Molecular epidemiology of *Rhodococcus equi* in slaughtered swine, cattle and horses in Poland

Lucjan Witkowski¹*, Magdalena Rzewuska², Shinji Takai³, Magdalena Kizerwetter-Świda² and Jerzy Kita¹

**Abstract**

**Background:** *Rhodococcus equi* is an emerging zoonotic presumably foodborne pathogen. Since the data on the worldwide prevalence of *R. equi* in meat animals are scarce, the present study aimed to investigate the molecular epidemiology of *R. equi* in swine, cattle and horse carcasses intended for human consumption in Poland.

**Results:** Totally 1028 lymph node samples were examined. *R. equi* was isolated from 26.6 % (105/395) swine and 1.3 % (3/234) bovine healthy submaxillary lymph nodes. In horses, *R. equi* was isolated only from 0.5 % (1/198) samples of middle tracheo-branchiales lymph node while no lymphocentrum retropharyngeum sample was positive (0/198). The purulent lesions were observed only in 0.8 % swine submaxillary lymph nodes samples (3/398) and in two of them *R. equi* was detected.

All bovine and most of swine isolates (98.1 %) were *vapB*-positive. 87.9 % of swine isolates carried 95-kb type 5 plasmid, 3.7 % type 1 and plasmid types: 4, 7, 10, 11, 21, 31 were carried by a single isolate (0.9 %). All bovine isolates carried VAPB type 26. Single horse isolate was *vapA*-positive and carried plasmid VAPA 85-kb type I.

**Conclusions:** The prevalence of *vapB*-positive *R. equi* in investigated healthy swine intended for human consumption was very high.

Not only swine, but also even apparently healthy cattle or horse carcasses should be considered as a potential source of *R. equi* for humans, especially in countries where undercooked or raw beef or horsemeat is traditionally consumed.

**Keywords:** *R. equi*, Swine, Equine, Meat, Slaughterhouse, Foodborne pathogen

---

**Background**

*Rhodococcus equi* is a Gram-positive bacterium widespread in the environment of grazing farms. It is common in the feces of wildlife and farm animals including swine, cattle, horses and others [1–5]. The virulence of *R. equi* is determined by the virulence-associated plasmids (VAPs) which harbor the pathogenicity islands that contain *vap* genes encoding the virulence-associated proteins (*Vaps*) [6, 7]. In virulent strains isolated from horses the *vapA* gene encoding virulence-associated 15–17-kDa protein (*VapA*) is predominant, whereas in swine isolates the *vapB* gene of the virulence-associated 20-kDa protein (*VapB*) is most prevalent. Bovine isolates usually carry the *vapN* gene however some isolates carry the *vapB* gene. The aforementioned three genes are located on plasmids VAPA, VAPB, and VAPN, respectively. Interestingly, pVAPN is a 120-kb invertron-like linear replicon unrelated to the circular virulence plasmids associated with equine (pVAPA) and swine (pVAPB) and harbors new *vap* multigene family members (*vapN* to *vapS*). Avirulent, “plasmidless” strains, widespread in the environment, mainly in soil show no evidence of these plasmids but are positive for a *traA* plasmid conjugal transfer gene marker [8–10]. The “TRAVAP” typing scheme classifies *R. equi* isolates into 4 categories: *traA*<sup>+</sup>/A<sup>+</sup>B<sup>−</sup> “horse-type”, *traA*/A<sup>+</sup>B<sup>+</sup> “pig-type”, *traA*/A<sup>−</sup>B<sup>−</sup> “bovine-type” and *traA*/A<sup>−</sup>B<sup>−</sup> plasmid-less type [10].

Using an unified nomenclature, plasmids were designated as pVAPA, pVAPB, and pVAPN (for “noA-noB”),
respectively [7, 9]. Analysis of restriction enzyme digestion patterns revealed several distinct VAPA plasmid types and over 20 types of VAPB plasmid but the digestion patterns of VAPN have not been so far recognized [3, 11, 12].

In horses, R. equi is a well-known pathogen responsible for foal rhodococcosis – highly fatal disease typically manifesting itself as pyogranulomatous bronchopneumonia. The disease usually affects foals aged up to six months whereas it rarely occurs in adult horses [13, 14]. Sporadically, the bacterium can prove pathogenic for other animals. In swine, R. equi was isolated for the first time in 1930s of the XX century, from purulent lesions in lymph nodes [15]. Recent studies have revealed that R. equi not previously suspected Mycobacterium spp., is the primary causal agent of lymphadenitis [16–21]. The disease was rarely described in ruminants such as camels [22], llamas [23] and mostly in goats [24]. Reports from cattle are sparse and all describe isolation from purulent lesions in the lymph nodes, mostly in animals with suspected tuberculosis [20, 25–27]. In wild animals R. equi was isolated from purulent lesions in the lymph nodes of wild boar [11, 28] and American bison as a co-infection with Mycobacterium avium subsp. paratuberculosis [29]. The clinical cases of pyogranulomatous skin disease and pneumonia associated with R. equi infection were also occasionally observed in cats and dogs [30].

Recently, R. equi infection in other species than horse has aroused considerable interest due to its frequent isolation from the lymph nodes and other tissues of apparently healthy animals intended for human consumption. The prevalence of R. equi in swine carcasses varies from 0 % to over 20 % [1, 2, 11, 18–20, 28, 31–33]. In wild boar the prevalence ranges from 0 % to even 52 % hunted animals [34–37], whereas in the roe deer and red deer it is lower than 1 % [37]. However, the data on the occurrence of R. equi in slaughtered apparently healthy cattle or horses are lacking.

The increasing number of R. equi infections in humans has been observed for several years and now R. equi is considered an emerging zoonotic pathogen. The clinical cases in humans are rare and so far have been usually diagnosed in immunocompromised patients [38]. Sources and routes of human infection remain unclear. It has been suggested that the contact with farm animals or their environment may plays a role in some cases of infection but a foodborne transmission seems to be the most likely route, especially the exposure by the consumption of raw, undercooked or contaminated meat [5, 10, 38, 39]. This theory is supported by the epidemiological relationship between human and animal R. equi infections. The strains of swine or bovine type have been isolated from human cases more often than equine or avirulent environmental strains [3, 8, 10, 39]. Furthermore, a pattern of geographical distribution of strains is similar in humans and animals. For example VAPB type 5 is predominant among isolates from humans and animals in Europe [12, 36], type 8 in the South America [5, 11, 39], while type 1 and 2 in Asia [1, 35].

Regardless of the significant progress, relatively little is known about types of virulence plasmids of R. equi isolated from the meat animals. The problem is important, as there is increasing trend for diagnosing R. equi infections in humans and growing rate of R. equi isolation from tissues of slaughtered or hunted animals. These facts may result from the infection running rife as well as from increasing awareness and improved diagnostics.

Thus, the aim of this study was to estimate the prevalence of R. equi in swine, cattle and horse carcasses intended for human consumption in Poland and characterize virulence plasmid types in the isolates.

Results

The results of R. equi isolation are presented in Table 1. Totally, 107 R. equi strains were isolated from swine, 105 strains from lymph nodes without any purulent lesions (26.6 % of swine) and 2 other from lymph nodes with purulent lesions. At least several colonies of R. equi were observed in all positive cultures from swine samples. The purulent lesions (which varied in size from 1 to 5 millimeters in wide) were observed only in 3 (0.8 %; CI 95 %: 0.3–2.2 %) samples and two of them were R. equi positive. No purulent lesions were observed in any of the samples from cattle or horses. R. equi in horses was isolated only once from the middle tracheo-bronchial lymph nodes of a two year-old stallion. The results of detection of plasmid genes vapa and vapb are presented in Table 1. The choE gene specific for R. equi was detected in all 111 isolates. All cattle and 98.1 % of swine isolates were vapb-positive. A single horse isolate was vapA-positive. Ten distinct VapB plasmid types were found in 10 trae-positive isolates: 9 in swine and 1 in cattle (Table 2). VAPB 95-kb type 5 was most prevalent in swine isolates and was detected in 94 of the vapB-positive isolates. All cattle isolates carried plasmid type 26. The horse strain carried 85-kb type 1 plasmid (Table 2).

Discussion

Our results confirmed the presence of R. equi in submaxillary lymph nodes of apparently healthy swine intended for the human consumption. Moreover, the prevalence of R. equi in healthy swine (26.58 %) appeared very high, when compared with the data from most previous studies which reported 0 to 3 % [1, 16, 28, 31, 32]. Only two reports, one from Hungary [33] and one from Japan [2] presented the results closer to our findings (14 and 21 %, respectively).

It is interesting why R. equi prevalence among swine from different countries varied so much. The methodology of R. equi isolation from swine was similar in all the aforementioned studies. For example, CAZ-NB used in our study
was widely used in similar studies and is commonly considered as the best selective medium for the isolation of \textit{R. equi} [5]. It has been suggested that contamination of slaughtered swine carcasses with pathogenic \textit{R. equi} might occur through feces [5]. It didn’t occur in this study due to food safety and quality procedures in the slaughterhouse and moreover, lymph nodes were collected with surrounding tissues and separated in the laboratory in sterile conditions. The \textit{R. equi} infection in humans is usually diagnosed in immunocompromised patients [38]. In animals (wild boars and cattle) the prevalence is higher in young than in older ones [27, 35]. Perhaps in swine also some factors predisposing for \textit{R. equi} infection exist, however they are not known.

Granulomatous lesions were rare in the investigated submaxillary lymph nodes from swine and \textit{R. equi} was isolated in two of three samples. This finding corresponds with the previous studies reporting such lesions in approx. 0.5 % of lymph nodes [16, 21]. The first \textit{R. equi} isolation from granulomatous lesions in lymph nodes in swine was described over 50 years ago, but its role still remains unclear [15]. One study revealed a very high prevalence of \textit{R. equi} in submaxillary lymph nodes with purulent lesions, the bacteria alone was isolated from 45 % and together with \textit{Mycobacterium avium} subsp. \textit{avium} from next 55 % of samples [16]. Most of the studies reported isolation of \textit{R. equi} alone from approx. 20 % of samples and together with mycobacteria from next 5 % of samples [19–21, 28]. The examination of lymphoid tissue from various organs of slaughtered swine with suspected \textit{Mycobacterium} spp. infection showed similar proportion of \textit{R. equi} infections alone (18 %), but much higher proportion of the mixed infections with mycobacteria (62 %) [17]. Moreover, the data on the role of \textit{R. equi} in lymphadenitis in wild boar are inconclusive. Although in Brazil \textit{R. equi} was isolated from 6.6 % of wild boar lymph nodes with lymphadenitis [28], no \textit{R. equi} was isolated from purulent lesions in Poland [37]. Similarly, the studies dealing with the prevalence of \textit{R. equi} in lesion-free wild boar submaxillary lymph nodes do not lead to clear conclusions. The reported prevalence varied from 0 % in Brazil [28], 5 % in Poland [37], 12 % in Hungary [34] to 52 % in Japan [35].

In our study almost all \textit{R. equi} isolates obtained from swine were \textit{vapB}-positive. Likewise, high prevalence of intermediate virulent strains in swine was described previously [31, 32]. However, other studies on swine isolates reported much lower percentage of \textit{vapB}-positive strains, in approx. 30 % [18, 33] or 70 % of isolates [1, 19]. The \textit{vapB}-positive \textit{R. equi} strains accounted for 25 to 70 % of isolates in wild boar [11, 34–36]. Interestingly, only in Japan the \textit{vapA}-positive \textit{R. equi} strains typical for the horse were rarely isolated also from swine and wild boar [32, 35].

Our results revealed the presence of 95-kb type 5 plasmid in most of \textit{vapB}-positive swine isolates. This plasmid type seems to be specific for \textit{R. equi} isolates from Europe because it has thus far been detected in most of European swine and wild boar isolates [18, 34, 36]. The 95-kb type 1 \textit{VAPB} plasmid, detected in 3.7 % of investigated swine strains in our study, was detected with similar frequency in wild boar \textit{R. equi} isolates in Hungary [34] and swine isolates in Brazil [11]. In contrast, this \textit{VAPB} type is predominant in Asia where was detected

| Table 1 Prevalence of \textit{R. equi} in lesion-free lymph nodes of slaughtered swine, cattle and horses |
|-----------------------------------------------|
| Animal species (specimen) | No. of samples | No. of isolates | Prevalence (%) | 95 % CI (%) | No. of VapA positive isolates | No. of VapB positive isolates | % of Vap positive isolates |
|-----------------------------------------------|
| Swine (submaxillary lymph nodes) | 395 | 105 | 26.6 | 22.5–31.1 | 0 | 103 | 98 |
| Cattle (submaxillary lymph nodes) | 234 | 3 | 1.3 | 0.5–3.7 | 0 | 3 | 100 |
| Horse (lymphocentrum retropharyngeum) | 198 | 0 | 0 | 0 | 0 | 0 | 0 |
| Horse (middle tracheo-branchiales lymph nodes) | 198 | 1 | 0.5 | 0.1–2.8 | 1 | 0 | 100 |
| Total | 1025 | 109 | | | | | 106 |

| Table 2 Plasmid profiles of \textit{R. equi} isolates from slaughtered swine, cattle and horses in Poland |
|-----------------------------------------------|
| Animal species | Virulence-associated plasmid | Plasmid type | No. of isolates (total No.111) | % of isolates |
|-----------------------------------------------|
| Swine | VAPB | 1 | 3.7 |
| | | 4 | 0.9 |
| | | 5 | 94 | 87.9 |
| | | 6 | 0.9 |
| | | 7 | 0.9 |
| | | 10 | 0.9 |
| | | 11 | 0.9 |
| | | 21 | 0.9 |
| | | 31 | 0.9 |
| | | 1 | 1.9 |
| Cattle | VAPB | 26 | 3 | 100 |
| Horses | VAPA | 85-1 | 1 | 100 |
in approx. 30 % of swine and wild boar isolates [31, 35]. Other VAPB types of investigated R. equi swine isolates (0.9 % of investigated isolates each) were described in 5 to 10 % of cases, worldwide: type 4 in wild boar isolates in Japan [35], type 6 and 7 in swine isolates in Thailand [31], type 7 and 11 in wild boar isolates in Poland [36], type 10 in swine isolates in Brazil [11] and type 21 in wild boar and swine isolates in Hungary and Slovenia [18, 34]. Type 8 was not detected in any of investigated isolates. However, this type is predominant in swine and wild boar isolates in Brazil [5, 11].

R. equi seems to have minor clinical importance in cattle. Nevertheless, it was isolated (alone or together with mycobacteria) from 1–3 % [20, 26] or even 23.5 % [27] of lymph nodes with lesions in slaughtered cattle suspected of Mycobacterium spp. infection. Currently, Mycobacterium spp. infection is an important problem in some countries, but granulomatous lesion in bovine lymph nodes are observed rarely. Thus, the prevalence of R. equi in population of slaughtered cattle was described as very low [27]. However, the isolation of R. equi from 1.3 % of lesion-free lymph nodes in our study contradicts previous data. Furthermore, all 3 bovine isolates were vapB-positive and carried plasmid type 26, found previously in isolates from lesion-free lymph nodes of a wild boar in Hungary [34]. Unfortunately, it is difficult to compare the molecular data of R. equi epidemiology in cattle because virulence of isolates was investigated only in a few studies on meat animals and plasmid types were rarely defined [20, 26]. No vapB-positive strains were detected and from the cases of lymphadenitis in cattle in Ireland [27] and Germany [25]. Surprisingly 9 % of R. equi strains isolated from cattle feces in Germany were vapA-positive [4]. However, further analysis of 25 bovine strains from Ireland [27] showed that 72.0 % of isolates had the specific plasmid type associated with the bovine host traA+/vapAB− (pVAPN in new nomenclature) [10].

All samples examined in our study were collected in slaughterhouses during a normal work day. Whole sampling procedure had to be non-disturbing and adapted to routine procedures in the facility. Submaxillary lymph nodes were easily available in swine and cattle carcasses but were not available in horses because were removed with the skin during flaying. Therefore, in horses we decided to collect lymphocentrum retropharyngeum as a lymph node representative for the head region. Additionally, we decided to collect middle tracheo-branchial lymph nodes. Interestingly, in this study no R. equi strains were isolated from lymphocentrum retropharyngeum of horses and only a single R. equi strain was isolated from middle tracheo-branchiales lymph nodes. This is surprising, because horse is the only animal species in which R. equi infection poses a serious clinical problem. However, only foals up to six months of age have a unique susceptibility to clinical disease, and the clinical cases in adult horses were described very rare. Those cases are probably associated with the immuno-suppression and activation of persistent infection of R. equi in lymph nodes [13, 14]. Thus, much higher prevalence of R. equi in investigated equine samples was expected. Isolated strain contains 85-kb type I VAPA plasmid which has been found in most of isolates from horses in Europe [12, 40].

Conclusions
This study confirms the occurrence of R. equi in healthy swine intended for human consumption. However, the very high prevalence of vapB-positive R. equi strains in investigated apparently healthy animals rises a questions of predisposing factors for R. equi infection in swine.

To the best of authors’ knowledge this is the first report of R. equi isolation from slaughtered, apparently healthy cattle and the first report of R. equi in meat horses at all. The low prevalence of R. equi in lymph nodes in these species suggests that their meat is unlikely to be the main source of R. equi infection for humans.

Not only swine, but also even apparently healthy cattle or horse carcasses should be considered as a potential source of R. equi for humans, especially in countries where undercooked or raw beef or horsemeat is traditionally consumed.

Methods
The study was approved by the 3rd Local Commission for Ethics in Animal Experiments (Decision No. 44/2009). The population of investigated animals was estimated on the basis of the data of The Agency for Restructuring and Modernization of Agriculture, Central Statistical Office in Poland and Polish Horse Breeders Association. Sample size was designed for each animal species according to a commonly used epidemiological method [41]. In order to estimate a prevalence with an accepted error of 5 % in swine, and 10 % in cattle and horses (level of confidence of 95 %, expected prevalence of 50 %). A 95 % confidence interval (95 % CI) for proportions was calculated using Wilson score method. The calculations were performed in Win Episcope 2.0 [41]. In 2011 year 19 675 000 swine, 1 372 000 cattle and 60 000 horses were slaughtered in Poland.

Samples were collected in 3 slaughterhouses from carcasses accepted for human consumption in 2011 and 2012. Submaxillary lymph nodes were obtained from swine (n = 398) and cattle (n = 234). From horses (n = 198) lymphocentrum retropharyngeum and middle tracheo-branchial lymph nodes were collected. A total number of 1028 lymph node samples were evaluated:
isolates from foals, pigs, humans and soil in Hungary. Clinical manifestation, virulence, and immunity. J Vet Microbiol. (2016) 16:98.

choE, traA, vapA genes, “equi factor” was studied in CAMP test. The presence of vap pathogenicity island seen through comparison of isolates using an alkaline lysis method of intermediate virulence isolated from patients with R. equi isolation, phenotypic and genotypic identification of isolates was conducted as described previously [36]. Briefly, thawed lymph nodes were cut into small pieces using sterile scissors. Then, one gram of tissue was added to 3 ml of sterile 0.9 % saline and was homogenized using PRO200 homogenizer Multi-Gen 7 (PRO Scientific Inc., USA). Finally, 100 µl of homogenized tissue was cultured in selective medium. For the bacteria isolation modified CAZ-NB medium was used, biochemical properties of isolates were determined using API Coryne test (bioMerieux, France) and the presence of “equi factor” was studied in CAMP test. The presence of four R. equi genes, choE, traA, vapA and vapB, was determined by PCR.

Plasmid DNA was isolated from the vapA and vapB-positive R. equi isolates using an alkaline lysis method with some modification and digested with restriction endonucleases EcoRI, EcoT221, HindIII and BamHI as described previously [36].

Abbreviations
VAP, virulence-associated plasmid; Vap, virulence-associated protein.

Acknowledgments
The authors thank Barbara Chojnacka, Alicja Grzechnik, Teresa Zygmanska and Beata Kowalkowska for excellent technical assistance.

Funding
The work was supported by a grant from the National Science Centre in years 2010–2013 as a research project No. N N308 131638.

Availability of data and material
All data and materials are available in Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, 02–776 Warsaw, Poland.

Authors’ contributions
LW acquisition of funding, conceiving, designing and coordination of the study, general supervision of the research group, participation in material and data collection, participation in laboratory analysis, drafting the manuscript. MR participation in acquisition of funding, participation in conceiving and designing the study, participation in laboratory analysis and assistance in drafting the manuscript. ST participation in laboratory analysis and assistance in drafting the manuscript. MRK participation in laboratory analysis and assistance in drafting the manuscript. JK participation in laboratory analysis and assistance in drafting the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study was approved by the 3rd Local Commission for Ethics in Animal Experiments (Decision No. 44/2009), Warsaw University of Life Sciences – SGGW, Ciszewskiego 8, 02–786 Warsaw, Poland.

Author details
1Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, 02–776 Warsaw, Poland.
2Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Ciszewskiego 8, 02–776 Warsaw, Poland.
3Department of Animal Hygiene, School of Veterinary Medicine, Kitasato University, Higashi 23–35-1, Towada, Aomori 034-8628, Japan.

Received: 8 September 2015 Accepted: 19 May 2016

References
1. Takai S, Tharavichitkul P, Takam P, Khantawa B, Tamura M, Tsukamoto A, Takayama Y, Yamatoda N, Kimura A, Sasaki Y, et al. Molecular epidemiology of Rhodococcus equi of intermediate virulence isolated from patients with and without acquired immune deficiency syndrome in Chiang Mai, Thailand. J Infect Dis. 2003;188(11):1717–23.
2. Takai S, Tsukub S. The incidence of Rhodococcus (Corynebacterium) equi in domestic animals and soil. Jpn J Vet Sci. 1985;47(1):493–6.
3. Takai S, Tharavichitkul P, Sasaki C, Onishi Y, Yamanaka T, Kakuda T, Tsukub S, Trikrong C, Rojanasthien S, Srimalaisuwan A, et al. 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. Ann J Trop Med Hyg. 2002;66(1):52–7.
4. Soedarmanto I, Zhicai W, Setyamahani A, Lammler C, Pheno- and genotyping of Rhodococcus equi isolated from faeces of healthy horses and cattle. Res Vet Sci. 1998;64(3):181–5.
5. Lara GH, Takai S, Sasaki Y, Kakuda T, Liston FJ, Rissett RM, de Morais AB, Ribeiro MG. VapB type 8 plasmids in Rhodococcus equi isolated from the small intestine of pigs and comparison of selective culture media. Lett Appl Microbiol. 2015;61(3):305–10.
6. Coulson GB, Aggarwal S, Hondalus MK. Characterization of the role of the pathogenicity island and Vap in the virulence of the intracellular actinomycete pathogen Rhodococcus equi. Infect Immun. 2010;78(8):3323–34.
7. Lettek M, Ocampo-Sosa AA, Sanders M, Fogarty U, Buckley T, Leadon DP, Gonzalez P, Scortti M, Meijer WG, Parkhill J, et al. Evolution of the Rhodococcus equi vap pathogenicity island seen through comparison of host-associated vapA and vapB virulence plasmids. J Bacteriol. 2008;190(17):5797–805.
8. Vazquez-Boland JA, Giaguere S, Hapeshi A, MacArthur I, Anastasi E, Valero-Reillo A. Rhodococcus equi: the many facets of a pathogenic actinomycete. Vet Microbiol. 2013;167(1–2):29–39.
9. Valero-Reillo A, Hapeshi A, Anastasi E, Alvarez S, Scortti M, Meijer WG, MacArthur I, Vazquez-Boland JA. An invertion-like linear plasmid mediates intracellular survival and virulence in bovine isolates of Rhodococcus equi. Infect Immun. 2015;83(7):2725–37.
10. Ocampo-Sosa AA, Lewis DA, Navas J, Quigley F, Callejo R, Scortti M, Leadon DP, Fogarty U, Vazquez-Boland JA. Molecular epidemiology of Rhodococcus equi based on traA, vapA, and vapB virulence plasmid markers. J Infect Dis. 2007;196(5):763–9.
11. Ribeiro MG, Takai S, Guazzelli A, Lara GH, da Silva AV, Fernandes MC, Condas LA, Siqueira AK, Salerno T. Virulence genes and plasmid profiles in Rhodococcus equi isolates from domestic pigs and wild boars (Sus scrofa) in Brazil. Res Vet Sci. 2011;91(3):478–81.
12. Makrai L, Takai S, Tamura M, Tsukamoto A, Sekimoto R, Sasaki Y, Kakuda T, Tsukub S, Varga J, Fodor L, et al. Characterization of virulence plasmid types in Rhodococcus equi isolates from foals, pigs, humans and soil in Hungary. Vet Microbiol. 2008;128(4):377–84.
13. Giaguere S, Cohen ND, Chaffin MK, Hines SA, Hondalus MK, Prescott JF, Slosvik NM. Rhodococcus equi clinical manifestations, virulence, and immunity. J Vet Intern Med. 2011;25(6):1221–30.
14. Monevsey PR, Waldridge BM. Successful treatment of Rhodococcus equi pneumonia in an adult horse. J Vet Intern Med. 2010;24(2):436–8.

15. Karlson AG, Moses HE, Feldman WH. Corynebacterium equi (Magnusson, 1923) in the submammary lymph nodes of swine. J Infect Dis. 1940;67:248–51.

16. Komijn RE, Wisselink HJ, Rijssman WM, Stockholte-Zunwieden N, Bakker D, van Zijderveeld FG, Eger T, Wagenaar JA, Putriulan FF, Ulfings BA. Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for Mycobacterium avium subsp. avium and positive for Rhodococcus equi. Vet Microbiol. 2007;120(3–4):352–7.

17. Shitaye JE, Parmova I, Matlova L, Dvorska L, Horvathova A, Vrbs V, Pavlik I. 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. 2006;51(11):497–511.

18. Pate M, Ocepek M, Zdovc I, Minato C, Ohmsy T, Matsuoka M, Honda Y, Hashimoto L, Sasaki Y, Kakuda T, et al. Intermediately virulent Rhodococcus equi isolates from pigs in Slovenia: discovery of new plasmid types and assessment of genetic diversity by pulsed-field gel electrophoresis. Vet Med. 2009;54(3):111–7.

19. Pate M, Zdovc I, Pars T, Krt B, Ocepek M. Isolation and characterisation of Mycobacterium avium and Rhodococcus equi from granulomatous lesions of swine lymph nodes in Slovenia. Acta Vet Hung. 2004;52(2):143–50.

20. Dvorska L, Parmova I, Lavickova M, Bartl J, Vrbs V, Pavlik I. Isolation of Rhodococcus equi and atypical mycobacteria from lymph nodes of pigs and cattle in herds with the occurrence of tuberculous gross changes in the Czech Republic over the period of 1996–1998. Vet Med. 1999;44(11):321–30.

21. Pavlik I, Matlova L, Dvorska L, Bartl J, Okrakova L, Dockel J, Parmova I. Tuberculous lesions in pigs in the Czech Republic during 1990–1999: occurrence, causal factors and economic losses. Vet Med. 2003;48(5):113–25.

22. Kinne J, Madarame H, Takai S, Jose S, Wernery U. Disseminated Rhodococcus equi infection in dromedary camels (Camelus dromedarius). Vet Microbiol. 2011;149(1–2):269–72.

23. Hong CB, Donahue JM. Rhodococcus equi-associated necrotizing lymphadenitis in a llama. J Comp Pathol. 1995;113(1):185–8.

24. Jeckel S, Holmes P, King S, Whatmore AM, Kirkwood I. Disseminated Rhodococcus equi infection in goats in the UK. Vet Rec. 2011;169(2):56.

25. Soedarmanto I, Oliveira R, Lammler C, Durling H. Identification and epidemiological relationship of Rhodococcus equi isolated from cases of lymphadenitis in cattle. Zentralblatt Bakteriol. 1997;286(4):457–67.

26. Sahraoui N, Muller B, Guetarni D, Boulahbal F, Yala D, Ouzrout R, Berg S, Smith NH, Zinsstag J. Molecular characterization of Mycobacterium bovis strains isolated from cattle slaughtered at two abattoirs in Algeria. BMC Vet Res. 2009;5:4.

27. Flynns O, Quigley F, Costelloe E, O’Grady, Gogarty A, Mc Guirk J, Takai S. Virulence-associated protein characterisation of Rhodococcus equi isolated from bovine lymph nodes. Vet Microbiol. 2001;78(3):221–8.

28. Ara GH, Ribeiro MG, Leite CQ, Paes AC, Guazzelli A, da Silva AV, Santos AC, Listoni FJ. Occurrence of Mycobacterium spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (Sus scrofa). Res Vet Sci. 2011;90(2):185–8.

29. Buergelt CD, Layton AW, Ginn PE, Taylor M, King JM, Habecker PL, Mauldin E, Whitlock R, Rosser C, Collins MT. The pathology of spontaneous paratuberculosis in the North American bison (Bison bison). Vet Pathol. 2000;37(5):428–38.

30. Takai S, Mathers RJ, Julian A, Garcia Ribeiro M, Rodrigues de Farias M, Sasaki Y, Inuzuka K, Kakuda T, Tsukubai S, Prescott JF. Virulence of Rhodococcus equi isolated from cats and dogs. J Clin Microbiol. 2003;41(9):4468–70.

31. Pooknhet C, Chumning S, Waiwajkul W, Minato C, Otsu Y, Takai S. Plasmid profiles and prevalence of intermediately virulent Rhodococcus equi from pigs in Nakhonpathom Province, Thailand: identification of a new variant of the 70-kb virulence plasmid, type 18. Vet Med Int. 2010;2010:491624.

32. Takai S, Fukunaga N, Ochi S, Imai Y, Sasaki Y, Tsukubai S, Sekizaki T. Identification of intermediately virulent Rhodococcus equi isolates from pigs. J Clin Microbiol. 1996;34(4):1034–7.

33. Makrai L, Takayama S, Denes B, Hajtos I, Sasaki Y, Kakuda T, Tsukubai S, Major A, Fodor L, Varga J, et al. Characterization of virulence plasmids and serotypes of Rhodococcus equi isolates from submaxillary lymph nodes of pigs in Hungary. J Clin Microbiol. 2005;43(3):1246–50.

34. Makrai L, Kobayashi A, Matsuoka M, Sasaki Y, Kakuda T, Denes B, Hajtos I, Revesz I, Janosi K, Fodor L, et al. Isolation and characterisation of Rhodococcus equi from submaxillary lymph nodes of wild boars (Sus scrofa). Vet Microbiol. 2008;131(3–4):318–23.

35. Sakai M, Ohno R, Higuchi C, Sudo M, Suzuki K, Sato H, Maeda K, Sasaki Y, Kakuda T, Takai S. Isolation of Rhodococcus equi from wild boars (Sus scrofa) in Japan. J Wildl Dis. 2012;48(3):815–7.

36. Rzewuska M, Witkowski L, Cieśk AA, Stefanska I, Cheboka D, Stefaniuk E, Kizewitter-Swida M, Takai S. Characterization of Rhodococcus equi isolates from submaxillary lymph nodes of wild boars (Sus scrofa), red deer (Cervus elaphus) and roe deer (Capreolus capreolus). Vet Microbiol. 2014;172(1–2):272–8.

37. Witkowski L, Rzewuska M, Cieśk AA, Chrobak-Chmiel D, Kizewitter-Swida M, Czopowicz M, Welz M, Kita J. Prevalence and genetic diversity of Rhodococcus equi in wild boars (Sus scrofa), roe deer (Capreolus capreolus) and red deer (Cervus elaphus) in Poland. BMC Microbiol. 2015;15(1):110.

38. Yamashchikov AV, Schuetz A, Lyon GM. Rhodococcus equi infection. Lancet Infect Dis. 2010;10(5):350–9.

39. Ribeiro MG, Takai S, de Vargas AC, Mattos-Guaraldi AL, Camello TCF, Ohno R, Okano H, da Silva AV. Short report: identification of virulence-associated plasmids in Rhodococcus equi in humans with and without acquired immunodeficiency syndrome in Brazil. Am J Trop Med Hyg. 2011;85(3):510–3.

40. Venner M, Meyer-Hamme B, Venspohl J, Hatori F, Shimizu N, Sasaki Y, Kakuda T, Tsukubai S, Takai S. Genotypic characterization of VapA positive Rhodococcus equi in foals with pulmonary affection and their soil environment on a warmblood horse breeding farm in Germany. Res Vet Sci. 2007;83(3):311–7.

41. Thrusfield MV. Veterinary Epidemiology. 3rd ed. Oxford: Blackwell Science; 2007.