



2012-09-05-136 CGN minireviews on mycobacteria: (02) Milk
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To: (04) Food-borne, water-borne and air-borne diseases; (05) Zoonoses, general; (08) Mycobacterial diseases; (22) Veterinary administration

CGN minireviews on mycobacteria as a public health risk

A new series, aimed at stimulating discussion on published literature dealing with the threat to public health posed by mycobacteria. Although some information of global significance has been known for decades, the risk posed by mycobacteria remains underestimated.

Prepared by the [Reference Laboratory for Paratuberculosis and Avian Tuberculosis](#) World Organization for Animal Health (OIE) and [Biomedical Technology, Epidemiology and Food Safety Global Network](#) operating in the Veterinary Research Institute, Brno, Czech Republic
We believe in the [One Health Initiative](#)

(02) Mycobacteria are present in milk and dairy products, including dried milk for formula feeding

The raw milk can be definitively contaminated by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) [1, 2]. Although the effectiveness of pasteurization has been confirmed by well performed experiments, many screenings in different countries resulted in the finding of culturable MAP cells in about 2% of retail milk and dairy products [3, 4, 5, 6]. MAP DNA has been found in 10% to 40% of retail milk samples. MAP cells cannot disappear from contaminated milk and although their presence cannot be proven by culture, their “death” need not be definitive [7]. As much as 10⁴ MAP cells have been found in dried milk for babies [8, 9]. The cell wall constituent muramyl dipeptide, which has potent immunomodulatory properties, is ingested from the first days of life, if a newborn baby is fed using formula preparations. Later, when breastfeeding has been terminated, babies continue in the ingestion of MDP from milk and dairy products and meat. There is no doubt that MDP of mycobacterial origin can affect immunomodulatory processes and trigger pro-inflammatory pathways by similar mechanisms as the synthetic pure MDP (a special minireview will be published).

Selected references

- [1] **Taylor, T.K., Wilks, C.R., Mcqueen, D.S. (1981)**
Isolation of Mycobacterium-Paratuberculosis from the Milk of A Cow with Johnes Disease
Veterinary Record, 109, 532-533
- [2] **Collins, M.T. (1997)**
Mycobacterium paratuberculosis: A potential food-borne pathogen?
Journal of Dairy Science, 80, 3445-3448
Mycobacterium paratuberculosis commonly infects dairy cattle, leading to Johnes's disease, which is also known as paratuberculosis. The infection is chronic, progressive, and incurable. As the infection progresses, excretion of M. paratuberculosis in feces and milk occurs, and the bacterium spreads through the blood to multiple internal organs. Consequently, raw products originating from cattle may harbor M. paratuberculosis. Thermal treatments, such as pasteurization, are commonly relied on to kill foodborne bacterial pathogens that can infect humans. The small number of studies conducted to determine the thermal resistance of M. paratuberculosis suggest that it is less susceptible to destruction by heat killing than are milkborne zoonotic bacterial pathogens such as Listeria spp. or Mycobacterium bovis. Published reports concerning the thermal resistance of M. paratuberculosis in milk are reviewed herein, and key issues concerning the efficacy of pasteurization for elimination of M. paratuberculosis from milk are summarized
- [3] **Ellingson, J.L.E., Anderson, J.L., Koziczowski, 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

Cattle with Johne's disease can shed live *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in their milk, and MAP can survive under simulated commercial pasteurization conditions. In several studies conducted in the United Kingdom and Canada, MAP DNA has been detected in retail pasteurized milk samples; however, in one study in the United Kingdom viable MAP was identified in commercially pasteurized milk. A double-blind study involving two laboratories was undertaken to evaluate retail pasteurized whole milk in the United States. Marshfield Clinic Laboratories used solid culture medium (Herrold's egg yolk agar slants with mycobactin J and amphotericin B, nalidixic acid, and vancomycin), and TREK Diagnostic Systems, Research and Development used liquid culture medium (ESP culture system). Cultures at both laboratories were confirmed by PCR. A total of 702 pints of retail whole milk were purchased in three of the top five milk-producing states (233 from California, 234 from Minnesota, and 235 from Wisconsin) over a 12-month period and were tested for the presence of viable MAP. The criteria used for identifying samples as positive for viable MAP were similar to those followed by most laboratories (positive culture with PCR confirmation). The combined data from the two laboratories revealed the presence of viable MAP in 2.8% of the retail whole milk pints tested. Although the number of samples containing viable MAP was similar among states (P & GT; 0.05), there was a seasonal effect on the presence of viable MAP in retail milk (P = 0.05). More MAP-positive samples were identified during the third quarter of the year (July through September). Of the 22 brands of retail milk tested, 12 (55%) yielded at least one sample positive for viable MAP

[4] **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

Over a 17-month period (March 1999 to July 2000), a total of 814 cows' milk samples, 244 bulk raw and 567 commercially pasteurized (228 whole, 179 semiskim, and 160 skim), from 241 approved dairy processing establishments throughout the United Kingdom were tested for the presence of *Mycobacterium paratuberculosis* by immunomagnetic PCR (to detect all cells living and dead) and culture (to detect viable cells). Overall, *M. paratuberculosis* DNA was detected by immunomagnetic PCR in 19 (7.8%; 95% confidence interval, 4.3 to 10.8%) and 67 (11.8%; 95% confidence interval, 9.0 to 14.2%) of the raw and pasteurized milk samples, respectively. Confirmed *M. paratuberculosis* isolates were cultured from 4 (1.6%; 95% confidence interval, 0.04 to 3.1%) and 10 (1.8%; 95% confidence interval, 0.7 to 2.8%) of the raw and pasteurized milk samples, respectively, following chemical decontamination with 0.75% (wt/vol) cetylpyridinium chloride for 5 h. The 10 culture-positive pasteurized milk samples were from just 8 (3.3%) of the 241 dairy processing establishments that participated in the survey. Seven of the culture-positive pasteurized milk samples had been heat treated at 72 to 74degreesC for 15 s; the remainder had been treated at 72 to 75degreesC for the extended holding time of 25 s. When typed by restriction fragment length polymorphism and pulsed-field gel electrophoresis methods, some of the milk isolates were shown to be types distinct from those of laboratory strains in regular use within the testing laboratory. From information gathered at the time of milk sample collection, all indications were that pasteurization had been carried out effectively at all of the culture-positive dairies. That is, pasteurization time and temperature conditions complied with the legal minimum high-temperature, short-time process; all pasteurized milk samples tested phosphatase negative; and postprocess contamination was considered unlikely to have occurred. It was concluded that viable *M. paratuberculosis* is occasionally present at low levels in commercially pasteurized cows' milk in the United Kingdom

[5] **Ikonomopoulos, J., Pavliik, 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

We investigated the presence of *Mycobacterium avium* subsp. *paratuberculosis* in retail cheeses from Greece and the Czech Republic. We found that 31.7% and 3.6% of our samples reacted positive by PCR and culture, respectively. Consumption of these cheeses is likely to result in human exposure to *M. avium* subsp. *paratuberculosis*, albeit at a low level for viable cells.

[6] **Kim, J.H., Griffiths, M.W. (2011)**

Emerging Pathogenic Bacteria: *Mycobacterium avium* subsp *paratuberculosis* in Foods

Korean Journal for Food Science of Animal Resources, 31, 147-157

Mycobacterium avium paratuberculosis (MAP), the cause of Johne's disease in animals, may be a causative agent of Crohn's disease (CD) in humans, but the evidence supporting this claim is controversial. Milk, meat, and water could be potential sources of MAP transmission to humans. Thus, if the link between MAP and Crohn's disease is substantiated, the fact that MAP has been detected in retail foods could be a public health concern. The purpose of the present study was to review the link between MAP and CD, the prevalence of MAP in foods, heat inactivation, control of MAP during food processing, and detection methods for MAP. Although MAP positive rates in retail milk in nine countries ranged from 0 to 2.9% by the culture method and from 4.5 to 15.5% by PCR, high temperature short time pasteurization can effectively control MAP. The effectiveness of pasteurization to inactivate MAP depends on the initial concentration of the MAP in raw milk. Development of highly sensitive and specific rapid detection methods for MAP may enhance investigation into the relationship between MAP and CD, the prevention of the spread of MAP, and problem-solving related to food safety. Collaboration and efforts by government agencies, the dairy industry, farmers, veterinarians, and scientists will be required to reduce and prevent MAP in food.

[7] **Grant, I.R. (2004)**

Inactivation of *Mycobacterium avium* subsp *Paratuberculosis* in milk during commercial HTST pasteurization

Bulletin of the International Dairy Federation No 392/2004, 62-67

The thermal inactivation of 11 strains of *Mycobacterium paratuberculosis* at pasteurization temperatures was investigated, Cows' milk inoculated with *M. paratuberculosis* at two levels (10(7) and 10(4) CFU/ml) was pasteurized



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in the laboratory by (i) a standard holder method (63.5 degrees C for 30 min) and (ii) a high-temperature, short-time (HTST) method (71.7 degrees C for 15 s). Additional heating times of 5, 10, 15, 20, and 40 min at 63.5 degrees C were included to enable the construction of a thermal death curve for the organism. Viability after pasteurization was assessed by culture on Herrold's egg yolk medium containing mycobactin J (HEYM) and in BACTEC Middle-brook 12B radiometric medium supplemented with mycobactin J and sterile egg yolk emulsion. Confirmation of acid-fast survivors of pasteurization as viable *M. paratuberculosis* cells was achieved by subculture on HEYM to indicate viability coupled with PCR using *M. paratuberculosis*-specific IS900 primers. When milk was initially inoculated with 10(6) to 10(7) CFU of *M. paratuberculosis* per ml, *M. paratuberculosis* cells were isolated from 27 of 28 (96%) and 29 of 34 (85%) pasteurized milk samples heat treated by the holder and HTST methods, respectively. Correspondingly, when 10(3) to 10(4) CFU of *M. paratuberculosis* per ml of milk were present before heat treatment, *M. paratuberculosis* cells were isolated from 14 of 28 (50%) and 19 of 33 (58%) pasteurized milk samples heat treated by the holder and HTST methods, respectively. The thermal death curve for *M. paratuberculosis* was concave in shape, exhibiting a rapid initial death rate followed by significant "tailing." Results indicate that when large numbers of *M. paratuberculosis* cells are present in milk, the organism may not be completely inactivated by heat treatments simulating holder and HTST pasteurization under laboratory conditions.

- [8] **Hruska, K., Bartos, M., Kralik, P., Pavlik, I. (2005)**
Mycobacterium avium subsp paratuberculosis in powdered infant milk: paratuberculosis in cattle - the public health problem to be solved

Veterinari Medicina, 50, 327-335 ([Open access journal - full paper available](#))

Fifty one products of dried milk baby food purchased from 10 producers from seven countries available on the Czech market have been tested. IS900, the specific fragments for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) have been detected using PCR in 25 samples (49.0 %) and fragment f57 by real time PCR in 18 samples (35.3%). These results correspond to the epidemiological situation in Europe and are not unexpected. Paratuberculosis in cattle was almost unknown in the Czech Republic until 1990. An increase in the number of cows with paratuberculosis found in slaughterhouses and the incidence of Crohn's disease in the last decade is evident. The possible risk of MAP dead cells or bacterial structures in food is discussed in respect to autoimmune Crohn's disease. The national programmes of paratuberculosis control and certification of paratuberculosis-free herds should be strongly supported to decrease the risk for children and other people under higher risk. Producers should use MAP free milk for baby food production on a voluntary basis.

- [9] **Hruska, K., Slana, I., Kralik, P., Pavlik, I. (2011)**
Mycobacterium avium subsp paratuberculosis in powdered infant milk: F57 competitive real time PCR

Veterinari Medicina, 56, 226-230 ([Open access journal - full paper available](#))

Mycobacterium avium subsp. *paratuberculosis* (MAP) in concentrations from 48 to 32 500 cells per gram of powdered infant milk were found in 18 out of 51 investigated samples (35%) in this study. More than 10 000 cells per gram were present in four samples (7.8%). Such concentrations mean that one package of milk contains 5 million MAP cells, which are ingested by a bottle-fed baby over the course of several days. Premature babies and bottle-fed newborns can be affected by pro-inflammatory triggers from a huge number of mycobacteria despite not suffering from infection with bacteria or viruses often linked with the etiology of Crohn's disease.

Search for "paratuberculosis AND milk" in the Web of Science database has retrieved 690 records
See [CGNI web page](#) for minireviews in pdf and the references with abstracts.

Next minireviews

Mycobacteria ...

- ... in water
- ... can be found all around, in every nook and cranny
- ... are distributed in bottled water
- ... play a role in an Island story
- ... even after their death can modulate inflammatory cytokines by means of their cell wall components
- ... were used for immunomodulation in Freund adjuvans already 65 years ago
- ... are pathogens as well as allergens or immunomodulators
- ... could be a missing environmental factor in many etiological hypothesis
- ... are considerably heat and chlorine resistant
- ... have unusual characteristics of food, water, and air borne pathogens or immunomodulators similar to allergens

See the introductory documents

- [Paratuberculosis and Crohn's disease: Premises and open questions](#)
- [Infectious diseases incorporated FUIDI premises](#)

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