NOSOCOMIAL *PNEUMOCYSTIS JIROVECII* INFECTIONS

NEVEZ G.*, CHABÉ M.**, RABODONIRINA M.***, VIRMAUX M.*, DEI-CAS E.****, HAUSER P.M.***** & TOTET A.******

Summary:

Airborne transmission of *Pneumocystis* sp. from host to host has been demonstrated in rodent models and several observations suggest that interindividual transmission occurs in humans. Moreover, it is accepted that the *Pneumocystis* organisms infecting each mammalian species are host specific and that the hypothesis of an animal reservoir for *Pneumocystis jirovecii* (*P. jirovecii*), the human-specific *Pneumocystis* species, can be excluded. An exosaprophytic form of the fungus cannot be strictly ruled out. However, these data point toward the potential for the specific host to serve as its own reservoir and for *Pneumocystis* infection in humans as an anthroponosis with humans as a reservoir for *P. jirovecii*. This review highlights the main data on host-to-host transmission of *Pneumocystis* in rodent models and in humans by the airborne route and provides a rationale for considering the occurrence of nosocomial infections and measures for their prevention

KEY WORDS : *Pneumocystis jirovecii*, nosocomial infections, genotyping.

Pneumocystis jirovecii (human-specific *Pneumocystis* species) is an atypical fungus which causes severe pneumonia in immunocompromised patients (Stringer *et al.*, 2002). Profound deficiency of cell-mediated immunity as a consequence of HIV infection is a major host factor for developing *Pneumocystis* pneumonia (PCP) (Walzer *et al.*, 2005). The microorganism remains the most frequent AIDS defining opportunistic pathogen in France and other developed countries despite the decline in PCP incidence related to the wide use of highly active antiretroviral therapy (Institut de Veille Sanitaire, 2002). Treatments for transplantation, graft, malignancy, and connective tissue disease are also host factors for PCP occurrence in other immunocompromised patients not infected with HIV (Sepkowitz *et al.*, 1995).

Over the past fifteen years, long term pulmonary carriage of *P. jirovecii* in healthy persons has been re-evaluated and PCP is now frequently considered to result from *de novo* infection rather than from reactivation of latent infection. At the same time, PCR assays have revealed that persons having an unusual non-bilateral interstitial pneumonia due to *P. jirovecii* can be infected with only a few organisms (Peterson *et al.*, 2005). In this context, terms such as carriage or colonization are frequently used. For example, it has been reported that immunocompromised patients – and also immunocompetent patients who have acute or chronic lungs diseases – can frequently be colonized with the microorganism (Peterson *et al.*, 2005).

It is now accepted that *Pneumocystis* organisms infecting each mammalian species are host-specific and the hypothesis of an animal reservoir for *P. jirovecii* can be excluded (Stringer *et al.*, 2002). An exosaprophytic form of the fungus cannot be strictly ruled out. However, these data point toward the potential for the specific host to serve as its own reservoir and for *Pneumocystis* infection in humans as an anthroponosis with humans as a reservoir for *P. jirovecii*. Suspected cases of interindividual transmission of *P. jirovecii* has been reported since the fifties, due to the occurrence of PCP case clusters in hospitals (Vanek *et al.*, 1952). The results of recent studies based on observations of PCP case clusters combined with *P. jirovecii* typing plead in favor of this transmission (Rabodonirina *et al.*, 2004; Hocker *et al.*, 2005; de Boer *et al.*, 2007). Moreover, investigations of subjects colonized with *P. jirovecii,* based on a genotyping approach, have suggested that these populations have a role in the circulation or transmission of the microorganism (Totet *et al.*, 2004).

* Laboratoire de Parasitologie et Mycologie, Hôpital Augustin Morvan, CHU et Laboratoire de Parasitologie et Mycologie (EA 3882, Biodiversité et Écologie microbienne), Faculté de Médecine, Université de Bretagne Occidentale, (École doctorale, ED 373), Brest, France.
** Institut Pasteur de Lille, (EA3609, Ecologie du Parasitisme) et Laboratoire de Parasitologie, Faculté de Pharmacie, Lille France.
*** Laboratoire de Parasitologie et Mycologie, Hôpital de la Croix Rousse, CHU de Lyon et UMR 145, IRD/Université de Montpellier, France.
**** Institut Pasteur de Lille, (EA3609, Ecologie du Parasitisme) et Service de Parasitologie-Mycologie, Centre de Biologie-Pathologie, CHRU de Lille, France.
***** Institut de Microbiologie, Centre Hospitalier Universitaire Vaudois et Université de Lausanne, Suisse.
****** Laboratoire de Parasitologie et Mycologie médicales, (EA3609, Ecologie du Parasitisme) CHU et Université de Picardie Jules Verne, Amiens, France.
Correspondence: Gilles Nevez, Laboratoire de Parasitologie et Mycologie, Hôpital Augustin Morvan, CHU, F-29609 Brest, France.
Tel.: 33 (0)2 98 01 33 08 – Fax: 33(0)2 98 22 39 87.
E-Mail: gilles.nevez@chu-brest.fr

This review highlights the main data on host-to-host transmission of *Pneumocystis* in rodent models and in humans by the airborne route and provides a rationale for considering the occurrence of nosocomial infections and measures for their prevention.

AIRBORNE ACQUISITION AND TRANSMISSION OF *PNEUMOCYSTIS* SP. IN RODENT MODELS

Pneumocystis sp. infects numerous domestic or wild mammals causing severe pneumonia in those that are immunodeficient or experimentally immunosuppressed (Dei-Cas *et al.*, 1998). Because clinical presentation of PCP in animals is close to that of immunocompromised humans, experimental PCP has been studied. The contribution of animal models to the knowledge of *Pneumocystis* epidemiology appears to be major.

Pneumocystis acquisition by the airborne route in rats was clearly established in the early 1980's (Hughes, 1982; Hughes *et al.*, 1983). It was observed that no germ-free rats immunocompromised with corticosteroid developed PCP when maintained in germ-free isolators. Conversely, exposure of these animals to ambient air from animal facilities, led to PCP occurrence, as did the introduction of a conventional rat into isolators. *Pneumocystis* is a highly transmissible microorganism in mice. A one day-exposure is enough for airborne transmission of the infection either from mice with corticosteroid-induced PCP to susceptible SCID mice (Soulez *et al.*, 1991) or from SCID mice with PCP to immunocompetent mice (Soulez *et al.*, 1991; Dumoulin *et al.*, 2000). Immunocompetent animals are able to harbor low parasite rates for weeks without developing acute PCP (Dumoulin *et al.*, 2000; Chabe *et al.*, 2004). Moreover, using the experimental SCID-Balb/c mouse transmission model, it has been proven that *Pneumocystis*-carrying Balb/c mice are able to transmit the infection either to susceptible SCID mice or to naive healthy mice. The secondly exposed "healthy" hosts are able to transmit the fungus to susceptible hosts which developed PCP (Dumoulin *et al.*, 2000; Gigliotti *et al.*, 2003; Chabe *et al.*, 2004).

THE INFECTIVE FORM OF *PNEUMOCYSTIS* SP.

Although *Pneumocystis* DNA has been detected in air of the Oxfordshire country (Wakefield, 1996) and in pond water (Casanova-Cardiel *et al.*, 1997), no exosaprophytic form consistent with an environmental reservoir has been discovered yet. At the same time, the airborne transmission of the fungus from host-to-host has been demonstrated in models as described above. However, the infective form acquired in natural conditions remains unknown. In Hughes's studies, exposure of the immunocompromised germ-free rats to *Pneumocystis* infected lung tissues into the isolator did not result in PCP (Hughes, 1982; Hughes *et al.*, 1983). The authors concluded to a low viability of known stages, i.e. the cysts, intracystic bodies and trophic forms, in the environment or an exosaprophytic form yet to be discovered. Creusy and colleagues had visualized a mature cyst containing intracystic bodies in the bronchial lumen of a SCID mouse developing experimental PCP suggesting that it may be exhaled by the infected subject in its air environment (Creusy *et al.*, 1996). However, the freshly intra cystic bodies, i.e. the small trophic forms, rather than the entire cyst may be released in the lumen, expectorated *via* the Pflugge's droplets and further transmitted to susceptible contact hosts. It is noteworthy that the small trophic form has the same size from 1 to 3 µm as other pathogens such as *Mycobacterium tuberculosis* that are successfully spread deep into the lung via this transmission route. This was previously postulated by Ng and colleagues (Ng *et al.*, 1997).

AIRBORNE ACQUISITION AND TRANSMISSION OF *P. JIROVECII* IN HUMANS

Airborne acquisition of *P. jirovecii*

It is assumed that the acquisition of *P. jirovecii* organisms by humans takes place via the airborne route and inhalation. This theory is supported by experiments in animal models combined with the fact that *P. jirovecii* has a high tropism for the human lungs. However, the intake of the microorganism from the air has not been demonstrated. Tasci and colleagues recently reported an interesting observation of a patient with bronchial carcinoma who developed PCP (Tasci *et a*l., 2003). Computed tomography showed a stenosis of the bronchus–intermedius, infiltrates of the complete left lung and only the right upper lobe, and absence of infiltrates of the post obstructive areas. It was considered that the decreased ventilation due to the airway obstruction limited the spread of *P. jirovecii* and the development of pneumonia foci in these aeras. This is consistent with the fact that *P. jirovecii* is inhaled.

Airborne transmission of *P. jirovecii*

Although the *P. jirovecii* exit mechanism from the lungs has not firmly been demonstrated, the microorganism

References	No. of patients	Underlying conditions of patients	Genotyping (method)
Ruskin *et al*., 1968	2	Connective tissue diseases	–
Brazinski *et al*., 1969	2	Hematological malignancies No risk factor for PCP?	–
Perera *et al*., 1970	19	Cancers	–
Yates *et al*., 1975	2	Hematological malignancies	–
Singer *et al*., 1975	11	Hematological malignancies	–
Ruebush *et al*., 1978	10	Cancers	–
Goesch *et al*., 1990	2	Hematological malignancies	–
Bensousan *et al*., 1990	10	Renal transplantation	–
Chave *et al*., 1991	5	Renal transplantation	–
Jacobs *et al*., 1991	5	No risk factor for PCP? Hospitalization in ICU	–
Glotz *et al*., 1992	8	Renal transplantation	–
Cheung *et al*., 1994	3	Cancers	–
Hennequin *et al*., 1995	7	Renal transplantation	–
Helweg-Larsen *et al*., 1998	8 6	Hematological malignancies AIDS	+ (Analysis of the ITS1 and ITS2 loci)
Olsson *et al*., 2001	10 (3+7)[b]	Renal transplantation Hematological malignancies	+ (Analysis of the mtLSUrRNA locus)
Rabodonirina *et al*., 2004[b]	10	Renal transplantation	+ (SSCP using 4 loci analysis)
Hocker *et al*., 2005[b]	6 (3+3)	Renal transplantation in childhood	+ (SSCP using 4 loci analysis)
de Boer *et al*., 2007 b	22	Renal transplantation	+ (Analysis of the ITS1 and ITS2 loci)

Note: PCP, *Pneumocystis* pneumonia; ICU, intensive care unit; ITS, internal transcribed spacers of nuclear rRNA operon; mtLSUrRNA, mitochondrial large sub unit rRNA; SSCP, single strand comformation polymorphism; –, not done; +, done; [a] two different clusters; [b] results of these studies are consistent with nosocomial acquisition of *P. jirovecii* through interhuman transmission.

Table I. – Main clusters of *Pneumocystis* pneumonia in hospitals that were reported over the past five decades.

may be exhaled by infected patients in their air environment in the course of ventilation. This was suggested by the fact that *Pneumocystis* DNA has been detected in air filters placed in hospital rooms of patients who developed PCP, *P. jirovecii* genotypes in air samples matching those identified in corresponding patients (Bartlett *et al*., 1994; Olsson *et al*., 1998). Moreover, *P. jirovecii* DNA was detected in the air filter membrane of an intubation system from a ventilated patient with PCP. This observation established that *P. jirovecii* DNA can be exhaled by an infected patient (Sing *et al*., 1999a). *P. jirovecii* cDNA was also detected using RT-PCR in air samples from the rooms of patients who developed PCP, showing that the microorganism may still be viable and therefore potentially infectious in the patient's environment (Latouche *et al*., 2001; Maher *et al*., 2001). A report by Vargas and colleagues is consistent with transmission by the airborne route. *P. jirovecii* DNA was amplified using PCR in samples of upper respiratory tracts from health care workers in situations of close contact with a PCP patient. Nasopharyngeal aspirates of 3/3 PCP patient's contacts tested positive for *P. jirovecii* DNA (Vargas *et al*., 2000).

Clusters of *Pneumocystis* pneumonia in hospitals

Interindividual transmission of *P. jirovecii* has been suspected through the occurrence of PCP case clusters in hospitals. Main clusters that have been reported concerned a number of patients from 2 to 22. They have been observed in units of pediatrics, oncology, intensive care, renal transplantation and infectious diseases (Vanek *et al*., 1952; Hamperl, 1956; von Reitsetbauer *et al*., 1956; von Harnack, 1960; Post *et al*., 1964; Santiago-Delpin *et al*., 1988; Kovaleva *et al*., 1991; other references are reviewed in Table I).

Helweg Larsen and colleagues, and Olsson and colleagues used a genotyping approach for evaluation of PCP clusters. However, they did not provide evidence for single-source outbreaks because *P. jirovecii* genotypes from patients suspected to be the sources of the microorganism did not completely match those identified in patients suspected to be susceptible contacts (Helweg-Larsen *et al*., 1998; Olsson *et al*., 2001). Recent analyses at the molecular level strongly suggested that interhuman transmission of *P. jirovecii* occurred in two clusters among renal transplant recipients, one in an adult unit (Rabodonirina *et al*., 2004), and the other in a pediatric unit (Hocker *et al*., 2005). The multi-target PCR-single-strand conformation polymorphism method was used to type *P. jirovecii* organisms. In the first cluster, nosocomial transmission from HIV-positive patients with PCP to ten patients who developed PCP over a 3-year period was suspected because the two categories of patients shared the same hospital building, were not isolated, and were receiving no or suboptimal anti-PCP prophylaxis. Among the 45 patients with PCP hospitalized during the 3-year period, eight renal transplant recipients and six HIV-infected patients may have encountered at least one (range 1-7) patient with active PCP within the three months prior to the diagnosis of their own PCP episode. In six instances (five renal transplant recipients, one HIV-infected patient), molecular typing supported the occurrence of interhuman transmission because the cases harbored the same *P. jirovecii* molecular type as that found in the encountered PCP patients. In the second cluster, three pediatric patients acquired the same two strains of *P. jirovecii,* an infant with mitochondriopathy and PCP being the probable index patient. Transmission events would have occurred in the absence of prophylaxis during hospitalization in rooms eight to ten meters apart, on the same floor, and in one instance during a summer camp organized by the pediatric nephrology unit. The results of a recent study by de Boer and colleagues (de Boer *et al.*, 2007) who investigated a PCP case cluster in renal transplant recipients using *P. jirovecii* genotyping at the internal transcribed spacers (ITSs) of the nuclear rRNA operon and analysis of encounters between patients are compatible with interhuman transmission.

Clusters of *Pneumocystis* pneumonia within households

Interindividual transmission of *P. jirovecii* has also been suspected through the occurrence of case clusters within households (Watanabe *et al.*, 1965; Latouche *et al.*, 1997; Miller *et al.*, 2002). Particularly, Miller and colleagues investigated the case of a mother and her 4.5 week-old infant who had PCP contemporaneously. The time course of clinical symptoms combined with *P. jirovecii* genotyping which showed the same genotype in samples from the two patients rendered the transmission of the microorganism from mother to infant highly probable (Miller *et al.*, 2002).

Colonized subjects as potential sources of *P. jirovecii*

Until the early nineties, PCP in immunocompromised patients was thought to arise through reactivation of latent infection early acquired in childhood. This hypothesis was challenged and now PCP is considered to result frequently from *de novo* acquisition of the microorganism (Morris *et al.*, 2002). Today, instead of long term pulmonary carriage in the general population, incidental and transient colonization may be the rule (Stringer *et al.*, 2002). Indeed, several populations have been identified as being colonized by the fungus. Low burden of *P. jirovecii* organisms have been detected using PCR assays in patients with various levels of immunodeficiency (Nevez *et al.*, 1999a; Nevez *et al.*, 1999b), with acute or chronic pulmonary diseases (Calderon *et al.*, 1996; Armbruster *et al.*, 1997; Sing *et al.*, 1999b; Nevez *et al.*, 2002), immunonaive infants with *P. jirovecii* primary infection (Nevez *et al.*, 2001a, Vargas *et al.*, 2001), pregnant women with physiological immunity changes (Vargas *et al.*, 2003), and health care workers in contact with patients with PCP (Miller *et al.*, 2001, Durand-Joly *et al.*, 2003, reviewed in Peterson *et al.*, 2005). In fact*, Pneumocystis* infections can have a large spectrum of presentations, of which PCP in immunocompromised patients may represent only a part, while mild infections such as colonization may constitute the major part. Investigations of the human reservoir of *P. jirovecii* by genotyping have been based on analyses of *P. jirovecii* organisms from PCP patients (Helweg-Larsen *et al.*, 1998; Olsson *et al.*, 2001; Rabodonirina *et al.*, 2004; Hocker *et al.*, 2005). Other investigations have considered the potential role of human populations that develop mild *Pneumocystis* infections (Hauser *et al.*, 2000; Miller *et al.*, 2001; Nevez *et al.*, 2001b; Nevez *et al.*, 2003; Totet *et al.*, 2003a; Totet *et al.*, 2003b). For example, Nevez and colleagues observed shared features of *P. jirovecii* ITS types in PCP patients, in colonized adult patients, and in infants with *Pneumocystis* primary infection from the same area, consistent with their contribution to a common reservoir for the microorganism (Nevez *et al.*, 2003; Totet *et al.*, 2003a, Totet *et al.*, 2003b). In the study by Miller and colleagues, *P. jirovecii* ITS genotypes in health care workers partly matched those found in PCP patients (Miller *et al.*, 2001). For these reasons, the role of health care workers in *P. jirovecii* circulation in the hospital was discussed.

Transmission of possible resistant *P. jirovecii* organisms in human populations

The analysis of dihydropteroate synthase (DHPS) locus of *P. jirovecii* may serve as a useful circulation marker of the microorganism in the human reservoir. DHPS is the enzymatic target of sulfonamides, which are the major drugs for PCP prophylaxis and treatment. *P. jirovecii* organisms with nonsynonymous mutations at positions 165 and 171 of the DHPS locus have been detected in PCP patients. It is assumed that these mutations result in reduction the affinity of DHPS with receptors for sulfonamides and therefore in lower sensitivity or even resistance of mutant *P. jirovecii* organisms to these drugs. Prior exposure to sulfonamide drugs has been identified as a predictor of mutant genotypes (reviewed in Totet *et al.*, 2004). In addition the city in which a patient resides has also been identified as an independent risk factor (Huang *et al.*, 2000; Kazanjian *et al.*, 2000), a factor that supports the hypothesis that *P. jirovecii* is transmitted from infected treated patients to susceptible untreated patients, either directly or through a common environmental source. Totet and colleagues identified *P. jirovecii* genotypes at the DHPS locus in immunocomptetent infants with primary infection and immunosuppressed adults with PCP (Totet *et al.*, 2004). Both groups of patients were monitored in the same city. The results pointed out identical features of *P. jirovecii* DHPS genotypes in the two groups providing additional arguments in favor of the fungus circulation within a reservoir made up of persons with different clinical presentations of *P. jirovecii* infection. In fact, all persons parasitized by *P. jirovecii* whatever their risk factor for infection and the form of parasitism they have, may act as interwoven circulation networks of *P. jirovecii*. This hypothesis is consistent with available data on *Pneumocystis* sp. transmission in animal models as described above.

CONCLUSIONS

Recent data support the concept of nosocomial *P. jirovecii* infection and permit to establish a new policy for isolation of patients as standard practice. Indeed, prophylaxis of PCP based on chemotherapy among patients known to be immunosuppressed and consequently at risk for the infection remains essential. However, given the potential resistance to anti *Pneumocystis* drugs, it may not be sufficient to achieve prevention. At least, it seems now prudent for a patient at risk for PCP to not share a hospital room with a patient who has active PCP.

Further studies to establish the role of colonized patients as infective sources of *P. jirovecii* are needed. Likewise, investigations are needed to delineate the role of *P. jirovecii* in disease of colonized subjects. It seems appropriate to expect that the infection provokes at least mild or moderate respiratory illness, with the microorganism acting as a co-morbidity factor. In this context, the use of specific treatments for *P. jirovecii* would be justified and reduce not only the global morbidity due to the microorganism but above all its reservoir.

ACKNOWLEDGEMENTS

This review is partly supported by the "Agence Française de Sécurité Sanitaire de l'Environnement et du Travail. AFSSET", contract N° EST-2006/1/41 (AT, GN), the Swiss National Science Foundation, grant 310000-112360 (PMH), the FIS-Europe "Carlos III" Institute of Health 03/1743 and ANR projects: "*Pneumocystis* PathoGenoMics" (ERA-NET 2006-2009) and "Community ecology of rodents and their pathogens in South-East Asia: effects of biodiversity changes and implications for health ecology" (CeroPath) (MC, EDC).

REFERENCES

ARMBRUSTER C., HASSL A. & KRIWANEK S. *Pneumocystis carinii* colonization in the absence of immunosuppression. *Scandinavian Journal of Infectious Diseases,* 1997, *29*, 591-593.

BARTLETT M.S., LEE C.H., LU J.J., BAUER N.L., BETTZ J.F., MCLAUGHLIN G.L. & SMITH J.W. *Pneumocystis carinii* detected in air. *The Journal of Eukaryotic Microbiology*, 1994, *41*, 75S.

BENSOUSAN T., GARO B., ISLAM S., BOURBIGOT B., CLEDES J. & GARRE M. Possible transfer of *Pneumocystis carinii* between kidney transplant recipients. *Lancet,* 1990, *336*, 1066-1067.

de BOER M.G., BRUIJNESTEIJN VAN COPPENRAET L.E., GAASBEEK A., BERGER S.P., GELINCK L.B., van HOUWELINGEN H.C., van den BROEK P., KUIJPER E.J., KROON F.P. & VANDENBROUCKE J.P. An outbreak of *Pneumocystis jiroveci* pneumonia with one predominant genotype among renal transplant recipients: interhuman transmission or a common environmental source? *Clinical Infectious Diseases*, 2007, *44*, 1143-1149.

BRADZINSKI J.H. & PHILLIPS J.E. *Pneumocystis* pneumonia transmission between patients with lymphoma. *The Journal of the American Medical Association*, 1969, *209*, 1527.

CALDERON E.J., REGORDAN C., MEDRANO F.J., OLLERO M. & VARELA J.M. *Pneumocystis carinii* infection in patients with chronic bronchial disease. *Lancet,* 1996, *347*, 977.

CASANOVA-CARDIEL L. & LEIBOWITZ M.J. Presence of *Pneumocystis carinii* DNA in pond water. *The Journal of Eukaryotic Microbiology*, 1997, *44*, 28S.

CHABÉ M., DEI-CAS E., CREUSY C., FLEURISSE L., RESPALDIZA N., CAMUS D. & DURAND-JOLY I. Immunocompetent hosts as a reservoir of *Pneumocystis* organisms: histological and RT-PCR data demonstrate active replication. *European Journal of Clinical Microbiology and Infectious Diseases*, 2004, *3*, 89-97

CHAVE J.P., DAVID S., WAUTERS J.P., VAN MELLE G. & FRANCIOLI P. Transmission of *Pneumocystis carinii* from AIDS patients to other immunosuppressed patients: a cluster of *Pneumocystis carinii* pneumonia in renal transplant recipients. *AIDS*, 1991, *5*, 927-932.

CHEUNG Y.F., CHAN C.F., LEE C.W. & LAU Y.L. An outbreak of *Pneumocystis carinii* pneumonia in children with malignancy. *Journal of Paediatrics and Child Health*, 1994, *30*, 173-175.

CREUSY C., BAHON-LE CAPON J., FLEURISSE L., MULLET C., DRIDBA M., CAILLIEZ J.C., ANTOINE M., CAMUS D. & DEI-CAS E. *Pneumocystis carinii* pneumonia in four mammal species: histopathology and ultrastructure. *The Journal of Eukaryotic Microbiology*, 1996, *43*, 47S-48S.

DEI-CAS E., BRUN-PASCAUD M., BILLE-HANSEN V., ALLAERT A. & ALIOUAT E.M. Animal models of pneumocystosis. *FEMS Immunology and Medical Microbiology*, 1998, *22*, 163-168.

DUMOULIN A., MAZARS E., SEGUY N., GARGALLO-VIOLA D., VARGAS S., CAILLIEZ J.C., ALIOUAT E.M., WAKEFIELD A.E. & DEI-CAS E. Transmission of *Pneumocystis carinii* disease from immunocompetent contacts of infected hosts to susceptible hosts. *European Journal of Clinical Microbiology & Infectious Diseases*, 2000, *19*, 671-678.

Durand-Joly I., Soula F., Chabe M., Dalle J.H., Lafitte J.J., Senechal M., Pinon A., Camus D. & Dei-Cas E. Long-term colonization with *Pneumocystis jirovecii* in hospital staffs: a challenge to prevent nosocomial pneumocystosis. *The Journal of Eukaryotic Microbiology,* 2003, *50*, 614S-615S.

Gigliotti F., Harmsen A.G. & Wright T.W. Characterization of transmission of *Pneumocystis carinii* f. sp. *muris* through immunocompetent BALB/c mice. *Infection and Immunity*, 2003, *71*, 3852-3856.

Glotz D., Kazatchkine M., Vincent F., Haddad H., Duboust A., Druet P. & Bariety J. *Pneumocystis carinii* pneumonia in a kidney transplantation center in the proximity of an AIDS care unit. *Presse Medicale*, 1992, *21*, 2154.

Goesch T.R., Gotz G., Stellbrinck K.H., Albrecht H., Weh H.J. & Hossfeld D.K. Possible transfer of *Pneumocystis carinii* between immunodeficient patients. *Lancet*, 1990, *336*, 627.

Hamperl H. *Pneumocystis* infection and cytomegaly of the lungs in the newborn and adult. *The American Journal of Pathology*, 1956, *2*, 11-13.

von Harnack G.A. Organisatorische Probleme bei der Bekämbfung der interstitiellen Pneumonie. *Monatsschrift für Kinderheilkunde*, 1960, *108*, 159-164.

Hauser P.M., Blanc D.S., Bille J., Nahimana A. & Francioli P. Carriage of *Pneumocystis carinii* by immunosuppressed patients and molecular typing of the organisms. *AIDS*, 2000, *14*, 461-463.

Helweg-Larsen J., Tsolaki A.G., Miller R.F., Lundgren B. & Wakefield A.E. Clusters of *Pneumocystis carinii* pneumonia: analysis of person-to-person transmission by genotyping. *The Quarterly Journal of Medicine*, 1998, *91*, 813-820.

Hennequin C., Page B., Roux P., Legendre C. & Kreis H. Outbreak of *Pneumocystis carinii* pneumonia in a renal transplant unit. *European Journal of Clinical Microbiology & Infectious Diseases*, 1995, *14*, 122-126.

Hocker B., Wendt C., Nahimana A., Tonshoff B. & Hauser P.M. Molecular evidence of *Pneumocystis* transmission in pediatric transplant unit. *Emerging Infectious Diseases*, 2005, *11*, 330-332.

Huang L., Beard C.B., Creasman J., Levy D., Duchin J.S., Lee S., Pieniazek N., Carter J.L., del Rio C., Rimland D. & Navin T.R. Sulfa or sulfone prophylaxis and geographic region predict mutations in the *Pneumocystis carinii* dihydropteroate synthase gene. *The Journal of Infectious Diseases,* 2000, *182*, 1192-1198.

Hughes W.T. Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. *The Journal of Infectious Diseases*, 1982, *145*, 842-848.

Hughes W.T., Bartley D.L. & Smith B.M. A natural source of infection due to *Pneumocystis carinii*. *The Journal of Infectious Diseases*, 1983, *147*, 595.

Institut de Veille Sanitaire. Surveillance du SIDA en France : situation au 31 mars 2000. *Bulletin Epidémiologique Hebdomadaire,* 2002, *27*, 133-139.

Jacobs J.L., Libby D.M., Winters R.A., Gelmont D.M., Fried E.D., Hartman B.J. & Laurence J.A. Cluster of *Pneumocystis carinii* pneumonia in adults without predisposing illnesses. *The New England Journal of Medicine,* 1991, *324*, 246-250.

Kazanjian P., Armstrong W., Hossler P.A., Burman W., Richardson J., Lee C.H., Crane L., Katz J. & Meshnick S.R. *Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. *The Journal of Infectious Diseases,* 2000, *182*, 551-557.

Kovaleva E.P., Borisova N.K., Ivanenko I.P. & Morozova N.A. *Pneumocystis carinii* infection in a pediatric tuberculosis hospital. *Antibiotics and Chemoterapy,* 1991, *36*, 43-45.

Latouche S., Poirot J.L., Maury E., Bertrand V. & Roux P. *Pneumocystis carinii hominis* sequencing to study hypothetical person-to-person transmission. *AIDS*, 1997, *11*, 549.

Latouche S., Totet A., Lacube P., Bolognini J., Nevez G. & Roux P. Development of an RT-PCR on the heat shock protein 70 gene for viability detection of *Pneumocystis carinii* f. sp. *hominis* in patients with pneumocystosis and in air sample. *The Journal of Eukaryotic Microbiology,* 2001, (Suppl.), 176S-177S.

Maher N.H., Vermund S.H., Welsh D.A., Dillon H.K., Awooda A. & Unnasch T.R. Development and characterization of a molecular viability assay for *Pneumocystis carinii* f. sp. *hominis*. *The Journal of Infectious Diseases,* 2001, *183*, 1825-1827.

Miller R.F., Ambrose H.E. & Wakefield A.E. *Pneumocystis carinii* f. sp. *hominis* DNA in immunocompetent health care workers in contact with patients with *P. carinii* pneumonia. *Journal of Clinical Microbiology,* 2001, *39*, 3877-3882.

Miller R.F., Ambrose H.E., Novelli V. & Wakefield A.E. Probable mother-to-infant transmission of *Pneumocystis carinii* f. sp. *hominis* infection. *Journal of Clinical Microbiology,* 2002, *40*, 1555-1557.

Morris A., Beard C.B. & Huang L. Update on the epidemiology and transmission of *Pneumocystis carinii*. *Microbes and Infection,* 2002, *4*, 95-103.

Nevez G., Raccurt C., Jounieaux V., Dei-Cas E. & Mazars E. Pneumocystosis versus pulmonary *Pneumocystis carinii* colonization in HIV-negative and HIV-positive patients. *AIDS,* 1999a, *13*, 535-536.

Nevez G., Raccurt C., Vincent P., Jounieaux V. & Dei-Cas E. Pulmonary colonization with *Pneumocystis carinii* in human immunodeficiency virus-negative patients: assessing risk with blood $CD4^+$ T cell counts. *Clinical Infectious Diseases,* 1999b, *29*, 1331-1332.

Nevez G., Totet A., Pautard J.C. & Raccurt C. *Pneumocystis carinii* detection using nested-PCR in nasopharyngeal aspirates of immunocompetent infants with bronchiolitis. *The Journal of Eukaryotic Microbiology,* 2001a, 122S-123S.

Nevez G., Guyot K., Totet A., Raccurt C. & Dei-Cas E. Pulmonary colonisation with *Pneumocystis carinii* in an immunosuppressed HIV-negative patient: detection and typing of the fungus by PCR. *Journal of Medical Microbiology,* 2001b, *50*, 198-200.

Nevez G., Totet A., Ganry O., Dorion I., Tabuteau S., Leclercq F., Jounieaux V. & Raccurt C. *Pneumocystis jiroveci* detection using the polymerase chain reaction in patients with sarcoidosis. *Journal de Mycologie Médicale*, 2002, *12*, 183-186.

NEVEZ G., TOTET A., JOUNIEAUX V., SCHMIT J.L., DEI-CAS E. & RACCURT C. *Pneumocystis jiroveci* Internal transcribed spacer types in patients colonized by the fungus and in patients with Pneumocystosis from the same French geographic region. *Journal of Clinical Microbiology,* 2003, *41*, 181-186.

NG V.L., YAJKO D.M. & HADLEY W.K. Extrapulmonary pneumocystosis. *Clinical Microbiology Reviews,* 1997, *10*, 401-418.

OLSSON M., LIDMAN C., LATOUCHE S., BJORKMAN A., ROUX P., LINDER E. & WAHLGREN M. Identification of *Pneumocystis carinii* f. sp. *hominis* gene sequences in filtered air in hospital environments. *Journal of Clinical Microbiology,* 1998, *36*, 1737-1740.

OLSSON M., ERIKSSON B.M., ELVIN K., STRANDBERG M. & WAHLGREN M. Genotypes of clustered cases of *Pneumocystis carinii* pneumonia. *Scandinavian Journal of Infectious Diseases,* 2001, *33*, 285-289.

PERERA D.R., WESTERN K.A., H.D. J., W.W. J., SCHULTZ M.G. & AKERS P.V. *Pneumocystis carinii* pneumonia in a hospital for children. Epidemiologic aspects. *The Journal of the American Medical Association,* 1970, *214*, 1074-1078.

PETERSON J.C. & CUSHION M.T. *Pneumocystis:* not just pneumonia. *Current Opinion in Microbiology,* 2005, *8*, 393-398.

POST C., DUTZ W. & NASARIAN I. Endemic *Pneumocystis carinii* pneumonia in south Iran. *Archives of Disease in Childhood,* 1964, *35*, 35.

RABODONIRINA M., VANHEMS P., COURAY-TARGE S., GILLIBERT R.P., GANNE C., NIZARD N., COLIN C., FABRY J., TOURAINE J.L., VAN MELLE G., NAHIMANA A., FRANCIOLI P. & HAUSER P.M. Molecular evidence of interhuman transmission of *Pneumocystis* pneumonia among renal transplant recipients hospitalized with HIV-infected patients. *Emerging Infectious Diseases,* 2004, *10*, 1766-1773.

VON REITSETBAUER E. & MORITSCH H. Epidemiologische un serologische Untersuchungen bei intertitieller plasmacellulärer Frühgeburtenpneumonie. *Monatsschrift für Kinderheilkunde,* 1956, *104*, 41-46.

RUEBUSH T.K., WEINSTEIN R.A., BAEHNER R.L., WOLFF D., BARTLETT M., GONZLES-CRUSSI F., SULZER A.J. & SCHULTZ M.G. An outbreak of *Pneumocystis* pneumonia in children with acute lymphocytic leukaemia. *American Journal of Diseases of Children,* 1978, *132*, 143-148.

RUSKIN J. & REMINGTON J.S. The compromised host and infection. *The Journal of the American Medical Association,* 1968, *202*, 1070-1074.

SANTIAGO-DELPIN E.A., MORA E., GONZALEZ Z.A., MORALES-OTERO L.A. & BERMUDEZ R. Factors in an outbreak of *Pneumocystis carinii* in a transplant unit. *Transplantation Proceedings,* 1988, *20*, 462-465.

SEPKOWITZ K.A., BROWN A.E. & ARMSTRONG D. *Pneumocystis carinii* pneumonia without acquired immunodeficiency syndrome. More patients, same risk. *Archives of Internal Medicine,* 1995, *155*, 1125-1128.

SING A., WONHAS C., BADER L., LUTHER M. & HEESEMANN J. Detection of *Pneumocystis carinii* DNA in the air filter of a ventilated patient with AIDS. *Clinical Infectious Diseases,* 1999a, *29*, 952-953.

SING A., ROGGENKAMP A., AUTENRIETH I.B. & HEESEMANN J. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. *Journal of Clinical Microbiology,* 1999b, *37*, 3409-3410.

SINGER C., ARMSTRONG D., ROSEN P.P. & SCHOTTENFELD D. *Pneumocystis carinii* pneumonia: a cluster of eleven cases. *Annals of Internal Medicine,* 1975, *82*, 772-777.

SOULEZ B., PALLUAULT F., CESBRON J.Y., DEI-CAS E., CAPRON A. & CAMUS D. Introduction of *Pneumocystis carinii* in a colony of SCID mice. *The Journal of Protozoology,* 1991, *38*, 123S-125S.

STRINGER J.R., BEARD C.B., MILLER R.F. & WAKEFIELD A.E. A new name (*Pneumocystis jiroveci*) for *Pneumocystis* from humans. *Emerging Infectious Diseases*, 2002, *8*, 891-896.

TASCI S., EWIG S., BURGHARD A. & LUDERITZ B. *Pneumocystis carinii* pneumonia. *Lancet,* 2003, *362*, 124.

TOTET A., RESPALDIZA N., PAUTARD J.C., RACCURT C. & NEVEZ G. *Pneumocystis jiroveci* genotypes and primary infection. *Clinical Infectious Diseases,* 2003a, *36*, 1340-1342.

TOTET A., PAUTARD J.C., RACCURT C., ROUX P. & NEVEZ G. Genotypes at the internal transcribed spacers of the nuclear rRNA operon of *Pneumocystis jiroveci* in nonimmunosuppressed infants without severe pneumonia. *Journal of Clinical Microbiology,* 2003b, *41*, 1173-1180.

TOTET A., LATOUCHE S., LACUBE P., PAUTARD J.C., JOUNIEAUX V., RACCURT C., ROUX P. & NEVEZ G. *Pneumocystis jirovecii* dihydropteroate synthase genotypes in immunocompetent infants and immunosuppressed adults, Amiens, France. *Emerging Infectious Diseases,* 2004, *10*, 667-673.

VANEK J. & JIROVEC O. Parasitäre Pneumonie "Interstitielle" Plasmazellenpneumonie der Frühgeboreren, verursacht durch *Pneumocystis carinii. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene,* 1952, *158*, 120-128.

VARGAS S.L., PONCE C.A., GIGLIOTTI F., ULLOA A.V., PRIETO S., MUNOZ M.P. & HUGHES W.T. Transmission of *Pneumocystis carinii* DNA from a patient with *P. carinii* pneumonia to immunocompetent contact health care workers. *Journal of Clinical Microbiology,* 2000, *38*, 1536-1538.

VARGAS S.L., HUGHES W.T., SANTOLAYA M.E., ULLOA A.V., PONCE C.A., CABRERA C.E., CUMSILLE F. & GIGLIOTTI F. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. *Clinical Infectious Diseases,* 2001, *32*, 855-861.

VARGAS S.L., PONCE C.A., SANCHEZ C.A., ULLOA A.V., BUSTAMANTE R. & JUAREZ G. Pregnancy and asymptomatic carriage of *Pneumocystis jiroveci. Emerging Infectious Diseases,* 2003, *9*, 605-606.

WAKEFIELD A.E. DNA sequences identical to *Pneumocystis carinii* f. sp. *carinii* and *Pneumocystis carinii* f. sp. *hominis* in samples of air spora. *Journal of Clinical Microbiology,* 1996, *34*, 1754-1759.

WALZER P.D. & CUSHION M.T. *Pneumocystis carinii* pneumonia. Marcel Dekker (eds), 2005, 715 p.

WATANABE J.M., CHINCHINIAN H., WEITZ C. & MCILVANIE S.K. *Pneumocystis carinii* pneumonia in a family. *The Journal of the American Medical Association*, 1965, *193*, 119-120.

YATES J.W., ELLISON R.R. & PALGER J. *Pneumocystis carinii* in a husband and wife. *Lancet,* 1975, *2*, 610.