# The occurrence of *Mycobacterium avium* in samples of meat and meat products from the retail network in the Czech Republic

Barbora Zalewska, Iva Slaná

Department of Food and Feed Safety
Veterinary Research Institute
Brno, Czech Republic

**Abstract**

The aim of this study was to examine meat and meat products originating from the Czech market and to determine whether the presence of *Mycobacterium avium* could be confirmed or not. Analysis of raw, heat treated and fermented meat products was performed using a quantitative real-time PCR method (qPCR) for the detection of specific insertion sequences for selected subspecies of *M. avium – M. avium* subsp. *paratuberculosis* (*MAP*), *M. avium* subsp. *avium* (*MAA*) and *M. avium* subsp. *hominissuis* (*MAH*). A total number of 77 meat products were tested. The presence of at least one type of *M. avium* was detected in 27 (35%) of these products. Seventeen (22%) products contained *MAP* DNA, four products (5%) contained *MAA* DNA, and twelve (16%) contained *MAH* DNA. The concentration of *MAP* and *MAH* DNA in certain samples exceeded $10^4$ genomes·g$^{-1}$. Analysis of meat and meat products, in some of which DNA of *M. avium* subspecies unusual for the type of meat tested was detected, demonstrated secondary contamination or cross contamination during processing. This finding may represent a potential risk for immunocompromised consumers.

*Food safety, meat products, Mycobacterium avium, real-time PCR*

## Introduction

The species *Mycobacterium avium,* a representative of the *M. avium* complex, is considered as a main human and animal pathogen in this group. The species *M. avium* is divided into four subspecies: *M. avium* subsp. *paratuberculosis* (*MAP*), *M. avium* subsp. *avium* (*MAA*), *M. avium* subsp. *hominissuis* (*MAH*) and *M. avium* subsp. *silvaticum*. The subspecies *MAP* causes paratuberculosis in ruminants (Johne's disease) and there are also suspicions that it may be one of the factors contributing to the development of Crohn's disease in man – chronic inflammatory disease of the gastrointestinal tract (Bull et al. 2003 and Chiodini et al. 2012). The subspecies *MAA* and *MAH*, which commonly occur in the environment around us, may cause tuberculous changes, dermatological and urogenital infections, osteomyelitis and lymphadenitis in immunecompromised patients (Griffith et al. 2007).

Mycobacteria have been detected in various foods, primarily in raw food and food that is only slightly cooked. The possible risk of their transmission from food to man has been associated with commodities such as unpasteurised milk, cheeses, baby food, fish, fruit juices and vegetables. Mycobacteria have, however, also been detected by molecular and cultivation methods in the tissues of infected cattle whose milk was intended for distribution to the market network (Alonso-Hearn et al. 2009). In contrast, another study that tested two hundred samples of beef meat destined for hamburger production did not confirm the presence of mycobacteria in any of the tested samples (Jaravata et al. 2007).

Contamination of meat and meat products may occur either by primary contamination by means of the dissemination of *MAP* in the tissues of an infected animal or by means of secondary contamination – faecal contamination of meat – or cross contamination during the subsequent processing of meat products (Elthworth et al. 2009). Studies show, however, that there should be no great risk to the consumer following thorough heat treatment of muscle, lymphatic or parenchymatous tissues even from animals at an advanced stage of paratuberculosis (Mutharia et al. 2010).

**Address for correspondence:**
MSc. Barbora Zalewska, Ph.D.
Mycobacteriology Unit
Department of Food and Feed Safety
Veterinary Research Institute
Hudcova 70, Brno, Czech Republic

Phone: +420 732 874 753
E-mail: zalewska@vri.cz
www.maso-international.cz

## Materials and Methods

### The origin of the samples

Totally 77 samples were purchased in three supermarkets and one butcher's shop on the Czech retail network. The samples were divided into three groups: raw meat (n = 54; 70%), heat treated meat products (n = 5; 7%) and fermented meat products (n = 18; 23%). The individual groups were then subdivided according to the species – beef, pork, chicken, duck, lamb and mixed (Plate X, Fig. 1).

### The DNA isolation and qPCR

The DNA was isolated from meat products with the use of a commercially available DNeasy Blood & Tissue kit (Qiagen) according to a previously published modified methodology (Slaná et al. 2010). A duplex qPCR system for detection of a specific IS*900* insertion sequences was used for quantification of *MAP* with the use of an internal amplification control to differentiate negative and inhibited samples (Slaná et al. 2008). *MAA* and *MAH* quantification was performed with the use of a triplex qPCR system for detection of IS*901* and IS*1245*, likewise with an internal amplification control (Slaná et al. 2010).

## Results and Discussion

Many previous pieces of research have shown that real-time PCR is an extremely rapid and sensitive detection method. Our study revealed the presence of certain representatives of the species *M. avium* in 35% of the samples of meat and meat products analysed. A total of 77 products, including both heat untreated products and products subjected to various types of heat treatment, were tested. Seventeen products (22%) were found to be positive for the presence of *MAP* DNA, four products (5%)contained *MAA* DNA and the presence of *MAH* DNA was confirmed in 12 products (16%) (Plate X, Fig. 2).

The heat treatment undergone by meat products is not sufficient to eliminate the DNA. No particularly significant differences were observed between the heat-treated products, fermented products and raw meat (Plate X, Fig. 1).

The largest amounts of DNA were detected in fermented meat products made of pork and beef meat, present at a quantity of as much as $10^4$ *MAP* genomes·$g^{-1}$. As much as $10^4$ *MAH* DNA genomes.$g^{-1}$ was also detected in raw pork meat, which is not particularly surprising in view of the fact that *MAH* is the most commonly isolated mycobacterium in the pig-rearing environment (Plate X, Fig. 1).

Findings of mycobacterial species in meat and meat products with which they would not seem to be associated were, however, surprising. This related, first and foremost, to detection of *MAP* DNA, typical host cattle, in pork and chicken meat, and detection of *MAA*, typical host birds, in beef and pork meat. This fact indicates possible cross contamination in a plant processing meat and meat products. It may also indicate improper product handling during packaging, etc. It is important to note in this regard the necessity of observing correct hygiene principles during the course of the entire process relating to meat and meat products. Factors such as the cleanness of tools and working surfaces, which must be different for raw and heat-treated products, the hygiene of employees and the overall standard of production technology, are extremely important to the high quality of the final product presented to the consumer on the retail network (Elthworth et al. 2009).

## Conclusions

We demonstrated the DNA presence of selected mycobacteria in meat and meat products taken from the retail network in the Czech Republic with the use of molecular methods. In view of the fact that this is evidence of DNA and not a cultivation finding (evidence of viable bacteria) of *M. avium*, there is no great risk to the consumer of meat and meat products from the retail network. The finding that representatives of *M. avium* atypical for the given animal species were identified in meat products was, however, surprising.

This led to the conclusion that secondary contamination or cross contamination of meat and meat products had occurred during subsequent processing.

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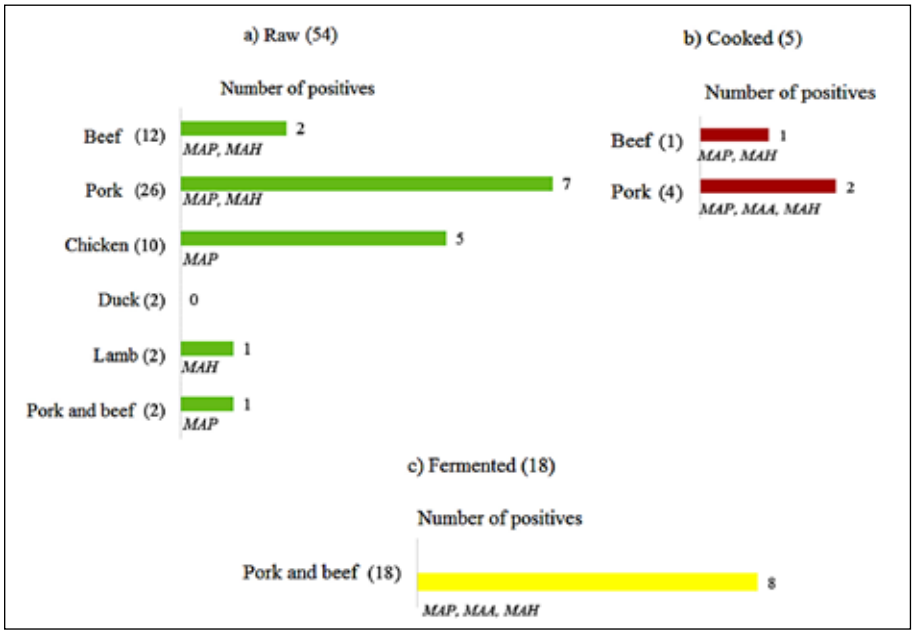


Fig. 1. Positive samples divided according to the type and the degree of processing (a, b, c). *Mycobacterium a.* subsp. *paratuberculosis (MAP)*, *M. a.* subsp. *avium (MAA), M. a.* subsp. *hominissuis (MAH)*

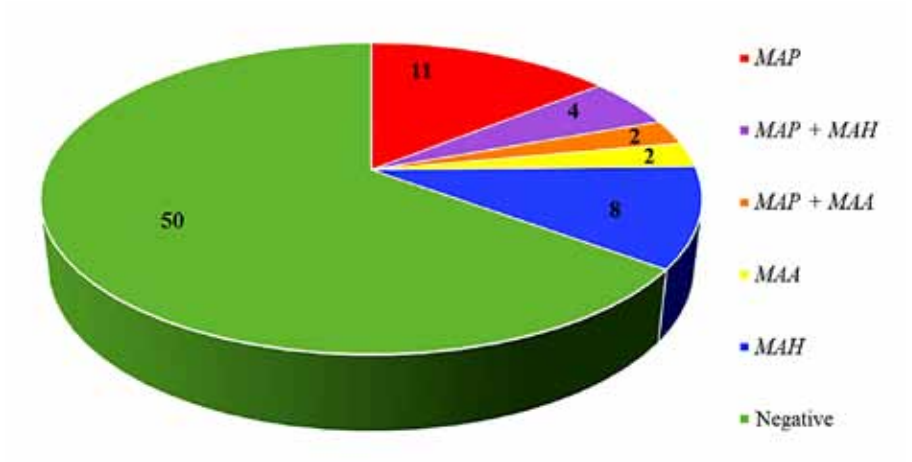


Fig. 2. A number of positive samples for *Mycobacterium avium* subsp. *paratuberculosis (MAP)*, *M. a.* subsp. *avium (MAA)* and *M. a.* subsp. *hominissuis (MAH)*