was deemed inappropriate. A structured narrative synthesis was performed, supported by comparative tables summarizing microbial reductions, F-values, and critical processing steps (Table 1). Studies were categorized by microorganism type and cheese variety to facilitate interpretation.

Quality control and reproducibility

All data extraction was independently verified by two reviewers (PB and AR) to ensure reproducibility, while F-value calculations were performed by a single reviewer (PB). Calculations were performed using experimental data reported in the studies included and D-values derived from the literature.

Results

Study selection and characteristics

The initial database search yielded 26,435 records. After screening titles and abstracts, 26,390 records were excluded, leaving 45 articles for full-text assessment. Of these, 38 were excluded due to lack of quantitative data, absence of full text, or non-compliance with inclusion criteria, resulting in 7 eligible studies (4 peer-reviewed articles and 3 conference papers) (Figure 1). The selected studies were conducted between 2000 and 2025, focusing on GP (n=3), PR (n=4), and Emmental (n=1) production. Despite differences in origin and certain technological practices, all cheeses shared key steps such as raw cow's milk usage, curd cooking, and extended ripening periods. The studies intentionally inoculated raw milk with high microbial loads (>4 log_{10} CFU/mL) to evaluate pathogen survival, except for *M. bovis*, which was introduced at 3 log_{10} CFU/mL. The main characteristics and microbial data of these studies are summarized in Table 1.

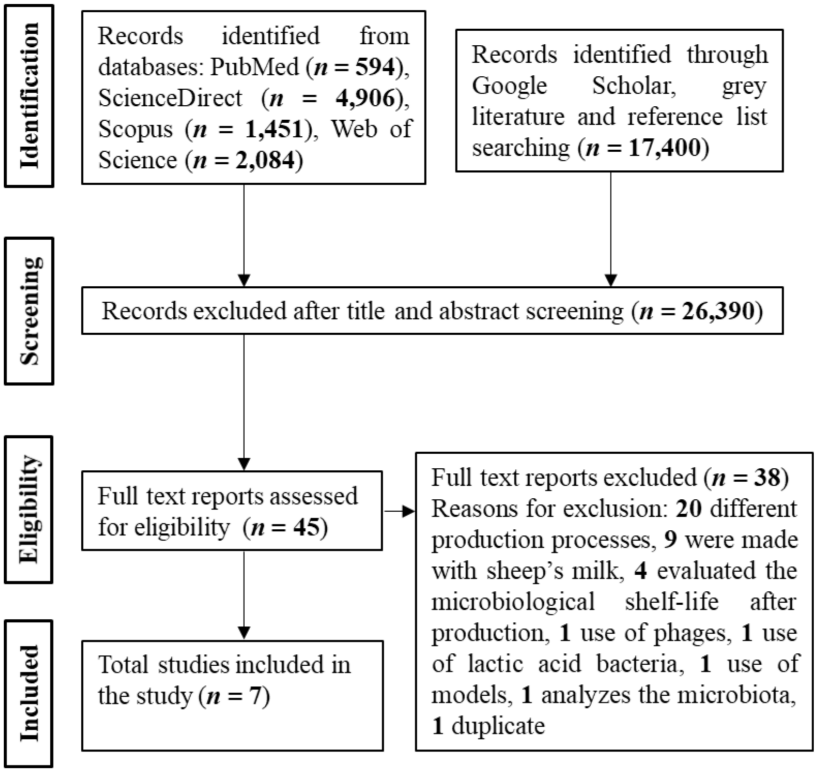

Figure 1. Flow chart summarizing the literature search of studies on the fate of pathogenic microorganisms in Grana-type cheese production.

Pathogen reduction during cheese production

Rapid and significant pathogen reductions were consistently observed during early stages of cheese production (Table 1). Panari *et al.* (2001, 2004) reported complete inactivation of *E. coli*, *L. monocytogenes*, *S.* Typhimurium, and *S. aureus* within 24 hours post-molding, corresponding to pH values of 5.2-5.4 and Aw below 0.97. The curd cooking step (53-56°C for 30-50 minutes) served as a primary critical control point, while acidification driven by lactic acid bacteria further suppressed pathogen survival. Early reductions exceeded 4 $\log_{10}$ CFU for all tested bacteria, demonstrating the synergistic effects of heat and acidification. Ercolini *et al.* (2005) highlighted the importance of temperature distribution within the curd. In GP production, the core, remaining above 50-55°C for several hours, allowed strong pathogen inactivation. Limited survival of O157:H7 was observed, while *L. monocytogenes*, *S.* Typhimurium, and *S. aureus* were rapidly reduced below detectable levels. In contrast, the outer layers cooled more rapidly, providing conditions in which some pathogens, particularly *S.* Typhimurium, could persist. Experimental inoculation combined with 3D thermal modeling confirmed that curd cooking and sustained core temperatures are necessary but not sufficient alone to guarantee complete safety.

Mycobacteria, such as *M. avium* subsp. *paratuberculosis* (MAP) and *M. bovis*, exhibited slower inactivation kinetics. Cammi *et al.* (2019) observed complete MAP elimination after 60-90 days of ripening at 18-22 , with pH stabilizing at approximately 5.3 and Aw around 0.94. Benedetti *et al.* (2003) reported that *M. bovis*, initially inoculated at 3 $\log_{10}$ CFU/mL, was fully inactivated within the first week of PR maturation, with no viable cells detected thereafter.

Spahr and Schafroth (2001) examined MAP in Emmental cheese. Inoculated milk (4-5 $\log_{10}$ CFU/mL) showed a ~2 $\log_{10}$ decline in the first 30 days, with complete inactivation in the core by day 90. Moreno *et al.* (2025) investigated avian influenza viruses (low-pathogenic H1N1 and highly pathogenic H5N1) during PR production. High viral loads (7.75 $\log_{10}$ EID_{50}/mL for H1N1; 6.75 $\log_{10}$ EID_{50}/mL for H5N1) were completely inactivated within 30 days of ripening. Key steps, including curd cooking, acidification, and prolonged ripening, lowered pH and Aw, creating an environment hostile to viral survival.

F-value calculation and equivalence assessment

To compare the Grana-type cheese production with standard pasteurization, equivalent F-values were calculated for all microorganisms reported in the reviewed studies (Table 2). Four studies (van Asselt and Zwietering, 2006; Saucier and Plamondon, 2011; Hammer *et al.,* 2015; Cui *et al.,* 2024) were used for D-value estimation because they provided experimentally determined thermal resistance data for relevant pathogens in milk or cheese matrices under controlled heating conditions.

For bacterial species such as *E. coli*, O157:H7, *S.* Typhimurium, and MAP, F-values consistently exceeded the HTST benchmark of 15 seconds, which represents the regulatory reference for effective milk pasteurization. For instance, *S.* Typhimurium showed F-values between 25.2 and 30.8 seconds, *E. coli*, O157:H7 between 35.7 and 37.9 seconds, and MAP between 28.0 and 29.6 seconds, all well above the HTST threshold, indicating that the thermal effect of production would be sufficient to achieve inactivation of these pathogens.

In contrast, certain microorganisms exhibited F-values close to or below the HTST standard. *L. monocytogenes* reached 12.4 seconds under one scenario, although alternative calculations yielded 16.5 seconds, surpassing the benchmark. *M. bovis* showed markedly low values (0.93 seconds), and avian influenza viruses (HPAIV H5N1, LPAIV H1N1) ranged from 13 to 15 seconds, consistently at or below the threshold.

Discussion and Conclusions

This review provides an overview of pathogen reduction during the production of Italian hard cheeses, specifically GP and PR. The analyzed studies consistently demonstrate that the production process, encompassing curd cooking, acidification, brining, and prolonged ripening, exerts a strong inhibitory effect on microbial populations, even under conditions of experimentally elevated contamination (Panari *et al.,* 2001, 2004; Ercolini *et al.,* 2005; Cammi *et al.,* 2019; Moreno *et al.,* 2025). Early stages of production are particularly effective in reducing the load of the main bacterial pathogens investigated in the reviewed studies. Curd cooking at 53-56°C for 30-50 minutes functions as the primary thermal stressor, achieving significant reductions in *E. coli*, *S.*

Table 1. Characteristics of eligible studies on the inactivation of pathogenic microorganisms in hard cheeses made from raw milk.

Study	Year	Cheese analyzed[a]	Microorganism[b]	Microbial load ($\log_{10}$ CFU or EID_{50}/mL)	Production stage[c]
Benedetti *et al.*	2003	GP	*M. bovis*	3.00	Beginning ripening
Cammi *et al.*	2019	PR, GP	MAP	5.19-5.28	Ripening
Ercolini *et al.*	2005	GP	O157:H7 *L. monocytogenes* *S. Typhimurium* *S. aureus*	4.57 4.86 4.67 4.45	Cooking the curd
Moreno *et al.*	2025	PR	LPAIV, H1N1 HPAIV, H5N1	7.75 6.75	Ripening
Panari *et al.*	2001 2004	PR	*E. coli* *L. monocytogenes* *S. Typhimurium* *S. aureus*	4.30 6.10 5.70 5.48	Cooking the curd
Spahr and Schafroth	2001	Emmentaler	MAP	4.00-5.00	Ripening

[a]Grana Padano, Parmigiano Reggiano; [b]*M. avium* subsp. *paratuberculosis* highly and low pathogenic avian influenza virus; [c]limit of detection of microorganism.

Typhimurium, *L. monocytogenes*, and *S. aureus*. The core of the curd, which retains higher temperatures for extended periods, ensures inactivation, while the outer layers may cool more rapidly, highlighting the importance of curd mass thermal dynamics (Pellegrino *et al.,* 1997; Ercolini *et al.,* 2005). Acidification by lactic acid bacteria rapidly lowers pH to values around 5.2-5.4, further inhibiting pathogen survival and contributing to early reductions exceeding 4 $\log_{10}$ CFU within 24-48 hours (Panari *et al.,* 2001, 2004). Resilient organisms, such as *M. bovis* and MAP, demonstrate slower inactivation kinetics and require prolonged maturation for complete elimination. Ripening induces gradual reductions in Aw and nutrient availability, while sustained low pH and microbial competition create an inhospitable environment for persistent pathogens (Spahr and Schafroth, 2001; Benedetti *et al.,* 2003; Cammi *et al.,* 2019). Similarly, avian influenza viruses (H1N1 and H5N1) are fully inactivated within 30 days of ripening, reflecting the combined effects of thermal stress, acidification, and reduced Aw (Moreno *et al.,* 2025).

The observed pathogen reduction can be interpreted through the lens of hurdle technology, which posits that microbial stability and food safety are achieved not through a single preservation factor, but via the synergistic interaction of multiple stressors (Leistner and Gorris, 1995; Roedel and Scheuer, 2007).

F-value calculations provide a quantitative framework to compare the lethality of cheese production with standard pasteurization (72°C for 15 seconds). Most bacterial pathogens in the reviewed studies exhibit F-values exceeding this benchmark, supporting the notion that traditional Grana-type cheese production achieves thermal effects at least comparable, or even exceeding, those of pasteurization in the first steps of production because of the combined effects of temperature and pH reduction. However, for highly resilient organisms such as mycobacteria, inactivation depends on the interplay of multiple processes, particularly ripening, rather than on heat and pH alone.

It is crucial to underline that when the initial inoculum is low, as in the case of *M. bovis* or HPAIV-H5N1, the observed reduction cannot be demonstrated as equivalent to HTST, since the limited starting population prevents achieving a comparable log-reduction.

European regulations maintain a precautionary approach, distinguishing between pasteurization and ripening. While ripening is recognized as a contributing factor to microbiological safety, it is not formally considered equivalent to pasteurization under EU law (European Commission, 2020). Only milk subjected to validated risk mitigation treatments, such as HTST pasteurization (72°C for 15 seconds), alone or combined with pH reduction or drying, is authorized for movement. This contrasts with assessments by Food Standards Australia New Zealand, which recognize the safety of certain raw-milk hard cheeses, including GP and PR, as equivalent to pasteurized milk products (FSANZ, 2002). The distinction reflects an evidence-based yet conservative approach, balancing traditional production practices with the need to mitigate potential public health risks.

The findings of this review align with the literature on raw-milk hard cheeses, demonstrating that extended ripening, combined with controlled thermal and acidification steps, effectively reduces pathogenic microorganisms. Also, observations from Swiss hard cheeses (*e.g.*, Emmental) highlight the role of hurdle technology and prolonged maturation in microbial safety (Spahr and Schafroth, 2001); moderate heat, a rapid pH drop (~5.3 within 24 hours), and extended ripening together contributed to pathogen elimination, emphasizing the critical importance of prolonged maturation for mycobacterial control. These data reinforce the principle that traditional processes, although non-thermal in isolation, can collectively achieve microbiological safety outcomes comparable to pasteurization. Despite certain limitations, including the small number of eligible studies and the heterogeneity of experimental designs, the available evidence provides a coherent basis for understanding microbial dynamics in Grana-type cheese production. Across all studies, the results were consistent and convergent, demonstrating substantial reductions in key pathogenic species. This consistency offers strong preliminary support for the hypothesis that traditional Grana-type cheese manufacture can achieve levels of microbial reduction comparable to pasteurization in terms of overall safety performance.

In conclusion, the accumulated evidence supports the microbiological safety of Grana-type cheeses, achieved without chemical preservatives but through the synergistic interaction of heat, acidification, osmotic stress, Aw reduction, and microbial competition. These findings provide a scientific foundation for discussions on regulatory equivalence with pasteurization. Ultimately, GP and PR represent a model of how traditional practices can ensure food safety through complex biological and technological interactions.

Table 2. F-values at 72°C equivalent to the cheese production process made from raw milk.

Microorganism[a]	D (sec)	D_{72}°C (sec)	$\log_{10}$ observed reduction (N_0/N)	F=D×$\log_{10}$ (N_0/N) (sec)	HTST equivalent (F=15 sec)[b]
S. Typhimurium	8.9[c]	5.4	4.67	25.2	> HTST
			5.70	30.8	> HTST
E. coli/O157:H7	12.8[c]	8.30	4.30	35.7	> HTST
			4.57	37.9	> HTST
L. monocytogenes	5.2[c]	2.7	4.86	12.4	na
			6.10	16.5	> HTST
MAP	12.0[d]	5.6	5.00	28	> HTST
			5.28	29.6	> HTST
M. bovis	14.5[e]	0.31	3.00	0.93	na
LPAIV, H1N1	1.9[f]	1.93	7.75	15	= HTST
HPAIV, H5N1			6.75	13	na

[a]*M. avium subsp. paratuberculosis*; Highly and low pathogenic avian influenza virus; [b]high-temperature short-time; na, not applicable because the inoculum was lower than necessary to establish the equivalence; [c]values of D_{70}°C from van Asselt and Zwietering, 2006; [d]values of D_{70}°C from Saucier and Plamondon, 2011; [e]values of $D_{62.5}$°C from Hammer *et al.*, 2015; [f]values of D_{72}°C from Cui *et al.*, 2024.

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Online supplementary material:
Supplementary Figure 1.Flow chart for Parmigiano Reggiano (PR[a]) and Grana Padano (GP[b]) cheese production.
Supplementary Table 1. Risk mitigation treatments for milk from areas subject to foot-and-mouth disease (FMV), Rift Valley fever (RFV), and lumpy skin disease (LSD) restrictions. Reproduced from: European Commission, 2020 (Annex VII, Delegated Regulation EU 2020/687).

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