Epidemiology of *Pneumocystis* infection in Human

Épidémiologie de l’infection par *Pneumocystis* chez l’homme

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**KEYWORDS**

*Pneumocystis*; Epidemiology; Transmission; Colonization

**Summary**  
*Pneumocystis jirovecii* (*P. jirovecii*) is an atypical fungus that causes pneumonia in immunosuppressed individuals and significant questions about its epidemiology and transmission remain unanswered. It is widely accepted that animal sources of *P. jirovecii* can be excluded because the *Pneumocystis* organisms that infect mammalian species are characterized by strong, close host species specificity. Similarly, an environmental reservoir of infection has not been found. Airborne transmission has been demonstrated in animal models and is assumed among humans. Highly sensitive PCR-technologies have allowed the detection of low numbers of *Pneumocystis* organisms in respiratory samples from colonized individuals who do not have *Pneumocystis* pneumonia. Studies have shown that individuals who have underlying Human immunodeficiency virus (HIV)-infection or other types of immunosuppression and those who are not immunosuppressed but have a chronic lung disease are often colonized by *P. jirovecii*. Further hypotheses claim that these groups may play a role in person-to-person transmission and that they may serve as reservoirs for future *Pneumocystis* infection in other susceptible individuals. On the other hand, *P. jirovecii* DNA was recently identified by molecular techniques in 35% of foetal lung and 5% of placenta samples from nonimmunodepressed women, who had a miscarriage, evidencing transplacental transmission in humans. Vertical transmission of *P. jirovecii* in humans could be an additional route of transmission of this stenoxenic microorganism that would ensure the persistence of *Pneumocystis* independent of environmental hazards. However, further studies are needed to confirm the role of this transmission route in the epidemiology of *Pneumocystis* infection in humans.

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**MOTS CLÉS**

*Pneumocystis* ; Épidémiologie ;

**Résumé**  
*Pneumocystis jirovecii* (*P. jirovecii*) est un champignon atypique responsable de pneumonie chez les sujets immunodéprimés. Des questions significatives sur l’épidémiologie et la transmission demeurent sans réponse. On accepte en général que les animaux ne constituent pas une source d’infection pour l’homme car les *Pneumocystis* des divers mammifères présen-

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Introduction

Organisms that are known today as Pneumocystis carinii (P. carinii) were thought to be life cycle stages of American trypanosomes in 1909, but 3 years later were identified as a different genus and species altogether. During the next two decades, these organisms remained in the realm of medical curiosities until they were linked to epidemics of plasmacellular interstitial pneumonia that plagued institutionalized premature and malnourished infants in Europe around World War II [8,10]. For many years, the entity named "P. carinii" was considered like a unique pulmonary pathogen able to cause pneumonia in immunosuppressed hosts. Only for the last years, marked genetic divergence was documented among the Pneumocystis strains of different mammals and now it is well-recognized that each form of Pneumocystis possesses unique genetic characteristics and strict host specificity [1]. The explanation for such strict host specificity has not been determined and is largely unprecedented in the fungi. More recently, the form that infects humans has been renamed Pneumocystis jirovecii (P. jirovecii) and P. carinii is now the name used to describe specifically the organism that infects rats [38,41].

The dramatic increase in the incidence of Pneumocystis Pneumonia (PCP) with the emergence of Human immunodeficiency virus (HIV) pandemic made Pneumocystosis a major medical and public health problem in the 1980s. During the 1990s, advances in the treatment of HIV reduced the frequency of PCP. Although at the beginning of the 21st century, the incidence of frank pneumonia caused by these organisms has decreased in developed countries, the prevalence of AIDS-related PCP in developing countries remains high and poorly controlled. Likewise, the number of patients who have an altered immune system or who are receiving chronic immunosuppressive medications and are thus at a risk for PCP is rapidly growing [16,27]. But at the beginning of the third millennium, the interest in Pneumocystis infection goes beyond PCP because a new spectrum of disease seems to emerge in immunocompetent hosts and mounting evidence points to new niches being exploited by these fungi. The presence of P. jirovecii in patients with underlying chronic diseases such as chronic obstructive pulmonary disease has been suggested to be a comorbidity factor [7,28].

The strategies used by these organisms to grow and survive in the context of an intact or debilitated host defenses are largely unknown and limited progress has been made in understanding its life cycle due in large part to the absence of a continuous in vitro culture system. Many questions about P. jirovecii epidemiology and transmission remain unanswered. Until now, human is the only known reservoir host for P. jirovecii and groups at risk for carriage probably represent a major species-specific reservoir of infection, which would allow P. jirovecii propagation [29].

Detection methods

As P. jirovecii cannot be grown in culture from clinical specimens, laboratory diagnosis of PCP has relied mainly upon microscopic visualization with conventional cytchemical or immunofluorescence staining of organisms in respiratory samples. These methods are useful when the organism burden is relatively high but they are insufficient for reliable detection when there is a small parasite load [18]. Although some investigators have detected Pneumocystis carriage by using traditional staining methods, these methods are generally not adequate for detection of Pneumocystis colonization, and researchers have turned to more sensitive molecular techniques [6,39].

Pneumocystis were first detected without the need to visualize the organisms through microscopic examination by the application of polymerase chain reaction (PCR) methods [51]. Nested PCR protocols soon supplanted standard single-cycle amplification owing to their increased sensitivity. More recently, real-time PCR has allowed levels of detection similar to the nested procedures, but is advantageous as it is performed in a one-step process [2,16]. Several strainotyping methods of amplified PCR products have allowed insight into epidemiology and transmission of P. jirovecii [4]. These sensitive techniques have enable detection of very low levels of P. jirovecii, not detectable by conventional histochemical
staining, in respiratory samples from individuals in whom it was not expected. It is thought that detection of *P. jirovecii* or its DNA in respiratory samples from individuals without signs or symptoms of pneumonia represents colonization with the organism, and accumulating evidence suggests that these groups of subjects may be important in the person to person transmission of *P. jirovecii*. Furthermore, they may be a major reservoir for future *Pneumocystis* infection in other susceptible individuals [29,33].

**Pneumocystis colonization in adult human**

HIV-positive individuals are the most studied population of *Pneumocystis* colonization, although estimates of the colonization rate vary considerably between reports from 10 to 68.8% (Table 1) [11,14,19,21,26,34,50]. The initial observations of a carrier state were obtained when PCR-based assays were evaluated for the diagnosis of PcP in HIV-positive patients. As there was an absence of a fulminant infection, but the presence of *P. jirovecii* was shown by PCR-based analyses, multiple laboratories independently arrived at the conclusion that this large population might serve as carriers [33].

In an interesting study, the length of carriage following an episode of AIDS-related PcP ranged from 2 week to 16 months. Importantly, two individuals were noted to have a change in genotype, which suggests that exogenous re-exposure, as opposed to latent infection, is the source of these organisms [50]. In a group of patients evaluated for PcP at San Francisco General Hospital, 68.8% of patients without PcP were colonized with *P. jirovecii*. No statistically significant differences were noted in demographic factors, CD4+ T cells, HIV RNA level, history of PcP, or use of PcP prophylaxis between carriers and non-carriers [14]. In a multicenter AIDS cohort study, colonization was detected in 46% of autopsy specimens and only smoking was associated with an increased risk of *Pneumocystis* colonization [26].

*Pneumocystis* colonization also occurs in non-HIV immunosuppressed patients [39]. Many non-HIV-positive people with impaired immunity, such as organ transplant recipients, patients with malignancies or those with connective tissue diseases, can develop PcP [16]. In the same way, these individuals are at risk of *Pneumocystis* colonization (Table 1) [13,20,25,31]. More subtle changes in immune function, such as those associated with pregnancy or chronic lung diseases (Table 2), have been related with *Pneumocystis* colonization as well [6,7,36,47,48].

There are some studies that have detected transient *Pneumocystis* colonization in healthy subjects, as such health care workers in contact with PcP patients [23,44]. There is also a study demonstrated the presence of *Pneumocystis* DNA among 20% adults that were otherwise healthy, although these data require independent verification [22].

**Pneumocystis colonization in children**

Primary exposure to *P. jirovecii* is common among infants, as is demonstrated by the increase in anti-*Pneumocystis* anti-

### Table 1

**Pneumocystis colonization in adults with immunosuppression.**

*Colonisation par *Pneumocystis* chez des adultes immunodéprimés.*

<table>
<thead>
<tr>
<th>Author, year [Reference]</th>
<th>Subjects no.</th>
<th>Sample</th>
<th>Diagnostic method</th>
<th>Population</th>
<th>Colonization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leigh et al., 1993 [19]</td>
<td>70</td>
<td>Induced sputum/BAL</td>
<td>PCR</td>
<td>HIV infected at different levels of CD4</td>
<td>10–40</td>
</tr>
<tr>
<td>Rabodonirina et al., 1997 [34]</td>
<td>80</td>
<td>BAL</td>
<td>Nested PCR</td>
<td>HIV infected with respiratory symptoms</td>
<td>25</td>
</tr>
<tr>
<td>Nevez et al., 1999 [31]</td>
<td>69</td>
<td>BAL</td>
<td>Heminested PCR</td>
<td>Various causes of immunosuppression</td>
<td>14</td>
</tr>
<tr>
<td>Matos et al., 2001 [21]</td>
<td>52</td>
<td>Induced sputum/Oropharyngeal washes</td>
<td>Nested PCR</td>
<td>HIV infected with respiratory symptoms</td>
<td>28.8</td>
</tr>
<tr>
<td>Helweg-Larsen et al., 2002 [13]</td>
<td>20</td>
<td>BAL, tracheal aspirate, sputum</td>
<td>Nested PCR</td>
<td>Patients with suspected pneumonia and receiving corticosteroids</td>
<td>60</td>
</tr>
<tr>
<td>Huang et al., 2003 [14]</td>
<td>32</td>
<td>Induced sputum/BAL</td>
<td>Nested PCR</td>
<td>HIV infected with respiratory symptoms</td>
<td>68.8</td>
</tr>
<tr>
<td>Wakefield et al., 2003 [50]</td>
<td>16</td>
<td>BAL</td>
<td>Nested PCR</td>
<td>HIV infected</td>
<td>43.8</td>
</tr>
<tr>
<td>Maskell et al., 2003 [20]</td>
<td>18</td>
<td>BAL</td>
<td>Nested PCR</td>
<td>Receiving prednisolone (&gt; 20 mg/day)</td>
<td>44</td>
</tr>
<tr>
<td>Morris et al., 2004 [26]</td>
<td>91</td>
<td>Autopsy lung</td>
<td>Nested PCR</td>
<td>HIV-infected men dying of non-PcP</td>
<td>46.2</td>
</tr>
<tr>
<td>Davis et al., 2008 [11]</td>
<td>172</td>
<td>Induced sputum/BAL</td>
<td>Nested PCR</td>
<td>HIV infected with non-<em>Pneumocystis</em> pneumonia</td>
<td>68</td>
</tr>
<tr>
<td>Mori et al., 2008 [25]</td>
<td>55</td>
<td>Induced sputum/BAL</td>
<td>PCR</td>
<td>Patients with rheumatoid arthritis receiving methotrexate, prednisolone or tacrolimus</td>
<td>10.9</td>
</tr>
</tbody>
</table>

BAL: bronchoalveolar lavage; Modified of Morris A, et al. JID 2008;197:10–7 [29].
body titers during the first few years of life. Among healthy, immunocompetent infants in Chile, the seroconversion rate reached 85% by 20 months of age and *Pneumocystis* DNA was found in 32% of infants studied [42]. In a study carried out among Spanish children, the overall seroprevalence of anti-*Pneumocystis* antibody was 73%. Furthermore, there was evidence of an age related increase in seroprevalence, from 52% at age 6 years to 66% at 10 years and to 80% at age 13 years [35]. These findings suggest the continuous exposure to the pathogen during the first and the second infancy and it is concordant with an airborne transmission. In this sense, the first molecular evidence of *P. jirovecii* transmission from colonized immunocompetent grandparents to their infant granddaughter has been recently provided [37].

*Pneumocystis* primary infection has been presumed to be an asymptomatic or mild nonspecific disease, however recent reports indicate that it can present clinically as a self-limiting upper or lower acute respiratory tract infection [17,40,42]. In a study carried out in Santiago de Chile, *Pneumocystis* DNA was detected by nested PCR in specimens from 45 (51.7%) of 87 infants who died unexpectedly in the community, but only 15% had pneumonia [46]. The significance of these findings is unclear. Some studies have suggested a likely association between primary *Pneumocystis* infection and sudden infant death syndrome but now, this association is not clear [43,45].

The early age of acquisition of *Pneumocystis* infection in different mammals, including humans, led scientists to think that vertical transmission could be as an additional route of transmission of *Pneumocystis*. In humans, transplacental transmission was first suggested by a few reports of PcP in neonates published before the AIDS epidemic [3,32]. A few years ago, a controversial case of vertical transmission of *P. jirovecii* was reported: an infection in the lungs of a fetus of an HIV-positive mother with PcP [30]. However, the study did not identify the organisms as *Pneumocystis*, and a subsequent fluorescein-labeled monoclonal antibody test yielded negative results [15]. Therefore, vertical transmission of *P. jirovecii* in humans has remained controversial. Only recently, the first molecular evidence of *P. jirovecii* transplacental transmission in humans has been provided [24]. In this study, tissues from human fetuses and placentas from nonimmunodepressed women, who had a miscarriage, were evaluated using the exquisitely sensitive detections method of PCR targeting the mtLSU and DHPS genes. *P. jirovecii* DNA was identified in 35% of foetal tissues and 5% of placenta samples, showing that transplacental transmission can occur in humans and that it is not a rare event [24].

### Conclusions

Although still impaired by the lack of a reliable and reproducible continuous in vitro cultivation system, research into the natural history of *P. jirovecii* has advanced owing to the expansion of molecular-based techniques [33]. Even though early studies described the identification of *Pneumocystis* DNA from the air surrounding apple orchards and the surface of pond water, no *Pneumocystis* forms were identified in environmental samples using microscopy, and it is uncertain whether there is an ecological niche for *Pneumocystis* outside the mammalian hosts [9,12,49]. Animal sources for *P. jirovecii* can be excluded because the *Pneumocystis* organisms that infect mammalian species are characterized by strong, close host species specificity [1]. So far, human is the only known reservoir host for *P. jirovecii* [29].

Highly sensitive PCR-technologies have allowed the detection of low numbers of *Pneumocystis* organisms in respiratory samples from colonized individuals who do not have PcP. Studies have shown that individuals who have underlying HIV-infection or other cause of immunosuppression and those who are not immunosuppressed but have chronic lung disease may often be colonized by *P. jirovecii* [29]. These groups at risk for carriage probably represent a major species-specific reservoir of infection, although transient *Pneumocystis* colonization has been also identified in healthy subjects that could behave as a sort of dynamic reservoir for future *Pneumocystis* infection in other susceptible individuals [22,23,44].

Recent demonstration of *P. jirovecii* transplacental transmission may explain the accumulating evidence that the primary infection is widely acquired very early in the life and support the view that human infants are a major natural reservoir for *P. jirovecii*, since they can remain colonized as their immune response matures [5,24].

The accumulating evidence suggests that *P. jirovecii* is a highly infectious organism with low virulence that takes advantage of hosts as temporary reservoirs of infection.
Probably, any person may be colonized by *Pneumocystis* at some stage in his life [10].

*P. jirovecii* is a highly adapted parasite that most likely circulates by active airborne horizontal and vertical (transplacental or aerial) transmission mechanisms among human populations developing mostly mild, though frequent, parasitism in the host lungs [1, 12]. The length of carriage and the possible detrimental effect of colonization in some populations as individuals with chronic lung diseases or infants remains to be defined.

There is growing evidence that *P. jirovecii* colonization is an important part of the organism’s life cycle and could have significant clinical consequences. Further investigation is needed to throw light on the role of *Pneumocystis* colonization in development and transmission of PCP and in other human diseases.

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