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IMMUNOBLOT PROFILES OF SERA FROM LABORATORY RATS NATURALLY INFECTED WITH *MYCOPLASMA PULMONIS* AND TECHNICIANS EXPOSED TO INFECTED ANIMAL FACILITIES

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ABSTRACT

Mycoplasma pulmonis have been isolated in about 10^5 CFU/mL from tracheal aspirates of rats from conventional animal facilities in São Paulo. The mycoplasma transmission by aerosol may happen from an infected rat to a healthy one at distances up to 120 cm. This condition also favors the technicians contamination. As this infection is unknown in humans, in this study the immunoblot profiles to *M. pulmonis* of sera from rats were compared to those presented by animal facility technicians. About 32 proteins from 11 to 230 kDa (kilodaltons) were recognized by the sera from rats naturally infected with *M. pulmonis.* Sera from technicians responsible for the cleaning and sanitation of cages of infected animals for more than seven years recognized about 10 proteins of this bacteria. Sera from individuals with shorter working time or that had never been exposed to such environment recognized few proteins. Proteins about 117 and 95 kDa were recognized by human and rat sera and by the negative controls. Although a positive human serum against *M. pulmonis* is unknown, this study established a temporary profile of protein recognition of human serum against such mycoplasma.

Key words: *Mycoplasma pulmonis*, human sera, animal facilities.

INTRODUCTION

Mycoplasma pulmonis is the most frequent mycoplasma isolated from rats and causes the Murine Respiratory Mycoplasmosis (MRM) that can be chronic or asymptomatic (8). In a previous study, about 10^5 CFU/ml of *M. pulmonis* were detected in tracheal aspirates of rats (18). MRM has been recognized as a major disease in laboratory rats and mice. Mycoplasma infection in laboratory animals interferes on the results of biomedical research (8,11). This issue has been documented in many aspects, but there is little information about immunoblot profiles of sera from rats naturally infected by *M. pulmonis.* The citations are related with immunoenzimatic tests of animal colony screenings (12).

The transmission of *M. pulmonis* by aerosols from an infected to a healthy rat by sneezing in a distance of about 120 cm (16) strongly suggests that such condition may happen between rats and humans (22). The technicians attending the cleaning of cages of facilities with rats infected with *M pulmonis* are the candidates to be infected with these bacteria. Exposure of humans to *M. pulmonis* was not considered yet because rodents are the natural hosts. Although mycoplasmas show specificity to their hosts, isolation of some mycoplasmas from unusual hosts was already reported (1, 2, 4, 14, 21, 24). In order to verify possible transmission of *M. pulmonis* to humans from rats, the present study has the goal to compare the immunoblot profiles of sera from technicians working at laboratory animal facilities and sera from rats naturally infected with *M. pulmonis*.

MATERIALS AND METHODS

Mycoplasma antigen

Two antigens were tested: the total lysate of *M. pulmonis* NCTC 10139 and the total lysate from the isolate Q10, obtained

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from a tracheal aspirate of naturally infected rat. Briefly, after growing in 1000 ml of SP4 medium(20), mycoplasma cells were concentrated by centrifugation at 20,449 *g* for 50 minutes, at 4ºC, washed three times by successive centrifugations (28,663 *g*, 30 minutes, 4ºC) and homogenized with PBS (15).

Sera

Forty sera from Wistar rats (*Rattus norvegicus* - 50% female and 50% male - 45 to 60 days old) of six different facilities were used. Nonclimate-controlled facilities in our region are defined as follows: light cycle of 12hs, without air-conditioning but ventilated; temperature about 24 to 28ºC and humidity from 40 to 80%. After euthanasia with an intraperitoneal overdose of pentobarbital, a sample of tracheal aspirate was obtained for mycoplasma isolation (18). Then, about 3 ml of blood was punctured from the heart and the serum was separated. Twenty-eight human (volunteers) sera were obtained from 10 mL of blood venipunctured from technicians working up to 15 years in different facilities with infected animals on the cleaning of rats cages. Twelve sera samples were from individuals that had never been in animal facilities. The human care and the use of animals were previously attested by the supervisors of each animal facility. The purpose of the study was previously explained to the supervisors and volunteers.

Electrophoresis and immunoblots

About 300 µg of antigen were electrophoresed on 12% SDS-PAGE and electrotransferred to nitrocellulose membranes. The membranes were blocked with TBS (20mMTris; 500mM NaCl, pH 7.5) with 5% nonfat milk and cut into 4mm width strips. Sera from rats, technicians and individuals who have never had contact with rats in animal houses were diluted 1:100 in TBS with 5% of nonfat milk and incubated for 2 hours with the strips. Hyperimmune horse serum to *M. pulmonis* was included as positive control. After washings, alkaline phosphatase-anti IgG conjugates against each specific serum were added and incubated for 2 hours. The recognized proteins on the membranes were detected by NBT/BCIP (Nitro-blue tetrazolium/ 5-bhromide 4-chloride 3-indolil phosphate) substrates and the reaction was interrupted with distilled water (3).

RESULTS

The most representative profiles obtained immunobloting human and rat sera to *M. pulmonis* NCTC 10139 are presented in Fig. 1. Results for the indigenous strain Q 10 were very similar and are not shown. All sera recognized bands about 117 and 95 kDa. Sera from infected rats recognized not identically about 33 proteins, ranging approximately from 11 to 230 kDa **(lanes 3 to 16)**. Most of the recognized bands were from 36 to 230 kDa. Serum from a non-infected rat **(lane 1)** recognized bands about

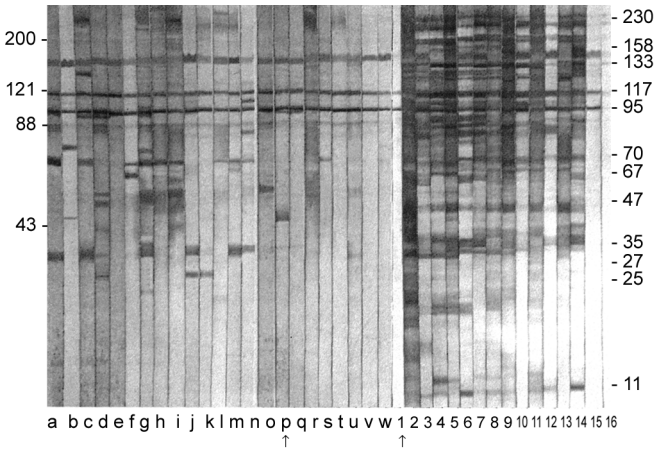

Figure 1: Immunoblot profiles of human and rat sera against *M. pulmonis* NCTC 10139. Sera on lanes ***a*** to ***p***: are from technicians with 7 to 15 years in contact with rats in animal facilities. Sera on lanes ***q*** to ***u***: are from individuals with less than 7 years of exposure. Lane ***w*** represent a serum from an individual without exposure to rats. Serum on lane ***1*** is from a non infected rat. On lane ***2*** is a horse hyperimmune serum to *M. pulmonis*. Sera on lanes ***3*** to ***16*** are from rats naturally infected with *M. pulmonis*. Numbers: molecular weight in kDa.

117 and 95 kDa and the hyperimmune horse serum **(lane 2)** recognized the higher number of bands. Although sera on **lanes 15** and **16** are from infected rats they recognized weakly a few bands.

Sera from technicians are on **lanes a** to **w**. Sera on **lanes a** to **p** are from individuals that have had contact with infected animals from 7 to 15 years, and recognized much less bands than rats. Most of the recognized bands by human sera ranged approximately from 25 to 200 kDa and sera from rats recognized bands with the same Molecular Weight. All human sera recognized a band of about 156 kDa. However this protein was not recognized by a noninfected rat as did some sera from infected rats (**lanes 1, 4, 8, 13** and **16**). The band about 70 kDa was the protein most frequently recognized by sera from technicians with more time of exposure to infected facilities. The band just below 95kDa was recognized by four sera shown on **lanes c**, **d, g** and **p**. A band between 117 and 95 kDa was recognized on **lane n** as did some sera from infected rats. Bands about 25 to 35 kDa and bands just above 47 kDa were recognized by sera from individuals with more time of exposure to infected facilities. This also happens not regularly with other MW proteins on **b, c, d, f, g, j** and **l**. Sera from individuals that had never been in animal facilities presented an identical profile as shown on **lane w.** The profile of this lane was obtained from an individual after one year of working in such environment.

DISCUSSION

M. pulmonis infects laboratory animals worldwide. Most of the animal facilities in Brazil do not control mycoplasmas by culture or serology (19). The recovery of *M. pulmonis* in about 10^5 CFU / mL from tracheal aspirates of rats and the aerosol generated by infected rats in animal facilities favor the transmission of this bacterium to humans. The culture of this specie from a colonized human mouth is very difficult because there are other mycoplasma species as normal flora.

The present study shows that sera from naturally infected rats by mycoplasma of six animal facilities recognized many proteins from the total lysate of *M. pulmonis* cells. Immunoblot profile differences can be justified by the variation on immune response of each rat, antigenic variation and different stages of the natural infection (6). A pattern of recognized bands of about ten or more proteins indicates that the animal could be infected by *M. pulmonis*. This is supported by the fact that the serum from an infected animal recognizes better and more proteins from the infected microorganism than from any other (12). Most of the immunoblot profiles obtained from sera of rats are coincident with the *M. pulmonis* isolations and at least 10 bands were recognized. The hyperimmune serum has a recognition profile similar to most naturally infected animals.

Sera from humans recognized less proteins to *M. pulmonis* than sera from rats, but human sera recognized some proteins with similar Molecular Weight that sera from infected rats. Sera from technicians with more than seven years of exposure to animal facilities recognized more and better *M. pulmonis* proteins than sera from individuals with shorter exposure time to infected rats or those that have never had contact with rodents. This indicates that the time of exposure of technicians to infected rats is related to the differences immunoblot profiles.

Differences on protein recognition profiles between humans and rats can be explained by the fact that the exposure to such agent by different hosts is not identical (7). Microbial infections on different anatomical sites may also result in different immune response to the same microorganism. Different hosts may recognize different epitopes of the same antigen, resulting on distinct recognition patterns (10). Adaptation to the host is another feature for antigenic variation in mycoplasmas and could mimicry the immune response. (5,23). Mycoplasmas also possess modulins that should be also considered in human exposure to these bacteria from other hosts.

Negative serum from a non infected rat and sera from all tested individuals recognized proteins of about 117 and 95 kDa. This indicates that these proteins are cross-reactive and should not be considered as a reference profile for rats and humans. However the sera from infected rats recognized these proteins more intensively.

Cross reactions among mycoplasmas and others microorganisms have already been described (9,13). *M. pulmonis* cross react with *M. arthritidis*, a specie less frequently isolated from murines (15). In this study, it was also observed that sera from rats and humans did not recognize proteins of total lysate of a reference strain of *M. arthritidis*-PG6 and this specie was not isolated from rats in this study.

Although humans have a mycoplasma flora on mucosal surfaces of oropharynx and genital tract, the cross reactions are described between *M. pneumoniae* and *M. genitalium* (17*)*. In addition rat sera did not recognize proteins of total lysates of reference strains of *M. pneumoniae*-FH, *M. hominis*-PG21, *M. orale*-CH19299, *M. buccale*-CH20247 and *M. salivarium* PG-20. Cross reactions in some animal origin mycoplasmas were reported but they are unknown to humans and vice-versa.

Cross reactions between human and murine mycoplasmas are also unknown but in the present study the proteins of about 117 and 95 kDa were shown to be cross reactive between rats and humans. The protein of about 156 kDa may also be considered a cross-reactive protein in human sera but not in all sera from rats.

Although a human serum positive to *M. pulmonis* is unknown and so are studies on this topic, the present results indicate that this mycoplasma promotes distinct immunoblot profile of sera from technicians exposed for more than seven years to this mycoplasma. For a better comprehension of this issue, studies with larger numbers of samples of sera from technicians exposed to infected animal facilities must be performed.

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RESUMO

Imunoeletroforese do soro de ratos naturalmente infectados com *Mycoplasma pulmonis* e bioteristas expostos a biotérios infectados

Mycoplasma pulmonis foi isolado em aproximadamente 10^5 UFC/mL do lavado traqueal de ratos mantidos em biotérios convencionais da cidade de São Paulo. A transmissão do micoplasma por aerossol pode ocorrer entre os animais em até 120 cm. Esta condição favorece a sua transmissão para os bioteristas que também são expostos a este microrganismo. Como esta colonização é desconhecida em humanos, as imunoeletroforeses dos soros destes indivíduos foram comparados à com os dos ratos. Aproximadamente 32 proteínas de 11 a 230 kDa foram reconhecidas pelo soros dos ratos naturalmente infectados com *M. pulmonis.* Os soros dos bioteristas que estão envolvidos por mais de 7 anos na higenização das caixas com animais infectados reconheceram

cerca de 10 proteínas deste microrganismo. O soro de indivíduos com menos tempo de serviço neste ambiente ou aqueles que nunca estiveram em biotérios reconhecerem poucas proteínas. As proteínas de aproxi-madamente 117 and 95 kDa foram reconhecidas pelo soro de ratos, humanos e soros controle negativos. Embora desconhece-se um soro humano positivo contra *M. pulmonis*, este estudo apresenta o perfil imunoeletroforético dos indivíduos expostos a este microrganismo.

Palavras-chave: *Mycoplasma pulmonis*, soro humano, biotérios.

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