Detection of *Pneumocystis* in lungs of bats from Brazil by PCR amplification

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*Pneumocystis* has been isolated from a wide range of unrelated mammalian hosts, including humans, domestic and wild animals. It has been demonstrated that the genome of *Pneumocystis* of one host differs markedly from that of other hosts. Also, variation in the chromosome and DNA sequence of *Pneumocystis* within a single host species has been observed. Since information about the occurrence and nature of infections in wild animals is still limited, the objective of this work was to detect the presence of *Pneumocystis* sp. in lungs of bats from two states from Brazil by Nested-PCR amplification. The bats, captured in caves and in urban areas, were obtained from the Program of Rabies Control of two States in Brazil, Mato Grosso and Rio Grande do Sul, located in the Mid-Western and Southern regions of the country, respectively. DNAs were extracted from 102 lung tissues and screened for *Pneumocystis* by nested PCR at the *mtLSU rRNA* gene and *small subunit of mitochondrial ribosomal RNA (mtSSU rRNA)*. Gene amplification was performed using the *mtLSU rRNA*, the primer set pAZ102H - pAZ102E and pAZ102X - pAZY, and the *mtSSU rRNA* primer set pAZ102 10FRI - pAZ102 10R-RI and pAZ102 13RI - pAZ102 14RI. The most frequent bats were *Tadarida brasiliensis* (25), *Desmodus rotundus* (20), and *Nyctinomops laticaudatus* (19). *Pneumocystis* was more prevalent in the species *Nyctinomops laticaudatus* (26.3% = 5/19), *Tadarida brasiliensis* (24% = 6/25), and *Desmodus rotundus* (20% = 4/20). Besides these species, *Pneumocystis* also was detected in lungs from *Molossus molossus* (1/11, 9.1%), *Artibeus fimbriatus* (1/1, 100%), *Sturnira lilium* (1/3, 33.3%), *Myotis levis* (2/3, 66.7%) and *Diphylla ecaudata* (1/2, 50%). PCR products which could indicate the presence of *Pneumocystis* (21.56%) were identified in DNA samples obtained from 8 out of 16 classified species from both states (5 bats were not identified). This is the first report of detection of *Pneumocystis* in bats from Brazil.

**INDEX TERMS:** *Pneumocystis* sp., bats, Nested-PCR.

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RESUMO.- [Detecção de Pneumocystis em pulmões de morcegos no Brasil por Nested-PCR.] Pneumocystis tem sido isolado de uma grande variedade de hospedeiros mamíferos, incluindo humanos, animais domésticos e selvagens. Tem-se demonstrado que o genoma do Pneumocystis de um hospedeiro difere marcadamente do de outros, assim como há variação no cromossomo e na sequência de DNA dentro de uma única espécie de hospedeiro. Sabendo que a informação da ocorrência e natureza da infecção em animais silvestres ainda é limitada, o objetivo do trabalho foi detectar, por Nested-PCR, a presença de Pneumocystis sp. em pulmões de diferentes espécies de morcegos de dois estados do Brasil. Estes mamíferos voadores foram capturados em cavernas, áreas florestadas, de campo e urbanas pelo Programa de Controle da Raiva do Mato Grosso (região Centro-Oeste) e do Instituto de Pesquisas Veterinárias Desidério Finamor (RS) e Instituto Sauver no Rio Grande do Sul (região Sul). Os DNAs foram extraídos de 102 pulmões e realizado Nested-PCR utilizando os primers pA2102H-pA2102E e pA2102X/R1-pA2Y/R1 para amplificação do gene mtLSU-rRNA, e pA2102 10F-R1 - pA2102 10R-R1 e pA2102 13-R1 - pA214-R1 para amplificação do gene mtSSU-rRNA. As espécies mais frequentes foram Tadarida brasilensis (25), Desmodus rotundus (20) e Nyctinomops laticaudatus (19). Pneumocystis foi detectado com maior prevalência nas Nyctinomops laticaudatus (26,3% = 5/19), Tadarida brasilensis (24% = 6/25) e Desmodus rotundus (20% = 4/20). Além destas espécies, Pneumocystis foi também detectado nos pulmões de Molossus molossus (1/11, 9,1%), Antibeus fimbriatus (1/1, 100%), Stumira lilium (1/3, 33%), Myotis levis (2/3, 66,7%) e Diphylla ecaudata (1/2, 50%). Os produtos de PCR indicaram a presença de Pneumocystis (21,56%) em amostras obtidas de 8 das 16 espécies classificadas para ambos os estados (cinco morcegos não foram classificados). Este é o primeiro registro de detecção de Pneumocystis em morcegos no Brasil.

TERMOS DE INDEXAÇÃO: Pneumocystis sp., bats, Nested-PCR.

INTRODUCTION

Pneumocystosis is the disease caused by infection with Pneumocystis sp., irrespective of species and host. The host species is usually identified or implicated because of the species specificity of Pneumocystis spp (Frenkel 1999). Members of the genus Pneumocystis exhibit strict host specificity with at least one distinct species resident in the lungs of every mammalian host examined for its presence (Cushion 1998, Icenhour et al. 2006). This organism is considered an opportunistic pathogen, and causes a kind of pneumonia which may be lethal if the host reaches a state (Frenkel 1999). Pneumocystis pneumonia is characterized by intense neutrophilic lung inflammation associated with diffuse alveolar damage, gas exchange impairment and respiratory failure (Wright et al. 1999, Beck et al 2001).

The genus Pneumocystis is complex, containing many species of unusual, ascomycetous yeasts, each of which can colonize the lungs of a different mammal (Stringer 2002). Pneumocystis spp infection has been reported in many mammalian hosts, such as in animals with acquired immunodeficiency or malnourishment, including foals (Perryman et al. 1978, Marrs 1987, Ewing et al. 1994, Peters et al. 1994b, Jensen et al. 2001), piglets (Bille-Hansen 1990, Kondo et al. 1993, Christensen 1996, Wakefield et al. 1997, Ramos-Vara et al. 1998, Jensen et al. 2001, Cavallini Sanches et al. 2006, 2007), dogs (Farrow et al. 1972, Hagiwara et al. 2001), cats (Yuezhong et al. 1996), monkeys (Durand-Joly et al. 2000, Demanche et al. 2001), shrews (Peters et al. 1994a, Laakonen and Sukura 1997), rabbits (Guillot et al. 1999, Dei-Cas et al. 2006), ferrets (Banerji et al. 1994), rats (Cushion 2004), and bats (Guillot et al. 2001, Akabar et al. 2008). Morphologic, antigenic and genetic differences can be detected across isolates of Pneumocystis extracted from the infected lungs of different mammalian host species (Eddind et al. 1992, Banerji et al. 1995, Mazars et al. 1995, Cushion 1998). Studies conducted in the past ten years have revealed that the genus Pneumocystis contains many distinct organisms. Differences have been observed for their cell surfaces, the conditions required for their growth, and their genomes. The genetic and functional diversity exhibited by these organisms indicates that Pneumocystis contains more than one species (Keely et al. 2003, Cushion 2004). Controversy surrounds these issues because Pneumocystis cannot be cultured, and mating experiments cannot be performed (Keely et al. 2003). However, the development of a molecular typing method for the uncultivable fungus Pneumocystis is described, and researchers have devised typing methods that rely on the amplification by PCR of variable regions of the genome, followed by the detection of the polymorphisms using DNA sequencing or various gel-based techniques (Hauser 2004). The degree of sequence variation seen between several gene orthologues suggested that DNA could be utilized to diagnose Pneumocystis species (Keely et al. 2003). The purpose of this article was to determine the prevalence of Pneumocystis DNA in bats captured in caves and in urban areas, from the Program of Rabies Control from Mato Grosso and Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

Animals. The bats, captured in caves, forests, and urban areas, were obtained from the Program of Rabies Control of two States in Brazil, Mato Grosso and research institutions of Rio Grande do Sul, located respectively in the Mid-Western and Southern regions of the country. The bats from the state of Mato Grosso were provided by Instituto de Defesa Agropecuária do Estado do Mato Grosso (INDEA/MT) and Laboratório de Apoio à Saúde Animal (LASA), while the bats from the state of Rio Grande do Sul were offered by the Instituto de Pesquisas Veterinárias Desidério Finamor and by the Instituto Sauver. The bats were captured by these same institutions, according to the guidelines in the rabies control manual for herbivores, standardized by the Ministério da Agricultura e Pecuária.
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The most frequent bat species were *Tadarida brasiliensis* (25), *Desmodus rotundus* (20), and *Nycinomops laticaudatus* (19). *Pneumocystis* was more prevalent in the species *Nycinomops laticaudatus* (26.3% = 5/19), *Tadarida brasiliensis* (24.0% = 6/25) and *Desmodus rotundus* (20.0% = 4/20). Besides these species, *Pneumocystis* was also detected in lungs from *Molossus molossus* (1/1), *Artibeus fimbriatus* (1/1, 100%), *Stumira lilium* (1/3, 33%), *Myotis levis* (2/3, 66.7%) and *Diphylla ecaudata* (1/2, 50%). PCR products of which could indicate the presence of *Pneumocystis* (21.56%) were identified DNA in samples obtained from 8 out of 16 classified species from both states (5 bats were not identified). The prevalence between wild and urban bats was (25.5% [13/51] versus 17.5% [7/40] respectively). Eleven bats were not identified for their origin.

**DISCUSSION**

Several PCR assays have been developed in the past 15 years. PCR tool revealed to be highly efficient in detecting *Pneumocystis* DNA in lung samples from domestic or wild mammals (Mazars et al 1997, Peters at al 1994), as well as in the air (Bartlett et al. 1997, Peters 1996) or water in the environment, and sequences of selected *Pneumocystis*-DNA amplified fragments are used to identify *Pneumocystis* species or genotypes (Casa-Nova-Cardiel & Leibowitz 1997). In a review, Cushion listed more than 30 different mammals from disparate groups such as rodents, carnivores, ungulates, lagomorphs, primates, insectivores and marsupials (Cushion 1998). In 2001 Guillot et al. confirmed a coevolution process observed in *Pneumocystis* species from mammalian hosts. They compared mitochondrial large subunit (mtLSU) and small subunit (mtSSU) ribosomal RNA sequences of *Pneumocystis* organisms from 24 different host species representing the following major mammalian groups: primates (12 species), rodents (5 species), carnivores (3 species), chiropters (one species), lagomorphs (one species), marsupials (one species), and ungulates (one species). With regard to bats, in a recent study (Akabar et al 2008), it was reported 29% (38/131) of *Pneumocystis* carriage, being out of: 6 from

![Fig.1. Polymerase chain reaction (PCR) products of DNA extracted from bat lungs infected with *Pneumocystis*. A nested PCR protocol was used to amplify a fragment of the *Pneumocystis* mtLSU rRNA gene (Lanes1,3) and mtSSU rRNA gene (Lane 2). Lane 4 negative control (water), M1, markers (100-bp ladder) and M2 (50-bp ladder).](image-url)
French Guiana, 13 from France and 19 from Mexico. Our percentage of Pneumocystis (21.6%) and the bat species were different from those reported in the study mentioned above. The prevalence of Pneumocystis between wild and urban bats in Brazil was 25.5% (13/51) versus 17.5% (7/40) respectively. The present study evaluates the presence of a pneumonia causal agent (Pneumocystis sp.) in Brazilian bats transmitted by the air, which promotes the dissemination inside a colony and placing at risk the development of the community. This is the first report to detect Pneumocystis in Brazilian bats, and afford to know the pathological impact on the lungs of these animals, the extension of the presence of the fungal agent in Chiroptera populations, the description of new species of the pathogen. Furthermore, the sequencing of Pneumocystis in bats could be associated within the French Guiana group and correlated with the phylogenetic analysis.

REFERENCES


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