健康豚のリンパ節からのRhodococcus(Corynebacterium) equiおよび非定型抗酸菌の分離

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Isolation of *Rhodococcus (Corynebacterium) equi* and Atypical Mycobacteria from the Lymph Nodes of Healthy Pigs

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ABSTRACT. The prevalences of *Rhodococcus (Corynebacterium) equi* and mycobacteria in the submaxillary and mesenteric lymph nodes of apparently healthy pigs were studied. *R. equi* and atypical mycobacteria were isolated from 11 (7.4%) and 7 (4.7%), respectively, of 148 submaxillary lymph nodes, and 0 (0%) and 17 (18.9%), respectively, of 90 mesenteric lymph nodes. *R. equi* was isolated from 3 (2.0%) pigs with atypical mycobacteria, and from 8 (5.4%) pigs without atypical mycobacteria. Atypical mycobacteria was isolated from 4 (2.4%) pigs without *R. equi*. It seems likely that lymph nodes of pigs are a suitable environment for *R. equi* and atypical mycobacteria to harbour.—KEY WORDS: lymph node, pig, *Rhodococcus equi*.

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Rhodococcus (Corynebacterium) equi is an animal pathogen, especially for foals, in which it causes a suppurative bronchopneumonia and enteritis [1, 16]. *R. equi* has also been isolated from the lymph nodes of swine, with and without tuberculous lesions in several countries including Japan [2, 3, 5, 13, 14, 18]. A significant etiologic role of *R. equi* in the production of tuberculosis-like lesions in the lymph nodes is still controversial [2, 3, 5, 12, 13, 14, 18]. Previously, we reported that *R. equi* was frequently isolated from the submaxillary lymph nodes of apparently healthy pigs [13]. A reasonable interpretation of the existance of *R. equi* in the normal lymph nodes of pigs seems to be that the lymph nodes of pigs are suitable habitat for *R. equi* [13, 14].

Recently, the prevalence of tuberculous infections in the mesenteric lymph nodes of pigs due to atypical mycobacteria has been reported [6, 7, 9, 10, 20]. There was a high incidence of atypical mycobacteria isolated from submaxillary and mesenteric lymph nodes of randomly selected pigs at abattoirs in Hokkaido, Japan [7].

The purpose of this study was to reveal the prevalence of *R. equi* and atypical mycobacteria in the submaxillary and mesenteric lymph nodes of apparently haelthy pigs.

Submaxillary and mesenteric lymph nodes were collected from apparently healthy pigs at an abattoir in Aomori prefecture. One portion of each lymph node was placed in a sterile dish for transportation to the laboratory. All fat was removed from the tissues. Each tissue was cut up finely with sterile scissors and then the pieces were placed in a homogenizer. On the macroscopical examination of the lymph nodes, there were no tuberculosis-like lesions. The tissue suspension was divided into two parts. One was placed onto the selective medium for isolation of *R. equi* as described previously [13]. The plate was incubated at 37°C for 2 to 3 days, and isolates were identified according to the accepted criteria [11]. The other part was centrifuged at 2,000 rpm for 20 minutes, and then the sediment was processed with five fold volume of one per cent NaOH for 10

minutes, and was inoculated onto two slants of Ogawa egg medium. The inoculated mediums were kept at 37°C and observed each week for growth. The identification of acid-fast isolates was done according to the following 27 parameters [15]: growth at 25, 37, and 45°C; growth rate; colonial morphology; pigmented colonies in dark; photochromogenicity; niacin production; nitrate reduction; catalase; thermostable catalase; arylsulphatase; Tween 80 hydrolysis; tolerance to 0.2% *p*-aminosalicylate; tolerance to 125, 250, and 500 μg of $NH_2OH \cdot HCl$ per m*l*; resistance to ethanbutanol (5 μg/m*l*); tolerance to *p*-nitrobenzoic acid (0.5 mg/m*l*); resistance to rifampicin (25 μg/m*l*); growth on $NaNO_2$ agar (0.1%); growth on picric acid agar (0.1%); growth on Sauton agar; growth on 0.1% Tween 80 Sauton agar; and growth on 0.1% glocose agar.

R. equi and mycobacteria were isolated from 11 (7.4%) and 7 (4.7%), respectively, of 148 submaxillary lymph nodes of apparently healthy pigs, and 0 (0%) and 17 (18.9%), respectively, of 90 mesenteric lymph nodes of apparently healthy pigs (Table 1). The number of *R. equi* isolates from submaxillary lymph nodes of pigs was significantly greater than that from mesenteric lymph nodes, and the number of mycobacteria isolates from mesenteric lymph nodes was significantly greater than that from submaxillary lymph nodes of pigs.

The number of pigs in which *R. equi* and mycobacteria were isolated from the submaxillary lymph nodes is shown in Table 2. *R. equi* was isolated from 3 (2.0%) pigs with mycobacteria and from 8 (5.4%) pigs without mycobacteria. Mycobacteria was isolated from 4 (2.7%) pigs without *R. equi*. There were no significant differences among the incidences of the occurrence of *R. equi* in the submaxillary lymph nodes with or without mycobacteria. Isolates of mycobacteria from the pigs with *R. equi* were identified as *M. chelonei* subsp. *chelonei*, *M. fortuitum* and *M. xenopi*. Twenty four isolates of mycobacteria from the submaxillary and mesenteric lymph nodes were also identified as atypical mycobacteria as shown in Table 3.

Table 1. Isolation of *R. equi* and mycobacteria from submaxillary and mesenteric lymph nodes of apparently healthy pigs

Lymph nodes	No. of tested	*Rhodococcus equi*		Mycobacteria	
		No. of isolates	%	No. of isolates	%
Submaxillary	148	11[a)]	7.4	7	4.8
Mesenteric	90	0	0	17[b)]	18.9

a) $p<0.05$ compared with mesenteric lymph nodes by chi-square test.
b) $p<0.01$ compared with submaxillary lymph nodes by chi-square test.

Table 2. Number of pigs in which *R. equi* and mycobacteria were isolated from the submaxillary lymph nodes[a)]

R. equi	Mycobacteria	No. of pigs	%	Note
+	−	8	5.4	
+	+	3	2.0	*M. chelonei* subsp. *chelonei*, *M. fortuitum*, *M. xenopi*
−	+	4	2.7	

a) One hundred and fourty eight submaxillary lymph nodes of pigs were examined.

Table 3. Identification of mycobacteria isolated from the submaxillary and mesenteric lymph nodes (L. N.) of apparently healthy pigs

Species	No. of isolates from	
	Submaxillary L. N.	Mesenteric L. N.
M. aurum	1	0
M. chelonei		
subsp. *abscessus*	0	2
subsp. *chelonei*	1	8
M. fortuitum	2	3
M. kansassi	0	1
M. phlei	0	1
M. smegmatis	1	0
M. terrae	1	0
M. xenopi	1	2
Total	7	17

Present study indicates that the submaxillary lymph nodes of pigs are a suitable environment for *R. equi* and atypical mycobacteria to harvour. Mesenteric lymph nodes provide a better environment for atypical mycobacteria to harbour, but do not provide a suitable environment for *R. equi*. The prevalence of *R. equi* in the submaxillary lymph nodes of pigs was not significantly dependent on the presence of atypical mycobacteria and vice versa. It seems doubtful that *R. equi* and atypical mycobacteria cooperate in producing caseous adenitis in pigs. McKenzie and Donald [8] speculated that *R. equi* could cause the tuberculous lesions in cattle without the presence of mycobacteria. However, it has been known that *R. equi* can be isolated from the normal cervical lymph nodes of pigs, and from a varying percentage of tuberculous-like lesions of pigs [2, 3, 5, 12, 13, 14, 18]. The evidence obtained from our present study is also in favour of the view that *R. equi* is not associated aetiologically with tuberculous like lesions. The difference between *R. equi*'s ability to cause tuberculous lesions in cattle and in pigs is not known. The isolation rate of *R. equi* from the submaxillary lymph nodes of pigs in the present study was lower than that in the previous study [13]. The reasons for the difference are not known. This might be partly caused by the difference among pig farms studied in the prevalence of *R. equi*.

R. equi and numbers of rapid growing mycobacteria are thought to be a saprophytic habitant of soil and has been isolated from the soil of domestic animals' feeding grounds [13, 16, 17, 19].. They are common in the alimentary tracts of herbivorous animals, and rapid mycobacteria have also been found on the mucous membrane and skins of animals [4]. There is little doubt that *R. equi* and atypical mycobacteria have access to submaxillary and mesenteric lymph nodes of pigs as a result of ingestion. Further work is needed to clarify the ecology of *R. equi* and atypical mycobacteria in pigs and their environment.

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REFERENCES

1. Cimprich, R. E., and Rooney, J. R. 1977. *Vet. Pathol.* 14: 95–102.
2. Cotchin, E. 1943. *J. Comp. Pathol. Ther.* 53: 298–309.
3. Feldman, W. H., Moses, H. E., and Karlson, A. G. 1940. *Cornell Vet.* 30: 465–481.
4. Gillespie, J. H., and Timoney, J. F. 1981. Hagan and Bruner's infectious diseases of domestic animals, 7th ed., Cornell university Press, Ithaca and London.
5. Karlson, A. G., Moses, H. E., and Fredman, W. H. 1940. *J. Infect. Dis.* 67: 243–251.
6. Karlson, A. G., and Thoen, C. O. 1971. *Am. J. Vet. Res.* 32: 1257–1261.
7. Masaki, S., Shimizu, K., Cho, N., and Hirose, T. 1982. *Jpn. J. Vet. Sci.* 44: 213–221.
8. McKenzie, R. A., and Donald, B. A. 1979. *J. Comp. Pathol.* 89: 31–38.
9. Miyashita, T., Yachida, S., Nara, H., Shimizu, K., and Ono, T. 1972. *J. Jpn. Vet. Med. Assoc.* 25: 76–84.
10. Nemoto, H., Hatakeyama, K., Matsui, K., and Ugi, H. 1969. *J. Jpn. Vet. Med. Assoc.* 22: 114–117.
11. Rogosa, M., Cummins, C. S., Lelliott, R. A., and Keddie, R. M. 1974. *In*: Buchanan, R. E., and Gibbons, N. E. (Editors), Bergey's manual of Determinative Bacteriology (8th edition), Wil-

liams and Wilkins, Baltimore, 606–607.
12. Smith, J. E. 1966. *J. Appl. Bacteriol.* 29: 119–130.
13. Takai, S., and Tsubaki, S. 1985. *Jpn. J. Vet. Sci.* 47: 493–496.
14. Takeuchi, S., Azuma, R., Ogimoto, K., Suto, T., Mochizuki, M., and Nakamura, T. 1967. *Bull. Natl. Inst. Anim. Hlth.* 55: 40–44 (in Japanese).
15. Tsukamura, M., 1967. *Tubercle* 48: 311–338.
16. Wilson, M. M. 1955. *Aust. Vet. J.* 31: 175–181.
17. Wolinsky, E., and Rynearson, T. K. 1968. *Am. Rev. Respir. Dis.* 97: 1032–1037.
18. Woodroofe, G. M. 1950. *Aust. J. Exp. Biol. Med. Sci.* 28: 399–409.
19. Woolcock, J. B., Mutimer, M. D., and Farmer, A-M. T. 1980. *Res. Vet. Sci.* 28: 87–90.
20. Yachida, S., and Shimizu, K. 1973. *Jpn. J. Vet. Sci.* 35: 459–471.

要　約

健康豚のリンパ節からの *Rhodococcus* (*Corynebacterium*) *equi* および非定型抗酸菌の分離(短報): 高井伸二・竹内泰造・椿　志郎 (北里大学獣医畜産学部獣医衛生学教室)——健康豚の顎下リンパ節148検体から *R. equi* を11株(7.4%), 非定型抗酸菌を7株(4.7%), および, 腸間膜リンパ節90検体から, 非定型抗酸菌のみを17株(18.9%), それぞれ分離した。顎下リンパ節148検体のうち, 3検体(2.0%)から *R. equi* と非定型抗酸菌が同時に分離され, *R. equi* あるいは非定型抗酸菌のみが分離されたのは, それぞれ, 8検体(5.4%), および4検体(2.7%)であった。豚のリンパ節は, *R. equi* および非定型抗酸菌の存在に好適な環境であることが示唆された。