



Review article

Mycobacterium avium subsp. *paratuberculosis* in food and options for intervention

Anja Buschulte^{1*} and Philipp Hammer²

¹ German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Max-Dohrn-Str. 8-10, D - 10589, Berlin, Germany

² Max Rubner-Institute (MRI), Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology, Hermann-Weigmann-Straße 1, D-24103 Kiel, Germany

**Article History:**

Received: 21-Jun-2022

Accepted: 21-Jul-2022

***Corresponding author:**

Anja Buschulte

Anja.Buschulte@bfr.bund.de**Abstract**

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Paratuberculosis in cattle and other domestic ruminants. Due to similarities in pathophysiology, there is an ongoing discussion on whether MAP may be a causative agent for Crohn's disease (CD) in humans as well. One aspect of this discussion includes the significance of food as a possible vector for transmission. The aim of this review was to collect information on the occurrence of MAP in food and on available intervention options for reduction or elimination during processing to follow precautionary principles in case a zoonotic role of MAP would be evidenced. Except for research on the occurrence and treatment of cow's milk, studies for MAP in food are rare. Investigations dealing with intervention measures are often based on very few or even single studies with a few number of test strains and repetitions. As an essential research need, the development and validation of accurate and robust detection methods for live MAP cells in food and human samples were identified. Such methods are needed to i) conduct systematic and representative surveys on the occurrence of MAP in food, ii) assess the risk of MAP transmission via food, and iii) evaluate experimental or commercial food processing for their efficacy in controlling MAP. Furthermore, for experimental setup, standardized protocols and technologies are crucial.

Keywords: *Mycobacterium paratuberculosis*, Food, Crohn's disease, Control, Intervention

Citation: Buschulte, A. and Hammer, P. *Mycobacterium avium* subsp. *paratuberculosis* in food and options for intervention. Ger. J. Microbiol. 2022. 2(2): 16-27. <https://doi.org/10.51585/gjm.2022.2.0015>

Introduction

The German Federal Institute for Risk Assessment (BfR) and the Max Rubner Institute (MRI) were asked by decree of the German Federal Ministry of Food and Agriculture (BMEL) to provide a report on the possible association between the causative agent of bovine Paratuberculosis (Johne's disease; JD) and Crohn's disease (CD) in humans with regard to the relevance of food. This article presents the results of the report with a compilation of data on the occurrence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in food, options for intervention to reduce or eliminate the pathogen during processing, and respective research needs. The occurrence and spread of MAP in the environment, in feed, in livestock, as well as animal health aspects and the pathogenesis of CD without reference to food and the diagnostic and therapy of CD were not subject to the report.

MAP has been known as the causative agent of Paratuberculosis in cattle since at least 1894 (Johne

and Frothingham, 1895). Besides the occurrence in cattle and domestic ruminants like goats and sheep, numerous wildlife animal species, e.g., deer (farmed especially), rabbits, wood mice, foxes, stoats, badgers, but also birds are affected by this disease (Gould et al., 2005). Paratuberculosis is on the World Organization for Animal Health (OIE) list of notifiable animal diseases and is endemic in most countries of the world. As a chronic, progressive and untreatable disease, Paratuberculosis is of particular economic importance in ruminant livestock as it causes major losses.

Due to similarities in pathophysiology, there is since more than a century an ongoing discussion on whether MAP may be a causative agent for CD in humans as well (Dalziel, 1913). One aspect of this discussion includes the significance of food as a possible vector for transmission. Regarding a link between MAP and CD, the first food item coming into focus for a possible transmission was pasteurized milk, based on studies showing inadequate heat inactivation (Chiodini and

Hermon-Taylor, 1993). This may explain the up-to-date unbalanced number of scientific reports regarding the occurrence, inactivation, and survival of MAP in milk and milk products compared to other types of food addressed here.

One objective of this review was to collect information on the occurrence of MAP in food, particularly on studies in which viable MAPs were detected, to identify a possible potential for transmission. More comprehensive reviews covering the issues of presence in food and respective detection methods for viable MAP and MAP DNA were published, for example, by Eltholth et al. (2009), the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (NACMCF, 2010), Waddell et al. (2016b), and recently by Foddai and Grant (2020), and Grant (2020). Another objective of this review was to identify intervention measures to reduce or eliminate MAP in food processing, as those measures would play an important role if a zoonotic potential for MAP would be confirmed. In addition, research needs regarding intervention options and detection methods are addressed.

Materials and methods

The literature search was carried out in PubMed, Google Scholar, Scopus, and public databases (last search March 2022). The focus was on intervention options for control of MAP in food processing, the occurrence of MAP in food, and respective detection methods. Therefore, unless otherwise noted, only studies reporting the occurrence/behavior/detection of viable MAP were used for this review to assess the potential for foodborne transmission. Literature that focused on the occurrence and spread of MAP in the environment (including the role of Amoebae), in feed, in livestock as well as animal health aspects and the pathogenesis of CD without reference to food, as well as the diagnostics and therapy of the disease only, were excluded. The remaining articles, news, or information were evaluated regarding their relevance to the topic of this article by examining the abstracts, texts, and keywords. After that, a full-text search was conducted to check for relevant secondary (cited) references.

Occurrence in food and options for intervention

Regarding the occurrence of MAP in food, currently, no systematic, representative, and comparable surveys are available, which is mainly due to the lack of a standardized, internationally accepted detection method of viable MAP cells and inconsistent study designs. In general, regarding the occurrence and behavior of MAP in food, it must be noted that the organism is unable to multiply outside a host organism due to its dependency on mycobactin as a growth factor. Accordingly, along the food chain in most cases, the numbers of MAP cells will not increase (except for example, during cheese-making – see below) but will probably rather decrease due to dilution and inactivation.

Milk and milk products

Fluid milk

The presence of MAP in raw cow milk is well documented (Bharathy et al., 2017). High loads are thought

to be due to fecal contamination (super-shedders may contribute up to 10⁸ colony forming units (CFU)/g feces) and poor milking hygiene, whereas secretory input was assumed to be low (Nauta and van der Giessen, 1998). More recent studies, however, conclude that MAP does not primarily enter the milk through fecal contamination but rather is shed directly into the milk within the udder (Gerrard et al., 2018). As far as quantitative data were generated, authors reported low numbers in a range between 1 CFU/50 mL and 7 CFU/mL based on samples of quarter milk, composite milk, or bulk tank milk (Sweeney et al., 1992; Hammer, 1999; Grant et al., 2002; O'Reilly et al., 2004; Foddai and Grant, 2017).

Except for cattle, no quantitative data are available for other milk-producing animals. It can be assumed that for industrial milk processing considerable dilution of the input will occur during milk collection. The dilution will depend on the number of shedders in a herd, their individual level of shedding, and the herd size. Further dilution will occur during milk collection in the respective truck tanks, and at the dairy depending on the size of the tanks and storage silos (Cerf et al., 2007; Boulais et al., 2011). For artisanal milk production and processing, only dilution at the farm level applies.

Although the performance criteria for high-temperature short-time treatment (HTST, 72°C for 15s) require a 5 Log₁₀ reduction of vegetative bacterial cells (CAC, 2009), MAP-positive samples were reported in the range of 1 to 10% in milk that had undergone this form of heat treatment (Grant et al., 2002; Ayele et al., 2005; Ellingson et al., 2005; Paolicchi et al., 2005; Gerrard et al., 2018). Quantification was not performed in these studies; the results are related to retail packs found positive. Presence in retail milk heated at even higher temperatures was reported by Paolicchi et al. (2005) (138°C, 30s) and McDonald et al. (2005) (78°C, 15s). Findings of MAP in heat-treated milk at retail are hard to explain, taking the efficacy of HTST as demanded in the Codex standard as given. Gerrard et al. (2018) suggested that MAP in raw milk is mainly found in somatic cells and that the survival of low levels of MAP during the pasteurization of naturally infected milk may be due to their intracellular location. The authors assume that the somatic cells provide sufficient protection to prevent complete inactivation of the bacteria by heat treatment, although further experiments are needed to confirm this hypothesis.

The formation of spores or a spore-like morphotype in MAP cells could serve as another possible explanation for heat resistance (Lamont et al., 2012). However, spore formation in Mycobacteria, in general, is discussed controversially (Traag et al., 2010; Abecasis et al., 2013). In the study of Lamont et al. (2012), strong evidence for the presence of spores was presented, and the authors applied several tests to exclude contamination in the long-term cultures of MAP. However, the following statements require further explanation: i) MAP was grown for sporulation on Arret-Kirshbaum agar without mycobactin within 72 h, ii)

MAP grew on potato-extract agar with mycobactin within 2 weeks, iii) spores did survive heat treatment at 70°C but not at 90°C. Speed of growth on both agars is quite untypical for MAP, even at the elevated incubation temperature of 39°C. Growth on agar without the addition of mycobactin can hardly be explained, as well as the low heat resistance of the spores.

According to a modeling approach, the presence of survivors in retail packs would generally be feasible (Cerf et al., 2007; Boulais et al., 2011). By application of Monte Carlo simulations, the authors estimated contamination of milk with MAP at the farm level, during milk collection, and at the dairy level after pasteurization. Many variables such as the number and infection status of herds, the intrinsic or fecal contamination of milk, the MAP concentration in bulk milk in the collection and dairy tanks, and the efficacy of pasteurization were considered. For the annual detection rate in 50 mL samples of pasteurized milk, the most probable scenario predicted 0.12% positive samples. This is much lower than reported in the above-cited studies on detection rates for milk at the retail level. According to Cerf et al. (2007), possible explanations for this discrepancy may be artifacts due to i) intra-laboratory contamination, ii) leakage in pasteurizer plates (which could be detected by determination of numbers of Enterobacteriaceae – a test which was not used by the authors reporting contamination of retail milk with MAP), and iii) reduced pasteurizer efficacy due to prolonged operation.

Regarding experimental setup for determination of heat inactivation of MAP in terms of standardization, the same as for the detection methods apply - none available (Condrón et al., 2015; Robertson et al., 2017). Laboratory methods normally cannot mimic the situation in practice. For the application of HTST in a pilot plant, a sufficiently turbulent flow, the application of overpressure in the product flow (to avoid the influence of plate leakage), a minimum residence time (to ensure that the fastest particle in the product flow is heated adequately) and a back-flow valve (for control of insufficient heating time or temperature – which is mandatory in Germany) are necessary (Condrón et al., 2015; Robertson et al., 2017; Mullan, 2019). Considering studies performed with pilot plants covering at least some of these necessities, an inactivation of MAP during HTST treatment of >4 to >6 Log₁₀ is likely to be achieved (Robertson et al., 2017; Mullan, 2019).

In dairy practice, bacteria are also removed by physical methods. Microfiltration will remove 2-3 Log₁₀ of the initial load (Hoffmann et al., 2006). Centrifugal cleaning and bactofugation will lead to a reduction at levels of 50-60% and >99%, respectively (Alfa Laval personal communication). This should apply to MAP as well. Only one study for MAP dealing with simulated bactofugation and filtration was found (Grant et al., 2005a). The authors used a laboratory centrifuge and membrane filters, achieving 95–99.9% removal of MAP cells for each technique. As continuous centrifugation with disc stack centrifuges and deep-bed filtration are applied instead in dairy practice, re-

search using adequate technology is needed. Another physical factor, which might lead to inactivation due to shear forces, is homogenization. For MAP, (Grant et al., 2005b) reported a “beneficial effect” at a holding time of 25s for in-hold homogenization of milk at 2.500 lb/in² (corresponds to 1.72 MPa) at 72.5° and 76.5°C. Hammer et al. (2014), however, did not find enhancement of inactivation during up-stream, in-hold, and down-stream homogenization at 20 MPa at temperatures of 72° and 68°C and a holding time of 28s. Further options for the control of MAP in fluid milk, which, however, currently have no relevance for practice are i) UV-irradiation, ii) high-pressure treatment, iii) pulsed electric fields, and iv) γ -irradiation. UV-irradiation of 1.000 mJ/mL led to a 1 Log₁₀ reduction at maximum (Altic et al., 2007; Donaghy et al., 2009). High-pressure treatment at 5.000 bar for 10 min achieved a 4-6 Log₁₀ reduction (López-Pedemonte et al., 2006; Donaghy et al., 2007). A 5.9 Log₁₀ reduction was observed by application of pulsed electric fields at 2.5000 pulses of 30 kV/cm for 8.5 min (Rowan et al., 2001). Highly effective was γ -irradiation at 1.2 kGy/h resulting in complete inactivation of up to 10⁸ CFU/mL of two MAP strains (Stabel et al., 2001).

Results for the detection of MAP from raw milk, heat-treated milk, and heat-inactivation experiments are hardly comparable. Up to date, no methods, especially for detection of viable MAP-laid down in an internationally accepted standard are available (for a comprehensive review of methods, see (National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2010)). This means that results for cultural detection may be biased in terms of, for example, the method applied, sample size, decontamination protocol, incubation time, and media used. The same problem regarding standardization is due to molecular or phage-based methods (Robertson et al., 2017; Butot et al., 2019; Mullan, 2019). One of the first efforts to solve this problem was performed by launching a ring trial involving four participating laboratories in 2008 (Donaghy et al., 2008).

Cultural and molecular methods were applied to detect MAP in raw milk artificially contaminated with Map-containing feces. The overall result was that cultural methods were less sensitive than molecular methods; however, laying down ISO 16140-2 (ISO, 2016a) as a benchmark for evaluation of alternative microbiological methods – should be applicable though reference methods are not available - the number of matrices, test strains, and participating laboratories was too low. Butot et al. (2019) published a more recent study addressing further requirements of ISO 16140-2. The main deviations from the requirements of the ISO standard were the number of strains used for testing inclusivity and exclusivity (4 instead of 50 were used for inclusivity; none instead of 30 for exclusivity) and the number of participating laboratories (2 instead of 10). Cultural, molecular, and phage assay-based methods were investigated for the detection of MAP in raw milk, heat-treated milk, and milk powder. In terms of sensitivity, among 432 true positive

samples, 94% were correctly assigned by IS900 qPCR, 83% by culture, 76% by f57 qPCR, and 40% by a peptide-mediated magnetic separation (PMS) phage-based assay. Correct identification out of 720 positive and negative samples (trueness) was achieved for 93% by IS900 qPCR, 89% by culture, 85% by f57 qPCR, and 49% by the PMS-phage. Though cultural methods are still the gold standard for the detection of viable MAP (Donaghy et al., 2008; Foddai and Grant, 2017; Robertson et al., 2017), a major disadvantage is the incubation times required to obtain either positive or negative results. A recent study showed a pathway for improvement of cultural methods by application of a factorial design and response surface methodology approach (Dane et al., 2022).

Phage assays, in addition to being enforced by PMS, as developed and improved for more than a decade by the working group of the Queen's University Belfast (QUB) (for example, Foddai et al. (2010); Foddai and Grant (2017)) would provide an alternative, though the performance of the method available in 2017 was low compared to cultural methods and PCR as proved in a ring-trial (Butot et al., 2019). A recent publication of this group concerning further development of the phage assay (Foddai and Grant, 2020) sounds promising in terms of speed, simplification, and standardization of the test approach. However, the authors acknowledged that for preceding tests, technology transfer to other laboratories needed considerable training and troubleshooting by the QUB team. So, the performance of the new development in terms of general and easy applicability needs to be shown and validated according to ISO 16140-2 (ISO, 2016b).

Milk products

Cheese: As MAP has been detected several times in raw milk and even in pasteurized milk from cattle, sheep, and goats (see above), its presence in cheese can be expected. Several studies based on the application of PCR or qPCR methods show respective evidence (Clark et al., 2006; Stephan et al., 2007; Albuquerque et al., 2019); however, reports based on cultural methods are rare. Cultural detection of MAP was achieved in 2 of 42 samples of Greek Feta-cheese made from pasteurized milk (Ikonomopoulos et al., 2005). Milk for production was pasteurized at 72-74°C for 15s. At the end of production, the pH of the cheeses was below 4.5, and the content of salt was $\geq 2\%$. In the same study, several cheese types made in the Czech Republic produced from pasteurized cow's milk were tested. MAP was detected in one out of 23 samples of hard cheese. Unfortunately, no information on ripening time was given. At the time of testing, the range of pH was 5.4-5.9, and the content of salt was 1-1.2%.

In a study from Scotland, 25 samples of artisanal cheese were tested (Williams and Withers, 2010). The cheeses were produced with either raw or pasteurized milk from different species and covered all types from soft to hard cheese. By cultural examination, MAP

was isolated from six kinds of cheese; all of them were ripened >3 months. A similar heterogeneous collection of cheese was tested in Cyprus with a phage assay and conventional culture methods. None of the tested 28 samples showed a positive result (Botsaris et al., 2010). Artisanal cheese from raw goats and sheep milk from Italy was tested by culture. MAP was not detected in 25 samples of sheep's milk, whereas one of seven samples of goat's milk was positive (soft cheese with a pH of 5.5) (Galiero et al., 2016). Testing of 30 samples of semi-hard cheese in Brazil (Coalho) from retail showed one positive result by culture and three positive results by IS900-based PCR (Faria et al., 2014). No details for the processing history of the respective samples were given.

During cheese production, the amount of MAP will be influenced by a combination of the following factors, which ideally work together in the sense of a hurdle concept: heat treatment of the cheese milk, pH, starter cultures, burning, salt content, and ripening time. However, it must be noted that at the beginning of the cheese production process, a concentration of MAP in the curd takes place (Donaghy et al., 2004; Badr, 2011). D-values (time needed under defined conditions – i.e., temperature, aw-value, pH, storage time – to reduce the initial colony count of a microorganism by 1 Log_{10} step), not related to a single factor, was reported in older studies as follows for i) fresh cheese: 36.5-59.9 days (Sung and Collins, 2000), ii) Emmentaler: 27.8 days and Tilsiter: 45.5 days (Spahr and Schafroth, 2001), iii) Cheddar: 90-120 days (Donaghy et al., 2004), iv) Danish blue and Gouda: 39.6 and 22.8 days respectively (Donaghy personal communication). The inactivation of several strains of MAP during the production of Parmigiano Reggiano and Grana Padano was investigated more recently (Cammi et al., 2019). Sampling was performed over a period of 12 months (months 2, 3, 4, 5, 6, 8, 10, 12), the aw-value was 0.90, and the pH was 5.3 at the end of ripening. No information on salt content is provided. After three months of ripening, all strains were no longer detectable. For Lighvan cheese, an Iranian semi-hard raw milk cheese produced from a mixture of ewes and goats' milk, Hanifian (2020) reported survival of MAP up to 10 months of storage as detected by cultural methods.

The cheeses were produced with three MAP strains (one clinical strain each from sheep and goats' origin and a reference strain) added to the cheese milk at two different inocula (2 and 3 log cell/ml). D-values for the clinical strains at an initial count of 10^3 CFU/ml of cheese milk were 109-112 days. After the 5th day of processing, all cheeses displayed a salt content of $>4\%$, moisture $<63\%$, and a pH of around 4.5. In addition to the mentioned factors above, Badr (2011) tested γ -irradiation during the production of fresh cheese also. Reductions of the test strain by 3 Log_{10} at 1 kGy, 5 Log_{10} at 1.5 kGy, and 7 Log_{10} at 2 kGy were achieved. Presently γ -irradiation is not allowed to be used in the European Union during cheese production.

Table 1: Research needs and data requirements (extracted/summarized from the above text without reference to distinct papers, only interventions already applied in practice regarded)

Food commodity	Options for intervention	Estimated efficacy	Database sufficiency	Remarks/research needs
Milk and milk products				
Fluid milk	HTST*	4-6 log ₁₀ reduction	Yes	Several studies utilizing adequate technology available
	Filtration	2-3 log ₁₀ reduction	No	only one study, laboratory filter was utilized; sufficient number of studies using deep-bed filtration
	Centrifugation	2-3 log ₁₀ reduction	No	Only one study, laboratory centrifuge was utilized; sufficient number of studies using stack centrifuges
	Homogenization	Not clear	No	Only two studies, one supporting and one denying efficacy; more studies on shear forces needed
Cheese	Hurdle concept	D-values 23-120 days, strongly depending on cheese type	No	HTST (if applied) + burning + competing starter culture (incl. bacteriocin production) + decrease of pH-value + decrease of aw-value + storage time, only six studies, all for different types of cheese; more studies covering aspects of cheese-making to develop improved database
Yoghurt and similar products	Hurdle concept decrease of pH-value <4	0.1-3.8 log ₁₀ reduction, strongly depending on combination of pH-value and starter culture	No	HTST (if applied) + decrease of pH-value + competing starter culture (incl. bacteriocin production), only three studies; more studies covering aspects of fermented fresh produce to develop improved database needed
Dried milk	Hurdle concept	No data	No	HTST + concentration + spray drying may lead to additive efficacy, input of ingredients for infant formula unclear; whole field needs to be investigated
Meat and meat products				
Meat for direct consumption	Heat treatment (cooking/frying)	D70°C-value 12-13s; log ₁₀ -reduction 2-7, depending on size and cooking time	No	Only two studies, done for ground beef; for marination and similar no data; studies on all aspects of (dish) meat preparations needed
Processed raw meat products	Hurdle concept decrease of pH-value	Up to 1.2 log ₁₀ reduction	No	One study only; whole field needs to be investigated Thermally processed meat products thermal treatment no data no i.e. heat-treated sausages; whole field needs to be investigated
Vegetables				
Tomatoes	No fertilization with manure or irrigation water containing MAP	Unknown	No	Only one study; whole field needs to be investigated

*HTST = high-temperature short time treatment

Yogurt and similar products

The reduction of MAP in these products is influenced by pH and the influence of starter cultures. Production of yogurt from milk inoculated with MAP before adding the starter cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* resulted in a final pH of 4.5 and no reduction of MAP count after six weeks of storage (Van Brandt et al., 2011). In the same study, five different fermented milk products (one yogurt and four probiotic milk drinks) were produced with one or mixtures of different probiotic starter cultures (Bifidobacteria and Lactobacilli) were obtained from retail sale and afterward inoculated with MAP. After storage at 6°C, with a pH of around 4.0 for six weeks, D-values of 16-36 days were determined. Interestingly depending on the starter culture present in the product and the tested MAP strain, a higher inactivation of 1.2 up to ≥ 3.8 log units was observed in these products.

Klanicova et al. (2012) investigated the behavior of MAP during the production of yogurt, sour milk, and Kefir. Two test strains were used at initial counts of 10^6 CFU/mL. Yogurts made with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* or *S. thermophilus* only reached pH values of 5.9 or 4.4 after fermentation for 18 h. For the production of sour milk, *L. helveticus* was used, resulting in a pH of 3.5, and for Kefir, a mixture of 7 starter cultures, some of them producing bacteriocins, led to a final pH of 4.1 after 18 h. After six weeks of storage, a reduction of MAP to a non-detectable level was observed for sour milk. For both yogurts, 0.1-0.5 Log₁₀ reduction was achieved, whereas, for Kefir, 1.9-2.3 log reduction was obtained, possibly due to the lower pH. The authors confirm that MAP can survive a pH reduction, but longer exposure to a pH below 4 in these products seems to contribute to inactivation. They, therefore, suggest that probiotic cultures that can lower the pH below 4 during fermentation will achieve better inactivation of MAP in soured milk products. From these data, the conclusion can be drawn that for substantial reduction of MAP a pH below 4 should be reached.

Kralik et al. (2018) tested the efficacy of bacteriocin-producing starter cultures on three strains of MAP *in-vitro* in the same laboratory. If the compounds enterocin and plantaricin were produced by *Lactobacillus* spp. more than 2 Log₁₀ reductions were achieved, compared to less than 1 Log₁₀ reduction if they originated from *Enterococcus* spp. Nisin from *Lactococcus* spp. led to a reduction of less than 1 Log₁₀ too. Interestingly, seven of the starter cultures tested here were also used during the production of Kefir in the earlier study without any effect on MAP. Further research on this discrepancy is needed, especially in terms of the characterization of bacteriocin-producing starter cultures for their suitability in controlling MAP.

Dried milk

Only a few data are published on the occurrence of MAP in products for human consumption containing dried milk. Negative results based on culture were ob-

tained for 122 samples of infant formula (12 producers from nine countries) collected in Australia. Among them, four showed positive results based on IS900 PCR (Acharya et al., 2017). A group of 51 samples of infant formula (10 producers from seven countries) collected in the Czech Republic was tested twice (Hruska et al., 2011, 2012).

PCR based on IS900 and qPCR based on f57 fragments yielded 25 and 18 positive results, respectively. A positive result from culture was not verified by repeated testing. In the second round of testing 18 samples showed positive results with f57 qPCR in a range of 48 to 32,500 cells/g again. Botsaris et al. (2016), who tested 32 samples from 10 different producers collected in Cyprus, performed a similar study. The authors used a phage assay with subsequent PCR on material from the plaques, which indicates the presence of viable MAP. In addition, the cultural examination was applied. MAP was detected in three samples with both methods. According to the plaque counts, numbers of 10 to <100 CFU/g were estimated. In a survey performed in the USA, 68 samples of infant formulas from 18 countries, representing 40 brands, were tested by peptide-mediated separation followed by a phage amplification assay (Grant et al., 2014). The detection method was based on Foddai et al. (2010). Viable MAP was detected in 44.1% of the samples in numbers of 4-668 plaque-forming unit (PFU)/50 mL. In addition, in two soy-based products, MAP was detected. The authors noted that “those provocative findings need further validation” and that “those studies are in progress”. A subsequent publication on this issue could not be found.

With regard to an entry of MAP via milk powder as an ingredient of infant formula, positive results are difficult to explain. The combined effects of dilution during milk collection, inactivation during pasteurization, concentration, and spray drying should result in a considerable reduction (Robertson et al., 2017; Mullan, 2019). Although the dry matter content is increased during concentration, a starting temperature of at least 73°C during the multiple-step process should also reduce bacterial numbers (Azzaro-Pantel et al., 2022). Spray drying, even with hot air, is regarded to be of minor efficacy due to cooling by evaporation and other factors (Lang and Sant’Ana, 2021). The presence of a cultivable MAP in a finished product seems to be rather unlikely in this context. However, the composition of infant formula can be quite complex, containing up to 30-50 ingredients (Lang and Sant’Ana, 2021).

Besides milk powder, various ingredients can be included that cannot be heat-treated to achieve bacterial inactivation, such as vitamins, individual amino acids, etc. (reviewed by Kent et al. (2015)). In practice, these components are added in a dry mix process, wet mix process, or a combination of both, and the absence of MAP would be crucial for each compound. Presently, no information is available on the MAP load of these ingredients. Research in cooperation with industries will be necessary to identify critical ingredients with regard to the input of MAP.

Meat and meat products

Comparable to the situation in milk, MAP can be present in or on the meat and organs of slaughtered animals. Regarding detection methods, the same problems as for milk and milk products are due. According to European law, clinically ill animals must not be slaughtered for human consumption; however, cattle without clinical symptoms may show disseminated infection with MAP (Antognoli et al., 2008).

Cross-contamination at the slaughterhouse can occur as well (Wells et al., 2009). Detection of MAP in beef without providing quantitative data was reported by several authors (Rossiter and Henning, 2001; Alonso-Hearn et al., 2009; Elze, 2009; Wells et al., 2009; Gill et al., 2011; Okura et al., 2011; Chaubey et al., 2017). Besides qualitative detection in beef, Mutharia et al. (2010) reported the presence of MAP in lymph nodes associated with muscles at levels of 200 CFU/g. In another study, MAP was detected in the meat of a sick cow at a level of 60 CFU/g, in meat from a subclinically ill sheep at 8 CFU/g, and in meat from 37 clinically ill sheep at levels of 50 ± 8 CFU/g (Redd-cliff et al., 2010). The reported counts are most likely underestimated, as the samples were chemically decontaminated in advance.

During meat processing, MAP can enter the production chain through individual carcasses or parts thereof. Mixing this meat with that of healthy animals, e.g., during the production of sausages or minced meat, can lead to dilution and spread on a large scale. Two studies investigated the occurrence of MAP in minced meat. In the first, 200 samples from retail in the USA were tested for MAP by qPCR and culture without positive results (Jaravata et al., 2007). In the second investigation in Italy, 140 samples were obtained directly from a processing plant and also examined by qPCR (all samples negative) and cultivation in broth (two samples positive) (Savi et al., 2015). Another two studies dealt with the inactivation of MAP in minced meat. Saucier and Plamondon (2011) contaminated portions of 25 g of ground beef with 10^7 CFU/g of MAP. These portions were sealed in plastic bags and pressed to a layer of 2 mm. Heating was performed in a water bath at temperatures of 55, 60, 65, and 70°C until a 5 Log₁₀ reduction was achieved. From the necessary heating times, D-values were calculated. At 70°C, the D-values were 12-13s resulting in z-values of 5.6-5.7°C. The experimental design can be questioned as ground beef usually is cooked by frying. However, using these results for the preparation of meals like “chili con carne” or “sauce Bolognese” could be suitable.

For the second study, lean ground beef sterilized by γ -irradiation was used (Hammer et al., 2013). Three strains of MAP were used to produce contaminated Hamburger patties of 50 and 70 g, thickness 1 and 0.7 cm, respectively. Frying was performed in a pan adjusted to 177°C (temperature requirement of a global fast-food supplier). Temperatures during cooking were recorded by seven thermocouples placed at different locations within the patties. A reduction of more than 5 Log₁₀ of MAP was achieved for all 50 g patties after

cooking (including 1 flip) for 5 or 6 min, compared to more than 4 Log₁₀ reductions for all 70 g patties cooked for 6 min and 2 to 7 Log₁₀ reduction after 5 min of cooking. The distribution of inactivation efficacy in 70 g patties cooked for 5 min was possibly due to the wide variation of temperatures achieved during the heating process recorded by the different thermocouples. This was most likely due to the non-homogeneous nature of the beef patties.

Regarding meat products other than minced meat, a series of publications by a laboratory from the Czech Republic are available. MAP was detected by qPCR in beef, pork, and chicken (with and without bones), ham, and pork sausages from retail (Klanicova et al., 2011). In a subsequent study, testing performed again only by qPCR, MAP was detected in liver dumplings, sausages, and Teewurst (beef, pork, or mixed) (Lorencova et al., 2014). It should be questioned whether positive results for products originating from pork and chicken could be due to cross-contamination during processing or retailing as, to date, these animals have not been recognized as hosts for MAP. The inactivation of MAP during the production of dry fermented sausage (DFS) and Teewurst was tested by the same laboratory (Lorencova et al., 2019). Two MAP strains were used at initial counts of about 2.5×10^4 CFU/g. No reduction was observed during the three-day production period for Teewurst. After four weeks storage, the final pH was 4.6 and aw-value was 0.92, which resulted in a reduction of MAP by 0.8-1.2 Log₁₀ cycles. The production time for DFS was 13 days, with no MAP detected on and after day 7. At that point of time, the pH was 4.96 and aw-value was 0.95. Compared to milk products, a higher pH seems to be effective in MAP reduction, possibly augmented by decrease of aw-value.

Miscellaneous

Water

Viable MAP has been detected by culture in surface water (lakes, rivers, catchment areas) and drinking water (Aboagye and Rowe, 2011; Gill et al., 2011; Chaubey et al., 2017; Sousa et al., 2021). In terms of potable water preparation, different applications of chlorination were investigated. The most effective treatment (inactivation achieved: 2.82 Log₁₀) was reached at a chlorine concentration of 2 μ g/mL for a duration of 30 min (Whan et al., 2001). For mycobacteria of the *Mycobacterium avium*-complex (MAC), it was shown that typical decontamination of potable water by irradiation with ultraviolet light resulted in a 4 Log₁₀ reduction (Hayes et al., 2008).

Vegetables

The presence of MAP on or in plants for food production cannot be excluded if contaminated surface water is used for irrigation or if manure from infected animals is used for fertilization. In this context, the NACMCF (2010) concluded that because MAP can survive in cattle feces, water, and soil and is present in many wild animals, farm runoff could potentially contaminate irrigation water that comes into contact with fruits and vegetables. No respective publications were found, how-

ever. The issue was addressed in a study where tomatoes were grown in contaminated soil under different experimental conditions (Kaevska et al., 2014). The soil was contaminated directly with cultures of MAP or with feces from an infected cow. Samples of stems, leaves, pollen, and fruits as far as available were collected at different time intervals and tested by culture and qPCR. Many samples showed positive PCR results, whereas only one sample from a stem after four weeks of growth was positive by culture.

Relevance of MAP in food for human health

Crohn's disease (CD) is a human inflammatory bowel disease (IBD) that can occur in any part of the gastrointestinal tract (Friedman and Blumberg, 2020). The acute or chronic phase, relapsing or recurrent course of the disease can severely impair the quality of life for patients. The etiology of the disease is still unknown; an autoimmune disease, a genetic disposition, and an infectious cause are discussed (McNees et al., 2015). Genetic and environmental factors appear to be involved in both the etiology of CD and immune dysregulation (Patel and Shah, 2011).

Due to the similarity of the pathognomonic changes in human CD and JD in cattle, the hypothesis came up that MAP could be causally related to CD. The detection of MAP in Crohn's patients is another reason for this hypothesis. A connection between MAP and CD has therefore been discussed since 1913 (Dalziel, 1913). A correlation between the increase in incidences of CD and JD over the past 100 years has indirectly contributed to this discussion. Because of the high importance of CD, numerous studies have therefore been carried out in recent decades to investigate the role of MAP in the development of CD.

Liverani et al. (2014) compared and evaluated the evidence for and against MAP involvement in the etiology of CD. They concluded that the available data do not prove that MAP is the causative agent of CD, but a certain degree of involvement of this bacterium in the physio-pathological steps of the disease seems justified. According to the authors, the literature contains conflicting and contradictory evidence for this association, with a lack of uniformity in the materials and methods used for MAP detection and inappropriate parameters for selecting patients and controls. Variability in the quality and quantity of samples analyzed and possible contamination during sample collection, transport, and/or preparation further biased the studies. Waddell et al. (2015) performed a meta-analysis on the zoonotic potential of MAP and its possible association with CD, evaluating 108 relevant studies. They revealed a significant positive association between JD and CD (odds ratio (OR) range 4.26-8.44). Great variability was found with regard to the selection of patient and control groups, sample selection, detection methods, and epidemiological level of evidence. Therefore, and due to the existing knowledge gaps, they judged the available evidence as not strong enough to assess the potential public health impact of exposure to MAP.

In a review, Robertson et al. (2017) addressed a

possible link between MAP and CD and concluded that a microbial contribution to the complex etiology of CD could not be ruled out; however, no causal link has been established yet. Regarding the more specific question of whether MAP in pasteurized milk poses a risk to food safety, the authors stated that the weight of evidence indicates that commercial pasteurization of milk substantially inactivates MAP, and levels of consumer exposure via dairy products would be very low. In a recent publication, Agrawal et al. (2021) addressed common questions about the contribution of MAP to the pathophysiology of CD. They reviewed the available information and evidence from different disciplines, including microbiology and veterinary medicine. The conclusion was that although much progress has been made in the evaluation of the links between MAP and CD, the true behavior of MAP in humans has not been fully understood or elucidated. They further concluded that simple microbe-host interaction can probably be excluded, however, MAP can cause dysbiosis of the gut microbiome which may lead to inflammatory cascades. "Infection" does not necessarily mean disease but may lead to colonization, persistence, or latency in this concern.

In summary, numerous scientific studies and publications have investigated possible causal relationships between MAP and CD. The results are ambiguous and partly contradictory. The studies themselves are hardly comparable because they differ in methodology and design. Scientific evidence proving that the intake of MAP via food in humans is causally involved in the development of CD in humans is, therefore, to be regarded as insufficient at present.

Research needs

For all issues addressed above, common and essential research needs development of accurate and robust detection methods for viable MAP cells in food and human samples were identified (Robertson et al., 2017; Mullan, 2019; Agrawal et al., 2021). These methods should be evaluated according to internationally accepted standards, i.e., ISO 16140 (ISO, 2016a). Although phage-based methods are a promising approach, the current application is not yet validated as an international standard and, therefore, is not ready for routine use. Standardized methods are crucial to obtain valid, reliable data on the occurrence of MAP in food, e.g., in the context of systematic and representative surveys, as well as to assess the risk of MAP transmission via food and to evaluate experimental or commercial food process measures on their efficacy in controlling MAP. This is particularly important if a zoonotic potential of MAP becomes evident. These considerations are supported by the results of Waddell et al. (2016a). They point out that differences in the laboratory protocols of the various studies are an important source of heterogeneity.

Also, Condron et al. (2015) and Robertson et al. (2017) emphasize that standardized protocols and technologies should be used for experimental setups to allow the comparison of results. Waddell et al. (2016a)

furthermore noted that only a few studies provided results estimating the average concentrations in contaminated samples, but this information is needed to develop a quantitative human exposure assessment model that could help interpret the relative importance of the different sources of MAP. Besides, fluid milk, studies on the occurrence and control of MAP in food are rare and sometimes utilize an inappropriate number of strains and repetitions. The whole field of seafood is not covered presently (Waddell et al., 2016b). In addition, the lack of standardized test protocols and detection methods makes comparisons difficult and often impossible, which is true for the data for fluid milk as well. An interesting aspect for further research would be the influence of pH in combination with factors increasing or decreasing its efficacy in any fermentation process.

Conclusion

In general, effective control and eradication of Paratuberculosis in ruminant livestock will reduce the risk of human exposure to MAP to levels causing no problems (NACMCF, 2010; Waddell et al., 2016b). However, if control of MAP during food production would become necessary due to a proven link to CD, several options for intervention are available (Table 1). Unfortunately, all data, except for heat inactivation in fluid milk, are based on very few or even single studies with a few test strains and repetitions. Therefore, substantial efforts to standardize testing protocols for intervention measures in the laboratories and practice, as well as detection methods of viable MAP cells, are required.

Furthermore, surveys on the occurrence of MAP in food are necessary to determine the dimension for the risk of transmission via food if the organism ever will be regarded as a human pathogen. This is particularly essential for exposure assessment in the context of risk assessment, to identify the major sources of risk and develop suitable risk mitigation strategies. More research is needed to evaluate options for intervention during food processing and to establish valid concepts, including hurdle technology.

Article Information

Funding. No funds, grants, or other support were received.

Conflict of Interest. The authors declare no conflict of interest.

References

- Abecasis, A.B., Serrano, M., Alves, R., Quintais, L., Pereira-Leal, J.B., Henriques, A.O., 2013. A genomic signature and the identification of new sporulation genes. *Journal of Bacteriology* 195, 2101–2115. [10.1128/JB.02110-12](https://doi.org/10.1128/JB.02110-12).
- Aboagye, G., Rowe, M.T., 2011. Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in raw water and water treatment operations for the production of potable water. *Water Research* 45, 3271–3278. [10.1016/j.watres.2011.03.029](https://doi.org/10.1016/j.watres.2011.03.029).
- Acharya, K.R., Dhand, N.K., Whittington, R.J., Plain, K.M., 2017. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in powdered infant formula using IS900 quantitative PCR and liquid culture media. *International Journal of Food Microbiology* 257, 1–9. [10.1016/j.ijfoodmicro.2017.06.005](https://doi.org/10.1016/j.ijfoodmicro.2017.06.005).
- Agrawal, G., Aitken, J., Hamblin, H., Collins, M., Borody, T.J., 2021. Putting Crohn's on the MAP: Five common questions on the contribution of *Mycobacterium avium* subspecies *paratuberculosis* to the pathophysiology of Crohn's disease. *Digestive Diseases and Sciences* 66, 348–358. [10.1007/s10620-020-06653-0](https://doi.org/10.1007/s10620-020-06653-0).
- Albuquerque, P., Cezar, R., Pinheiro Junior, J., Grazielle Nascimento, G., Santos, A., Mota, R., 2019. Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in coalho cheese in the State of Pernambuco, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 71, 1917–1921. [10.1590/1678-4162-10754](https://doi.org/10.1590/1678-4162-10754).
- Alonso-Hearn, M., Molina, E., Geijo, M., Vazquez, P., Sevilla, I., Garrido, J.M., Juste, R.A., 2009. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from muscle tissue of naturally infected cattle. *Foodborne Pathogens and Disease* 6, 513–518. [10.1089/fpd.2008.0226](https://doi.org/10.1089/fpd.2008.0226).
- Altic, L.C., Rowe, M.T., Grant, I.R., 2007. UV light inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk as assessed by FASTPlaqueTB phage assay and culture. *Applied and Environmental Microbiology* 73, 3728–3733. [10.1128/AEM.00057-07](https://doi.org/10.1128/AEM.00057-07).
- Antognoli, M.C., Garry, F.B., Hirst, H.L., Lombard, J.E., Dennis, M.M., Gould, D.H., Salman, M.D., 2008. Characterization of *Mycobacterium avium* subspecies *paratuberculosis* disseminated infection in dairy cattle and its association with antemortem test results. *Veterinary Microbiology* 127, 300–308. [10.1016/j.vetmic.2007.08.017](https://doi.org/10.1016/j.vetmic.2007.08.017).
- Ayele, W.Y., Svastova, P., Roubal, P., Bartos, M., Pavlik, I., 2005. *Mycobacterium avium* subspecies *paratuberculosis* cultured from locally and commercially pasteurized cow's milk in the Czech Republic. *Applied and Environmental Microbiology* 71, 1210–1214. [10.1128/AEM.71.3.1210-1214.2005](https://doi.org/10.1128/AEM.71.3.1210-1214.2005).
- Azzaro-Pantel, C., Madoumier, M., Gésan-Guiziou, G., 2022. Development of an ecodesign framework for food manufacturing including process flowsheeting and multiple-criteria decision-making: Application to milk evaporation. *Food and Bioproducts Processing* 131, 40–59. [10.1016/j.fbp.2021.10.003](https://doi.org/10.1016/j.fbp.2021.10.003).
- Badr, H.M., 2011. Inactivation of *Mycobacterium paratuberculosis* and *Mycobacterium tuberculosis* in fresh soft cheese by gamma radiation. *Radiation Physics and Chemistry* 80, 1250–1257. [10.1016/j.radphyschem.2011.05.013](https://doi.org/10.1016/j.radphyschem.2011.05.013).
- Bharathy, S., Gunaseelan, L., Porteen, K., 2017. Exploring the potential hazard of *Mycobacterium avium* subspecies *paratuberculosis* as a cause for Crohn's disease. *Veterinary World* 10, 457–460. [10.14202/vetworld.2017.457-460](https://doi.org/10.14202/vetworld.2017.457-460).
- Botsaris, G., Slana, I., Liapi, M., Dodd, C., Economides, C., Rees, C., Pavlik, I., 2010. Rapid detection methods for viable *Mycobacterium avium* subspecies *paratuberculosis* in milk and cheese. *International Journal of Food Microbiology* 141 Suppl 1, S87–90. [10.1016/j.ijfoodmicro.2010.03.016](https://doi.org/10.1016/j.ijfoodmicro.2010.03.016).
- Botsaris, G., Swift, B.M.C., Slana, I., Liapi, M., Christodoulou, M., Hatzitofi, M., Christodoulou, V., Rees, C.E.D., 2016. Detection of viable *Mycobacterium avium* subspecies *paratuberculosis* in powdered infant formula by phage-PCR and confirmed by culture. *International Journal of Food Microbiology* 216, 91–94. [10.1016/j.ijfoodmicro.2015.09.011](https://doi.org/10.1016/j.ijfoodmicro.2015.09.011).
- Boulais, C., Wacker, R., Augustin, J.C., Cheikh, M.H.B., Peladan, F., 2011. Modeling the occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in bulk raw milk and the impact of management options for exposure mitigation. *Journal of Food Protection* 74, 1126–1136. [10.4315/0362-028X.JFP-11-005](https://doi.org/10.4315/0362-028X.JFP-11-005).
- Butot, S., Ricchi, M., Sevilla, I.A., Michot, L., Molina, E., Tello, M., Russo, S., Arrigoni, N., Garrido, J.M., Tomas, D., 2019. Estimation of performance characteristics of analytical methods for *Mycobacterium avium* subsp. *paratuberculosis* detection in dairy products. *Frontiers in Microbiology* 10, 509. [10.3389/fmicb.2019.00509](https://doi.org/10.3389/fmicb.2019.00509).
- CAC, 2009. Codex Alimentarius Commission. Code of hygienic practice for milk and milk products. CAC/RCP 57-2004. URL: http://www.fao.org/fileadmin/user_upload/livestockgov/documents/CXP_057e.pdf.
- Cammi, G., Ricchi, M., Galiero, A., Daminelli, P., Cosciani-Cunio, E., Dalzini, E., Losio, M.N., Savi, R., Cerutti, G., Garbarino, C., Leo, S., Arrigoni, N., 2019. Evaluation of *Mycobacterium avium* subsp. *paratuberculosis* survival during the manufacturing process of Italian raw milk hard cheeses (Parmigiano Reggiano and Grana Padano). *International Journal of Food Microbiology* 305, 108247. [10.1016/j.ijfoodmicro.2019.108247](https://doi.org/10.1016/j.ijfoodmicro.2019.108247).

- Cerf, O., Griffiths, M., Aziza, F., 2007. Assessment of the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in commercially pasteurized milk. *Foodborne Pathogens and Disease* 4, 433–447. [10.1089/fpd.2007.0028](https://doi.org/10.1089/fpd.2007.0028).
- Chaubey, K.K., Singh, S.V., Gupta, S., Singh, M., Sohal, J.S., Kumar, N., Singh, M.K., Bhatia, A.K., Dhama, K., 2017. *Mycobacterium avium* subspecies *paratuberculosis* - An important food borne pathogen of high public health significance with special reference to india: an update. *The Veterinary Quarterly* 37, 282–299. [10.1080/01652176.2017.1397301](https://doi.org/10.1080/01652176.2017.1397301).
- Chiodini, R.J., Hermon-Taylor, J., 1993. The thermal resistance of *Mycobacterium paratuberculosis* in raw milk under conditions simulating pasteurization. *Journal of Veterinary Diagnostic Investigation* 5, 629–631. [10.1177/104063879300500424](https://doi.org/10.1177/104063879300500424).
- Clark, D.L., Anderson, J.L., Koziczkowski, J.J., Ellingson, J.L.E., 2006. Detection of *Mycobacterium avium* subspecies *paratuberculosis* genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. *Molecular and Cellular Probes* 20, 197–202. [10.1016/j.mcp.2005.12.006](https://doi.org/10.1016/j.mcp.2005.12.006).
- Condon, R., Farrokh, C., Jordan, K., McClure, P., Ross, T., Cerf, O., 2015. Guidelines for experimental design protocol and validation procedure for the measurement of heat resistance of microorganisms in milk. *International Journal of Food Microbiology* 192, 20–25. [10.1016/j.ijfoodmicro.2014.09.027](https://doi.org/10.1016/j.ijfoodmicro.2014.09.027).
- Dalziel, T.K., 1913. Chronic interstitial enteritis. *The British Medical Journal* 2, 1068–1070. URL: <http://www.jstor.org/stable/25307529>.
- Dane, H., Koidis, A., Stewart, L.D., Grant, I.R., 2022. Optimization of the composition of a solid culture medium for *Mycobacterium avium* subsp. *paratuberculosis* using factorial design and response surface methodology. *Journal of Applied Microbiology* 132, 4252–4265. [10.1111/jam.15517](https://doi.org/10.1111/jam.15517).
- Donaghy, J., Keyser, M., Johnston, J., Cilliers, F.P., Gouws, P.A., Rowe, M.T., 2009. Inactivation of *Mycobacterium avium* ssp. *paratuberculosis* in milk by UV treatment. *Letters in Applied Microbiology* 49, 217–221. [10.1111/j.1472-765X.2009.02644.x](https://doi.org/10.1111/j.1472-765X.2009.02644.x).
- Donaghy, J.A., Linton, M., Patterson, M.F., Rowe, M.T., 2007. Effect of high pressure and pasteurization on *Mycobacterium avium* ssp. *paratuberculosis* in milk. *Letters in Applied Microbiology* 45, 154–159. [10.1111/j.1472-765X.2007.02163.x](https://doi.org/10.1111/j.1472-765X.2007.02163.x).
- Donaghy, J.A., Rowe, M.T., Rademaker, J.L.W., Hammer, P., Herman, L., De Jonghe, V., Blanchard, B., Duhem, K., Vindel, E., 2008. An inter-laboratory ring trial for the detection and isolation of *Mycobacterium avium* subsp. *paratuberculosis* from raw milk artificially contaminated with naturally infected faeces. *Food Microbiology* 25, 128–135. [10.1016/j.fm.2007.06.007](https://doi.org/10.1016/j.fm.2007.06.007).
- Donaghy, J.A., Totton, N.L., Rowe, M.T., 2004. Persistence of *Mycobacterium paratuberculosis* during manufacture and ripening of cheddar cheese. *Applied and Environmental Microbiology* 70, 4899–4905. [10.1128/AEM.70.8.4899-4905.2004](https://doi.org/10.1128/AEM.70.8.4899-4905.2004).
- Ellingson, J.L.E., Anderson, J.L., Koziczkowski, J.J., Radcliff, R.P., Sloan, S.J., Allen, S.E., Sullivan, N.M., 2005. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. *Journal of Food Protection* 68, 966–972. [10.4315/0362-028X-68.5.966](https://doi.org/10.4315/0362-028X-68.5.966).
- Eltholth, M.M., Marsh, V.R., Van Winden, S., Guitian, F.J., 2009. Contamination of food products with *Mycobacterium avium paratuberculosis*: A systematic review. *Journal of Applied Microbiology* 107, 1061–1071. [10.1111/j.1365-2672.2009.04286.x](https://doi.org/10.1111/j.1365-2672.2009.04286.x).
- Elze, J., 2009. Nachweis von. URL: https://elib.tiho-hannover.de/receive/etd_mods.00001327.
- Faria, A.C.S., Schwarz, D.G.G., Carvalho, I.A., Rocha, B.B., De Carvalho Castro, K.N., Silva, M.R., Moreira, M.A.S., 2014. Short communication: Viable *Mycobacterium avium* subspecies *paratuberculosis* in retail artisanal Coalho cheese from Northeastern Brazil. *Journal of Dairy Science* 97, 4111–4114. [10.3168/jds.2013-7835](https://doi.org/10.3168/jds.2013-7835).
- Foddai, A., Elliott, C.T., Grant, I.R., 2010. Maximizing capture efficiency and specificity of magnetic separation for *Mycobacterium avium* subsp. *paratuberculosis* cells. *Applied and Environmental Microbiology* 76, 7550–7558. [10.1128/AEM.01432-10](https://doi.org/10.1128/AEM.01432-10).
- Foddai, A.C.G., Grant, I.R., 2017. Sensitive and specific detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in raw milk by the peptide-mediated magnetic separation-phage assay. *Journal of Applied Microbiology* 122, 1357–1367. [10.1111/jam.13425](https://doi.org/10.1111/jam.13425).
- Foddai, A.C.G., Grant, I.R., 2020. A novel one-day phage-based test for rapid detection and enumeration of viable *Mycobacterium avium* subsp. *paratuberculosis* in cows' milk. *Applied Microbiology and Biotechnology* 104, 9399–9412. [10.1007/s00253-020-10909-0](https://doi.org/10.1007/s00253-020-10909-0).
- Friedman, S., Blumberg, R.S., 2020. Chronisch entzündliche darmerkrankungen, in: *Harrisons Innere Medizin*. 20. ed. Thieme Verlag, Germany. volume 3, pp. 2807–2828.
- Galiero, A., Fratini, F., Mataragka, A., Turchi, B., Nuvoloni, R., Ikonomopoulos, J., Cerri, D., 2016. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in cheeses from small ruminants in Tuscany. *International Journal of Food Microbiology* 217, 195–199. [10.1016/j.ijfoodmicro.2015.10.029](https://doi.org/10.1016/j.ijfoodmicro.2015.10.029).
- Gerrard, Z.E., Swift, B.M.C., Botsaris, G., Davidson, R.S., Hutchings, M.R., Huxley, J.N., Rees, C.E.D., 2018. Survival of *Mycobacterium avium* subspecies *paratuberculosis* in retail pasteurised milk. *Food Microbiology* 74, 57–63. [10.1016/j.fm.2018.03.004](https://doi.org/10.1016/j.fm.2018.03.004).
- Gill, C.O., Saucier, L., Meadus, W.J., 2011. *Mycobacterium avium* subsp. *paratuberculosis* in dairy products, meat, and drinking water. *Journal of Food Protection* 74, 480–499. [10.4315/0362-028X.JFP-10-301](https://doi.org/10.4315/0362-028X.JFP-10-301).
- Gould, G., Franken, P., Hammer, P., Mackey, B., Shanahan, F., 2005. *Mycobacterium avium*. *Food Protection Trends* 25, 268–297.
- Grant, I., Foddai, A., Kunkel, B., Collins, M., 2014. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* (map) in infant formula, in: 2th International Colloquium on Paratuberculosis. Parma, Italy, 2014-06-22/2014-06-26 2014, International Association for Paratuberculosis, Curran Associates, Inc. p. 306. URL: <http://www.paratuberculosis.net/proceedings/proc12.pdf>.
- Grant, I.R., 2020. *Mycobacterium avium* subsp. *paratuberculosis* in animal-derived foods and the environment, in: Behr, M.A., Stevenson, K., Kapur, V. (Eds.), *Paratuberculosis Organism, Disease, Control*. 2nd ed. CAB International, Wallingford, Oxfordshire, pp. 14–28. [10.1079/9781845936136.0000](https://doi.org/10.1079/9781845936136.0000).
- Grant, I.R., Ball, H.J., Rowe, M.T., 2002. Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. *Applied and Environmental Microbiology* 68, 2428–2435. [10.1128/AEM.68.5.2428-2435.2002](https://doi.org/10.1128/AEM.68.5.2428-2435.2002).
- Grant, I.R., Williams, A.G., Rowe, M.T., Muir, D.D., 2005a. Efficacy of various pasteurization time-temperature conditions in combination with homogenization on inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Applied and Environmental Microbiology* 71, 2853–2861. [10.1128/AEM.71.6.2853-2861.2005](https://doi.org/10.1128/AEM.71.6.2853-2861.2005).
- Grant, I.R., Williams, A.G., Rowe, M.T., Muir, D.D., 2005b. Investigation of the impact of simulated commercial centrifugation and microfiltration conditions on levels of *Mycobacterium avium* ssp. *paratuberculosis* in milk. *International Journal of Dairy Technology* 58, 138–142. [10.1111/j.1471-0307.2005.00201.x](https://doi.org/10.1111/j.1471-0307.2005.00201.x).
- Hammer, P., 1999. *Mycobacterium paratuberculosis*. *Bulletin : International Dairy Federation (IDF)* , 19–22.
- Hammer, P., Kiesner, C., Walte, H.G.C., 2014. Short communication: Effect of homogenization on heat inactivation of *Mycobacterium avium* subspecies *paratuberculosis* in milk. *Journal of Dairy Science* 97, 2045–2048. [10.3168/jds.2013-7361](https://doi.org/10.3168/jds.2013-7361).
- Hammer, P., Walte, H.G.C., Matzen, S., Hensel, J., Kiesner, C., 2013. Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* during cooking of hamburger patties. *Journal of Food Protection* 76, 1194–1201. [10.4315/0362-028X.JFP-12-474](https://doi.org/10.4315/0362-028X.JFP-12-474).
- Hanifan, S., 2020. Behavior of *Mycobacterium avium paratuberculosis* in Lighvan cheese tracked by propidium monoazide

- qPCR and culture. *LWT* 133, 109886. [10.1016/j.lwt.2020.109886](https://doi.org/10.1016/j.lwt.2020.109886).
- Hayes, S.L., Sivaganesan, M., White, K.M., Pfaller, S.L., 2008. Assessing the effectiveness of low-pressure ultraviolet light for inactivating *Mycobacterium avium* complex (MAC) microorganisms. *Letters in Applied Microbiology* 47, 386–392. [10.1111/j.1472-765X.2008.02442.x](https://doi.org/10.1111/j.1472-765X.2008.02442.x).
- Hoffmann, W., Kiesner, C., Clawin-Rädecker, I., Martin, D., Einhoff, K., Lorenzen, P.C., Meisel, H., Hammer, P., Suhren, G., Teufel, P., 2006. Processing of extended shelf life milk using microfiltration. *International journal of dairy technology* 59, 229–235. [10.1111/j.1471-0307.2006.00275.x](https://doi.org/10.1111/j.1471-0307.2006.00275.x).
- Hruska, K., Bartos, M., Kralik, P., Pavlik, I., 2012. *Mycobacterium avium* subsp. *paratuberculosis* in powdered infant milk: Paratuberculosis in cattle – the public health problem to be solved. *Veterinárni Medicína* 50, 327–335. [10.17221/5631-VETMED](https://doi.org/10.17221/5631-VETMED).
- Hruska, K., Slana, I., Kralik, P., Pavlik, I., 2011. *Mycobacterium avium* subsp. *paratuberculosis* in powdered infant milk: F57 competitive real time PCR. *Veterinárni Medicína* 56, 226–230. [10.17221/1563-VETMED](https://doi.org/10.17221/1563-VETMED).
- Ikonomopoulos, J., Pavlik, I., Bartos, M., Svastova, P., Ayele, W.Y., Roubal, P., Lukas, J., Cook, N., Gazouli, M., 2005. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in retail cheeses from Greece and the Czech Republic. *Applied and Environmental Microbiology* 71, 8934–8936. [10.1128/AEM.71.12.8934-8936.2005](https://doi.org/10.1128/AEM.71.12.8934-8936.2005).
- ISO, 2016a. ISO 16140-1:2016 Microbiology of the food chain - Method validation. Part 1: Vocabulary. Last reviewed 2021. URL: <https://www.iso.org/standard/54869.html>.
- ISO, 2016b. ISO 16140-2:2016 Microbiology of the food chain - Method validation. Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. Last reviewed 2021. URL: <https://www.iso.org/standard/54870.html>.
- Jaravata, C.V., Smith, W.L., Rensen, G.J., Ruzante, J., Cullor, J.S., 2007. Survey of ground beef for the detection of *Mycobacterium avium paratuberculosis*. *Foodborne Pathogens and Disease* 4, 103–106. [10.1089/fpd.2006.54](https://doi.org/10.1089/fpd.2006.54).
- Johne, H., Frothingham, L., 1895. Ein eigenthümlicher fall von tuberculose beim rind. *Deutsche Zeitschrift für Thiermedizin* 21, 438–454.
- Kaevska, M., Lvonicik, S., Slana, I., Kulich, P., Kralik, P., 2014. Microscopy, culture, and quantitative real-time PCR examination confirm internalization of Mycobacteria in plants. *Applied and Environmental Microbiology* 80, 3888–3894. [10.1128/AEM.00496-14](https://doi.org/10.1128/AEM.00496-14).
- Kent, R.M., Fitzgerald, G.F., Hill, C., Stanton, C., Ross, R.P., 2015. Novel approaches to improve the intrinsic microbiological safety of powdered infant milk formula. *Nutrients* 7, 1217–1244. [10.3390/nu7021217](https://doi.org/10.3390/nu7021217).
- Klanicova, B., Slana, I., Roubal, P., Pavlik, I., Kralik, P., 2012. *Mycobacterium avium* subsp. *paratuberculosis* survival during fermentation of soured milk products detected by culture and quantitative real time PCR methods. *International Journal of Food Microbiology* 157, 150–155. [10.1016/j.ijfoodmicro.2012.04.021](https://doi.org/10.1016/j.ijfoodmicro.2012.04.021).
- Klanicova, B., Slana, I., Vondruskova, H., Kaevska, M., Pavlik, I., 2011. Real-time quantitative PCR detection of *Mycobacterium avium* subspecies in meat products. *Journal of Food Protection* 74, 636–640. [10.4315/0362-028X.JFP-10-332](https://doi.org/10.4315/0362-028X.JFP-10-332).
- Kralik, P., Babak, V., Dziedzinska, R., 2018. The impact of the antimicrobial compounds produced by lactic acid bacteria on the growth performance of *Mycobacterium avium* subsp. *paratuberculosis*. *Frontiers in Microbiology* 9, 638. [10.3389/fmicb.2018.00638](https://doi.org/10.3389/fmicb.2018.00638).
- Lamont, E.A., Bannantine, J.P., Armien, A., Ariyakumar, D.S., Sreevatsan, S., 2012. Identification and characterization of a spore-like morphotype in chronically starved *Mycobacterium avium* subsp. *paratuberculosis* cultures. *Plos One* 7, e30648. [10.1371/journal.pone.0030648](https://doi.org/10.1371/journal.pone.0030648).
- Lang, E., Sant'Ana, A.S., 2021. Microbial contaminants in powdered infant formula: What is the impact of spray-drying on microbial inactivation? *Current Opinion in Food Science* 42, 195–202. [10.1016/j.cofs.2021.06.007](https://doi.org/10.1016/j.cofs.2021.06.007).
- Liverani, E., Scaioli, E., Cardamone, C., Dal Monte, P., Belluzzi, A., 2014. *Mycobacterium avium* subspecies *paratuberculosis* in the etiology of Crohn's disease, cause or epiphenomenon? *World Journal of Gastroenterology* 20, 13060–13070. [10.3748/wjg.v20.i36.13060](https://doi.org/10.3748/wjg.v20.i36.13060).
- Lorencova, A., Babak, V., Kralova, A., Borilova, G., 2019. Survival of *Mycobacterium avium* subsp. *paratuberculosis* in raw fermented sausages during production and storage. *Meat Science* 155, 20–26. [10.1016/j.meatsci.2019.04.023](https://doi.org/10.1016/j.meatsci.2019.04.023).
- Lorencova, A., Vasickova, P., Makovcova, J., Slana, I., 2014. Presence of *Mycobacterium avium* subspecies and hepatitis E virus in raw meat products. *Journal of Food Protection* 77, 335–338. [10.4315/0362-028X.JFP-13-252](https://doi.org/10.4315/0362-028X.JFP-13-252).
- López-Pedemonte, T., Sevilla, I., Garrido, J.M., Aduriz, G., Guamis, B., Juste, R.A., Roig-Sagués, A.X., 2006. Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in cow's milk by means of high hydrostatic pressure at mild temperatures. *Applied and Environmental Microbiology* 72, 4446–4449. [10.1128/AEM.01924-05](https://doi.org/10.1128/AEM.01924-05).
- McDonald, W.L., O'Riley, K.J., Schroen, C.J., Condrón, R.J., 2005. Heat inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Applied and Environmental Microbiology* 71, 1785–1789. [10.1128/AEM.71.4.1785-1789.2005](https://doi.org/10.1128/AEM.71.4.1785-1789.2005).
- McNees, A.L., Markesich, D., Zayyani, N.R., Graham, D.Y., 2015. *Mycobacterium paratuberculosis* as a cause of Crohn's disease. *Expert Review of Gastroenterology & Hepatology* 9, 1523–1534. [10.1586/17474124.2015.1093931](https://doi.org/10.1586/17474124.2015.1093931).
- Mullan, W.M.A., 2019. Are we closer to understanding why viable cells of *Mycobacterium avium* subsp. *paratuberculosis* are still being reported in pasteurised milk? *International Journal of Dairy Technology* 72, 332–344. [10.1111/1471-0307.12617](https://doi.org/10.1111/1471-0307.12617).
- Mutharia, L.M., Klassen, M.D., Fairles, J., Barbut, S., Gill, C.O., 2010. *Mycobacterium avium* subsp. *paratuberculosis* in muscle, lymphatic and organ tissues from cows with advanced Johne's disease. *International Journal of Food Microbiology* 136, 340–344. [10.1016/j.ijfoodmicro.2009.10.026](https://doi.org/10.1016/j.ijfoodmicro.2009.10.026).
- NACMCF, 2010. The national advisory committee on microbiological criteria for foods. assessment of food as a source of exposure to *Mycobacterium avium* subspecies *paratuberculosis* (MAP). *Journal of Food Protection* 73, 1357–1397. [10.4315/0362-028X-73.7.1357](https://doi.org/10.4315/0362-028X-73.7.1357).
- Nauta, M.J., van der Giessen, J.W., 1998. Human exposure to *Mycobacterium paratuberculosis* via pasteurised milk: A modelling approach. *The Veterinary Record* 143, 293–296. [10.1136/vr.143.11.293](https://doi.org/10.1136/vr.143.11.293).
- Okura, H., Toft, N., Pozzato, N., Tondo, A., Nielsen, S.S., 2011. Apparent prevalence of beef carcasses contaminated with *Mycobacterium avium* subsp. *paratuberculosis* sampled from danish slaughter cattle. *Veterinary Medicine International* 2011, 152687. [10.4061/2011/152687](https://doi.org/10.4061/2011/152687).
- O'Reilly, C.E., O'Connor, L., Anderson, W., Harvey, P., Grant, I.R., Donaghy, J., Rowe, M., O'Mahony, P., 2004. Surveillance of bulk raw and commercially pasteurized cows' milk from approved Irish liquid-milk pasteurization plants to determine the incidence of *Mycobacterium paratuberculosis*. *Applied and Environmental Microbiology* 70, 5138–5144. [10.1128/AEM.70.9.5138-5144.2004](https://doi.org/10.1128/AEM.70.9.5138-5144.2004).
- Paolicchi, F., Cirone, K., Morsella, C., Gioffre, A., Romano, M., 2005. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from commercial pasteurized milk, in: 8th International Colloquium on Paratuberculosis. 14-18.08.2005, Copenhagen, Dänemark, International Association for Paratuberculosis, Copenhagen, Dänemark. p. 342.
- Patel, A., Shah, N., 2011. *Mycobacterium avium* subsp. *paratuberculosis*-incidences in milk and milk products, their isolation, enumeration, characterization, and role in human health. *Journal of Microbiology, Immunology, and Infection* 44, 473–479. [10.1016/j.jmii.2011.04.009](https://doi.org/10.1016/j.jmii.2011.04.009).
- Reddacliff, L.A., Marsh, I.B., Fell, S.A., Austin, S.L., Whittington, R.J., 2010. Isolation of *Mycobacterium avium* subspecies *paratuberculosis* from muscle and peripheral lymph nodes using acid-pepsin digest prior to BACTEC culture. *Veterinary Microbiology* 145, 122–128. [10.1016/j.vetmic.2010.03.011](https://doi.org/10.1016/j.vetmic.2010.03.011).
- Robertson, R.E., Cerf, O., Condrón, R.J., Donaghy, J.A., Heggum, C., Jordan, K., 2017. Review of the controversy over whether or not *Mycobacterium avium* subsp. *paratuberculosis* poses a food safety risk with pasteurised dairy products.

- International Dairy Journal 73, 10–18. [10.1016/j.idairyj.2017.04.009](https://doi.org/10.1016/j.idairyj.2017.04.009).
- Rossiter, C., Henning, W., 2001. Isolation of *Mycobacterium paratuberculosis* from thin market cows at slaughter. *Journal of Animal Science* 79, 113.
- Rowan, N.J., MacGregor, S.J., Anderson, J.G., Cameron, D., Farish, O., 2001. Inactivation of *Mycobacterium paratuberculosis* by pulsed electric fields. *Applied and Environmental Microbiology* 67, 2833–2836. [10.1128/AEM.67.6.2833-2836.2001](https://doi.org/10.1128/AEM.67.6.2833-2836.2001).
- Saucier, L., Plamondon, , 2011. Heat inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in aseptically prepared ground beef. *International Journal of Food Engineering* 7. [10.2202/1556-3758.2062](https://doi.org/10.2202/1556-3758.2062).
- Savi, R., Ricchi, M., Cammi, G., Garbarino, C., Leo, S., Pongolini, S., Arrigoni, N., 2015. Survey on the presence of *Mycobacterium avium* subsp. *paratuberculosis* in ground beef from an industrial meat plant. *Veterinary Microbiology* 177, 403–408. [10.1016/j.vetmic.2015.03.013](https://doi.org/10.1016/j.vetmic.2015.03.013).
- Sousa, T., Costa, M., Sarmiento, P., Manso, M.C., Abreu, C., Bull, T.J., Cabeda, J., Sarmiento, A., 2021. DNA-based detection of *Mycobacterium avium* subsp. *paratuberculosis* in domestic and municipal water from Porto (Portugal), an area of high IBD prevalence. *AIMS Microbiology* 7, 163–174. [10.3934/microbiol.2021011](https://doi.org/10.3934/microbiol.2021011).
- Spahr, U., Schaefroth, K., 2001. Fate of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss hard and semihard cheese manufactured from raw milk. *Applied and Environmental Microbiology* 67, 4199–4205. [10.1128/AEM.67.9.4199-4205.2001](https://doi.org/10.1128/AEM.67.9.4199-4205.2001).
- Stabel, J., Waldgren, C., Garry, F.B., 2001. Gamma-radiation effectively destroys. *J. Dairy Sci.* 84, 27.
- Stephan, R., Schumacher, S., Tasara, T., Grant, I.R., 2007. Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss raw milk cheeses collected at the retail level. *Journal of Dairy Science* 90, 3590–3595. [10.3168/jds.2007-0015](https://doi.org/10.3168/jds.2007-0015).
- Sung, N., Collins, M.T., 2000. Effect of three factors in cheese production (pH, salt, and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. *Applied and Environmental Microbiology* 66, 1334–1339. [10.1128/AEM.66.4.1334-1339.2000](https://doi.org/10.1128/AEM.66.4.1334-1339.2000).
- Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. *Journal of Clinical Microbiology* 30, 166–171. [10.1128/jcm.30.1.166-171.1992](https://doi.org/10.1128/jcm.30.1.166-171.1992).
- Traag, B.A., Driks, A., Stragier, P., Bitter, W., Broussard, G., Hatfull, G., Chu, F., Adams, K.N., Ramakrishnan, L., Losick, R., 2010. Do Mycobacteria produce endospores? *Proceedings of the National Academy of Sciences of the United States of America* 107, 878–881. [10.1073/pnas.0911299107](https://doi.org/10.1073/pnas.0911299107).
- Van Brandt, L., Coudijzer, K., Herman, L., Michiels, C., Hendrickx, M., Vlaemynck, G., 2011. Survival of *Mycobacterium avium* ssp. *paratuberculosis* in yoghurt and in commercial fermented milk products containing probiotic cultures. *Journal of Applied Microbiology* 110, 1252–1261. [10.1111/j.1365-2672.2011.04979.x](https://doi.org/10.1111/j.1365-2672.2011.04979.x).
- Waddell, L., Rajić, A., Stärk, K., McEwen, S.A., 2016a. *Mycobacterium avium* ssp. *paratuberculosis* detection in animals, food, water and other sources or vehicles of human exposure: A scoping review of the existing evidence. *Preventive Veterinary Medicine* 132, 32–48. [10.1016/j.prevetmed.2016.08.003](https://doi.org/10.1016/j.prevetmed.2016.08.003).
- Waddell, L.A., Rajić, A., Stärk, K.D.C., McEWEN, S.A., 2015. The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: A systematic review and meta-analysis of the evidence. *Epidemiology and Infection* 143, 3135–3157. [10.1017/S095026881500076X](https://doi.org/10.1017/S095026881500076X).
- Waddell, L.A., Rajić, A., Stärk, K.D.C., McEwen, S.A., 2016b. The potential public health impact of *Mycobacterium avium* ssp. *paratuberculosis*: Global opinion survey of topic specialists. *Zoonoses and Public Health* 63, 212–222. [10.1111/zph.12221](https://doi.org/10.1111/zph.12221).
- Wells, J.E., Bosilevac, J.M., Kalchayanand, N., Arthur, T.M., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M., 2009. Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in ileocecal lymph nodes and on hides and carcasses from cull cows and fed cattle at commercial beef processing plants in the United States. *Journal of Food Protection* 72, 1457–1462. [10.4315/0362-028x-72.7.1457](https://doi.org/10.4315/0362-028x-72.7.1457).
- Whan, L.B., Grant, I.R., Ball, H.J., Scott, R., Rowe, M.T., 2001. Bactericidal effect of chlorine on *Mycobacterium paratuberculosis* in drinking water. *Letters in Applied Microbiology* 33, 227–231. [10.1046/j.1472-765x.2001.00987.x](https://doi.org/10.1046/j.1472-765x.2001.00987.x).
- Williams, A.G., Withers, S.E., 2010. Microbiological characterisation of artisanal farmhouse cheeses manufactured in Scotland. *International Journal of Dairy Technology* 63, 356–369. [10.1111/j.1471-0307.2010.00596.x](https://doi.org/10.1111/j.1471-0307.2010.00596.x).