Journal of Animal and Veterinary Advances 5(5) : 390-394, 2006

Epidemiological Survey of Pneumocystis Carinii in Rodents of Sari, Northern Iran

M. Sharif, H. Ziaei, A. Daryani, [1]M. Nasrolahei and B. Lackterashi
Department of Parasitology and Mycology, School of Medicine,
Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran
[1]Department of Immunology and Microbiology, School of Medicine,
Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran

Abstract: *Pneumocystis carinii* (*P. carinii*) organisms constitute a large group of heterogenous atypical microscopic fungi that are able to infect immunocompromised mammals and proliferate in their lungs, inducing *Pneumocystis carinii* pneumonia. Despite intensive investigation in human and animal hosts, information on the occurrence and nature of infections in wild animals is scarce, although characterization of infections in wild-animal populations may help to elucidate the life-cycle and transmission of this elusive organism. Due to the interspecific with *P. carinii* in differences in prevalence and intensity of *P. carinii* infection and to the antigenic and genetic diversity of *P. carinii* organisms originating from various host species, which may affect the infectivity and pathogenicity of these organisms, we should be cautious when making generalizations about the nature of *P. carinii* infection. This study conducted to find out the prevalence of infection in wild rats in their natural habits and also in immunosuppressed rats in Sari, Mazandaran Province of Iran.

Key words: *Pneumocystis carinii*, rodents, epidemiological, northern Iran

INTRODUCTION

P. carinii is an opportunistic pathogen capable of causing life-threatening pneumonia (PCP) in patients with Aids and in other immunocompromised individuals[1-4]. Although *P. carinii* causes pneumonia in immunosuppressed individuals, serological data suggest that most healthy individuals have exposed to pneumocystis at an early age but do not develop clinically significant pneumonia[5,6]. Transmission of *P. carinii* is by airborne route. Apart from humans, *P. carinii* is found in a wide variety of other mammalian hosts[7] worldwide. By using Polymerase Chain Reaction (PCR) technique, *P. carinii* DNA has been detected in air samples[8], asymptomatic animals[9-13], normal healthy individuals[14] and immunocompetent patients with other diseases[14,15] even if the organism was not detected by light microscopy. Animal models been widely used as a source of *P. carinii* organisms and for studying many aspects of *P. carinii* infection. This is because sustained in vitro cultivation of *P. carinii* has not been possible[16,17], although recently a new method has been reported which is now being evaluated in a number of centers[18]. The rat model has been particularly useful in studies of epidemiology[19,20] drug sensitivity[21], immunology[22] and the biology of the organism[23]. Rat and human derived *P. carinii* organisms, however, are known to differ significantly in many respects. Considerable divergence has been shown between the genes of rat and human derived *P. carinii*[24,25] as well as antigenic[26] and Ultra structural[27] differences. Although the state of knowledge about *P. carinii* has improved, significant questions about its epidemiology and transmission remain unanswered. The aim of the present work was to study the prevalence of *P. carinii* in immunocompetent and immunosuppressed wild and house rats in northern Iran. This is the first report of detection of *P. carinii* in wild and house rats in northern Iran. Information on the occurrence of *P. carinii* in wild rats in this geographical area will provide information on the organism's distribution.

MATERIALS AND METHODS

During the period From December 2004-June 2005,158 urban and house rodents (Rattus rattus 64; Rattus norvegicus 83; Mus musculus 11) were live trapped at various locations throughout Sari in northern Iran. The 158 nonimmunosuppressed rats and mice were then housed singly or in groups of up to 3 per cage in the laboratory and were sacrificed by exsanguinations under deep chloroform anaesthesia at varying intervals throughout the study (0-58 days). The animals were all housed initially in a quarantine room and then transferred

Corresponding Author: M. Sharif, Department of Parasitology and Mycology, School of Medicine, Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran

to a test room, both of which were under negative pressure. There were no immunosuppressed animals housed in the same building. No immunosuppressive drugs were administered, nor were the rats treated with drugs against bacteria infections or worms. Fifteen wild rats were trapped, taken to a different animal facility and immunosuppressed with corticosteroids. Standard treatment regimens consist of cortisone acetate (125 mg kg^{-1}) subcutaneously injected twice weekly. The seven rats died after 5 and 12 days, but the remaining rats continued on immunosuppressive drugs until they were sacrificed at between 48 and 58 days. At necropsy, tissue samples of lung were taken and fixed in 10% formalin for 48-72 hrs, dehydrated, cleared in xylene and embedded in Paraffin wax. Sections were stained with haemotoxyllin and eosin according to the technique of[28]. The secretions in trachea and bronchi, lung were taken to make the imprints or smears which were stained with Gomori-Groccot's methenamine silver nitrate (GMS)[29] and Methanol-Giemsa methods[30]. *Pneumocystis carinii* cysts and trophozoites were examined under a Zeiss microscope. Also pieces of lung were minced and then homogenized in a homogenizer (Blaessing glass). The *P. carinii* organisms were separated from the homogenate by differential filtration, using, 10-, 5- and 3- μm pore size filters. The filtrate was centrifuged at 2.000x g for 10 min, the supernatant was discarded and the pellet was resuspended in sterile Phosphate-Buffered Saline (PBS). A 10 μL samples of this filtrate was placed on a 1-cm etched square on a slide, stained with Giemsa stain and evaluated by counting trophozoite to determine the intensity of infection.

RESULTS

To study Pneumocystis infection in healthy as well as immunodeficient animals, we developed 2 method of staining. Giemsa and GMS method of staining was used to search for *P. carinii* organism in sample extracted from the lungs. A total of 157 wild rats and 11 mice, live trapped at various locations in northern Iran were examined. By these 2 methods of staining, *P. carinii* not detected 0 out of 158 (0%) in impression smear and lung homogenate of immunocompetent rats and mice. We found *P. carinii* in 7 out of (100%) of rats which were under standard treatment regimen of corticosteroid. The extent of *P. carinii* infection observed in the lungs varied, depending the rat died or was killed after immunosuppression.The immunocompetent animals appeared healthy, while immunosuppressed one became chronically ill and wasted and lost about 30% of their original weight. Immunocompetent animals sacrificed at varying intervals did not show any greater tendency to acquire the infection over time. The intensity of *P. carinii* infection in the group of rats on the standard treatment regimen slowly increased over time, however, the intensity of the infection varied somewhat among these rats sacrificed at any given time intervals. *P. carinii* was light to moderate in most rats by week 4, moderate to heavy at week 6 and at peak intensity at week 7 to 8. Pathological changes of lung samples observed, when stained with haematoxyllin-eosin. In minimal or light infection, Pneumocystis organisms were found individually or in small groups along the walls of the alveoli. As the infection progressed, more alveoli became involved and began to be filled with clumps of organisms. Epithelial hyperplasia and thickened septum of pulmonary alveoli were observed in the lung tissue were found to be infiltrated by lymphocytes and macrophages. Immunocompetent rats and mice killed, had no evidence of Pneumonia throughout the lungs.

DISCUSSION

Special forms of *P. carinii* constitute a large group of heterogeneous atypical microscopic fungi able to infect immunocompromised hosts by an airborne route and to proliferate in their lungs, inducing *P. carinii pneumonia* (PCP). The understanding of *P. carinii* infection is a crucial epidemiological challenge, since the source of the infection in humans, the means by which humans become infected and the other key components of the natural history of PCP, such as the primary infection or the reservoir, have not yet been clearly established. The most important finding reported in this work was the non detection of *P. carinii* in immunocompetent rats and mice and interestingly the detection of *P. carinii* in the lungs of all immunodeppressed rats. The one condition required for proliferation sufficient to cause pneumonia in any recognized host appears to be suppression of the host immune system[31].It means that most laboratory rats have latent *P. carinii* infection and when animals are immunosuppressed by adrenal corticosteroids overt infection develops[32]. However, in the animal model, a prolonged period of immunodeficiency is required for the development of pneumocystis pneumonitis and this can be induced either by the administration of corticosteroids or by dietary deprivation of protein[33,34].Autopsy studies using microscopy or immunofluorescence, revealed no evidence or only a very low rate of prevalence of *P. carinii* (less than 1%) in adult without predisposing diseases[35-37]. Studies using both monoclonal antibodies and the more sensitive techniques of PCR also have failed to reveal evidence of pneumocystis colonization in lung

tissue obtained at post mortem or in broncho-alveolar lavage fluid from immunocompetent individuals[36,38,39]. In agreement with our work, multiple studies have failed to find the organism on autopsy, in lung tissue, or in respiratory samples from non- immunocompromised individuals[38,40,41]. Wakefield was unable to find evidence of *P. carinii* in BAL fluid from 10 healthy subjects using PCR[40]. Peters replicated this result in post-mortem lung tissue from 15 immunocompetent adults[38]. Contini and colleagues studied a group of children with normal immune systems and found that none of 15 subjects had *P. carinii* detected by PCR of nasopharyngeal aspirates[41]. Studies using PCR to detect *P. carinii* DNA in non- immunocompromised animals have been similarly unsuccessful[42,43]. Although small numbers of subjects may have resulted in sampling error, if long-term carriage of *P. carinii* occurred in the majority of people, then one would expect studies using these sensitive methods to detect the organism's DNA in at least some proportion of the population.

In contrast to the studies described mentioned, a number of studies have found *P. carinii* in respiratory specimens from non-immunocompromised hosts with no clinical evidence of PCP[44-46]. One PCR study of Bronchoalveolar Lavage (BAL) fluid detected *P. carinii* DNA in almost 20% of fluid samples from 174 HIV-negative subjects[44]. A similar percentage of BAL fluid samples (19%) from 63 non- immunocompromised patients with underlying lung disease tested positive for *P. carinii* by PVR and a recent series reported that among patients with cystic fibrosis, 7 of 95 (7.4%) had Pneumocystis in detectable by PCR in sputum samples[45,47]. Fifty-one of 161 autopsy specimens (32%) from a study of sudden infant death syndrome were found to contain Pneumocystis by microscopy[46]. These studies may support the hypothesis that *P. carinii* can exist in its host for long periods of time without resulting in clinical disease. Alternatively, the findings could imply that *P. carinii* is ubiquitous in the environment and that exposure in humans is characterized by intermittent colonization or sub-clinical infection.

We postulate that presumed immunocompetent members of mammalian populations could play a significant role in the circulation of Pneumocystis species in ecosystem. Serological surveys have shown that primary infection by *Pneumocystis carinii*, probably an asymptomatic event in most cases, is one of the most frequent infections in humans, affecting up to 94% of immunocompetent infants by the age of 30 months[48]. Before infants develop specific immunity leading to total eradication, parasites might proliferate transiently in their lung, being transmitted to ½susceptible½members of the population (immunocompetent infants without specific immunity against Pneumocystis, or immunosuppressed individuals). Thus, presumed immunocompetent infants and young children could be a previously unrecognized reservoir of *P. carinii* infection in the community.

CONCLUSION

This work demonstrates that immunocompetent hosts transiently parasitized with *P. carinii* can act as a source of infection for susceptible hosts. Moreover, these results suggest that immunocompetent host may play a role in the circulation of Pneumocystis in ecosystems.

REFERENCES

1. Huges, W.T., 1987. *Pneumocystis carinii Pneumonitis*. Boca Raton, FL: CRC Press, pp: 97-104.
2. Dei-Cas, E., B. Soulez, F. Palluault, P. Charet and D. Camus, 1990. *Pneumocystis carinii*, un defi pour le biologiste. Med. Sci., 6: 517-525.
3. Cailliez, J.C., N. Seguy, C.M. Denis, E.M. Aliouat, E. Mazars, L. Polonelly, D. Camus and E. Dei-Cas, 1996. *Pneumocystis carinii*: an atypical fungal micro-organism. J. Med. Vet. Mycol., 34: 227-239.
4. Laakonen, J., 1998. *Pneumocystis carinii* in wildlife. Int. J. Parasitol., 28: 241-252.
5. Bishop, L.R. and J.A. Kovacs, 2003. Quantitation of anti-Pneumocystis jiroveci antibodies in healthy persons and immunocompromised patients. J. Infect. Dis., 187: 1844-1848.
6. Peglow, S.W., A.G. Smulian and M.J. Linke, *et al*., 1990. Serologic responses to *Pneumocystis carinii* antigens in health and disease, 161: 296-306.
7. Kaneshiro, E.S., 1998. The lipid of *Pneumocystis carinii*. Clin. Microbiol. Rev., 11: 27-41.
8. Huges, W.T., 1987. Geographic distribution. In: *Pneumocystis carinii* pneumonitis, Boca Raton, FL: CRC Press, 1: 33-57.
9. Laakonen, J. and A. Sukura, 1997. *Pneumocystis carinii* of the common shrew. Sorex araneus, shows a discrete phenotype. J. Eukaryot. Microbiol., 44: 117-121.
10. Laakonen, J., A. Sukura, V. Haukisal and H. Hentonen, 1993. *Pneumocystis carinii* and helminth parasitism in shrews Sorex araneus and Sorex caesutiens. J. Wildlife. Dis., 29: 273-277.
11. Peters, S.E., A.E. Wakefield, K.E. Whiwell and J.M. Hopkin, 1994. *Pneumocystis carinii pneumonia* in Thoroughbred foals., identification of a genetically distinct organism by DNA amplification. J. Clinn. Microbiol., 32: 213-216.

12. Pifer, L.L., W.T. Huges, S. Stagno and D. Woods, 1978. *Pneumocystis carinii* infection: Evidence for high prevalence in normal and immunosuppressed children. Pediatrics., 61: 35-41.
13. Stringer, J.R. and P.D. Walzer, 1996. Molecular biology and epidemiology og *Pneumocystis carinii* infection in AIDS. AIDS., 10: 561-571.
14. Shiota, T., T. Kurimoto and T. Yoshida, 1986. prevalence of *Pneumocystis carinii* in wild rodents in Japan. Zentralbl. Bakteriol. Mikrobiol. Hyg., 261: 381-389.
15. Shimizu, A., F. Kimura and S. Kimura, 1985. Occurrence of *Pneumocystis carinii* in animals in Japan. Jpn. J. Vet. Sci., 47: 309-311.
16. Aliouat, E.M., E. Dei Cas, L. Dujardin, J.P. Tissier, P. Billaut and D. Camus, 1996. High infectivity of *Pneumocystis carinii* cultivated on L2 rat alveolar epithelial cells. J. Eukaryot. Microbiol., 43: 225.
17. Sloand, E., B. Laughon, M. Armstrong, M.S. Bartlett, W. Blumenfeld, M. Cushion, J.A. Kalica, W. Martin, E. Pitt, E.L. Pesanti, F. Richards, R. Rose and P. Walzer, 1993. The challenge of *Pneumocystis carinii* culyure. J. Eukaryot. Microbiol., 40: 188-195.
18. Merali, S., U. Frevert, J.H. Williams, K. Chin, R. Bryan and A.B. Clarkson, 1999. Continuous axenic cultivation of *Pneumocystis carinii*. Proc. Natl. Acad. Sci. USA., 96: 2402-2407.
19. Hughes, W.T., 1982. Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. J. Infect. Dis., 145: 842-848.
20. Vargas, S.L., W.T. Hughes, A.E. Wakefield and H.S. Oz, 1995. Limited persistence in and subsequent elimination of *Pneumocystis carinii* from the lungs after *P. carinii* pneumonia. J. Infect. Dis., 172: 506-510.
21. Walzer, P.D., J. Foy, P. Steele, C.K. Kim, M. White, R.S. Klein, B.A. Otter and C. Allegra, 1992. Activities of antifolate, antiviral and other drugs in an immunosuppressed rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents. Chemother., 36: 1935-1942.
22. Vasquez, J., A.G. Smulian, M.J. Linke and M.T. Cushion, 1996. Antigenic differences associated with genetically distinct *Pneumocystis carinii* from rats. Infect. Immun., 64: 290-297.
23. Stringer, J.R., 1996. *Pneumocystis carinii*. What is it, exactly? Clin. Microbiol. Rev., 9: 489-498.
24. Linclair, K., A.E. Wakefield, S. Banerji and J.M. Hopkin, 1991. *Pneumocystis carinii* organisms derived from rat and human hosts are genetically distinct. Mol. Biochem. Parasitol., 45: 183-184.
25. Stringer, J.R., S.L. Stringer, J. Zhang, R. Baughman, A.G. Smulian and M.T. Cushion, 1993. molecular genetic distinction of *Pneumocystis carinii* from rats and humans. J. Eukaryot. Microbiol., 40: 733-741.
26. Gigliotti, F., 1992. Host species-species antigenic variation of mannosylated surface glycoprotein of *Pneumocystis carinii*. J. Infect. Dis., 165: 329-336.
27. Creusy, C., J. Bahon le Capon, L. Fleurisse, C. Mullet, M. Dridba, J.C. Cailliez, M. Antoine, D. Camus and E. Dei Cas, 1996. *Pneumocystis carinii* pneumonia in four mammal species: histopathology and ultrastructure.J. Eukaryot. Microbiol., 43: 47-48.
28. Lillie, R.D., 1954. Histopathological Technique and Practical histochemistry, 2nd Edn. McGraw-hill, New York, pp: 47-51.
29. Grocott, R.G., 1955. A stain for fungi in tissue sections and smears. Using Gomori,s methenamine-silver nitrate technique. Am. J. Clin. Pathol., 25: 975-979.
30. Bessis, M., 1976. Reinterpretation des Frottis Sanguins, Masson, Paris, pp: 270.
31. Bartlet, M.S. and J.W. Smith, 1991. *Pneumocystis carinii*, an opportunist in immunocompromised patients. Clin. Microbiol. Rev., pp: 133-149.
32. Frenkel, J.K., J.T. Good and J.A. Schultz, 1966.Latent Pneumocystis infection of rats, relapse and chemotherapy. Lab. Invest., 15: 1559-1577.
33. Wakefield, A.E., 1995. Re-examination of epidemiological concepts. In: Bailliere,s Clinical Infectious Diseases.International Practice and Research (Sattler, F.R. and Walzer, P.D., Eds.), Bailliere Tindall, W. B. Saunders, London, 2: 431-438.
34. Huges, W.T., 1987. *Pneumocystis carinii* Pneumonitis. CRC Press, Boca Raton, FL.
35. Esterly, J.A., 1968. *Pneumocystis carinii* in lungs of adults at autopsy. Am. Rev. Respir. Dis., 97: 935-937.
36. Millard, P.R. and A.R. Heryet, 1988. Observation favouring *Pneumocystis carinii* pneumonia as a primary infection:a monoclonal antibody study on paraffin sections. J. Pathol., 154: 365-370.
37. Vogel, C.L., M.H. Cohen, R.D. Powell and D. Devitas, 1968. *Pneumocystis carinii* pneumonia. Ann. Intern. Med., 68: 97-108.
38. Peters, S.E., A.E. Wakefield and K. Sinclair, 1992. A Search for *Pneumocystis carinii* in post mortem lungs by DNA amplification. J. Pathol., 166: 195-198.
39. Matusiewicz, S.P., R.J. Ferguson and A.P. Greening, 1994. *Pneumocystis carinii* in bronchoalveolar lavage fluid and bronchial washings. Br. Med. J., 308: 1206-1207.
40. Wakefield, A.E., F.J. Pixley, S. Banerji, K. Sinclair, R.F. Miller, E.R. Moxon and J.M. Hopkin, 1990. Detection of *Pneumocystis carinii* with DNA amplification, Lancet., 336: 451-453.

41. Contini, C., R. Romani, R. Cultrera, E. Angelici, M.P. Villa and R. Ronchetti, 1997. Carriage of *Pneumocystis carinii* in children with chronic lung diseases. J. Eukaryot.Microbiol., 44: 155.
42. Chen, W., F. Gigliotti and A.G. Harmsen, 1993. Latency is not an inevitable outcome of infection with *Pneumocystis carinii*. Infect. Immun., 61: 5406-5409.
43. Sepkowitz, K., N. Schluger, T. Godwin, D. Armstrong, A. Cerami and R. Bukala, 1993. DNA amplification in experimental Pneumocystosis: characterization of serum *Pneumocystis carinii* DNA and potencial *P. carinii* carrier state. J. Infect. Dis., 168: 421-426.
44. Nevez, G., V. Jounieaux, M.D. Linas, K. Guyot, P. Leophonte, P. Massip, J.I. Schmit, J.P. Seguela, D. Camus, E. Dei-Cas, C. Raccurt and E. Mazars, 1997. High frequency of *Pneumocystis carinii* sp.f.hominis colonization in HIV-negative patients. J. Eukaryot. Microbiol., 44: 36.
45. Sing, A., A. Roggenkamp, I.B. Autenrieth and J. Heesemann, 1999. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J. Clin. Microbiol., 37: 3409-3410.
46. Vargas, S.L., C.A. Ponce, W.T. Huges, A.E. Wakefield, J.C. Weitz, S. Donoso, A.V. Ulloa, P. Madrid, S. Gould, J.J. Latorre and R. Avila, 1999. Association of Primary *Pneumocystis carinii* infection and sudden infant death syndrome. Clin. Infect. Dis., 29: 1489-1493.
47. Sing, A., A.M. Geiger, M. Hogardt and J. Heesemann, 2001. *Pneumocystis carinii* carriage among cystic fibrosis patients, as detected by nested PCR. J. Clin. Microbiol., 39: 2717-2718.
48. Peglow, S.L., A.G. Smulian, M.J. Linke, C.L. Pogue, S. Nurre, J. Crisler, J. Phair, J.W.M. Gold, D. Armstrong and P.Walzer, 1990. Serologic responses to *Pneumocystis carinii* antigens in health and disease. J. Infect. Dis., 161: 296-306.