NO EVIDENCE OF *RHODOCOCCUS EQUI* INFECTION IN PIGS FROM THE SOUTHEAST REGION OF BRAZIL

(AUSÊNCIA DE INFECÇÃO POR RHODOCOCCUS EQUI EM SUÍNOS DA REGIÃO SUDESTE DO BRASIL)

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SUMMARY

Pigs and their environment have been pointed out as a possible source of *Rhodococcus equi* infection in AIDS patients. Intermediately virulent *R. equi* were detected in AIDS cases from São Paulo, and to establish a possible epidemiological association with pigs we searched for *R. equi* in submaxillary lymph nodes of pigs slaughtered in this geographic area. Although we used several protocols of culture and selective media for *R. equi* isolation, no case of *R. equi* infection was detected in 1,068 pigs from 12 municipal districts in the Southeast region of Brazil. This result suggests another source of human *R. equi* infection in AIDS patients from São Paulo.

KEY-WORDS: *Rhodococcus equi*; pigs; Brazil.

RESUMO

Suínos e seu meio ambiente foram apontados como possíveis fontes de infeção por *Rhodococcus equi* para pacientes com AIDS. *R. equi* de virulência intermediária foram detectados em casos de AIDS de São Paulo, e para se estabelecer uma possível associação com a espécie suína foi pesquisado *R. equi* em linfonodos submaxilares de suínos. Apesar de terem sido usados vários protocolos de cultura e meios seletivos para o isolamento, nenhum caso de infecção por *R. equi* foi detectado em 1.068 suínos provenientes de 12 municípios da região sudeste do Brasil. Este resultado sugere uma outra fonte de infecção para a espécie humana pelo *R. equi* em pacientes com AIDS, no Estado de São Paulo.

PALAVRAS-CHAVE: *Rhodococcus equi*; suínos; Brasil

INTRODUCTION

*Rhodococcus equi* is a soil organism ingested by many herbivores and is widespread in their environment (WOOLCOCK et al., 1980; PRESCOTT et al., 1984; TAKAI & TSUBAKI, 1985). Virulent *R. equi* has been isolated from horse feces and from soil on horse-breeding farms in which episodes of chronic, suppurative bronchopneumonia and enteritis in foals were reported (TAKAI et al., 1991b).

The virulence-associated antigen of 15- to 17-kDa and 85-kb and 90-kb plasmids have been reported to be essential for virulence to foals and for pathogenicity to mice (10⁶ bacteria needed for mouse lethality) (TAKAI et al., 1991a,c; TKACHUK-SAAD & PRESCOTT, 1991;
TAKAI et al., 1995b; TAN et al., 1995). These epidemiological markers have been considered useful in identifying virulent \textit{R. equi} in horses and their environment (TAKAI, 1997).

In humans, \textit{R. equi} has been reported to be an important opportunistic pathogen in immunosuppressed patients, especially in AIDS cases (HARVEY & SUNSTROM, 1991). TAKAI et al. (1994) detected \textit{R. equi} in different virulence levels in immunosuppressed individuals: they isolated and characterized virulent strains similar to those of foals, along with intermittently virulent strains having a 20-kDa antigen that kill mice with $10^7$ cells, and avirulent strains that do not kill mice with $10^6$ cells or more. Nevertheless, the majority of \textit{R. equi} isolated from AIDS patients were intermittently virulent, presenting the virulence-associated antigen of 20-kDa associated with one of four distinct large plasmids of 79-100-kb (TAKAI et al., 1995a).

Since only one-third of humans infected with \textit{R. equi} reported contact with farm animals, Takai and his colleagues decided to investigate the route of \textit{R. equi} infection in human beings, firstly searching for \textit{R. equi} in soil and sand at parks and gardens in Japan, but no virulent strains were detected (TAKAI et al., 1996b). Afterwards, they tried to isolate \textit{R. equi} from the submaxillary lymph nodes of pigs with and without lesions resembling those of tuberculosis (TAKAI et al., 1996a). They isolated \textit{R. equi} from 56 (3.1%) of 1,832 pigs in Japan, and detected the presence of the 20-kDa antigen in all isolates. In addition, the authors demonstrated that the expression of the 20-kDa antigen and the \textit{R. equi} pathogenicity in mice were associated with the presence of five large, distinct plasmids of 79- to 95-kb (TAKAI et al., 1996a). Taking into account these data, they suggested that pigs and their environment could be associated with the source of \textit{R. equi} infection in some human cases.

In Brazil, only one study using pigs was conducted in the South, during the period from 1981 to 1992, and documented the presence of \textit{R. equi} infection in 6 out of 25 lymph nodes suspected of tuberculosis lymphadenitis (OLIVEIRA et al., 1995). The authors reported that these pigs were from 15 municipal districts in the State of Rio Grande do Sul, but they did not give the number of animals slaughtered during this period, so that the prevalence of \textit{R. equi} infection in pigs from this region of Brazil could not be calculated.

In a recent study conducted by us in São Paulo, Brazil, we detected \textit{R. equi} infection in AIDS patients, and characterized the majority of isolates as intermediately virulent (unpublished data). Taking into consideration the results of Takai and our data, we decided to search for the route of \textit{R. equi} transmission in AIDS patients from São Paulo.

A pig slaughterhouse in a municipal district of São Paulo that receives animals from several farms from the Southeast part of Brazil, and slaughters about 600 animals per day was contacted, and after consent of the Serviço de Inspeção Federal (SIF), submaxillary lymph nodes randomly obtained from 1,068 pigs were tested for the presence of \textit{R. equi}. The pigs came from twelve municipal districts located in the Southeast region of Brazil (Fig. 1), and the procedure for the isolation and identification of \textit{R. equi} were conducted at the Immunology Department and Culture Collection Sector of Instituto Adolfo Lutz, a Public Health Institute in São Paulo, Brazil.

A selective medium for \textit{R. equi} isolation, named modified-BHI-NANT agar, was employed (TAKAI et al., 1996a). The medium consisted of brain heart infusion agar supplemented with yeast extract and bacto peptase, and containing nalidixic acid ($20 \mu g/ml$), novobiocin ($2.5 \mu g/ml$), and potassium tellurite ($5 \mu g/ml$).

Submaxillary lymph nodes were removed from freshly slaughtered pigs, placed in a sterile recipient, and transported to the laboratory. The fat layer surrounding the lymph nodes was removed, and the lymph nodes were immersed in boiling water for 3 s prior to being cut up with sterile scissors. One thin fragment was triturated between emery slides, and the fluid obtained placed on a modified-BHI-NANT agar plate and incubated for 2 to 3 days at 30°C. All suspected \textit{R. equi} colonies were counted and subcultured, and then identified in our laboratory.

Unfortunately, using this approach we were unable to detect \textit{R. equi} in more than 200 attempts. We then decided to modify the methodology and add other steps to the culture procedure.

Briefly, we changed the temperature of culture incubation from 30°C to 37°C for 168 other lymph nodes tested. Indeed, instead of placing the lymph node tissues directly on modified BHI-NANT agar, we first cultured the tissue fragments in BHI broth, and after an incubation period of 24 h at 37°C, an aliquot of the broth was transferred to the modified BHI-NANT agar for 3 additional days of culture (400 lymph nodes analyzed). No improvement in the results was obtained.

Then, according to Takai’s personal recommendations, we reduced of half the concentration of antibiotics in the modified BHI-NANT agar, maintaining the same conditions as the standard culture (2 to 3 days of incubation at 30°C; 300 additional lymph nodes were evaluated). Concomitantly, for security, we placed a piece of each lymph node tissue in BHI-chocolate agar, and after 2 days of culture, colonies suspected of \textit{R. equi} were subcultured in modified BHI-NANT agar. Although some suggestive colonies of \textit{R. equi} were observed in this study,
they were not confirmed using the CAMP test and other biochemical tests for *Corynebacterium*.

Using all culture protocols previously reported we cultivated *R. equi* isolates from foals and humans in our laboratory, and *R. equi* ATCC 33701 and ATCC 33704 strains. The positive results obtained with these controls permitted us to confirm the absence of *R. equi* in pigs from the Southeast region of Brazil.

We do not know if our result represents simply no *R. equi* infections among pigs in some regions of Brazil, or if it is a consequence of antibiotic content in the ration used to feed pigs. But the results obtained stimulate us to continue this study searching for other routes of *R. equi* infection in humans in Brazil, possibly related to horses and cattle, and their environment.

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