pneumoniae in patients and asymptomatic healthy individuals. *Infect Control Hosp Epidemiol* 2007; 28: 1114–1116.
6. Rodríguez-Baño J, López-Cerero L, Navarro MD, de Alba PD, Pascual A. Faecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli*: prevalence, risk factors and molecular epidemiology. *J Antimicrob Chemother* 2008; 62: 1142–1149.
7. Briñas L, Moreno MA, Teshager T *et al.* Monitoring and characterization of extended-spectrum β-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrob Agents Chemother* 2005; 49: 1262–1264.
8. Carattoli A. Animal reservoirs for extended-spectrum β-lactamase producers. *Clin Microbiol Infect* 2008; 14 (suppl): 117–123.
9. Jouini A, Vinué L, Slama KB *et al.* Characterization of CTX-M and SHV extended-spectrum beta-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J Antimicrob Chemother* 2007; 60: 1137–1141.
10. Mesa RJ, Blanc V, Blanch AR *et al.* Extended-spectrum beta-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). *J Antimicrob Chemother* 2006; 58: 211–215.
11. Valverde A, Grill F, Coque TM *et al.* High rate of intestinal colonization with extended spectrum β-lactamases producing organisms in household contacts of infected community patients. *J Clin Microbiol* 2008; 46: 2796–2799.
12. Moubareck C, Daoud Z, Hakime NI *et al.* Countrywide spread of community- and hospital-acquired extended-spectrum β-Lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. *J Clin Microbiol* 2005; 43: 3309–3313.
13. Pallecchi L, Bartoloni A, Fiorelli C *et al.* Rapid dissemination and diversity of CTX-M extended-spectrum beta-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* 2007; 51: 2720–2725.
14. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing, 17th informational supplement Approved standard M100-S17*. Wayne, PA: Clinical and Laboratory Standards Institute, 2007.
15. Vinué L, Lantero M, Sáenz Y *et al.* Characterization of extended-spectrum β-lactamases and integrons in *Escherichia coli* isolates in a Spanish hospital. *J Med Microbiol* 2008; 57: 916–920.
16. Sáenz Y, Briñas L, Domínguez E *et al.* Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrob Agents Chemother* 2004; 48: 3996–4001.
17. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
18. Hopkins KL, Deheer-Graham A, Threlfall EJ, Batchelor MJ, Liebana E. Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum β-lactamase (CTX-M-40) isolated in the UK. *Int J Antimicrob Agents* 2006; 27: 572–575.
19. Bae IK, Lee YN, Lee WG, Lee SH, Jeong SH. Novel complex class 1 integron bearing an ISCR1 element in a *Escherichia coli* isolate carrying the $bla_{CTX\text{-}M\text{-}14}$ gene. *Antimicrob Agents Chemother* 2007; 51: 3017–3019.
20. Navarro F, Mesa RJ, Miró E, Gómez L, Mirelis B, Coll P. Evidence for convergent evolution of CTX-M-14 ESBL in *Escherichia coli* and its prevalence. *FEMS Microbiol Lett* 2007; 273: 120–123.
21. Eckert C, Gautier V, Arlet G. DNA sequence analysis of the genetic environment of various $bla_{CTX\text{-}M}$ genes. *J Antimicrob Chemother* 2006; 57: 14–23.
22. Cartelle M, Tomas MM, Molina F, Moure R, Villanueva R, Bou G. High-level resistance to ceftazidime conferred by a novel enzyme, CTX-M-32, derived from CTX-M-1 through a single Asp240-Gly substitution. *Antimicrob Agents Chemother* 2004; 48: 2308–2313.

Seasonal variation of *Pneumocystis jirovecii* infection: analysis of underlying climatic factors

A. Sing[1], S. Schmoldt[2], R. P. Laubender[3], J. Heesemann[2], D. Sing[4] and M. Wildner[1]

1) *Bavarian Health and Food Safety Authority, Oberschleißheim,* 2) *Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie,* 3) *Institute for Medical Informatics, Biometry and Epidemiology, Ludwig Maximilians-Universität München, München, Germany and* 4) *Human Services for Individuals with Special Needs, School of Applied Health and Social Sciences, Upper Austria University of Applied Sciences, Linz, Austria*

Abstract

Pneumocystis jirovecii causes severe pneumonia (PCP) in immunocompromised patients. Seasonal changes of PCP incidence may be associated with climate changes. In this first study using multiple linear regression statistics to assess monthly climatic data and *Pneumocystis*, PCP incidence was positively correlated with mean temperature, but not with rainfall or wind strength.

Keywords: Climate, environment and public health, epidemiology, incidence, infection, meteorological factors, *Pneumocystis jirovecii*, *Pneumocystis* pneumonia

Original Submission: 7 August 2008; **Revised Submission:** 13 October 2008; **Accepted:** 24 October 2008

Editor: S. Cutler

Article published online: 9 June 2009

Clin Microbiol Infect 2009; **15:** 957–960

10.1111/j.1469-0691.2009.02804.x

Corresponding author and reprint requests: A. Sing, Bavarian Health and Food Safety Authority, Veterinärstraße 2, 85764 Oberschleißheim, Germany

E-mail: andreas.sing@lgl.bayern.de

Present address: D. Sing, Catholic University of Applied Sciences, Benediktbeuern, Germany

Pneumocystis jirovecii pneumonia (PCP) is one of the most frequent and serious opportunistic infections. However, the modes of transmission of PCP remain unknown. Air-borne transmission from infected or colonized humans is the favoured model, and environmental sources are also considered [1].

Between January 1989 and December 2006, 757 PCP patients with pneumonia due to *P. jirovecii* were microscopically diagnosed, using mainly bronchoalveolar lavage specimens at the Max-von-Pettenkofer-Institute, which serves the major infectious disease hospitals in Munich (1.3 million inhabitants). In order to analyse climatic effects on PCP incidence, climate data concerning Munich [mean temperature (TMM), total precipitation representing rainfall activity (RSS), mean wind force (FMM), maximum wind-speed (FXX)] were obtained monthly from the German National Meteorological Service (http://www.dwd.de). In order to analyse seasonal effects, four groups (variables) were created: summer (May–August), winter (November–February), spring (March–April) and autumn (September–October) according to average monthly TMM.

Since climate data were available only for May 1992 to December 2006, the 576 PCP patients (77.4% HIV-positive) diagnosed during this period were included in a study of the influence of climatic factors on PCP incidence. Analysis took into consideration the pre- and post-HAART years (before and after January 1997).

The pre-HAART mean annual PCP incidence was nearly twice that of the post-HAART period (57.6 vs. 29.6). In May and August PCP incidence was highest (Fig. 1). Bivariate graphical exploration and regression modelling of the influence of weather parameters on monthly incidence rates as dependent variables, with adjustment for mean annual incidence and logarithmic transformation to achieve normal distribution, were performed [SPSS version 12.0 (SPSS Inc, Chicago, IL, USA), STATA 10.0 (StataCorp LP, College Station, TX, USA)]. Results of the linear regression modelling are summarized in Table 1. Fractional polynomial analysis did

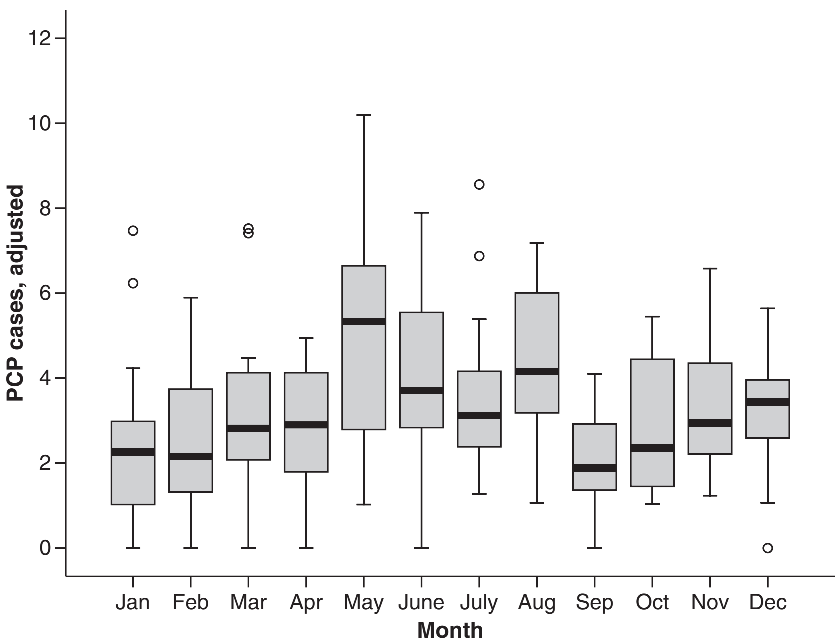

FIG. 1. PCP cases per month, on average (Munich, May 1992 to December 2006). Monthly PCP case numbers were adjusted for annual case numbers (adjustment factor: annual cases/average annual cases).

TABLE 1. Association of climatic factors with monthly PCP case numbers (Munich, May 1992 to December 2006)

	Bivariate				Multiple			
Variable	Coeff. β	95% CI	p	R2	Coeff. β	95% CI	p	R2
TMM	0.016	0.005–0.026	0.004	0.048	−0.005	−0.023–0.012	0.551	–
RSS	0.001	−0.001–0.003	0.215	0.009	−0.001	−0.003–0.002	0.641	–
FMM	−0.161	−0.389–0.067	0.166	0.011	0.062	−0.349–0.225	0.671	0.119
FXX	0.001	−0.014–0.017	0.862	<0.001	0.004	−0.014–0.023	0.665	–
HAART	−0.143	−0.309–0.023	0.090	0.016	−0.130	−0.291–0.030	0.111	–
Summer	**0.347**	**0.191–0.503**	**<0.001**	**0.099**	0.415	0.154–0.676	0.002	–

Bivariate and multiple linear regression models [dependent variable: logarithmic (ln) transformation of adjusted monthly cases + 1; bold: final model].
Variables: mean temperature 2 m above ground (TMM), total precipitation as a measurement for rainfall activity (RSS), mean wind-force (FMM); maximum wind speed at a altitude of 10 m (strongest gust) (FXX); post-HAART vs. pre-HAART (HAART); months May through August (summer).

not improve the model fit [2]. Although no significant correlation was found between PCP incidence and RSS, FMM or FXX, PCP incidence was positively correlated with TMM. Interestingly, the power of the variable 'summer' was much stronger than TMM in explaining PCP incidence. Additional inclusion of any single of the four weather parameters into the model did not have a robust effect on PCP incidence when compared with the variable 'summer' alone. Whereas PCP monthly incidence was independent of the pre- and post-HAART periods, the 'summer' effect was significantly more pronounced in the post-HAART period. Lag modelling considering a 4- or 8-week incubation period, based on animal and human outbreak studies [3–6], had no additional benefit in explaining PCP seasonality.

In contrast to mainly descriptive studies from Spain [7,8] and the UK [9], which showed PCP incidence to be maximal in the winter months, similar to the situation with other infectious respiratory diseases, this first study using multiple linear regression statistics to assess monthly climatic data and PCP incidence has revealed an opposite pattern, with PCP incidence being maximal in the summer months. Moreover, PCP incidence was positively correlated with the mean temperature, but not with rainfall activity or wind strength.

Most previous studies analysing seasonal variations in PCP incidence are mainly descriptive. A seasonal distribution with the highest PCP incidence in winter was observed in the first post-HAART study analysing 498 PCP patients from southern Spain within two consecutive years [7]. These findings were corroborated in another study from southern Spain analysing 536 PCP patients, from both the pre- and post-HAART eras [8]. A similar pattern in 792 patients, with highest PCP incidence in winter and spring, and the most pronounced peak in January, was reported from London between 1985 and 2000 [9]. Strikingly, this seasonal pattern is opposite to that seen in two other London-based studies [10,11]. A more heterogeneous incidence pattern was described in three pre-HAART studies from the USA, the UK and Switzerland, showing PCP peaks in the period March–June (Baltimore, Chicago, Los Angeles, Pittsburgh, 202 AIDS patients [12]), early summer (London, 137 HIV patients [10]) or summer and autumn (Geneva, 93 HIV patients [13]).

In contrast to these descriptive studies, no seasonal clustering of PCP episodes was found in 118 HIV patients from Ohio, USA during the period 1986–1989 [14].

In two of these studies, which additionally reported mean temperature and/or rainfall activity on a purely descriptive level, no clear association between climate factors and PCP incidence could be identified [10,13], while Lubis *et al.* [9] found the lowest rainfall activity in the month with the highest PCP incidence. So far, only one study has explored the relationship between PCP incidence and climatic factors using statistical methods, reporting an inverse correlation between PCP incidence and the mean temperature for southern Spain, but without influence of relative humidity on the number of PCP patients [8].

In comparison with these previous studies, the current study is based on a statistically more comprehensive and powerful methodology, using four different climatic factors as variables that may influence PCP incidence. In contrast to the studies from the UK and Spain, a clear positive correlation was found between PCP incidence and the mean monthly temperature, whereas rainfall and wind strength were not found to be correlated with PCP incidence. Therefore, these results do not support the concept of increased incidence of *Pneumocystis* infection during colder months, as is the case with other respiratory pathogens [8]. Also, the idea that prior upper respiratory infections might increase susceptibility to *P. jirovecii* [7,12] is not supported by our time lag analysis.

Strikingly, the influence of temperature on PCP incidence was by far exceeded by the seasonal effect of the variable 'summer' (TMM 14.2 ± 1.2–18.6 ± 1.7) . A possible explanation for the stronger influence of season, compared with that of single climate parameters, might be that season-dependent factors besides climate, such as human behaviour or type of leisure activity, might be relevant for *Pneumocystis* transmission.

In conclusion, this study and others indicate that seasonal variations in PCP incidence exist, albeit to different extents and with different tendencies. Among the analysed climatic parameters, TMM is the most strongly correlated factor—again to different extents and with different tendencies. However, other factors may better explain seasonal variations in PCP incidence. The controversial data from two London-based studies [9,10], i.e. the clustering of different *P. jirovecii* genotypes according to patients' locale in London [11,15], and the presence or absence of an association between certain *P. jirovecii* genotypes and climatic factors [16], suggest that local parameters may also be important.

Transparency Declaration

The authors declare the absence of financial support and no conflict of interest.

References

1. Thomas CF Jr, Limper AH. Current insights into the biology and pathogenesis of *Pneumocystis* pneumonia. *Nat Rev Microbiol* 2007; 5: 298–308.

2. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. *Appl Stat* 1994; 43: 429–467.
3. Vestereng VH, Bishop LR, Hernandez B, Kutty G, Larsen HH, Kovacs JA. Quantitative real-time polymerase chain-reaction assay allows characterization of pneumocystis infection in immunocompetent mice. *J Infect Dis* 2004; 189: 1540–1544.
4. An CL, Gigliotti F, Harmsen AG. Exposure of immunocompetent adult mice to *Pneumocystis carinii f. sp. muris* by cohousing: growth of *P. carinii f. sp. muris* and host immune response. *Infect Immun* 2003; 71: 2065–2070.
5. Miller RF, Ambrose HE, Novelli V, Wakefield AE. Probable mother-to-infant transmission of *Pneumocystis carinii f. sp. hominis* infection. *J Clin Microbiol* 2002; 40: 1555–1557.
6. Helweg-Larsen J, Tsolaki AG, Miller RF, Lundgren B, Wakefield AE. Clusters of *Pneumocystis carinii* pneumonia: analysis of person-to-person transmission by genotyping. *QJM* 1998; 91: 813–820.
7. Calderón EJ, Varela JM, Medrano FJ *et al.* Epidemiology of *Pneumocystis carinii* pneumonia in southern Spain. *Clin Microbiol Infect* 2004; 10: 673–676.
8. Varela JM, Regordán C, Medrano FJ *et al.* Climatic factors and *Pneumocystis jirovecii* infection in southern Spain. *Clin Microbiol Infect* 2004; 10: 770–772.
9. Lubis N, Baylis D, Short A *et al.* Prospective cohort study showing changes in the monthly incidence of *Pneumocystis carinii* pneumonia. *Postgrad Med J* 2003; 79: 164–166.
10. Miller RF, Grant AD, Foley NM. Seasonal variation in presentation of *Pneumocystis carinii* pneumonia. *Lancet* 1992; 339: 747–748.
11. Walzer PD, Evans HE, Copas AJ, Edwards SG, Grant AD, Miller RF. Early predictors of mortality from *Pneumocystis jirovecii* pneumonia in HIV-infected patients: 1985–2006. *Clin Infect Dis* 2008; 46: 625–633.
12. Hoover DR, Graham NM, Bacellar H *et al.* Epidemiologic patterns of upper respiratory illness and *Pneumocystis carinii* pneumonia in homosexual men. *Am Rev Respir Dis.* 1991; 144: 756–759.
13. Vanhems P, Hirschel B, Morabia A. Seasonal incidence of *Pneumocystis carinii* pneumonia. *Lancet* 1992; 339: 1182.
14. Dohn MN, White ML, Vigdorth EM *et al.* Geographic clustering of *Pneumocystis carinii* pneumonia in patients with HIV infection. *Am J Respir Crit Care Med* 2000; 162: 1617–1621.
15. Miller RF, Lindley AR *et al.* Genotypic variation in *Pneumocystis jirovecii* isolates in Britain. *Thorax* 2005; 60: 679–682.
16. Miller RF, Evans HE, Copas AJ, Cassell JA. Climate and genotypes of *Pneumocystis jiroveciii*. *Clin Microbiol Infect* 2007; 13: 445–448.