

2012-10-02-148 CGN minireviews on mycobacteria: (06) Mycobacteria in plants and vegetables
To: (04) Food-borne, water-borne and air-borne diseases; (05) Zoonoses, general; (08) Mycobacterial diseases;
(22) Veterinary administration (27) Scientific information

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.

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

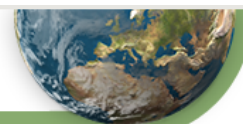
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(06) Mycobacteria in plants and vegetables (M. Kaevska, Brno)

The contamination of food of plant origin with mycobacteria has been reported already several decades ago (Nassal et al., 1974). Mycobacteria were found in fruits and vegetables, such as strawberries, radish, cucumbers etc. mainly in edible parts which were close to, or beneath the soil surface. Crucially, mycobacteria were present, although in smaller numbers, even after washing of the fruits. In the same study, the first experiments demonstrating the presence of bacteria inside the fruits were reported. This finding was explained by root uptake of bacteria. In the past couple of decades, the numbers of papers which have connected mycobacteria to food contamination and which have recognised its impact on animal and human health have been increasing (Kaevska and Hruska, 2010). The contamination of pre-harvest leaf lettuce with EHEC upon exposure to contaminate irrigation water has been described by Wachtel et al. (2002).

Mycobacteria were detected also in seven out of 121 vegetable samples which posed a danger to HIV-infected individuals (Yajko et al., 1995). A later study compared the genotypes of *M. avium* isolates from patients and foods and demonstrated a link between them (Yoder et al., 1999). Mycobacteria (predominantly *M. avium*) were isolated from 46 samples of salads, leak, lettuce, mushrooms, and other vegetables as well as apple juice and twenty nine isolates were tested (Argueta et al., 2000). Zwielehner et al. (2008) studied the microbial communities present in the phylosphere of lettuce leaves. After Denaturing Gradient Gel Electrophoresis and sequencing analyses, sequences from the genus *Mycobacterium* were found on leaves as well as soil samples. The sequence obtained from conventionally grown lettuce was most similar to *M. alvei*.

The adoption of the routine use of molecular biology methods, i.e., DNA isolation and PCR/real time PCR represents a major breakthrough in the detection of mycobacteria in general. These techniques enable considerably more rapid and sensitive detection of mycobacteria, with the possibility of quantification. Most of the methods developed so far, are used for the detection of *M. a. paratuberculosis* (MAP). Pribylova et al. (2011) detected MAP in grass samples using IS900 real time quantitative PCR. MAP-specific DNA was detected in 13 out of 19 samples examined (approx. 10² copies/g).



Selected references

- Argueta C, Yoder S, Holtzman AE, Aronson TW, Glover N, Berlin OGW, Stelma GN, Froman S, Tomasek P (2000)
Isolation and identification of nontuberculous mycobacteria from foods as possible exposure sources
Journal of Food Protection, 63, 930-933

A variety of foods collected from local supermarkets and produce stands were examined as possible sources of nontuberculous mycobacterial exposure. Food samples were combined with sterile ultrapure water and manually shaken. To remove large particles, the suspensions were filtered through a sterile strainer, centrifuged, and the supernatants were discarded. The food pellets were stored at -75 degrees C. The pellets were treated with either oxalic acid or sodium hydroxide-sodium citrate solutions to reduce contamination by nonmycobacterial organisms. Decontaminated pellets were cultured on both Middlebrook 7H10C agar and Middlebrook 7H10C agar with supplemental malachite green. Plates were observed for growth at 2 and 8 weeks. Isolates demonstrating acid-fastness were identified to species using polymerase chain reaction and restriction enzyme analysis. Nontuberculous mycobacteria (NTM) were recovered from 25 of 121 foods. Six different species of NTM were isolated, the most predominant being *Mycobacterium avium*.

- Kaevska M, Hruska K (2010)
Mycobacteria in water, feedstocks and food: analysis of publications
Veterinarni Medicina, 55, 571-580

Papers on mycobacteria in food, feed and water, published between 1945 and 2010 and indexed in the database Web of Science® (Thomson Reuters) were ranked according to authors, institutions, countries and source titles. The total number of papers on mycobacteria and food and mycobacteria and water were 1486 and 1419, respectively. More than 40% of papers have been published in the last five years. In addition to publications in peer reviewed journals the archives of ProMED-mail and the Rapid Alert System for Food and Feed of the European Union were also searched. It is evident that much attention is being paid to mycobacteria in food, feed and water as they likely pose a public health risk

- Nassal J, Breunig W, Schnedelbach U (1974)
Atypical mycobacteria in fruit, vegetables, and cereals (in German)
Prax Pneumologie 28, 667-674

Abstract not available.

- Pribylova R, Slana I, Kaevska M, Lamka J, Babak V, Jandak J, Pavlik I (2011)
Soil and Plant Contamination with *Mycobacterium Avium* subsp *Paratuberculosis* After Exposure to Naturally Contaminated Mouflon Feces
Current Microbiology, 62, 1405-1410.

The aim of this study was to demonstrate the persistence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in soil and colonization of different plant parts after deliberate exposure to mouflon feces naturally contaminated with different amounts of MAP. Samples of aerial parts of plants, their roots, and the soil below the roots were collected after 15 weeks and examined using IS900 real-time quantitative PCR (qPCR) and cultivation. Although the presence of viable MAP cells was not demonstrated, almost all samples were found to be positive using qPCR. MAP IS900 was not only found in the upper green parts, but also in the roots and soil samples (from 1.00×10^0 to 6.43×10^3). The level of soil and plant contamination was influenced mainly by moisture, clay content, and the depth from which the samples were collected, rather than by the initial concentration of MAP in the feces at the beginning of the experiment.



Wachtel, M.R., Whitehand, L.C., Mandrell, R.E. (2002)

Association of Escherichia coli O157 : H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water

Journal of Food Protection, 65, 18-25

Recent foodborne outbreak's have linked infection by enterohemorrhagic Escherichia coli (EHEC) serotype O157:147 to the consumption of contaminated lettuce. Contamination via food handler error and on-the-farm contamination are thought to be responsible for several outbreaks. Though recent studies have examined the application of EHEC to store-bought lettuce, little is known about the attachment of EHEC to growing plants. We investigated the association of lettuce seedlings with EHEC O157:H7 strains implicated in lettuce or fruit outbreaks using hydroponic and soil model systems. EHEC strains that express the green fluorescent protein were observed by stereomicroscopy and confocal laser scanning microscopy to determine adherence pattern on growing lettuce seedlings. Bacteria adhered preferentially to plant roots in both model systems and to seed coats in the hydroponic system. Two of five nonpathogenic E. coli strains showed decreased adherence to seedling roots in the hydroponic system. EHEC was associated with plants in as few as 3 days in soil, and contamination levels were dose-dependent. EHEC levels associated with young plants inoculated with a low dose suggested that the bacteria had multiplied. These data suggest that preharvest crop contamination via contaminated irrigation water can occur through plant roots

Yajko DM, Chin DP, Gonzalez PC, Nassos PS, Hopewell PC, Reingold AL, Horsburgh CR, Yakus MA, Ostroff SM (1995)

Mycobacterium-Avium Complex in Water, Food, and Soil Samples Collected from the Environment of Hiv-Infected Individuals

Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, 9, 176-182

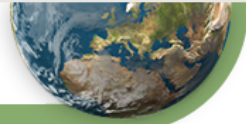
As part of an epidemiologic study of Mycobacterium avium complex (MAC) infection in San Francisco, water, food and soil samples were collected from the home environment of 290 persons with human immunodeficiency virus (HIV) infection and cultured for mycobacteria. Isolates recovered from the environment were compared with isolates cultured from study patients. Although mycobacteria were recovered from numerous environmental samples, isolates reactive with MAC-specific DNA probes were recovered from only four of 528 (0.76%) water samples and one of 397 (0.25%) food samples. The species M. avium was recovered from one water (0.19%) and one food sample. In contrast, MAC was recovered from 55% and M. avium from 27% of soil samples taken from potted plants in patients' home. Speciation of 76 MAC isolates from study patients showed all isolates belonged to the species M. avium. With use of serotype and multilocus enzyme electrophoresis analysis, some of the soil isolates were found to be similar to isolates recovered from study patients. The results of this study suggest that soil, rather than water, may be a significant reservoir of organisms causing MAC infection in San Francisco.

Yoder S, Argueta C, Holtzman A, Aronson T, Berlin OGW, Tomasek P, Glover N, Froman S, Stelma G (1999)

PCR comparison of Mycobacterium avium isolates obtained from patients and foods

Applied and Environmental Microbiology, 65, 2650-2653.

Mycobacterium avium is a cause of disseminated disease in AIDS patients. A need for a better understanding of possible sources and routes of transmission of this organism has arisen. This study utilized a PCR typing method designed to amplify DNA segments located between the insertion sequences IS1245 and IS1311 to compare levels of relatedness of M. avium isolates found in patients and foods. Twenty-five of 121 food samples yielded 29 mycobacterial isolates, of which 12 were M. avium. Twelve food and 103 clinical M. avium isolates were tested. A clinical isolate was found to be identical to a food isolate, and close relationships were found between two patient isolates and two food isolates. Relatedness between food isolates and patient isolates suggests the possibility that food is a potential



source of *M. avian* infection. This study demonstrates a rapid, inexpensive method for typing *M. avium*, possibly replacing pulsed-field gel electrophoresis.

Zwieblehner J, Handschur M, Michaelsen A, Irez S, Demel M, Denner EBM, Hasiberger AG (2008) **DGGE and real-time PCR analysis of lactic acid bacteria in bacterial communities of the phyllosphere of lettuce**
Molecular Nutrition & Food Research, 52, 614-623.

Food associated indigenous microbial communities exert antagonistic effects on pathogens and may routinely deliver health relevant microorganisms to the GI tract. By using molecular, culture independent methods including PCR-DGGE of 16S rDNA-coding regions and real-time PCR (RT-PCR) as well as BIOLOG metabolic fingerprinting, microbial communities on lettuce were analyzed in samples from fields, from supermarkets and soil. Amplified 16S rRNA gene sequences (57.7%) could be assigned to species previously reported as typical for the phyllosphere including *Pantoea agglomerans*, *Pseudomonas flavescens*, *Moraxella* spp., and *Mycobacterium* spp: 71.8% of the sequences obtained represented so far undescribed taxa. Principal component analysis of BIOLOG metabolic profiles indicated a seasonal variation in the lettuce phyllosphere microbial community structure. Various lactic acid bacteria were detected including several *Lactobacillus* and *Leuconostoc* species in particular on lettuce from organic farming. By RT-PCR lactobacilli were found with a range of abundances from 1×10^4 to 1×10^5 copies/g lettuce. Considering the importance of salad in many diets lettuce may contribute to a constant supply with LAB.

See [CGNI web page](#) for minireviews in pdf and the references with abstracts.

Next minireviews

Mycobacteria ...

- ... in water
- ... are distributed in bottled water
- ... play a role in an Island story (ovine paratuberculosis and Crohn's disease)
- ... in the Czech Republic (Crohn's disease after the Iron Curtain had been raised)
- ... in Sardinia (T1D)
- ... in Cardiff (river Taff contaminated by MAP)
- ... in Mankanto (ill-fated swimming)
- ... can be found in every nook and cranny
- ... as secret agents (formula feeding, cold chain hypothesis, hygiene hypothesis)
- ... can, even after their death 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 the missing environmental factor in many etiological hypotheses
- ... 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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