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Proliferation of mycobacteria in a piggery environment revealed by *mycobacterium*-specific real-time quantitative PCR and 16S rRNA sandwich hybridization

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Abstract

Pig mycobacteriosis is the most common animal mycobacterial disease in Finland with a long-term average prevalence of 0.34% and temporary peaks as high as 0.85%. In the current study *Mycobacterium*-specific real-time qPCR and 16S rRNA sandwich hybridization were utilized for culture-independent detection and measurement of potentially infectious mycobacteria in selected piggeries.

Participating herds ($n = 5$) were selected according to prevalence of tuberculous lesions (>4%) in slaughtered carcasses. When DNA extracted from piggery bedding materials was analyzed by *Mycobacterium*-targeted qPCR using the SYBR green I dye for detection of amplification products, 10^5 to 10^7 cell equivalents of mycobacterial DNA were detected in unused bedding materials and 10^8 to 10^{10} g^{-1} dry weight in used bedding materials. When *Mycobacterium*-specific hybridization probes were used for detection of amplification products, 10^5 to 10^7 cell equivalents of mycobacterial DNA g^{-1} dry weight were detected in unused bedding materials in four out of the five piggeries studied and up to 10^8 cell equivalents in used bedding material. The results were confirmed by the *Mycobacterium*-specific 16S rRNA sandwich hybridization assay.

The present results show, that mycobacteria occur in organic materials commonly used on pig farms, and may proliferate in bedding materials during use. We also show that DNA- and RNA-based methods may be utilized for detection of environmental reservoirs of mycobacteria causing porcine and human infection.

Keywords: Mycobacteria; Piggery environment; Pig mycobacteriosis; Real-time qPCR; Sandwich hybridization

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1. Introduction

Nearly half of the currently known environmental mycobacteria (114 species) are opportunistic pathogens (Biet et al., 2005; Howard and Byrd, 2000; Thorel et al., 2001) that infect humans and animals. Pig mycobacteriosis is the most common animal mycobacterial disease in Finland with a long-term average prevalence 0.34% (Ali-Vehmas et al., 2004). The prevalence of pig mycobacteriosis increased in Finland gradually from 0.07% in 1998 to a maximum of 0.85% in 2003 and remained as high as 0.61% in 2004. This causes economic losses as the result of condemnation of infected pork as conditionally edible after processing. Genetically similar *Mycobacterium avium* strains have been isolated from humans and pigs (Komijn et al., 1999; Ramasoota et al., 2001), suggesting pigs as a vehicle infecting humans or that pigs and humans are exposed to common sources or infection. The reservoirs of infective environmental mycobacteria are unclear but bedding materials have been suspected as sources of infection for pigs (Matlova et al., 2003, 2004, 2005; Windsor et al., 1984) and municipal waters and peat-rich soils for humans (Primm et al., 2004).

The potential risk caused by environmental mycobacteria to animal and public health, microbiological safety and economic profitability of pork production necessitates effective tools for detecting the reservoirs of infectious mycobacteria. Mycobacterial cultivation methods are slow, selective and poorly suited for quantitative analysis. A *Mycobacterium*-specific quantitative PCR assay based on amplification of the *hsp*-65 gene has been published (Khan and Yadav, 2004) but the *hsp*-65 primers cannot amplify *M. avium* and *M. intracellulare*, the principal pig pathogens.

In the present work a *Mycobacterium*-specific real-time qPCR assay was utilized in combination with *Mycobacterium*-specific 16S rRNA sandwich hybridization in culture-independent analysis of reservoirs of viable and potentially infectious mycobacteria in the piggery environment.

2. Materials and methods

2.1. Sampling

The selected birth to finish pig farms were sampled from June 21 to August 9, 2004 (Table 1) in the province of Ostrobothnia in Finland, each with a >4% prevalence of tuberculous lesions in meat control during one or more years between 2002 and 2004 (average 2.2% in 2003 in the provincial slaughterhouse in Nurmo). The farms housed from 15 to 102 sows and differed in the usage of bedding materials.

2.2. Reference strains

The strains *Mycobacterium fortuitum*^{T} (DSM 46621), *M. lentiflavum*^{T} (DSM 44418), *Mycobacterium botniense* E347^T, *Mycobacterium gordonae*^{T} (DSM 44160), *Mycobacterium chlorophenolicum* PCP-1^T (HAMBI 2278, DSM 43826) were grown at 28 °C on DSM65 agar (0.4% glucose, 0.4% yeast extract, 1% malt extract, 0.2% $CaCO_3$, 1.2% agar), with the exception of *M. lentiflavum* which was grown at 37 °C on Middlebrook 7H10 agar (Difco BD, Franklin Lakes, NJ, USA) with enrichment. *Corynebacterium glutamicum*^{T} (DSM 20300), *Gordonia terrae*^{T} (DSM 43249), *Rhodococcus opacus*^{T} (DSM 43205), *Bacillus cereus*^{T} (ATCC 14579) and *Pseu-*

Table 1
The prevalence of tuberculous lesions in slaughtered animals between the years 2002–2004 in five birth to finish farms

Piggery	Sows per farm	Prevalence of tuberculous lesions (%) in slaughtered animals						Bedding
		2002		2003		2004		
		No.	%	No.	%	No.	%	
1	102	514	3.3	2086	6.7	2371	5.9	Straw
2	35	180	0.0	207	4.5	204	2.5	Peat + straw
3	45	219	0.9	397	6.0	396	2.3	Straw + wood shavings
4	15	110	4.5	207	8.7	165	6.1	Straw + wood shavings
5	60	743	9.4	326	22	1100	8.3	Straw + wood shavings