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Short communication

Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for *Mycobacterium avium* subsp. *avium* and positive for *Rhodococcus equi*

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Abstract

The prevalence of granulomatous lesions in lymph nodes of pigs was studied. From January till August 2004 in two slaughterhouses in The Netherlands 2,116,536 pigs were examined for the presence of granulomatous lesions in the sub-maxillary lymph nodes. In 15,900 (0.75%) of these pigs, lesions could be detected. Nine farms with the highest incidence of lesions were selected for a more detailed pathological and bacteriological examination. On these farms, the prevalence of lesions in sub-maxillary lymph nodes ranged from 2.3 to 5.7% with a mean of 3.0%. From 1276 pigs that were sampled, 98 (7.7%) displayed granulomatous lesions in the sub-maxillary lymph nodes and one (0.1%) pig showed lesions in its mesenteric lymph node. *Mycobacterium avium* subsp. *avium* (MAA) could not be isolated from the lymph nodes of the 99 pigs with lesions and from a selection of lymph nodes ($n = 61$) of pigs without lesions. *Rhodococcus equi* was isolated from 44 out of 98 (44.9%) of the sub-maxillary lymph nodes with granulomatous lesions and from two mesenteric lymph nodes without lesions. A comparison of former studies and the current results indicate that the prevalence of MAA infections in slaughter pigs has strongly decreased over the last decade, whereas *R. equi* is highly prevalent. The high incidence of granulomatous lesions associated with the bacteriological presence of *R. equi* could be considered as a serious cause of misdiagnosis of MAA infections in cases where meat inspection is carried out by inspection for granulomatous changes of lymph nodes only.

Keywords: *Mycobacterium avium*; Swine mycobacteriosis; Lymphadenitis; Bacteria; Diagnosis; *Rhodococcus equi*

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1. Introduction

Mycobacterium avium subsp. *avium* (MAA) is a potential zoonotic pathogen, which belongs to *M. avium* complex bacteria (MAC). MAA can cause opportunistic infections in humans, especially in those suffering from a HIV infection (Wagner and Young, 2004; Biet et al., 2005). In addition, MAA can cause cervical lymphadenitis in young, otherwise healthy children between 0 and 4 years of age (Haverkamp et al., 2004). The reservoir for infection with MAA in humans is unknown. MAA is ubiquitous and can be isolated from water, soil, compost, bedding materials in stables and other environmental sources (Engel et al., 1978; Thoen, 1992; Matlova et al., 2003, 2004). MAA can also be isolated from animals, most frequently from birds and pigs (Thoen, 1992). Genotyping of MAA strains isolated from humans and pigs revealed that these strains have a high homology (Komijn et al., 1999). This could indicate that pigs are a source of infection for humans or that pigs and humans share common sources of infection, e.g. the environment.

In pigs, infections with MAA are usually limited to the lymph nodes. Especially the sub-maxillary and mesenteric lymph nodes are affected (Thoen, 1992). MAA infections in pigs have no apparent effect on the health of the animal and diagnosis by physical examination of the live pig is usually impossible. Since MAA is a potential zoonotic pathogen it is necessary to exclude MAA from the food chain. In accordance to European Union legislation (Regulation 2004/854/EC), infections caused by Mycobacteria in pigs are diagnosed presumptively in slaughter houses by meat inspectors. The sub-maxillary lymph nodes of slaughter pigs are incised and examined at post-mortem inspection for granulomatous lesions. Furthermore, the mesenteric lymph nodes are inspected for granulomatous lesions visually, by palpation and if necessary by incision.

It is considered that granulomatous lesions in lymph nodes are typical for an infection with mycobacteria (Brown and Neuman, 1979). However, *Rhodococcus equi* is also frequently isolated from lesions in sub-maxillary lymph nodes of pigs with granulomatous lymphadenitis (Prescott, 1991; Takai et al., 1996a; Hondalus, 1997; Dvorska et al., 1999). *R. equi* can cause disease in horses, especially in young foals. In humans, it mainly causes disease in those infected with HIV, and the infection occurs mainly in lungs (Prescott, 1991; Hondalus, 1997). The reservoir of the human infection is not elucidated. *R. equi* is a robust soil organism widespread in the environment and will potentially multiply in the presence of horse manure (Takai et al., 1996b). Prescott (1991) reviewed the history of 32 AIDS patients suffering from an infection with *R. equi* and found a possible animal source of infection for 12 of these patients, confirming the zoonotic potential of this species.

The prevalence of granulomatous lesions in the sub-maxillary and/or mesenteric lymph nodes of Dutch slaughter pigs was determined in 1996 to be 0.5% (Komijn et al., 1999). From 54.2% of these lesions, MAA was isolated. This study was performed to determine the prevalence of granulomatous lesions in pigs in The Netherlands in 2004 and to compare the results with the previous study performed in 1996. Furthermore, on selected farms, sub-maxillary and mesenteric lymph nodes with and without lesions were sampled at slaughter and examined bacteriologically for MAA and *R. equi*.

2. Materials and methods

2.1. Lesions of pigs at post-mortem meat inspection

The prevalence of granulomatous lesions in slaughter pigs was determined for the period January till August 2004. Two slaughterhouses (I and II), where a system was used to register lesions during the post-mortem meat inspection, were selected. Both slaughterhouses were located in the southern part of The Netherlands and in each slaughterhouse approximately 6000 pigs were slaughtered daily. The total number of pigs slaughtered and the number of pigs from which the heads were condemned for reasons of granulomatous lesions in the sub-maxillary lymph nodes were counted and prevalence of lesions was calculated.

2.2. Selection of farms and sampling

In order to obtain a considerable number of lymph nodes with granulomatous lesions for bacteriological

and pathological examination, farms were selected with a recent history for such lesions. Therefore data were used from the registration of lesions at post-mortem meat inspection in slaughterhouse I for the period September till December 2003. Nine farms were selected and in January and February 2004 in several deliveries from these farms the sub-maxillary and mesenteric lymph nodes were examined pathologically for granulomatous lesions at slaughter. From each delivery, at least five pigs without and all pigs with granulomatous lesions in the sub-maxillary lymph nodes were sampled for further examination.

2.3. Bacteriological examination

To culture for MAA the lymph nodes were ground, decontaminated by 1 M sodium hydroxide for 15 min at room temperature followed by a 5% oxalic acid treatment also for 15 min at room temperature. Samples were inoculated onto Löwenstein-Jensen medium, Stonebrink egg medium and Middlebrook 7H10 agar followed by incubation for 12 weeks at 37 °C. Ziehl-Neelsen stain was performed to identify acid-fast bacilli. To culture for *R. equi*, lymph nodes were inoculated onto normal blood agar plates supplemented with 5% sheep blood and incubated for 48 h at 37 °C. Suspected colonies were tested for a synergistic hemolytic reaction (CAMP test) with *Staphylococcus aureus* on 5% sheep blood agar plates, which is an essential criterion for identification of *R. equi* (Prescott, 1991). To confirm the identification of *R. equi*, 16S ribosomal sequencing was performed. In short: DNA was purified using QIAquick spin columns, according to the procedure described by the manufacturer (Qiagen). Target DNA sequence was amplified by PCR using universal primers 8FPL and 806R (Relman, 1993). DNA analysis was performed using an ABI carried out on 3100 Avant genetic analyzer and compared with the NCBI database using BLAST (Applied Biosystems).

3. Results

3.1. Prevalence of lesions

During meat inspection at two slaughterhouses in The Netherlands for the period January till August 2004 in total 2,116,536 pigs were examined for the presence of granulomatous lesions in the sub-maxillary lymph nodes. In 15,900 (0.75%) of these pigs, lesions were detected. The prevalence of granulomatous lesions in slaughterhouse I was higher than in slaughterhouse II. From 898,858 pigs slaughtered in slaughterhouse I 9649 (1.05%) pigs displayed lesions in the sub-maxillary lymph nodes whereas from the 1,217,678 pigs slaughtered in slaughterhouse II 6,251 (0.51%) pigs showed lesions.

3.2. Selection of farms and sampling

Nine farms with the highest incidence of lesions in the sub-maxillary lymph nodes were selected for a more detailed pathological and bacteriological examination. During the period September to December 2003 the prevalence of lesions in lymph nodes on these farms ranged from 2.3 to 5.7% with a mean of 3.0%. Prevalence on these farms was calculated on the basis of results at meat inspection in slaughterhouses of minimal 5 and maximal 27 successive deliveries of slaughter pigs, in total 111 deliveries and 18,855 pigs. In January and February 2004 the sub-maxillary- and mesenteric lymph nodes from 1276 pigs from these nine farms were sampled.

3.3. Pathological and bacteriological examination

The results of the pathological examination showed that 98 (7.7%) out of the 1276 examined pigs had granulomatous lesions in the sub-maxillary lymph nodes and only one pig had lesions in its mesenteric lymph node. The remaining 1177 (92.2%) pigs were free of lesions in their lymph nodes. Bacteriological examination of the lymph nodes of the 99 pigs with lesions and from a selection of lymph nodes ($n = 61$) of pigs without lesions showed that they were all negative for Mycobacteria, including MAA. However, *R. equi* was isolated from 44 out of 98 (44.9%) sub-maxillary lymph nodes with granulomatous lesions (Table 1). In sub-maxillary lymph nodes without lesions no *R. equi* was detected. From the 160 examined mesenteric lymph nodes, *R. equi* was isolated from two lymph nodes in which no lesions were detected during pathological examination (Table 1). *R. equi* was isolated from affected lymph

Table 1
Pathological and bacteriological examination from sub-maxillary and mesenteric lymph nodes of 160 pigs originating from nine farms with a recent history of granulomatous lesions

Lymph node	No. (%) of lymph nodes							
	Pathological positive				Pathological negative			
	MAA[a] positive	*R. equi*[b] positive	MAA and *R. equi* negative	Total	MAA positive	*R. equi* positive	MAA and *R. equi* negative	Total
Sub-maxillary	0 (0.0)	44 (44.9)	54 (55.1)	98 (100)	0 (0.0)	0 (0.0)	62 (100)	62 (100)
Mesenteric	0 (0.0)	0 (0.0)	1 (100)	1 (100)	0 (0.0)	2 (1.3)	157 (98.7)	159 (100)

[a] *Mycobacterium avium* subsp. *avium*.
[b] *Rhodococcus equi*.

nodes from all nine sampled farms (Table 2). The number of lymph nodes with lesions varied from 3 to 28 per farm and the number of isolations of *R. equi* from 3 to 12 (Table 2).

The isolated *R. equi* strains showed a synergetic hemolytic reaction on 5% sheep blood agar with *S. aureus*. To confirm the identification, from one isolate the 16S rDNA was amplified by PCR and sequenced. Its sequence showed that the isolate was identical to *R. equi*.

4. Discussion

In 1996 the prevalence of granulomatous lesions in lymph nodes of slaughter pigs in The Netherlands was 0.5% and in 54.2% of the cases MAA was isolated (Komijn et al., 1999). The results of this study showed that the prevalence of granulomatous lesions in lymph nodes in 2004 was 0.75%, an increase in comparison to the results of 1996. However, in contrast to the results of the study in 1996, in 2004 no MAA bacteria could be detected in lymph nodes after bacteriological examination. Apparently, the prevalence of MAA infections in The Netherlands in 2004 was considerably less when compared with the prevalence in 1996. One of the reasons for the decrease in prevalence of MAA infections could be the change in use of compost on pig farms. Pigs fed with compost can develop granulomatous lymphadenitis (Engel et al., 1978). In a search for possible sources of MAA infections in pigs on two farms of the survey of 1996 it appeared that

Table 2
Distribution of pigs with granulomatous lesions in sub-maxillary and mesenteric lymph nodes across farms and their outcome after bacteriological examination for *Mycobacterium avium* subsp. *avium* (MAA) and *Rhodococcus equi*

Farm	No. of examined pig carcasses	No. (%) of pigs with lesions					
		Sub-maxillary lymph nodes			Mesenteric lymph nodes		
		Pathological	Bacteriological		Pathological	Bacteriological	
			MAA	*R. equi*		MAA	*R. equi*
1	155	5 (3.2)	0 (0.0)	3 (1.9)	0	0 (0.0)	0 (0.0)
2	114	11 (9.6)	0 (0.0)	3 (2.6)	0	0 (0.0)	2 (0.0)[a]
3	68	3 (4.4)	0 (0.0)	2 (2.9)	0	0 (0.0)	0 (0.0)
4	117	7 (5.6)	0 (0.0)	3 (2.6)	1	0 (0.0)	0 (0.0)
5	69	3 (4.3)	0 (0.0)	3 (4.3)	0	0 (0.0)	0 (0.0)
6	153	14 (9.2)	0 (0.0)	7 (4.6)	0	0 (0.0)	0 (0.0)
7	139	19 (13.7)	0 (0.0)	12 (8.6)	0	0 (0.0)	0 (0.0)
8	235	28 (11.9)	0 (0.0)	8 (3.4)	0	0 (0.0)	0 (0.0)
9	226	8 (3.5)	0 (0.0)	3 (1.3)	0	0 (0.0)	0 (0.0)
Total	1276	98 (7.7)	0 (0.0)	44 (3.4)	1	0 (0.0)	2 (0.0)[a]

[a] No percentages are given because the two lymph nodes bacteriologically positive for *R. equi* showed no lesions after pathological examination.

samples of compost contained MAA bacteria (Komijn, 1999). At present no pig farms, except for organic pig farms in The Netherlands use compost anymore including the nine farms from which we sampled lymph nodes for bacteriological examination.

A difference in prevalence of granulomatous lesions between the two slaughterhouses was observed. A possible explanation for this finding is a true difference in prevalence of lesions in lymph nodes of pigs on farms. Another explanation may be a difference in methodology of scoring for lesions between slaughterhouses. Lesions are scored visually at slaughter and it cannot be excluded that such subjective observation will influence the outcome of the scoring.

R. equi was frequently isolated from granulomatous lesions in sub-maxillary lymph nodes (44 out of 98) and no other bacteria were detected. Apparently, in this survey *R. equi* was the most important bacterium in causing lymphadenitis in pigs. As *R. equi* is also known as a bacterial species with zoonotic potential, the presence of *R. equi* and the food borne attribution to human *R. equi* infections should be analysed in more detail.

The isolation of *R. equi* was nearly exclusively from the sub-maxillary lymph nodes (44 out of 160) and not from the mesenteric lymph nodes (2 out of 160). These findings are in agreement with reports of others indicating that isolation of *R. equi* was usually limited to respiratory tract lymph nodes (Prescott, 1991; Dvorska et al., 1999). Furthermore, we found that isolation of *R. equi* was nearly exclusively from lymph nodes with granulomatous lesions (44 out of 46). Several reports confirm these findings but and in contrast to our findings, *R. equi* may also be recovered from normal sub-maxillary lymph nodes in healthy pigs (Prescott, 1991; Takai et al., 1996a; Dvorska et al., 1999).

A high number of lymph nodes with granulomatous lesions (54 out of 98) was bacteriologically negative for MAA and *R. equi*. Similar observations have been made earlier in The Netherlands (Komijn et al., 1999), in the US (Brown and Neuman, 1979) and in Czech Republic (Dvorska et al., 1999). Reasons for these observations could be that the granulomatous lesion are merely aesthetic or that the process had healed and no living bacteria were present. Another possible explanation was given by Dvorska et al. (1999), who suggested that during the immune response of the host organism to the infection, the subsequent lesion forming results in a total devitalisation of the agent. Experimental infections with MAA in pigs with bacteriological, pathological and immunological examinations at different time intervals after infection might reveal whether this is the case.

The results from our study show that detection of granulomatous lesions in pig lymph nodes by eye is not a reliable diagnostic test to determine an infection with MAA. Furthermore, additional examinations by culture methods appear to be necessary to estimate the true prevalence of MAA infections in pigs. However, this approach is time-consuming and laborious. Therefore, other more fast and reliable tests for the detection of MAA infections in pigs are strongly needed. Finally, the high occurrence of *R. equi* in lymph nodes of pigs provokes the question to the risk of *R. equi* transmission from pigs to the human population.

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