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REGULAR ARTICLES

Identification of *Mycobacterium* species and *Rhodococcus equi* in peccary lymph nodes

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Abstract

Mycobacterium species and the virulence-associated proteins (*vapA*, *vapB*, and *vapN* genes) of *Rhodococcus equi* isolated from 330 lymph nodes of collared peccaries (*Tayassu tajacu*) and white-lipped peccaries (*Tayassu pecari*) intended for human consumption were investigated. Thirty-six (10.9%) *R. equi* strains were isolated; 3.3% ($n = 11/330$) were from white-lipped peccary lymph nodes, and 7.6% (25/330) were from collared peccary lymph nodes. Among the 11 isolates of *R. equi* from the white-lipped peccaries, 90.9% ($n = 10/11$) were obtained from the mesenteric lymph nodes, and only 9.1% ($n = 1/10$) were obtained from the mediastinal lymph nodes. In the 25 isolates of *R. equi* obtained from the collared peccaries, 40.0% ($n = 10/25$) were recovered from the mesenteric lymph nodes, 36% ($n = 9/25$) from the submandibular lymph nodes, and 24.0% ($n = 6/25$) from the mediastinal lymph nodes. No *vapA*, *vapB*, or *vapN* genes (plasmidless) or three host-associated types (pVAPA, pVAPB, and pVAPN) were identified among the *R. equi* isolates. *Mycobacterium* species were isolated in 3.03% ($n = 10/330$) of all the lymph nodes analyzed. Among the 10 mycobacterial isolates, 60% ($n = 6/10$) were from the white-lipped peccary lymph nodes, and 40% ($n = 4/10$) were from the collared peccary lymph nodes. Ten *Mycobacterium* species were detected by PCR-PRA with a predominance of *M. avium* type 1. Sequencing of the *hsp65* and *rpob* genes revealed mycobacteria that were saprophytic (*M. sinense* and *M. kumamotonense*) and potentially pathogenic (*M. colombiense* and *M. intracellulare*) to humans and animals. To our knowledge, this is the first description of *R. equi* and/or mycobacterial species identified in the lymph nodes of peccary specimens. *R. equi* (plasmidless) and the mycobacterial species described here have been reported as causes of pulmonary and extrapulmonary infections in both immunocompetent and immunocompromised humans.

Keywords Tayassuidae species · Collared peccary · White-lipped peccary · Mycobacteria · Rhodococcosis

Introduction

Mycobacterium species and *Rhodococcus equi* (*R. equi*) are major microorganisms recovered from both granulomatous/pyogranulomatous lesions and apparently normal lymph nodes of pigs worldwide (Takai et al. 1996; Makrai et al. 2002; Lara et al. 2015). Despite these findings, the impact of these pathogens in lymph node infections in the peccary species is poorly understood; at the same time, there has been an increase in human consumption of meat from this wildlife animal species (Miler and Fowler 2014).

The genus *Mycobacterium* includes several species that are classified into three groups of relevance for animal and public health: (i) obligatory pathogens of humans and animals; (ii) microorganisms that are potentially pathogenic to animals and humans (nontuberculous or opportunistic mycobacteria); and

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(iii) saprophytic, ubiquitous, or environmental species. *M. avium* subsp. *avium* (*M. avium*) belongs to the *Mycobacterium avium-intracellulare* complex (Stanford and Stanford 2012), which is classified as potentially pathogenic mycobacteria, and has been described as the main agent of lymphadenitis in pigs (Leão et al. 1999; Lara et al. 2011). In humans, *M. avium* predominantly causes pneumonia and, less frequently, extrapulmonary infections, and it mainly affects people who are living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (SOMOSKOVI and SALFINGER 2014).

R. equi infections are commonly restricted to the lymphatic system of pigs and cause submandibular and mesenteric lymphadenitis (Giguère and Prescott 1997). The occurrence of human rhodococcosis has increased globally, and the disease will represent an emergent health problem in the coming years (Shahani 2014). *R. equi* typically causes pyogranulomatous bronchopneumonia in both immunosuppressed and immunocompetent patients, especially among people who are living with HIV/AIDS (Takai et al. 2003).

The pathogenicity of *R. equi* has been attributed to the presence of plasmid-encoded virulence-associated proteins (Vap), which are apparently essential to the survival of the pathogen within phagocytic cells (Meijer and Prescott 2004). Vaps harbor pathogenicity islands that contain distinct genes (Vázquez-Boland et al. n.d.). Over the last few decades, pathogenic strains have been conventionally classified as virulent or intermediately virulent based on whether they harbor a circular large plasmid that encodes either virulence-associated protein A (VapA or pVAPA) or B (VapB or pVAPB), respectively (Meijer and Prescott 2004; MacArthur et al. 2017). Intermediately virulent or pVAPB *R. equi* strains are frequently found in the lymph nodes of pigs ("pig-type"), in wild boars (Ribeiro et al. 2011a), and, curiously, in humans, particularly in immunocompromised patients (Makrai et al. 2002; Takai et al. 2003; Ribeiro et al. 2011b). In turn, virulent or pVAPA *R. equi* strains are typical causal agents of a life-threatening suppurative pneumonia in foals up to 6 months of age ("horse-type"). Strains that lack *vapA* and *vapB* genes (plasmidless) are usually found in the feces of non-horse and non-pig hosts, respectively, and have been identified as a cause of human infections (Meijer and Prescott 2004; MacArthur et al. 2017). However, they may hypothetically harbor other unidentified Vaps (Ribeiro et al. 2017).

A novel host-adapted *R. equi* linear virulence plasmid that encodes virulence-associated protein N (VapN or pVAPN) has been described. This new type of protein that is associated with *R. equi* virulence has been isolated mainly from cattle ("bovine type") (Valero-Rello et al. 2015a, b). Nevertheless, the pathogenicity of pVAPN *R. equi* isolates to domestic animals, wildlife, and humans is still uncertain; moreover, pVAPN strains have recently been identified in the lymph nodes of slaughtered bovines intended for human consumption and in patients who are living with HIV/AIDS in Brazil (Ribeiro et al. 2017). In addition, similarities of *R. equi* pVAPB types (or variants) obtained from humans and pigs indicate that pig-to-human infection may occur by ingestion of pork products contaminated with pVAPB strains from the lymph nodes and/or feces, given the lack of contact between people with rhodococcosis and domestic pigs or pig breeding facilities (Takai et al. 2003; Ribeiro et al. 2011b; Lara et al. 2015). In this scenario, the presence of three host-associated types (pVAPA, pVAPB, and pVAPN) of *R. equi* as well as mycobacterial species recovered from the lymph nodes of slaughtered peccary species intended for human consumption was investigated.

Methods

Animals and bacterial strains

The samples were collected from two distinct peccary breeding facilities located in two Brazilian States (Sao Paulo e Goias) upon slaughter of the animals intended for human consumption. The sample collection was authorized by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA). Although 110 animals were sampled, the minimum number of peccary specimens estimated for the study was 69, which was based on the following: the total number of animals available for sampling (a finite population of 1000 animals), an estimated prevalence of 5%, a test sensitivity of 90%, and a 95% confidence level (Dohoo et al. 2010).

All of the animals enrolled in the study were bred in pens whose size ranged from 150 to 400 ha with access to soil and were fed mainly roots, fruits, and plants. The animals were slaughtered with approximately 200 days old when they weighed 25–45 kg and at the end of the year, which coincides with the period of major human consumption. All of the carcasses were inspected at slaughter, including the lymph nodes, for the presence of nodules.

A total of 330 lymph nodes (110 mesenteric, 110 mediastinal, and 110 submandibular) from 55 collared and 55 white-lipped peccaries were aseptically subjected to microbiological culture in CAZ-NB media for the selective isolation of *R. equi* (Lara et al. 2015) and in Stonebrink-Lesslie and Loewenstein-Jensen media for the selective isolation of mycobacteria. Fragments of the lymph nodes of all the animals were aseptically cultured in CAZ-NB media. The plates were incubated at 37 °C under aerobic conditions and were evaluated at 24, 48, and 72 h. For *Mycobacterium* isolation, fragments of all the lymph node samples were subjected to the Petroff decontamination method (Lara et al. 2011) with some modifications, and they were subsequently cultured in Löwenstein-Jensen and Stonebrink media. The samples were kept at 37 °C in

aerobic conditions for up to 90 days. The colonies compatible with mycobacteria were subjected to Ziehl-Neelsen staining for the identification of acid-fast bacillus (AFB). In addition, colonies indicative of *R. equi* in CAZ-NB and mycobacteria in Stonebrink-Lesslie and/or Loewenstein-Jensen media were subjected to conventional phenotypic classification (Quinn et al. 2011) and, subsequently, to further molecular analysis.

R. equi DNA isolation and detection of virulence-associated proteins

Genetic material of *R. equi* strains was amplified using 10 μl of DNA extracted in 50 μL of volume containing 10 mM Tris-HCl (pH 8.3 at 25 °C), 50 mM KCl, 0.2 mM deoxynucleotide triphosphates, 1.5 mM MgCl 2, 2.5 U Taq DNA polymerase, and 1 mM each primer. Samples were submitted to 30 cycles of amplification, according to the following time/temperature conditions (cycles): denaturation for 90 s at 94 °C, annealing for 1 min at 55 °C, and extension for 2 min at 72 °C. Detection of virulent (VapA) gene sequences (15–17 kDa) was carried out using primer 1 (5′-GACTCTTCACAAGACGGT-3 ′) and primer 2 (5′-TAGGCGTTGTGCCAGCTA-3′), targeting an amplification sequence between 569 and 552 base pairs (bp). For strains of intermediate virulence (VapB), primer 3 (5′-AACGTAGTCGCGGTGAGAA-3 ‘) and primer 4 (5′-ACCG AGACTTGAGCGACTA-3′) were used for the amplification of a gene between 240 and 258 bp (Takai et al. 2002) (Takai et al. 2003). For the detection of VapN or “bovine type” strains, primer 5 (5′–AGAGTTCATGCGTGACAACG–3′) and primer 6 (5′–GTCCACAGGTCACCGTTCTT–3′) were used (Valero-Rello et al. 2015a, b).

Mycobacterial DNA isolation and molecular diagnosis

Extraction and purification of the DNA from the *Mycobacteria* isolates was performed according to previous studies (Van Soolingen et al. n.d.; Bemer-Melchior and Drugeon 1999). Restriction enzyme polymerase chain reaction was used to identify the nontuberculous or opportunistic mycobacteria species (Telenti et al. 1993). For this technique, 2.5 μL of the extracted DNA was used and added 10 pmol of each primer, Tb11 (5′-ACCAACGATGGTGTGTCCAT-3′) and Tb12 (5′-CTTGTCGAACCGCATACCCT-3′). These primers targeted heat shock protein 65 (*hsp65*), resulting in a 441 bp product. The cycles were as follows: one cycle at 94 °C for 10 min; 35 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; and one final cycle at 72 °C for 10 min. The amplified fragment was digested by the enzymes BstEII (Promega, WI, USA) and HaeII (Invitrogen™, CA, USA). The profiles found were analyzed using the public domain bioinformatics tool PRASITE (http://app.chuv.ch/prasite/index.html). For the purification of the samples, a commercial kit was used (Illustra GFX PCR DNA and Gel Band Purification™), and the amplicon was quantified by a Nanodrop™. For all the samples, the sequencing was performed in forward and reverse directions in an ABI Prism 3500 automatic sequencer (Applied Biosystems, CA, USA). A phylogenetic tree was constructed based on the neighbor-joining method (Saitou and Nei 1987) with Mega7 software. As an internal root, sequences of *Mycobacteria* species previously identified as belonging to the *M. avium* complex were used. Sequences of *Corynebacterium bovis*, for the *rpob* gene, and *Nocardia* sp., for the *hsp65* gene, were used as outgroups. All the sequences were obtained from GenBank, and a nucleotide substitution model was then constructed (Tamura and Nei 1993). The statistical reliability was confirmed by the bootstrap method with 1000 replicates.

The chi-square test, Fisher’s exact test, the Cochran Q test, and the Wilcoxon method were performed to compare the presence of the different microorganisms isolated in the peccaries versus the detection of the virulence-associated proteins of *R. equi*. The analysis was performed using the statistical program R (R Development Core Team, 2015, version 3.2.3) at a significance level of 0.05.

Results

Thirty-six (10.9%) *R. equi* strains were isolated, of which 6.7% were from the white-lipped peccary lymph nodes (n = 11/165) and 15.1% (n = 25/165) were from the collared peccary lymph nodes. There was no significant difference ($p > 0.05$) in the isolation rates of *R. equi* in the collared peccaries compared with the white-lipped peccaries using Fisher’s tests. When Cochran’s Q test was used in the white-lipped peccaries and collared peccaries, it was possible to compare the dependent frequencies. There was a significant difference ($p < 0.05$) in the isolation of *R. equi* between the lymph nodes of the sampled animals. In contrast, the Wilcoxon method indicated that there was no significant difference ($p > 0.05$) when the submandibular and mediastinal lymph nodes were compared for the isolation of *R. equi*, but a significant difference ($p < 0.05$) existed between the submandibular lymph nodes and the mesenteric lymph nodes. Among the 11 *R. equi* strains recovered from the white-lipped peccaries, 90.9% (n = 10/11) were obtained from the mesenteric lymph nodes, whereas only 9.1% (n = 1/11) were obtained from the mediastinal lymph nodes. Among the 25 *R. equi* isolates from the collared peccaries, 40.0% (n = 10/25) were from the mesenteric lymph nodes, 36% (n = 9/25) from the submandibular lymph nodes, and 24.0% (n = 6/25) from the mediastinal lymph nodes. A significant difference ($p < 0.05$) was observed in the isolation of *R. equi* in the submandibular lymph nodes of collared peccaries compared with white-lipped peccaries using Fisher’s exact test. There was no significant difference between the two peccary species in the

isolation of *R. equi* in the mediastinal lymph nodes ($p > 0.05$) using Fisher's exact test or in the mesenteric lymph nodes ($p > 0.05$) using the chi-square test. Cochran's Q test was applied in the white-lipped peccary group and showed a significant difference ($p < 0.05$) in the isolation of *R. equi* between the lymph nodes of the sampled animals; however, the Wilcoxon method indicated no significant difference ($p > 0.05$) between the submandibular and the mediastinal lymph nodes in the isolation of *R. equi*. However, there was a significant difference ($p < 0.05$) between the submandibular lymph nodes compared with the mesenteric lymph nodes and the mediastinal lymph nodes. Using Cochran's Q test only in the group of collared peccaries, no significant difference ($p > 0.05$) was observed in the isolation of *R. equi* between the lymph nodes of the animals sampled. No pVAPA, pVAPB, or pVAPN genes were detected in the *R. equi* isolates that were considered avirulent (plasmidless).

Colonies compatible with *Mycobacteria* species were observed in 3.03% ($n = 10/330$) of the lymph nodes cultured. Among the 10 mycobacterial isolates, 60.0% ($n = 6/10$) and 40.0% ($n = 4/10$) of the lymph nodes were from the white-lipped peccaries and the collared peccaries, respectively. There was no significant difference ($p > 0.05$) in the isolation of mycobacteria in the white-lipped peccaries versus the collared peccaries using Fisher's exact test. Among the white-lipped peccaries, all the mycobacterial isolates were from the submandibular lymph nodes, whereas in the collared peccaries, all the strains were isolated from the mesenteric lymph nodes. There was no significant difference ($p > 0.05$) in the detection of *Mycobacterium* sp. in the submandibular lymph nodes of white-lipped peccaries compared with the collared peccaries using Fisher's exact test. Among the collared peccaries, all the strains were isolated from the mesenteric lymph nodes. There was no significant difference ($p > 0.05$) in *Mycobacterium* sp. identification in the mesenteric lymph nodes of the collared peccaries compared with the white-lipped peccaries using Fisher's exact tests. Cochran's Q test in the white-lipped peccaries and the collared peccaries showed no significant difference ($p > 0.05$) in the isolation of *Mycobacterium* sp. between the lymph nodes of the animals sampled. Likewise, using Cochran's Q test in the group of white-lipped peccaries and collared peccaries separately, no significant difference ($p > 0.05$) was observed in the isolation of *Mycobacterium* sp. between the lymph nodes of the animals sampled.

Of the 330 lymph node samples sampled, 13.9% ($n = 46/330$) showed isolation of *R. equi* and/or *Mycobacterium* species. There was no significant difference ($p > 0.05$) in the identification of the pathogens studied in the white-lipped peccary lymph nodes versus the collared peccary lymph nodes using Fisher's exact tests. Out of the total samples, 7.3% ($n = 24/330$) of the mesenteric lymph nodes were positive for at least one of the pathogens studied. From these, 4.5% ($n = 15/330$) of the submandibular lymph nodes and 2.1% ($n = 7/330$) of the mediastinal lymph nodes were positive. There was a bias to greater isolation of the pathogens studied in the mesenteric lymph nodes when compared to the mediastinal or the submandibular lymph nodes. Only one sample of the mesenteric lymph nodes from a collared peccary showed simultaneous isolation of *R. equi* and mycobacteria.

Ten *Mycobacterium* species were detected by PCR-PRA (Table 1), and sequencing of the genes *hsp65* and *rpob* showed a predominance of *M. avium* type 1, *M. colombiense*, *M. intracellulare*, and *M. kumamotonense* (Figs. 1 and 2).

Discussion

This study revealed a 10.9 and 3.03% rate of identification of *R. equi* and *Mycobacteria* species, respectively, from apparently normal lymph nodes of peccaries. To our knowledge, this is the first time this finding has been described. Despite the absence of virulence-associated genes, the identification of *R. equi* and nontuberculous mycobacteria is a public health concern, since these pathogens have increasingly been reported to cause pulmonary and extrapulmonary diseases, both in immunocompetent and immunocompromised people (Stanford and Stanford 2012; Vázquez-Boland et al. n.d.; SOMOSKOVI and SALFINGER 2014; WHO 2016).

A peculiar geographic distribution of the virulence plasmid profile of *R. equi* in swine species has been observed worldwide. In Europe, particularly in Hungary (Makrai et al. 2002, 2005) and Poland (Witkowski et al. 2016), a predominance of *R. equi* pVAPB (VapB) type 5 (or variant) in the submandibular lymph nodes of pigs and wild boars (*Sus scrofa*) has been described (Rzewuska et al. 2014). Conversely, *R. equi* pVAPB (VapB) types 1 and 2 were the most frequent types (variants) identified in Asia, Thailand (Takai et al. 2003) and Japan (SAKAI et al. 2012). In South America, more specifically in

Table 1 Molecular characterization by the PCR-PRA technique of species of the genus *Mycobacterium* in 10 isolates obtained from taiassuideos lymph nodes. Brazil, 2016–2017

Mycobacterium species	Collared peccary	White-lipped peccary	Total
M. avium type 1	1/10 (10.0%)	2/10 (20.0%)	3/10 (30.0%)
*M. kumamotonense*type 1	2/10 (20.0%)	1/10 (10.0%)	3/10 (30.0%)
*M. colombiense*type 1	–	3/10 (30.0%)	3/10 (30.0%)
*M. intracellulare*type 3	1/10 (10.0%)	–	1/10 (10.0%)
Total	4/10 (40.0%)	6/10 (60.0%)	10/10 (100.0%)

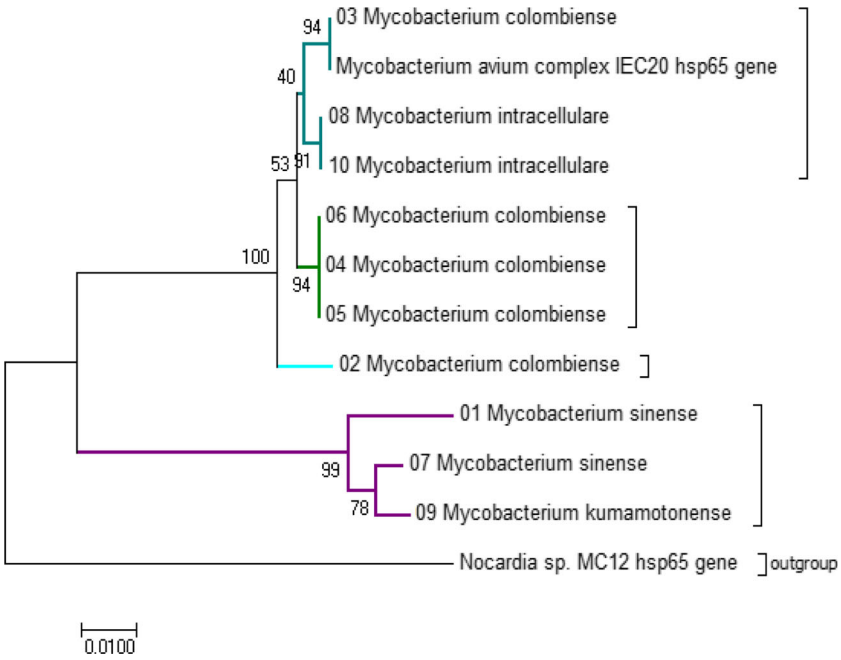

Fig. 1 Phylogenetic relationship of the *hsp*65 gene among mycobacteria species (*M. sinense*, *M. colombiense*, *M. intracellulare*, and *M. kumamotonense*) identified in the lymph nodes of Tayassuidae. Brazil, 2016–2017 (phylogenetic tree analyzed by the neighbor-joining method, with 1000 replications)

Brazil, a study that focused on the virulence characterization of *R. equi* isolated from the lymph nodes of pigs and wild boars with and without lymphadenitis described a predominance of pVAPB (VapB) type 8 strains, together with the detection of types(variants) 1 and 29 (Lara et al. 2011).

In the coming years, given the similarities of the pVAPB types (or variants) of *R. equi* strains in humans and pigs, it is hypothesized that pig-to-human infection might occur by ingestion of pork or pork products that are contaminated with intermediately virulent strains from the lymph nodes and/or feces, given the lack of contact between human patients with rhodococcosis and domestic pigs or their environment (Takai et al. 2003; Ribeiro et al. 2011b; Lara et al. 2015). In fact, this was the main motivation for the present study because, similar to what occurs in pigs (Takai et al. 2003; Ribeiro et al. 2011a; Lara et al. 2011, 2015), these pathogens may be transmitted from peccaries to humans by means of consumption of meat or meat products contaminated with lymph node contents. In the current study, no *vapA*, *vapB*, or *vapN* genes were detected in the *R. equi* strains recovered from the lymph nodes of the peccaries. Nevertheless, the identification of this pathogen in apparently normal lymph nodes represents a public health

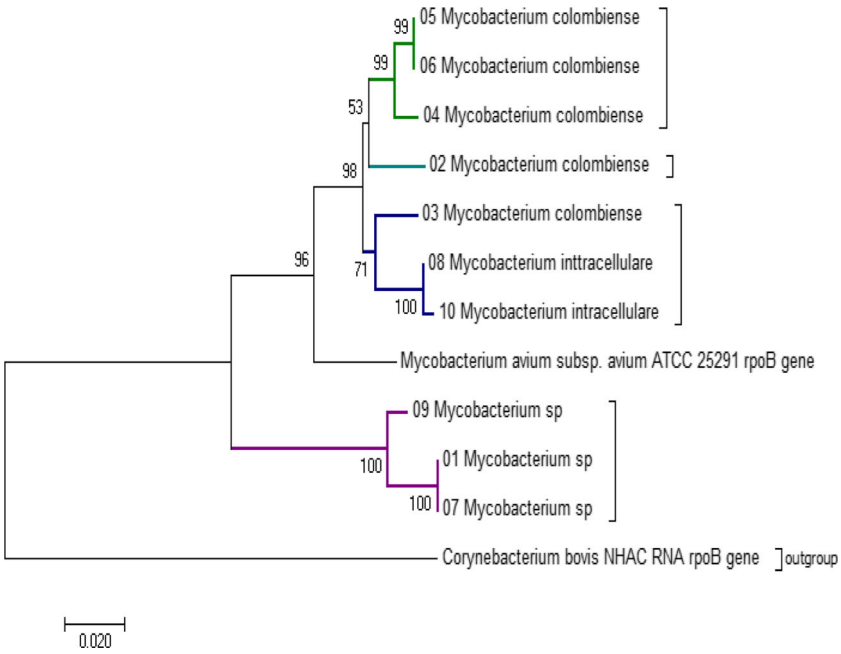

Fig. 2 Phylogenetic relationship of the *rpob* gene among mycobacteria species (*Mycobacterium* spp., *M. colombiense*, *M. intracellulare*, and *M. kumamotonense*) identified in the lymph nodes of Tayassuidae. Brazil, 2016–2017 (phylogenetic tree analyzed by the neighbor-joining method, with 1000 replications)

concern due to increasing human consumption of meat from this wildlife animal species (Miler and Fowler 2014). In addition, different countries, including the USA (Verville et al. 1994), Japan (Takai 1997), Thailand (Takai et al. 2002), Hungary (Makrai et al. 2002), and Brazil (Ribeiro et al. 2011a, b), have reported human rhodococcosis caused by avirulent (plasmidless) strains.

The pathogenicity of *R. equi* strains lacking the typical Vaps to humans may be related to other virulence factors, such as the capsule, the oxidase, and phospholipase C exoenzymes, as well as the resistance of the pathogen to conventional antimicrobials, particularly in immunosuppressed patients (Takai 1997; Meijer and Prescott 2004; Ribeiro et al. 2011b), or may be due to pathogens with unreported virulence-associated plasmid profiles.

Despite the absence of the typical Vaps in our isolates, studies in several countries focusing on the virulence plasmid profile of *R. equi* have contributed to the investigation of the pathogenicity, molecular epidemiology (with emphasis on the geographic distribution of the types), and risks that livestock, companion animals, and wildlife pose as sources of infection to humans (Takai 1997; Meijer and Prescott 2004; Vázquez-Boland et al. n.d.).

Mycobacterium species are major pathogen of submandibular and mesenteric lymphadenitis and the cause for condemnation of pig carcasses (Leão et al. 1999; Straw et al. 2006; Lara et al. 2011). In addition, evidence has supported that European wild boars (*Sus scrofa*) are important reservoirs of *Mycobacterium tuberculosis* complex, due to the identification of this pathogen in the lymph nodes of these animals (Naranjo et al. 2008). In this context, 10 mycobacteria were detected in the peccaries sampled in the present study, including 6 from the submandibular lymph nodes and 4 from the mesenteric lymph nodes that were apparently normal. This finding may be related to the transmission of mycobacteria mainly by oral route due to the wide distribution of the pathogen in the environment, leading contamination of the soil, water, food, and tools used in animal handling. The oral transmission of pathogen causes a predominance of infections in the digestive tract, including its lymph nodes (Straw et al. 2006).

Most of the mycobacterial species identified by PCR-PRA in this study belonged to the *M. avium* complex. Likewise, *M. avium* complex (MAC) has increasingly been reported in human mycobacteriosis, causing both pulmonary and extrapulmonary infections, especially in people who are living with HIV/AIDS (Stanford and Stanford 2012; SOMOSKOVI and SALFINGER 2014). *M. avium* type 1 was the causative agent of disease in up to 90% patients affected by clinical MAC complex infections showing mainly serious respiratory problems (Kiehn et al. 1985; Slutsky et al. 1994; Leão et al. 1999). Moreover, the MAC complex has been described as the major cause of tuberculosis in people who are living with HIV/AIDS elsewhere (INDERLIED et al. 1993).

Sequencing of the *hsp65* and *rpob* genes from our 10 isolates of mycobacteria revealed a concordance of 70.0% and identity > 96.0% compared with similar mycobacterial species deposited in GenBank. This molecular method enabled the identification of mycobacteria that are potentially pathogenic to humans and animals (*M. colombiense* and *M. intracellulare*), as well as environmental or saprophytic mycobacteria (*M. kumamotonense* and *M. sinense*) (Stanford and Stanford 2012). The phylogenetic tree showed four clusters in the two sequenced genes and predominantly confirmed mycobacteria belonging to the MAC complex (*M. intracellulare* and *M. colombiense*) and those of environmental or saprophytic origin (*M. kumamotonense* and *M. sinense*). *M. colombiense* and *M. intracellulare* have been related to pulmonary and disseminated infections in humans (Murcia et al. 2006), as well as other miscellaneous infections (osteomyelitis, synovitis, encephalitis, hepatitis), especially in patients who are living with HIV/AIDS (Stanford and Stanford 2012; SOMOSKOVI and SALFINGER 2014; WHO 2016). Similarly, *M. kumamotonense* and *M. sinense* have been reported to cause pulmonary infections in immunocompromised patients (Jarzembowski et al. 2008). Some discrepancy that was observed between the PCR-PRA results and the sequencing results may be attributed to point changes in nucleotide sequences (deletions, insertions, transitions) that interfered with the action of the restriction enzymes, leading to variations in the resulting fragments, which made it difficult to compare them with the standards in the PRA reference site (Ringuet et al. 1999).

Tayassu tajacu and *Tayassu pecari*, which are also called the collared peccary and the white-lipped peccary, respectively, are peccary species belonging to the Brazilian fauna (Oda et al. 2004). Globally, evidence has supported that, in addition to commercial pigs (Lara et al. 2011), wild boars (Meng et al. 2009) and feral pigs may be potential reservoirs of mycobacterial species (Straw et al. 2006), even in apparently asymptomatic animals. Based on the findings from this study, the identification of potentially pathogenic mycobacteria in the apparently normal lymph nodes of peccaries represents a public health concern because these animals are slaughtered for human consumption. In addition, similar to studies evaluating swine species, epidemiological studies focusing on the main pathogens that affect peccaries are of great importance for these commercially slaughtered wildlife, particularly in relation to identifying zoonotic pathogens, such as mycobacteria and *R. equi*.

Compliance with ethical standards

This study was carried out in accordance with the guidelines for the ethical use of animals approved by the Ethics Committee on Animal Use (CEUA-FMVZ-UNESP/Botucatu, state of São Paulo), Brazil, protocol number 101/2015/2013-CEUA.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Bemer-Melchior, P., Drugeon, H. B., 1999. Inactivation of *Mycobacterium tuberculosis* for DNA typing analysis, Journal of Clinical Microbiology, 37, 2350–2351.

Dohoo, I., Martin, W., Stryhn, H. 2010. Veterinary Epidemiologic Research Inc., (Canada; Charlottetown - Prince Edward Island).

Giguère, S., Prescott, J.F. 1997. Clinical manifestations, diagnosis, treatment, and prevention of *Rhodococcus equi* infections in foals. Veterinary Microbiology, 56(3-4), 313–334.

Inderlied, C.B., Kemper, C.A., Bermudez, L.E., 1993. The *Mycobacterium avium* complex, Clinical Microbioly Reviews, 6, 266–310.

Jarzembowski, J.A., Michael, B., Young, M.D. 2008. Nontuberculous mycobacterial infections, Archives of Pathology & Laboratory Medicine, 132, 1333–1341.

Kiehn, T.E., Edwards, F.F., Brannon, P., Tsang, A.Y., Maio, M., Gold, J.W., Whimbey, E., Wong, B., Mcclatchy, J.K., Armstrong, D., 1985. Infections caused by Mycobacterium avium complex in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroaglutination characteristics, Journal of Clinical Microbiology, 21, n.2, 168–175.

Lara, G.H.B., Ribeiro M. G., Leite, C. Q. F., Paes, A.C., Guazzellia, A., Silvac, A. V., Santos, A. C. B., Listoni, F.J.P., 2011. Occurrence of *Mycobacterium* spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (*Sus scrofa*), Research in Veterinary Science, 90, 185–188.

Lara, G.H.B., Takai, S., Sasaki, Y., Kakuda, T., Listoni, F.J.P., Risseti, R.M., de Morais, A.B.C., Ribeiro, M.G., 2015. VapB type 8 plasmids in *Rhodococcus equi* isolated from the small intestine of pigs and comparison of selective culture media, Letters in Applied Microbiology, 61, 306–310.

Leão, S.C., Briones, M., SircillI, M.P., Balian, S.C., Morés, N., Ferreira Neto, J.S., 1999. Identification of two novel Mycobacterium avium alleic variants by PCR-restriction enzyme analysis (PRA) in pig and human isolates from Brazil, Journal of Clinical Microbiology, 37, 2592–2597.

MacArthur, I., Anastasi, E., Alvarez, S., Scortti, M., Vázquez-Boland, J.A., 2017. Comparative genomics of *Rhodococcus equi* virulence plasmids indicates host-driven evolution of the vap pathogenicity island. Genome Biology and Evolution, 9, 1241–1247.

Makrai, L., Takai, S., Tamura, M., 2002. Characterization of virulence plasmids in *R. 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.

Meijer, W.G., Prescott, J.F., 2004. *Rhodococcus equi*, Veterinary Research, 35, 383–396.

Meng, W.J., Lindsay, D.S., Sriranganathan, N., 2009. Wild boars as sources for infections diseases in livestock and humans. Philosophical Transaction of the Royal Society of London Serie B Biological Sciences 364, 2697–2707

Miler, R.E.; Fowler, M.E. Fowler's Zoo and Wild Animal Medicine. 8th ed. 2014. 792p.

Murcia, M.I., Tortoli, E., Menendez, M.C., Palenque, E., Garcia, M.J. 2006. *Mycobacterium colombiense* sp. nov., a novel member of the *Mycobacterium avium* complex and description of MAC-X as a new ITS genetic variant, Internatioal Journal of Systematic and Evolutionary Microbiology, 56, 2049–2054.

Naranjo, V., Gortazar, C.J., De La Fuente, J., 2008. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. Veterinary Microbiology 127, 1–9.

Oda, S.H.I., Bressan, M.C., Miguel, G.Z., Vieira, J. O., Faria, P.B., Savian, T.V., Kabeya, D.M., 2004. Efeito do método de abate e do sexo sobre a qualidade da Carne de Capivara (*Hydrochaeris hydrochaeris*), Ciência e tecnologia de alimentos, 24, 236–242.

Quinn, P.J., Markey, B.K., Leonard, F.C., Fitzpatrick, E.S., Fanning, S., Hartigan, P.J., 2011. Veterinary Microbiology and Microbial Disease (UK: Wiley-Blackwell).

Ribeiro, M.G., Takai, S., Vargas, A. C., Mattos-Guaraldi, A.L., Camello, T. C. F., Ohno, R., Okano, H., Silva, A. V. 2011a. Identification of virulence associated plasmids in *Rhodococcus equi* in humans with and without acquired immunodeficiency syndrome in Brazil, The American Journal of Tropical Medicine and Hygiene, 85, 510–513.

Ribeiro MG, Lara GHB, Takai S, Guazzelli A, Silva AV, Fernandes MC, et al. Virulence genes and plasmid profile in *Rhodococcus equi* isolates from domestic pigs and wild boars (*Sus scrofa*) in Brazil. Res Vet Sci. 2011b.

Ribeiro, M.G., Lara, G.H.B., da Silva, P., Franco, M.M.J., de Mattos-guaraldi, A.L., de Vargas, A.P.C., Sakate, R.I., Pavan, F.R., Colhado, B.S., Portilho, F.V.R., Motta, R.G., Kakuda, T., Takai S. Novel bovine-associated pVAPN plasmid type in *Rhodococcus equi* identified from lymph nodes of slaughtered cattle and lungs of people living with HIV/AIDS. Transboundary and Emerging Diseases, 2017. https://doi.org/10.1111/tbed.12785.

Ringuet, H., C. Akoua-Koffi, S. Honore, A. Varnerot, V. Vincent, Berche, P., 1999. *hsp*65 sequencing for identification of rapidly growing, *Mycobacteria,* 37, 852–57.

Rzewuska, M., Witkowski, L., Cisek, A.A., Stefańska, I., Chrobak, D., Stefaniuk, E., Kizerwetter-Świda, M., Takai, S. 2014. Characterization of *Rhodococcus equi* isolates from submaxillary lymph nodes of wild boars (*Sus scrofa*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). Veterinary Microbiology, 172(1-2), 272–278.

Saitou, N., Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees, Molecular Biology and Evolution, 4, 406–425.

SAKAI, M.; OHNO, R.; HIGUCHI, C.; SUZUKI, K.; MAEDA, K.; SASAKI, Y.; KAKUDA, T.; TAKAI, S. Isolation of Rhodococcus equi from wild boars (Sus scrofa) in Japan. Journal of Wildlife Diseases, 48, 3, 815–817, 2012.

Shahani L. *Rhodococcus equi* pneumonia and sepsis in al allogenic haematopoietic stem cell transplant recipient. BMJ Case Report. 2014. https://doi.org/10.1136/bcr-2014-204721

Slutsky, A.M., Arbeit, R.D., Barber, T.W., Rich, J.; Von Reyn, C.F., Pieciak, W., Barlow, M.A., Maslow, J.N., 1994. Polyclonal infections due to *Mycobacterium avium* complex in patients with AIDS detected by pulsed-field gel electrophoresis of sequential clinical isolates, Journal of Clinical Microbiology, 32, 1773–1778.

SOMOSKOVI, A.; SALFINGER, M. Nontuberculous mycobacteria in respiratory infections: advances in diagnosis and identification. Clinics in Laboratory Medicine, Philadelphia, 34, 2, 271–295, 2014

Stanford, J., Stanford C., 2012. Mycobacteria and their world, International Journal of Mycobacteriology,1, 1–12.

Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J., 2006. Diseases of Swine, ninth ed. Blackwell Publishing, Ames, p. 1153.

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

Takai, S., Fukunga, N., Ochiai, S. 1996. Identification of intermediately virulent *Rhodococcus equi* isolates from pigs, Journal of Clinical Microbiology, 34, 1034–1037.

Takai, S., TharavicchitkuL, 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 Hygiene, 66, 52–55.

Takai, S., Tharavichitkul, P., Takarn, P., Khantawa, B., Tamura, M., Tsukamoto, A., Takayama, S., Yamatoda, N., Kimura, A., Sasaki, Y., Kakuda, T., Tsubaki, S., Maneekarn, N., Sirisanthana, T., Kirikae, T., 2003. Molecular epidemiology of *Rhodococcus equi* of intermediate virulence isolated from patients with and without acquired immune deficiency syndrome in Chiang Mai, Thailand. The Journal of Infectious Diseases,188, 1717–1723.

Tamura, K.; Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Molecular Biology and Evolution, 10, 512–526.

Telenti, A., Marchesi, F., Balz, M., Bally, F., Böttger, E. C., Bodmer, T. 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis, Journal of Clinical Microbiology, 31, 175–178.

Valero-Rello, A., Hapeshi, A., Anastasi, E., Alvarez, S., Scortti, M., Meijer, W.G., Macarthur, I., Vazquez-Boland, J.A., 2015a. An invertron-like linear plasmid mediates intracellular survival and virulence in bovine isolates of Rhodococcus equi, Infection and Immunity, 83, 2725–2737.

Valero-Rello, A., Hapeshi, A., Anastasi, E., Alvarez, S., Scortti, M., Meijer, W.G., MacArthur, I., Vázquez-Boland, J. A., 2015b. An invertron-like linear plasmid mediates intracellular survival and virulence in bovine isolates of *Rhodococcus equi*. Infection and Immunity, 83, 2725–2737.

Van Soolingen, D., de Haas, P. E. W., Hermans P. W. M., VAN Embden, J. D., DNA fingerprinting of *Mycobacterium tuberculosis*, Methodsin Enzymology, 235, 196–205.

Vázquez-Boland JA, Giguère S, Hapeshi A, MacArthur I, Anastasi E, Valero-Rello A. Rhodococcus equi: the many facets of a pathogenic actinomycete. Veterinary Microbiology, 167, 9–33.

Verville, T.D., Huycke, M.M., Greenfield, R.A., Fine, D.P., Kuhls, T.L., Slater, L.N., 1994. *Rhodococcus equi* infections in humans. Medicine, 73, 119–132.

Witkowski, L., Rzewuska, M., Takai, S., Kizerwetter-Świda, M., Kita, J. 2016. Molecular epidemiology of *Rhodococcus equi* in slaughtered swine, cattle and horses in Poland. BMC Microbiology, 16(1), 98.

WORLD HEALTH ORGANIZATION - WHO. Global tuberculosis report. 20th edition. p.204, 2016.