2012-09-25-144 CGN minireviews on mycobacteria: (04) How to assess MAP in retail milk
To: (04) Food-borne, water-borne and air-borne diseases; (05) Zoonoses, general; (08) Mycobacterial diseases; (22) Veterinary administration
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CGN minireviews on mycobacteria as a public health risk

A new series, aimed at stimulating discussion on published literature dealing with the threath 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 support the One Health Initiative

(04) **How to assess MAP in retail milk (**K. Hruska, Brno, Czech Republic)

Already since 1981 it is known that MAP can be isolated from milk [1]. It does not matter if 10% or 20% of samples have been positive in 100 or 300 of retail milk samples. Different, non-significant results could come from the other 300 samples, collected next week or next month from the same dairies. Some consumers are certainly exposed to dead or temporarily devitalised MAP. This is a fact that does not require repeated confirmation and expensive research for further analysis. Negative culture is of negligible importance, because even in raw milk or in faeces MAP cells are not always culturable [2]. Pasteurization is not fully efficient if milk is highly contaminated with MAP [3, 4]. Moreover, some MAP cells undergo resuscitation several days after pasteurization [5]. Only MAP DNA-free batches of dairy products or products prepared from MAP DNA-free milk or milk with low concentrations of MAP can eliminate or decrease the risk for sensitive consumers. The use of a reliable quantitative assay with high sensitivity is necessary to protect consumers. A legal limit of contamination should be established.

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

Abstract not available.

[2] Gao, A.L., Odumeru, J., Raymond, M., Hendrick, S., Duffield, T., Mutharia, L. (2009)
Comparison of milk culture, direct and nested polymerase chain reaction (PCR) with fecal culture based on samples from dairy herds infected with Mycobacterium avium subsp paratuberculosis
Canadian Journal of Veterinary Research-Revue Canadienne de Recherche Veterinaire, 73, 58-64

Mycobacterium avium subsp. paratuberculosis (MAP) is the etiologic agent of Johne's disease in cattle and other farm ruminants, and is also a suspected pathogen of Crohn's disease in humans. Development of diagnostic methods for MAP infection has been a challenge over the last few decades. The objective of this study was to investigate the relationship between different methods for detection of MAP in milk and fecal samples. A total of 134 milk samples and 110 feces samples were collected from 146 individual cows in 14 MAP-infected herds in southwestern Ontario. Culture, IS900 polymerase chain reaction (PCR) and nested PCR methods were used for detecting MAP in milk; results were compared with those of fecal culture. A significant relationship was found between milk culture, direct PCR, and nested PCR ($P < 0.05$). The fecal culture results were not related to any of the 3 assay methods used for the milk samples ($P > 0.10$). Although fecal culture showed a higher sensitivity than the milk culture method, the difference was not significant ($P = 0.2473$). The number of MAP colony-forming units (CFU) isolated by culture from fecal samples was, on average, higher than that isolated from milk samples ($P = 0.0083$). There was no significant correlation between the number of CFU cultured from milk and from feces (Pearson correlation coefficient = 0.1957, N = 63, P = 0.1243). The animals with high numbers of CFU in milk culture may not be detected by fecal culture at all, and vise versa. A significant proportion (29% to 41%) of the positive animals would be missed if only 1 culture method, instead of both milk and feces, were to be used for diagnosis. This suggests that the shedding of MAP in feces and milk is not synchronized. Most of the infected cows were low-level shedders. The proportion of low-level shedders may even be underestimated because MAP is killed during decontamination, thus reducing the chance of detection. Therefore, to identify suspected Johne's-infected animals using the tests in this study, both milk and feces samples should be collected in duplicate to enhance the diagnostic rate. The high MAP kill rate identified in the culture methods during decontamination may be compensated for by using the nested PCR method, which had a higher sensitivity than the IS900 PCR method used

[3] Van Brandt, L., Van der Plancken, I., De Block, J., Vlaemynck, G., Van Coillie, E., Herman, L., Hendrickx, M. (2011)
Adequacy of current pasteurization standards to inactivate Mycobacterium paratuberculosis in milk and phosphate buffer
International Dairy Journal, 21, 295-304

Mycobacterium avium subspecies paratuberculosis (MAP) inactivation kinetics was studied to assess whether current legislative pasteurization prescriptions are sufficient to reduce this bacterium to an acceptable level in raw milk. To assess possible protective effects of milk components during pasteurization, raw milk and phosphate-buffered saline (PBS) were compared. Analyzing data from three replicate experiments in milk separately gave D-60 degrees C-values ranging from 114.3 to 244.5 s and z-values ranging from 4.2 to 6.8 degrees C; in PBS the ranges were 162.4 to 353.3 s and 4.0 to 9.0 degrees C, respectively. No statistically significant difference was observed between the heat resistance of MAP in milk versus PBS. The currently prescribed minimum HTST pasteurization conditions (71.7 degrees C, 15 s) were found to be insufficient to kill MAP in milk in 2 out of 6 replicate experiments, while LTLT pasteurization (minimum 62.7 degrees C, 30 min) was effective, based on extrapolation of the inactivation data obtained. (c) 2011 Elsevier Ltd. All rights reserved

[4] Van Brandt, L., Vlaemynck, G., Herman, L., Hendrickx, M. (2011)
Letter to the editor: High-temperature short-time pasteurization of milk inactivates Mycobacterium avium ssp paratuberculosis: A comment on Van Brandt et al. (2011) Response
International Dairy Journal, 21, 510-512

Abstract not available.

[5] 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

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
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