The occurrence of *Mycobacterium avium* subsp. *avium* in the muscle tissue of pigs intended for consumption

Marija Kaevská, Petr Kříž, Iva Slaná

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

Abstract

The aim of this study was to determine the occurrence and quantity of *Mycobacterium avium* subsp. *avium* (*M. a. avium*) in lymph nodes and diaphragm pillars in samples taken from naturally infected pigs intended for consumption. A *post-mortem* analysis was performed on 40 pigs, and tuberculoid changes to the lymph nodes found in 17 of them. Samples of various lymph nodes and diaphragm pillars were taken from these pigs, while submandibular lymph nodes and diaphragm pillars were taken from pigs without tuberculoid lesions. A total of 107 samples were tested by quantitative real-time PCR. *M. a. avium* was detected in three samples of diaphragm pillars at a quantity of as much as 10^3 cells per gram of muscle tissue. Quantitative real-time PCR is a useful method for detecting and quantifying mycobacteria in foods.

Mycobacteria, M. avium, pork meat, qPCR

Introduction

The genus *Mycobacterium* is made up of more than 160 species of non-motile, acid-alcohol-fast rods that include both obligate pathogens (the *M. tuberculosis* complex, *M. leprae*) and conditionally pathogenic species (*M. avium, M. chelonae, M. marinum*, etc.). According to the current taxonomy of mycobacteria, the species *M. avium* is divided into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* (Mijs et al. 2002). These subspecies have differing pathogenicity and virulence in respect of various hosts. *M. a. avium* is an agent of avian tuberculosis, a chronic disease in birds that occurs primarily on small poultry farms (Shitaye et al. 2006). In addition to its occurrence in various species of bird, *M. a. avium* has also been isolated from the tissues of pigs, sheep, cattle and horses (Pavlik et al. 2000). The most frequent types of illness caused by *M. avium* in humans are chronic lung infections and lymphadenitis, which occur most commonly in children (Griffith et al. 2007). A finding of tuberculoid lesions in pig organs during veterinary inspection at the slaughterhouse is reason for condemning the meat in part or whole. This causes considerable economic losses for pig farms. Although *M. a. hominissuis* is the commonest agent of these lesions in pigs, *M. a. avium* has also been isolated in as many as a third of cases (Pavlik et al. 2003; Shitaye et al. 2006).The occurrence of an agent of avian tuberculosis in pig meat may be a risk factor for human infection. The data on the extent of mycobacterium contamination of meat in the available literature is inadequate. It is difficult to obtain cultivation proof of mycobacteria in view of their slow growth and special requirements for the growth medium, and this means their occurrence is underestimated in practice. Detection of mycobacteria by molecular-biological methods is much more sensitive and faster (Kralik et al. 2011a).Only a few studies concerned with the possible occurrence of mycobacteria and their contamination of meat and meat products have been published. Representatives of the species *M. avium* have been detected both in meat from artificially infected pigs (Slana et al. 2010) and in meat and meat products on the retail network in the Czech Republic (Shitaye et al. 2009; Klanicova et al. 2011). *M. a. hominissuis* has been found to survive in homemade pork sausages

Address for correspondence:
Ing. Marija Kaevská, Ph.D.
Veterinary Research Institute
Hudcova 70, 621 00
Brno, Czech Republic

Phone: +420 533 331 618
E-mail:kaevska@vri.cz
www.maso-international.cz

following cold smoking, though not following the heat treatment of sausages (Kralik et al. 2011b).

This study was performed with the aim of determining the occurrence and quantity of *M. a. avium* in samples of lymph nodes and diaphragm pillars from slaughtered pigs from a farm on which the occurrence of avian tuberculosis had been proven by cultivation.

Materials and Methods

Forty slaughtered pigs came from a farm on which an agent of avian tuberculosis had been diagnosed by cultivation. The pigs were subjected to the usual veterinary inspection at the slaughterhouse and a total of 107 samples of tissue were taken. Samples of lymph nodes (submandibular, mediastinal, mesenteric, inguinal and hepatic) and diaphragm were taken from 17 pigs in which tuberculoid changes had been found. Samples of submandibular and mesenteric lymph nodes and samples of diaphragm were taken from pigs without tuberculoid findings (a total of 23). DNA was isolated from all the samples with a modified commercial kit (DNeasy Blood and Tissue Kit, Qiagen). Detection and quantification of *M. a. avium* was performed by triplex quantitative real-time PCR (qPCR) with an internal amplification control to differentiate inhibited and truly negative samples according to a previously described method (Slana et al. 2010).

Results

The DNA specific for *M. a. avium* was proven in a total of 20 pigs (half of the animals inspected). Mycobacterial DNA was also proven by qPCR in at least one sample taken from each of 15 pigs with tuberculous lesions. Mycobacterial DNA was also found in 5 pigs with no macroscopic findings. The presence of DNA from *M. a. avium* was proven in a total of 26 (24.3%) of all the tissue samples examined. Mycobacterial DNA occurred most frequently in the submandibular lymph nodes, followed by the mesenteric lymph nodes (Figure 1).

The quantity of this DNA ranged from 10^3 to 10^8 cells per gram of tissue, and was higher in samples taken from tissues with macroscopic findings (Table 1). DNA from *M. a. avium* was also proven in 3 samples of diaphragm pillars taken from animals with pathological-anatomical changes.

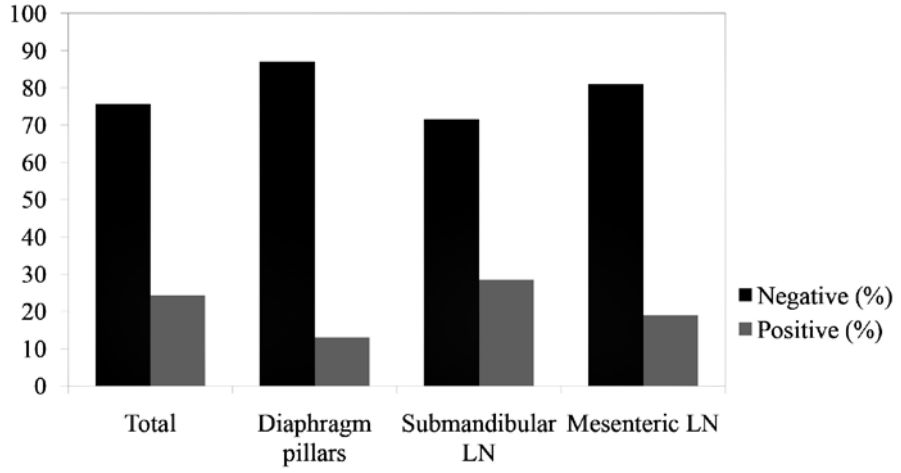

Fig. 1. Results of tests on samples by tissue type

Table 1. Detection of *Mycobacterium avium* subsp. *avium* in slaughtered pigs

Group	No of pigs	Positive pigs	Tissue tested	No of samples tested/positive	qPCR cells/gram*
TB+	17	15	Diaphragm pillars	17/3	8.54×10^2
			Submandibular LN	9/8	6.14×10^7
			Mesenteric LN	7/6	6.22×10^6
			Mediastinal LN	1/0	
			Hepatic LN	3/3	3.65×10^6
			Inguinal LN	1/1	9.68×10^7
TB-	23	5	Diaphragm pillars	23/0	
			Submandibular LN	23/4	2.19×10^3
			Mesenteric LN	23/1	1.23×10^3
Total	40	20		107/26	

TB+ pigs with pathological-anatomical changes, TB- pigs without change, LN lymph nodes; *average value given

Discussion

Although mycobacteria are not a significant agent of alimentary diseases, their occurrence in food may represent a risk, particularly for immunosuppressed individuals. *M. a. avium* may be detected in the intestinal mucosa of patients with a compromised immune system (Sun et al. 2005; Kaevska et al. 2014). Pork meat and products may be a source of significant human pathogens such as bacteria of the genera *Salmonella*, *Yersinia*, *Campylobacter*, *Listeria* and others if they are not subjected to adequate heat treatment (Fosse et al. 2008). A clear change in human eating habits has been seen in developed countries in recent years. Steak tartare containing pork meat, for example, is available on the Czech and Central European market. There is inadequate information available on the occurrence of mycobacteria in pork meat, although direct evidence of mycobacteriosis caused by consumption of pork is yet to be described (EFSA 2011). Many studies concerning mycobacterial infections in pigs have been published (Komijn et al. 1999; Pavlik et al. 2003; Miranda et al. 2012). Mycobacteria have been detected most commonly in lymph nodes (sampled in view of the higher probability of detection). Detecting mycobacteria in the lymph nodes is unsuitable from the viewpoint of food safety as such results cannot be generalised or extrapolated to provide data corresponding to the occurrence of mycobacteria in meat (the lymph nodes are not consumed). The possibility of secondary contamination of meat by mycobacteria from the lymph nodes or intestines (sites of primary occurrence of mycobacteria) during slaughter and veterinary inspection cannot, however, be ruled out. In this study, we compared the occurrence in lymph nodes and in muscle tissue, and selected the diaphragm pillars as a muscle with a relatively good blood supply. *M. a. avium* was detected most frequently in the submandibular and mesenteric lymph nodes (Table 1), as has been the case in other studies (Komijn et al. 1999; Pavlík et al. 2003). The DNA from *M. a. hominissuis* has been detected in the diaphragm pillars and leg muscle in a previous study into the occurrence and dissemination of agents of mycobacteriosis in the organs of artificially infected pigs, though not from *M. a. avium* (Slana et al. 2010). *M. a. avium* and other representatives of the species *M. avium* have been detected in various kinds of meat products. Specific DNA has been detected in raw, but also heat-treated and fermented products (Klanicova et al. 2011).

The results of our study confirm contamination of meat in naturally infected animals from which the infection agent may find its way to consumers. One of the disadvantages

of DNA detection by the qPCR method is the impossibility of distinguishing between live and dead bacteria. A system for the rapid and reliable detection of viable mycobacterial cells in foodstuffs must be developed in the future for the purposes of comprehensive risk assessment in relation to food safety.

Acknowledgements

The results were obtained with the financial support of the project LO1218 of the Ministry of Education within the framework of National Sustainability Programme I. The study obtained financial support in the form of a grant from the Ministry of Agriculture of the Czech Republic (No. QJ1210113 – The National Agency for Agricultural Research).

References

EFSA 2011: Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), and Animal Health and Welfare (AHAW). Scientific opinion on the public health hazards to be covered by inspection of meat (swine). EFSA **9**: 2351

Fosse J, Seegers H, Magras C 2008: Foodborne zoonoses due to meat: a quantitative approach for a comparative risk assessment applied to pig slaughtering in Europe. Vet Res **39**: 1

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, Von Reyn CF, Wallace RJ, Winthrop K 2007: An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Resp Crit Care Med **175**: 367-416

Kaevska M, Sterba J, Svobodova J, Pavlik I 2014: *Mycobacterium avium* subsp. *avium* and *Mycobacterium neoaurum* detection in an immunocompromised patient. Epidemiol Infect **142**: 882-885

Klanicova B, Slana I, Vondruskova H, Kaevska M, Pavlik I 2011: Real-time quantitative PCR detection of *Mycobacterium avium* subspecies in meat products. J Food Prot **74**: 636-640

Komijn RE, De Haas PEW, Schneider MME, Eger T, Nieuwenhuijs JHM, Van den Hoek RJ, Bakker D, Van Zijderveld FG, Van Soolingen D 1999: Prevalence of *Mycobacterium avium* in slaughter pigs in the Netherlands and comparison of IS*1245* restriction fragment length polymorphism patterns of porcine and human isolates. J Clin Microbiol **37**: 1254-1259

Kralik P, Slana I, Kralova A, Babak V, Whitlock RH, Pavlik I 2011a: Development of a predictive model for detection of *Mycobacterium avium* subsp. *paratuberculosis* in faeces by quantitative real time PCR. Vet Microbiol **149**: 133-138

Kralik P, Vondruskova H, Slana I, Moravkova M, Pavlik I 2011b: Survival of *Mycobacterium avium* subsp. *hominissuis* in homemade smoked pork sausages. Czech J Food Sci **29**: 547-553

Mijs W, De Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, Van Soolingen D 2002: Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp *avium* for bird-type isolates and *'M. avium* subsp *hominissuis*' for the human/porcine type of *M. avium*. Int J Syst Evol Microbiol **52**: 1505-1518

Miranda C, Matos M, Pires I, Correia-Neves M, Ribeiro P, Alvares S, Vieira-Pinto M, Coelho AC 2012: Diagnosis of *Mycobacterium avium* complex in granulomatous lymphadenitis in slaughtered domestic pigs. J Comp Pathol **147**: 401-405

Pavlik I, Svastova P, Bartl J, Dvorska L, Rychlik I 2000: Relationship between IS*901* in the *Mycobacterium avium* complex strains isolated from birds, animals, humans, and the environment and virulence for poultry. Clin Diagn Lab Immunol **7**: 212-217

Pavlik I, Matlova L, Dvorska L, Bartl J, Oktabcova L, Docekal J, Parmova I 2003: Tuberculous lesions in pigs in the Czech Republic during 1990-1999: occurrence, causal factors and economic losses. Vet-Med Czech **48**: 113-125

Shitaye JE, Parmova I, Matlova L, Dvorska L, Horvathova A, Vrbas V, Pavlik I 2006: Mycobacterial and *Rhodococcus equi* infections in pigs in the Czech Republic between the years 1996 and 2004: the causal factors and distribution of infections in the tissues. Vet-Med Czech **51**: 497-511

Shitaye JE, Horvathova A, Bartosova L, Moravkova M, Kaevska M, Donnelly N, Pavlik I 2009: Distribution of non-tuberculosis mycobacteria in environmental samples from a slaughterhouse and in raw and processed meats. Czech J Food Sci **27**: 194-202

Slana I, Kaevska M, Kralik P, Horvathova A, Pavlik I 2010: Distribution of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by culture and IS*901* and IS*1245* quantitative Real Time PCR. Vet Microbiol **144**: 437-443

Sun HY, Chen MY, Wu MS, Hsieh SM, Fang CT, Hung CC, Chang SC 2005: Endoscopic appearance of GI mycobacteriosis caused by the *Mycobacterium avium* complex in a patient with AIDS: case report and review. Gastrointestinal Endoscopy **61**: 775-779