Intermediately virulent *Rhodococcus equi* isolates from pigs in Slovenia: discovery of new plasmid types and assessment of genetic diversity by pulsed-field gel electrophoresis

M. Pate[1], M. Ocepek[1], I. Zdovc[1], C. Minato[2], Y. Ohtsu[2], M. Matsuoka[2], Y. Honda[2], L. Hashimoto[2], Y. Sasaki[2], T. Kakuda[2], S. Takai[2]

[1]Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]School of Veterinary Medicine, Kitasato University, Towada, Aomori, Japan

ABSTRACT: The presence of large plasmids in 30 *Rhodococcus equi* strains isolated from pig lymph nodes with granulomatous changes was investigated. Plasmid DNAs were isolated and digested with the restriction endonucleases *Bam*HI, *Eco*RI, *Eco*T22I and *Hind*III for detailed comparison and estimation of plasmid sizes. A total of nine isolates were identified as intermediately virulent (VapB-positive), harbouring large plasmids of type 5 (n = 5) and four new variants that we tentatively designated as type 19 (n = 1), 20 (n = 1), 21 (n = 1) and 24 (n = 1). All isolates were subjected to genotyping with pulsed-field gel electrophoresis (PFGE). High genetic diversity was observed: 21 distinct genotypes were detected; five were found in multiple isolates and the others were unique. Isolates of the same plasmid type exhibited different PFGE profiles and vice versa. In a few cases, multiple strains from certain farms were analysed, the majority of which exhibited diverse PFGE profiles. Our findings demonstrate the presence of a wide variety of *R. equi* strains even in small confined environments such as farms. This is the first molecular epidemiology study of intermediately virulent *R. equi* isolates from Slovenian pigs.

Keywords: genotyping; molecular epidemiology; restriction enzyme analysis; virulence-associated genes

Rhodococcus equi is a well-recognized pathogen in veterinary medicine. This aerobic gram-positive facultative intracellular coccobacillus is largely a soil organism and causes chronic bronchopneumonia, lymphadenitis and enteritis in foals younger than 6 months old. Infection can also occur in a wide variety of other mammals, usually due to immunosuppression (Prescott, 1991). *R. equi* is frequently isolated from the lymph nodes of pigs (Barton and Hughes, 1980; Katsumi et al., 1991; Prescott, 1991; Takai et al., 1996; Pate et al., 2004; Makrai et al., 2005; Shitaye et al., 2006; Komijn et al., 2007); it also seems to be gaining importance in human health (Jones et al., 1989; Kedlaya et al., 2001; Weinstock and Brown, 2002; Torres-Tortosa et al., 2003; Ulivieri and Oliveri, 2006). Increased numbers of human *R. equi* infections might be a consequence of the AIDS epidemic but may also reflect the increasing awareness of this opportunistic pathogen (Prescott, 1991).

Based on virulence-associated antigens and virulence plasmids, *R. equi* strains are classified as virulent, intermediately virulent and avirulent. Virulent strains are identified by the presence of virulence-associated 15- to 17-kDa antigens (VapA) and virulence plasmid DNAs of 85 to 90 kb in size.

Supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia (Grant No. L4-6081-0406-04/4.04) and by the Ministry of Education, Science, Sports and Culture of Japan (Grant-in-aid for general scientific research, Takai: 18405038).

Currently, at least 12 different virulence plasmids in VapA-positive *R. equi* have been isolated from horses worldwide (Ribeiro et al., 2005). Intermediately virulent strains are characterized by the presence of a virulence-associated 20-kDa antigen (VapB) and virulence plasmids of 79 to 100 kb found in pig, wild boar and human isolates; 23 distinct plasmid types have been reported to date (Takai et al., 1995; Fukunaga et al., 1999; Makrai et al., 2008). Avirulent *R. equi* contains neither virulence-associated antigens nor plasmid DNA and is widespread in soil (Takai, 1997).

In pigs, the lesions from which *R. equi* has been isolated strikingly resemble those caused by tuberculosis and have therefore raised considerable interest among veterinarians. However, the organism can be isolated with similar frequency from the lymph nodes and tonsils of otherwise healthy pigs (Karlson et al., 1940; Barton and Hughes, 1980; Takai et al., 1996; Makrai et al., 2005). The causative role of *R. equi* in granulomatous lymphadenitis in swine is unclear. In some cases the tuberculous lesions can be explained by the concurrent presence of various *Mycobacterium* species (Dvorska et al., 1999; Pate et al., 2004; Shitaye et al., 2006). Efforts to reproduce the lesions in swine submaxillary lymph nodes by feeding the pigs with cultures of *R. equi* have not been successful (Karlson et al., 1940).

In a previous study, we characterized *R. equi* strains isolated from granulomatous lesions of pig lymph nodes by testing the isolates for the presence of genes encoding virulence-associated antigens VapA and VapB (Pate et al., 2004). The aim of this study was to further investigate the characteristics of intermediately virulent *R. equi* isolates and to determine their genetic diversity on the basis of plasmid typing and pulsed-field gel electrophoresis (PFGE).

MATERIAL AND METHODS

R. equi isolates

A total of 30 *R. equi* strains from pigs were investigated. Isolates were obtained from the lymph nodes with granulomatous lesions, collected over a 4-year period during routine abattoir inspections targeted at detecting tuberculosis. Slaughtered animals were bred on two large (≥ 1 000 pigs) and 20 small farms (< 1 000 pigs). The origin of the strains in terms of geographic location is shown in Table 1. The high prevalence of isolates from the north-eastern region correlates with the highest density of piggeries in this part of Slovenia. The isolates were identified as *R. equi* on the basis of colony morphology, biochemistry (API Coryne kit, BioMerieux, France) and a 16S rRNA-based polymerase chain reaction (PCR) described previously (Bell et al., 1996).

VapA and VapB PCR

Template DNA was extracted with a simplified boiling method: *R. equi* colonies scraped from sheep blood agar plates were suspended in 100 µl PCR-grade water, incubated at 95°C for 15 min and centrifuged for 2 min at 11 000 × g. The supernatant was used for PCR without further purification. Testing for the virulence-associated genes encoding virulence-associated antigens VapA and VapB was performed with primer sets and amplification protocols described previously (Takai et al., 1995; Makrai et al., 2002).

Restriction enzyme analysis of plasmid DNA

Plasmid DNA was extracted by the alkaline lysis method (Birnboim and Doly, 1979) with some modifications as described previously (Takai et al., 1993), and digested with the restriction endonucleases *Bam*HI, *Eco*RI, *Eco*T22I and *Hind*III for detailed comparison and estimation of plasmid sizes (Takai et al., 1999). Samples of the plasmid preparations were separated in 0.7% or 1.0% agarose gels at approx. 5 V/cm for 2 h.

Pulsed-field gel electrophoresis

R. equi colonies were transferred to brain heart infusion (BHI) broth and incubated for 36 h at 30°C in a shaking incubator. Bacterial cells were washed and resuspended in SE buffer (10mM Tris, 1M NaCl; pH 7.6). Agarose plugs were made from a 1 : 1 mixture of 2% low-melting-point agarose and the cell suspension that was transferred into disposable plug molds (Bio-Rad, USA). The plugs were lysed for 18 h at 37°C in a resuspension buffer containing lysozyme (20 mg/ml). The cells were then treated for 24 h at 50°C with a solution (50mM Tris-HCl, 50mM EDTA, 1% v/v lauroyl sarcosine;

pH 9.0) containing proteinase K (300 µg/ml) and washed three times with Tris-EDTA buffer. *Vsp*I (12 IU/plug, MBI Fermentas, USA) was used for digestion according to the manufacturer's instructions. The fragments were resolved in 1% electrophoresis-grade agarose gel using a CHEF-MAPPER system (Bio-Rad, USA) with the following parameters: time 22 h, temperature 14°C, voltage 200 V, angle 120°. During the first block (7 h), the initial and final pulse times were 6 s and 15 s, respectively; during the second block (15 h), the initial pulse time was 23 s and the final pulse time was 40 s. The gels were stained with ethidium bromide (0.5 µg/ml), de-stained in distilled water and photographed under UV light. A MidRange PFG Marker II (New England BioLabs, USA) was used for estimation of the molecular sizes of the fragments.

Table 1. Origin and results of VapB-PCR, plasmid typing and PFGE of 30 *R. equi* isolates from pig lymph nodes, collected in 2000–2003

No.	Strain code	Origin	Town	Region	PCR	Plasmid type	PFGE type
1	32/00	SF1	a	NE	positive	24	B
2	52/00	SF2	b	NE	negative	–	F
3	65/00	SF3	a	NE	negative	–	G
4	67/00	SF4	c	NE	positive	5	A
5	70/00	LF1	d	C	negative	–	H
6	78/00	LF2	e	NE	positive	19	C
7	101/00	SF5	f	NE	negative	–	I
8	108/00	SF6	g	NE	negative	–	D
9	138/00	SF7	h	NE	negative	–	J
10	140/00	SF8	i	NE	negative	–	E
11	143/00	SF9	j	NE	negative	–	K
12	157/00	SF10	k	NE	negative	–	A
13	158/00	SF11	l	NE	negative	–	A
14	160/00	nn	nn	nn	negative	–	E
15	166/00	SF12	m	NE	positive	5	L
16	172/00	SF3	a	NE	positive	5	M
17	181/00	SF13	n	NE	negative	–	D
18	187/00	SF14	o	NE	positive	5	B
19	18/01	SF5	f	NE	positive	5	N
20	20/01	SF8	i	NE	negative	–	O
21	35/01	SF15	p	NE	negative	–	A
22	37/01	SF15	p	NE	negative	–	P
23	39/01	SF16	r	NE	negative	–	Q
24	50/01	SF17	s	NE	negative	–	R
25	51/01	LF2	e	NE	positive	20	C
26	55/01	LF1	d	C	positive	21	S
27	56/01	SF18	t	NE	negative	–	T
28	60/01	SF19	u	NE	negative	–	A
29	96/02	SF20	v	NE	negative	–	U
30	56/03	LF1	d	C	negative	–	A

SF = small farm, LF = large farm, nn = not known, NE =north-east, C = central

RESULTS

Among 30 isolates, nine were positive for the *vapB* gene and none for the *vapA* gene. All VapB-positive isolates were tested for the presence of virulence plasmids. On the basis of restriction enzyme analysis, five distinct plasmid types were found. Besides the already described type 5, which was found to be the most prevalent, four new types were discovered and designated as 19, 20, 21 and 24 (Table 1, Figure 1).

All the isolates included in this study were also subjected to genotyping with PFGE. A total of 21 different PFGE profiles were found (designated A–U); five profiles (A–E) were discovered in multiple isolates. Profile A was the most prevalent one as it was found in six isolates, followed by the profiles B, C, D and E which were found in two isolates each. In six cases, multiple isolates from certain farms (LF1, LF2, SF15, SF3, SF5, SF8) obtained in the same year or over a few (1–3) years were investigated. The profiles of the isolates collected from each respective farm differed from each other with the exception of two isolates from the farm LF2, which were both profile C but exhibited different plasmid types (19 and 20).

Isolates containing the same plasmid type (i.e. type 5) exhibited different PFGE profiles and vice versa – isolates with identical PFGE profiles contained different virulence plasmids (Table 1).

DISCUSSION

The presence of *R. equi* in pigs was first demonstrated over half a century ago, but its role in granulomatous lymphadenitis still remains unclear. The studies on this topic report conflicting results: some authors recovered *R. equi* from the lymph nodes of healthy pigs (Takai et al., 1996; Madarame et al., 1998; Dvorska et al., 1999; Makrai et al., 2005) while others reported the isolation of

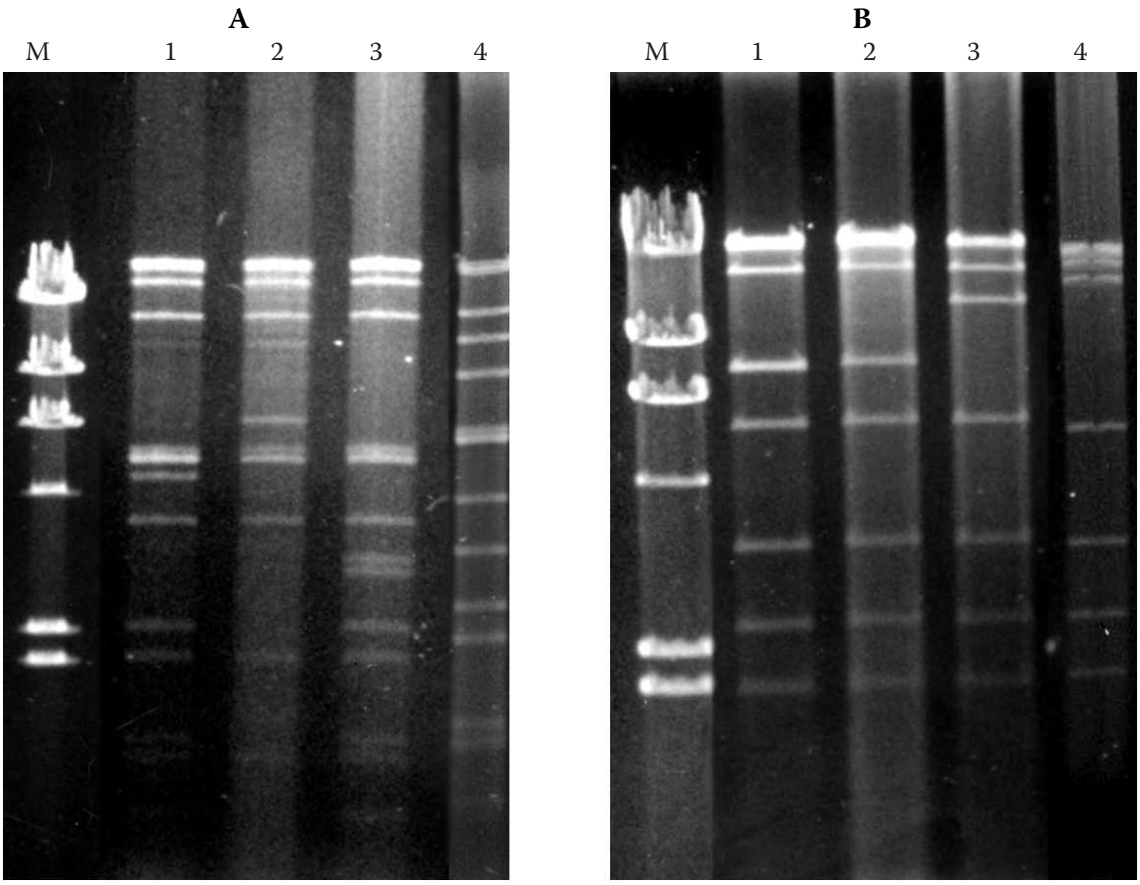

Figure 1. *Eco*RI (A) and *Eco*T22I (B) restriction fragments of the new plasmid types of *R. equi* isolates of intermediate virulence. Lane 1 = strain 78/00 (plasmid type 19); lane 2 = strain 51/01 (plasmid type 20); lane 3 = strain 55/01 (plasmid type 21); lane 4 = strain 32/00 (plasmid type 24). Markers (M) are *Hind*III digestion products of bacteriophage lambda DNA

R. equi almost exclusively from lymph nodes with granulomatous lesions, identifying it as the causative agent of lymphadenitis in pigs (Komijn et al., 2007). In the scope of one study, *R. equi* was found in pigs with and without caseous lymphadenitis, but the isolation rate was reported to be five times higher in the lymph nodes with lesions (Katsumi et al., 1991).

In our previous study (Pate et al., 2004) *R. equi* was isolated from swine lymph nodes with lesions alone (27.3%), or together with mycobacteria (5.4%). A similar Czech study showed that *R. equi* alone was isolated from 7.4% of pigs and as part of a mixed infection with atypical mycobacteria in another 2.3% of pigs (Dvorska et al., 1999). Takai and Tsubaki (1985) and Takai et al. (1996) found *R. equi* in 21.7% and 3.1% of investigated submaxillary lymph nodes of apparently healthy pigs, respectively. Recently, an isolation rate of 14.0% was reported from swine submaxillary lymph nodes without macroscopic lesions in Hungary (Makrai et al., 2005).

Although there seems to be a increasing trend for isolation of *R. equi* from swine lymph nodes (Shitaye et al., 2006; Komijn et al., 2007), either due to a true increase of infections or as a consequence of improved diagnostics in terms of awareness of this pathogen, relatively little research has been carried out with respect to characterization of its virulence plasmid types. It has been demonstrated that *R. equi* strains from pigs may express VapA or VapB, which are characteristic for strains from AIDS patients (Takai et al., 1996; Makrai et al., 2002; Takai et al., 2002). This raised questions about the zoonotic potential of *R. equi* and increased interest in further research. The abovementioned Hungarian study (Makrai et al., 2005) reported six plasmid types in 26.8% VapB-positive isolates. Furthermore, VapB-positive *R. equi* was isolated with a similar isolation rate (25.6%) also from submaxillary lymph nodes of Hungarian wild boars (Makrai et al., 2008). The isolation of strains containing a 95-kb plasmid type 5 in pigs, wild boars and immunocompromised humans supports the hypothesis that there is an epidemiological relationship between human *R. equi* infections and the presence of intermediately virulent *R. equi* strains in pigs and wild boars (Makrai et al., 2005, 2008).

Twenty-three distinct plasmids of 79 to 100 kb that are associated with the expression of the 20-kDa antigen (VapB) in isolates from pigs, wild boars and humans have been discovered so far. Of the representative plasmid types, eight have been found in Japanese pig isolates (types 2–4, 6–9 and 12; Fukunaga et al., 1999), eight in human isolates from France and Thailand (types 1, 5, 10, 11, 13–16; Takai et al., 1995), one in a Hungarian pig isolate (type 17; Makrai et al., 2005), three in pig isolates from Thailand (types 18, 22, 23; Takai, personal communication) and three in wild boar isolates from Hungary (types 25–27; Makrai et al., 2008).

Herein, we describe the further investigation of *R. equi* strains recovered from swine lymph nodes during our previous study (Pate et al., 2004). Even though all 30 strains expressed VapB when tested previously, it was impossible to detect it in 21 strains when retested two years later for the purpose of this study. This is most probably a consequence of frequent subculturing which very likely resulted in plasmid curing. Thus, only nine strains could be investigated for the presence of large plasmids and five plasmid types were identified with type 5 being the most prevalent. The same plasmid type has been found in the majority of pig and wild boar isolates in Hungary (Makrai et al., 2005, 2008) but in a much lower proportion (2.9–9.9%) in isolates from Japan (Fukunaga et al., 1999). In addition, four isolates exhibited different restriction patterns from the ones discovered before. Considered new, they were tentatively designated as types 19, 20, 21 and 24. Thus, there are currently 27 known variants of 79–100 kb plasmids in VapB-positive *R. equi*.

Insight into the genetic structure of the strains was also obtained using PFGE analysis. Genotyping revealed considerable heterogeneity as 70% of the isolates exhibited unique patterns. Multiple strains of the same origins had diverse PFGE profiles in the majority of cases. Even greater diversity was observed by comparing the results of both typing methods: the isolates of the same plasmid type could be further subtyped with PFGE and vice versa. The combination of both methods thus proved useful to increase discrimination between the isolates.

Our findings demonstrate the presence of a wide variety of *R. equi* strains in pigs, not only in different geographical regions but also in smaller, confined environments such as farms. This suggests that the infection is presumably not transmitted between the animals on the farms. It seems that, unlike *Mycobacterium avium*, another ubiquitous opportunistic pathogen frequently encountered in pigs, *R. equi* is not likely to cause outbreaks of lymphadenitis in piggeries. In the case of *M. avium* subsp. *avium*, strains of the same genotype were isolated from the animals involved in the outbreaks

of mycobacteriosis on the respective Slovenian farms in the past years (Pate, personal communication). The outbreaks were hypothetically a consequence of the multiplication of mycobacteria in the contaminated feedstuff or water. *R. equi*, being a soil organism, is obviously capable of surviving for a long time in contaminated soil and other environmental sources and opportunistically infect susceptible hosts, but the immunocompetence of individual animals seems to play an important role in the development of the infection.

In conclusion, this study revealed a high genetic diversity of *R. equi* in pigs. PFGE results demonstrated that pigs are obviously susceptible to infection with a wide variety of *R. equi* strains that persist in the environment and that there is apparently no animal-to-animal transmission of infection on the farms. Analysis of plasmid types showed that Slovenian *R. equi* isolates contain two of the plasmids that have been previously identified in isolates from other countries and novel plasmid types that we describe for the first time. The discovery of plasmid types 19–21 and 24 is a new contribution to the knowledge about virulence plasmids in intermediately virulent *R. equi* strains. Considering the small number of isolates that tested positive for large plasmids, the discovery of four new plasmid types among them indicates the possibility of a considerable variety in plasmids. Investigation of a larger panel of strains would probably result in a broader spectrum of plasmid variants. Because of seemingly high rate of presumed plasmid curing, it would be interesting to investigate this phenomenon more thoroughly. The significance of intermediately virulent *R. equi* in Slovenian pigs with regard to its pathogenicity, zoonotic potential and *R. equi* infection in humans remains to be explored in the future.

REFERENCES

Barton M.D., Hughes K.L. (1980): *Corynebacterium equi*: a review. Veterinary Bulletin, 50, 65–80.

Bell K.S., Philp J.C., Christofi N., Aw D.W. (1996): Identification of *Rhodococcus equi* using the polymerase chain reaction. Letters in Applied Microbiology, 23, 72–74.

Birnboim H.C., Doly J. (1979): A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research, 7, 1513–1523.

Dvorska L., Parmova I., Lavickova M., Bartl J., Vrbas V., Pavlik I. (1999): Isolation of *Rhodococcus equi* and atypical mycobacteria from lymph nodes of pigs and cattle in herds with the occurrence of tuberculoid gross changes in the Czech Republic over the period of 1996–1998. Veterinarni Medicina, 44, 321–330.

Fukunaga N., Okoda T., Katsumi M., Takai S. (1999): Restriction cleavage patterns of plasmid DNA of intermediately virulent *Rhodococcus equi* isolates from the mandibular lymph nodes of pigs in Kagoshima, Aomori and Miyagi Prefectures and the environment of pig farms. Journal of the Japan Veterinary Medical Association, 52, 789–792.

Jones M.R., Neale T.J., Say P.J., Horne J.G. (1989): *Rhodococcus equi*: an emerging opportunistic pathogen? Australian and New Zealand Journal of Medicine, 19, 103–107.

Karlson A.G., Moses H.E., Feldman W.H. (1940): *Corynebacterium equi* (Magnusson, 1923) in the submaxillary lymph nodes of swine. Journal of Infectious Diseases, 67, 243–251.

Katsumi M., Kodama N., Miki Y., Hiramune T., Kikuchi N., Yanagawa R., Nakazawa M. (1991): Typing of *Rhodococcus equi* isolated from submaxillary lymph nodes of pigs in Japan. Zentralblatt fur Veterinarmedizin B, 38, 299–302.

Kedlaya I., Ing M.B., Wong S.S. (2001): *Rhodococcus equi* infections in immunocompetent hosts: case report and review. Clinical Infectious Diseases, 32, E39–E46.

Komijn R.E., Wisselink H.J., Rijsman V.M., Stockhofe-Zurwieden N., Bakker D., van Zijderveld F.G., Eger T., Wagenaar J.A., Putirulan F.F., Urlings B.A. (2007): Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for *Mycobacterium avium* subsp. *avium* and positive for *Rhodococcus equi*. Veterinary Microbiology, 120, 352–357.

Madarame H., Yaegashi R., Fukunaga N., Matsukuma M., Mutoh K., Morisawa N., Sasaki Y., Tsubaki S., Hasegawa Y., Takai S. (1998): Pathogenicity of *Rhodococcus equi* strains possessing virulence-associated 15- to 17-kDa and 20-kDa antigens: experimental and natural cases in pigs. Journal of Comparative Pathology, 119, 397–405.

Makrai L., Takai S., Tamura M., Tsukamoto A., Sekimoto R., Sasaki Y., Kakuda T., Tsubaki S., Varga J., Fodor L., Solymosi N., Major A. (2002): Characterization of virulence plasmid types in *Rhodococcus equi* isolates from foals, pigs, humans and soil in Hungary. Veterinary Microbiology, 88, 377–384.

Makrai L., Takayama S., Denes B., Hajtos I., Sasaki Y., Kakuda T., Tsubaki S., Major A., Fodor L., Varga J., Takai S. (2005): Characterization of virulence plasmids and serotyping of *Rhodococcus equi* isolates from submaxillary lymph nodes of pigs in Hungary. Journal of Clinical Microbiology, 43, 1246–1250.

Makrai L., Kobayashi A., Matsouka M., Sasaki Y., Kakuda T., Denes B., Hajtos I., Revesz I., Janosi K., Fodor L., Varga J., Takai S. (2008): Isolation and characterization of *Rhodococcus equi* from submaxillary lymph nodes of wild boars (*Sus scrofa*). Veterinary Microbiology, doi:10.1016/j.vetmic.2008.04.009.

Pate M., Zdovc I., Pirs T., Krt B., Ocepek M. (2004): Isolation and characterisation of *Mycobacterium avium* and *Rhodococcus equi* from granulomatous lesions of swine lymph nodes in Slovenia. Acta Veterinaria Hungarica, 52, 143–150.

Prescott J.F. (1991): *Rhodococcus equi*: an animal and human pathogen. Clinical Microbiology Reviews, 4, 20–34.

Ribeiro M.G., Seki I., Yasuoka K., Kakuda T., Sasaki Y., Tsubaki S., Takai S. (2005): Molecular epidemiology of virulent *Rhodococcus equi* from foals in Brazil: virulence plasmids of 85-kb type I, 87-kb type I, and a new variant, 87-kb type III. Comparative Immunology, Microbiology and Infectious Diseases, 28, 53–61.

Shitaye J.E., 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. Veterinarni Medicina, 51, 497–511.

Takai S. (1997): Epidemiology of *Rhodococcus equi* infections: a review. Veterinary Microbiology, 56, 167–176.

Takai S., Tsubaki S. (1985): The incidence of *Rhodococcus (Corynebacterium) equi* in domestic animals and soil. Japanese Journal of Veterinary Science, 47, 493–496.

Takai S., Watanabe Y., Ikeda T., Ozawa T., Matsukura S., Tamada Y., Tsubaki S., Sekizaki T. (1993): Virulence-associated plasmids in *Rhodococcus equi*. Journal of Clinical Microbiology, 31, 1726–1729.

Takai S., Imai Y., Fukunaga N., Uchida Y., Kamisawa K., Sasaki Y., Tsubaki S., Sekizaki T. (1995): Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. Journal of Infectious Diseases, 172, 1306–1311.

Takai S., Fukunaga N., Ochiai S., Imai Y., Sasaki Y., Tsubaki S., Sekizaki T. (1996): Identification of intermediately virulent *Rhodococcus equi* isolates from pigs. Journal of Clinical Microbiology, 34, 1034–1037.

Takai S., Shoda M., Sasaki Y., Tsubaki S., Fortier G., Pronost S., Rahal K., Becu T., Begg A., Browning G., Nicholson V.M., Prescott J.F. (1999): Restriction fragment length polymorphisms of virulence plasmids in *Rhodococcus equi*. Journal of Clinical Microbiology, 37, 3417–3420.

Takai S., Tharavichitkul P., Sasaki C., Onishi Y., Yamano S., Kakuda T., Tsubaki S., Trinarong C., Rojanasthien S., Sirimalaisuwan A., Tesaprateep T., Maneekarn N., Sirisanthana T., Kirikae T. (2002): Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with acquired immune deficiency syndrome and prevalence of virulent *R. equi* in soil collected from domestic animal farms in Chiang Mai, Thailand. American Journal of Tropical Medicine and Hygiene, 66, 52–55.

Torres-Tortosa M., Arrizabalaga J., Villanueva J.L., Galvez J., Leyes M., Valencia M.E., Flores J., Pena J.M., Perez-Cecilia E., Quereda C. (2003): Prognosis and clinical evaluation of infection caused by *Rhodococcus equi* in HIV-infected patients: a multicenter study of 67 cases. Chest, 123, 1970–1976.

Ulivieri S., Oliveri G. (2006): Cerebellar abscess due to *Rhodococcus equi* in an immunocompetent patient: case report and literature review. Journal of Neurosurgical Sciences, 50, 127–129.

Weinstock D.M., Brown A.E. (2002): *Rhodococcus equi*: an emerging pathogen. Clinical Infectious Diseases, 34, 1379–1385.

Received: 2008–07–03
Accepted: 2009–03–18

Corresponding Author:

Prof. Shinji Takai, PhD., Kitasato University, School of Veterinary Medicine, Department of Animal Hygiene, Higashi 23-35-1, Towada, Aomori 034-8628, Japan
Tel. +81 176 24 9458, Fax +81 176 23 8703, E-mail: takai@vmas.kitasato-u.ac.jp