

## Transmission of *Pneumocystis carinii* from patients to hospital staff

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### Abstract

**Background** – An extrahuman reservoir of human pathogenic *Pneumocystis carinii* remains unknown. Host to host transmission has been described in animal studies and in cluster cases among immunodeficient patients. *P carinii* DNA has recently been detected in air filters from inpatient and outpatient rooms in departments of infectious diseases managing patients with *P carinii* pneumonia (PCP), suggesting the airborne route of transmission. Exposure of staff to *P carinii* may occur in hospital departments treating patients with PCP.

**Methods** – Exposure to *P carinii* was detected by serological responses to human *P carinii* by ELISA, Western blotting, and indirect immunofluorescence in 64 hospital staff with and 79 staff without exposure to patients with PCP from Denmark and Sweden. DNA amplification of oropharyngeal washings was performed on 20 Danish staff with and 20 staff without exposure to patients with PCP.

**Results** – There was no significant difference in the frequency or level of antibodies to *P carinii* between staff exposed and those unexposed to patients with PCP. None of the hospital staff had detectable *P carinii* DNA in oropharyngeal washings. **Conclusions** – There is no difference in antibodies and no detectable *P carinii* DNA in oropharyngeal washings, which suggests that immunocompetent staff treating patients with PCP are not a potentially infectious source of *P carinii* for immunocompromised patients.

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Keywords: *Pneumocystis carinii*, transmission, serology, polymerase chain reaction.

Serological studies suggest that most children have been exposed to *Pneumocystis carinii*<sup>1-3</sup> and it has previously been thought that *P carinii* pneumonia in the immunocompromised host is a result of reactivation of a latent infection acquired asymptotically in childhood. However, DNA amplification in necroscopic lung specimens from immunocompromised patients has failed to detect *P carinii* DNA,<sup>4</sup> nor has *P carinii* been identified in immunosuppressed HIV infected patients without prior pulmonary disease.<sup>5,6</sup> Animal studies with immunosuppressed rats have previously suggested an airborne route of infection.<sup>7,8</sup> Host to host transmission has been described in rats and rhesus macaques,<sup>8,9</sup> as well as in cluster cases among

immunodeficient patients.<sup>10-13</sup> *P carinii* DNA has recently been detected in air filters from animal facilities housing immunosuppressed rats with *P carinii* pneumonia (PCP) as well as from air filters from inpatient and outpatient rooms in hospital departments treating patients with PCP.<sup>14,15</sup>

Raised *P carinii* antibody titres, detected by immunofluorescence, have previously been described in serum samples from staff in close contact with patients with PCP compared with staff without contact with AIDS patients.<sup>16</sup> These results raise the question whether staff may transmit the microorganism from one patient to another, and whether the patient should be placed in respiratory isolation. In order to contribute to this debate we investigated the possible transmission of *P carinii* from patients to staff by studying the serological responses and DNA amplification of oropharyngeal samples in hospital staff exposed and unexposed to patients with PCP.

### Methods

Two groups of hospital staff exposed or unexposed to patients with PCP from Copenhagen, Denmark and Stockholm, Sweden were studied. The Danish group exposed to patients with PCP consisted of 38 doctors and nurses (17 women) of mean age 38 years who were handling patients with documented PCP at two infectious diseases departments in Copenhagen, each treating 20-40 patients with PCP per year. The Swedish group consisted of 26 nurses (21 women) of mean age 37 years working at one infectious diseases department in Stockholm where about 20 patients with PCP were diagnosed and treated yearly. The staff had worked at the infectious diseases departments for at least six months and were in close contact with patients with PCP during diagnostic bronchoscopy and in the daily handling and care of the patients. The Danish staff unexposed to patients with PCP were 40 staff members (29 women) of mean age 37 years from a blood bank and a surgery gastroenterological department at a hospital without an infectious diseases department. The corresponding Swedish group were 39 staff members (27 women) of mean age 35 years from a surgery department at the same hospital as the exposed staff. The unexposed staff had not previously been employed in an infectious diseases department. All 143 subjects were healthy and received no immunosuppressive drugs. Specimens of serum were drawn from all subjects and stored at -20°C for antibody testing. Oropharyngeal washings were obtained from

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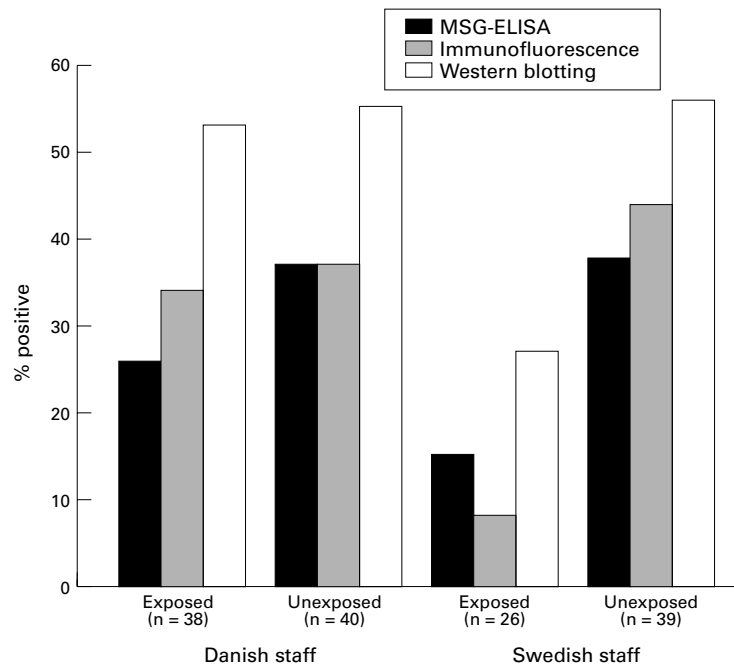


Figure 1 Percentage of subjects with antibodies to *P. carinii* detectable by MSG-ELISA, immunofluorescence, and Western blotting among hospital staff exposed and unexposed to patients with *P. carinii* pneumonia in Denmark and Sweden. No significant differences were found between the Danish groups of staff.

20 subjects in the exposed and unexposed group from Denmark by rinsing and gargling their mouths with 10 ml sterile saline within two days of diagnosing a patient with PCP. All specimens were randomised and coded to ensure blind testing.

#### DETECTION OF ANTIBODIES

Antibody responses to human *P. carinii* antigens of 95 kD (the major surface antigen, MSG), 60 kD, and 40 kD were detected by Western blotting using serum diluted 1/100.<sup>17</sup> Indirect immunofluorescence was performed using paraffin sections of human lung infected with *P. carinii* as antigen and serum specimens diluted 1/10 as previously described.<sup>18</sup> Specimens resulting in fluorescing *P. carinii* were positive. The titre of the specimen was the highest dilution of serum giving a positive result. Using a previously described MSG-ELISA<sup>19-21</sup> IgG antibodies to MSG of *P. carinii* were detected using serum diluted 1/100. A positive sample had an optical density of >0.20.

#### DNA AMPLIFICATION

DNA was extracted from 2 ml of oropharyngeal washings using proteinase K digestion followed by phenol chloroform extraction. The poly-

merase chain reaction using *P. carinii* specific mitochondrial ribosomal RNA primers was carried out as previously described.<sup>22</sup> Appropriate positive and negative controls were run in each experiment.

#### DATA ANALYSIS

The Mann-Whitney test was used to test for differences in levels of antibodies and the  $\chi^2$  test for differences in frequency of antibodies between groups.

#### Results

The percentage of serum specimens with antibodies to *P. carinii* detected by three different immunological techniques (ELISA, immunofluorescence, and Western blotting) in Danish and Swedish staff exposed and unexposed to patients with PCP is shown in fig 1. There were no significant differences in the frequency of antibodies to MSG or human *P. carinii* between Danish exposed staff and Danish and Swedish unexposed staff members ( $p > 0.1$ ). When the Swedish exposed staff members were compared with the Swedish unexposed staff members significantly fewer had detectable antibodies against MSG ( $p < 0.05$ ) and against *P. carinii* by immunofluorescence ( $p < 0.05$ ), but not by Western blotting ( $p > 0.1$ ).

In those with detectable antibodies, no significant differences were found in the level of antibodies detected by MSG-ELISA and immunofluorescence among the four groups of staff studied (table 1).

Of the total 143 serum samples, all three tests were positive in 27 (19%) and negative in 66 (46%) samples. Of the remaining samples, 32 had concordance between two of the tests (all positive by Western blotting, 17 MSG-ELISA, and 15 immunofluorescence). In 18 samples only one test was positive (13 Western blotting and five immunofluorescence). Thus, all MSG-ELISA positive serum samples were confirmed by the 95 kD band in Western blots whereas the 40 kD and/or the 60 kD bands confirmed 38 of 47 immunofluorescence positive samples. Only four of the immunofluorescence positive samples were confirmed exclusively by the 95 kD band.

Oropharyngeal washings obtained from 20 Danish exposed staff members and 20 samples from Danish unexposed staff members were examined by a previously described polymerase chain reaction technique using *P. carinii* specific mitochondrial ribosomal RNA primers.<sup>22</sup> In none of the 40 oropharyngeal washings were *P. carinii* specific amplification products seen.

#### Discussion

Leigh *et al* have previously reported increased titres of *P. carinii* antibodies using immunofluorescence in 24 hospital staff (mean age 24 years) in close contact with patients with PCP compared with 24 staff at a nursing home (mean age 42 years) and found an overall prevalence of antibodies in the 48 staff members of 96%.<sup>16</sup> These results contrast with our

Table 1 Levels of antibodies to *Pneumocystis carinii* detected by MSG-ELISA (OD) and immunofluorescence (titre) in staff members with detectable antibodies exposed and unexposed to *P. carinii* pneumonia

	Median antibody levels (number of patients)			
	Exposed staff		Unexposed staff	
	Danish	Swedish	Danish	Swedish
MSG-ELISA (OD)	0.49 (10)	0.33 (4)	0.35 (15)	0.32 (15)
Immunofluorescence (titre)	10 (13)	30 (2)	20 (15)	20 (17)

No significant differences were found between the groups of staff ( $p > 0.1$ ).

findings where only 36% of the 78 Danish hospital staff and 29% of the 65 Swedish hospital staff had antibodies to *P. carinii* detected by immunofluorescence. Despite using three different methods, we were unable to show either a significantly higher frequency or level of antibodies to *P. carinii* in exposed than in unexposed staff members. Variation of seroprevalence in the background population, in populations of different age groups, and possible differences in the numbers of subjects studied may explain the discrepancies between the studies. One explanation for the fact that staff members exposed to patients with PCP did not develop an increased antibody titre to *P. carinii* is that the immunocompetent subjects eliminate the *P. carinii* organisms without mounting a measurable antibody response. Alternatively, the staff member may not have had a high enough exposure (number of *P. carinii* organisms) to mount the antibody response.

Different prevalences of antibodies to *P. carinii*, varying from 3% to 90% in the adult population, have been reported.<sup>21,23,24</sup> The differences may be explained by geographical regional variation and the use of different immunological methods and antigens.<sup>21,23-26</sup> The frequency of antibodies to MSG determined by ELISA found in our study correlates with previous results where 33% of healthy controls had antibodies.<sup>19</sup> A variation was also found in our study population. The most likely explanation for this is the relatively small number of subjects tested in the group in which the lowest frequency of antibodies was detected – that is, the Swedish exposed staff members. In general there was good agreement between the three immunological tests used. The results of immunofluorescence and MSG-ELISA were complementary, MSG-ELISA detecting only the 95 kD band whereas immunofluorescence reacts preferably with the 40 kD and 60 kD bands.

It has previously been shown that *P. carinii* specific amplification products can be detected in oropharyngeal washings from HIV infected patients with verified PCP by the polymerase chain reaction with a sensitivity of 72% compared with the corresponding results in bronchoalveolar lavage fluid.<sup>22</sup> Furthermore, *P. carinii* specific DNA has been detected in air filters in hospital rooms housing patients with PCP.<sup>14</sup> We therefore investigated oropharyngeal washings from 20 Danish staff members exposed to patients with PCP and 20 unexposed staff members. *P. carinii* DNA was not detected in any of the 40 specimens. It is possible that *P. carinii* is not transmitted to the pharynx in levels detectable by the polymerase chain reaction. However, it is also possible that the oropharyngeal washings were not obtained soon enough after exposure to the patients with PCP, and rapid elimination of the organism may explain the negative results of the polymerase chain reaction.

In conclusion, we have been unable to show that *P. carinii* is transmitted from patient to staff to such an extent that it results in increased antibody responses to the microorganism or sufficient *P. carinii* specific DNA to be detected by polymerase chain reaction in oropharyngeal

washings. Our results therefore suggest that immunocompetent individuals are probably unable to spread the organism to immunodeficient patients.

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