Seasonal dynamics of *Pneumocystis carinii* in the field vole, *Microtus agrestis*, and in the common shrew, *Sorex araneus*, in Finland

J. LAAKKONEN[1]*, H. HENTTONEN[2], J. NIEMIMAA[2] *and* T. SOVERI[3]

[1] *Section of Anatomy and Embryology, Faculty of Veterinary Medicine, P.O. Box 57, FIN-00014 Helsinki University, Helsinki, Finland*
[2] *Finnish Forest Research Institute, Vantaa Research Center, P.O. Box 18, FIN-01301 Vantaa, Finland*
[3] *Section of Physiology, Faculty of Veterinary Medicine, P.O. Box 57, FIN-00014 Helsinki University, Helsinki, Finland*

(*Received 30 April 1998; revised 23 June 1998; accepted 24 June 1998*)

SUMMARY

Seasonal dynamics of *Pneumocystis carinii* in the field vole, *Microtus agrestis*, and in the common shrew, *Sorex araneus*, were investigated in southern and central Finland by microscopical examination of methenamine silver-stained tissue sections. In both host species at both localities the number of *P. carinii* cyst forms was highest in late autumn (November). In *S. araneus*, prevalence was higher than in *M. agrestis* during all seasons. None of the animals was heavily infected or apparently ill, and neither species showed any extrapulmonary dissemination. In this study covering an increase phase and 4 peak host-density phases of the vole cycle, the occurrence of *P. carinii* seemed to be related to the population density of *M. agrestis*.

Key words: *Pneumocystis carinii*, *Microtus agrestis*, *Sorex araneus*, *seasonality*, *transmission*.

INTRODUCTION

Pneumocystis carinii is an opportunistic pulmonary pathogen capable of causing fatal pneumonia in immunocompromised hosts, including humans. The transmission of this atypical fungus (Stringer, 1996), previously considered to be a protozoan, is known to be airborne (Hughes, 1982), but the infective form has not yet been identified. Two distinct developmental stages occur in the mammalian alveolus: the trophozoite, which is the proliferating form, and the cyst form. Different phases of cyst formation (e.g. the pre-cyst) can be separated by maturation of the cyst.

Although *P. carinii* has been found in many wild mammal species (Laakkonen, 1998), recent studies with rodents and shrews indicate considerable interspecific differences in prevalence between small mammal species (see Laakkonen, 1998). Recent reports have also shown antigenic (Laakkonen & Sukura, 1997) and genetic diversity (Peters *et al*. 1994; Bishop *et al*. 1997; Mazars *et al*. 1997) among organisms infecting different species of wild animals. *Pneumocystis carinii* has been reported to be exceptionally common in *Sorex araneus* (see Laakkonen, 1998). In contrast, low-protein feeding trials in *Microtus agrestis* have indicated that *P. carinii* may be more opportunistic in laboratory rats of the subfamily Murinae than it is in voles of the subfamily Arvicolinae (Laakkonen *et al*. 1995). Still, *P. carinii* has been found to be common in the late fall of peak years in the cyclic *M. agrestis* populations in southern and central Finland (Laakkonen *et al*. 1995). It is not known, however, whether the high host density contributes to the high prevalence through improved possibilities for transmission or by increased susceptibility of the host due to overcrowding-related stress. Furthermore, since the life-cycle and ecological requirements of *P. carinii* remain poorly known, we have no knowledge of the factors influencing the survival of *P. carinii* in the environment.

In this study we monitored the prevalence of *P. carinii* in *M. agrestis* during 1 increase, and 4 peak host-density phases of the cycle, and compared those prevalences with the occurrence of *P. carinii* in syntopic *S. araneus* in the same period. Our aim was to see whether occurrence of this elusive organism in wild small mammals depends mainly on the host species, on season, on locality, or whether the decisive factor is population density of the host.

MATERIALS AND METHODS

Study localities

The material was collected from Evo (61° 10′ N, 25° 03′ E) in southern Finland, and from Luhanka (61° 48′ N, 25° 45′ E) in central Finland. In these areas the vole densities vary in similar, but asyn-

* Corresponding author: Section of Anatomy and Embryology, Faculty of Veterinary Medicine, P.O. Box 57, FIN-00014 Helsinki University, Helsinki, Finland. Tel: +358 9 70849796. Fax: +358 9 70849799. E-mail: Juha.Laakkonen@helsinki.fi

https://doi.org/10.1017/S0031182098003497 Published online by Cambridge University Press

chronic 3-year periods. The study areas comprised several old fields surrounded by coniferous forest. The climate was characterized by clear seasonality (Fig. 1E and F). Mean temperatures (°C) and precipitation (mm) of the trapping months in Fig. 1 are from meteorological stations at Lammi (precipitation data), 15 km south of the Evo study site, at Lahti (mean temperatures) 45 km east of Evo, and at Jyväskylä, 45 km north of the Luhanka study site (source: The Monthly Report of the Finnish Meteorological Institute).

Host animals

Animals were caught with 'Ugglan Special' live traps checked at 6-h intervals.

(1) *Evo*. In total 150 field voles, *Microtus agrestis*, were trapped from a population at peak density (September, $n = 51$ and November, $n = 50$) and immediately before the crash (January, $n = 49$) in 1992–1993. A total of 159 common shrews, *Sorex araneus*, were caught at the same time with traps used for vole-trapping. An additional sample ($n = 31$) from the same site from the autumn of 1989 was also included in the analyses.

(2) *Luhanka* (*central Finland*). Sixty field voles were trapped during the autumn (September, $n = 24$ and November $n = 36$) of an increase year in 1993, and 96 field voles were trapped at peak density in November 1994 ($n = 96$); 143 common shrews were trapped in 1991 and 1992 during the monitoring of the previous peak-density phase of voles in Luhanka (see Laakkonen *et al*. 1995).

Additional data from our earlier study (Laakkonen *et al*. 1995) were included in some analyses of this paper in order to extend the data set into 2 peaks in each study site (included in Fig. 1A and B).

Protocols for processing samples

In the laboratory, the voles were killed with ether and autopsy was performed immediately after each trapping period. Shrews were found dead in live traps, and frozen (−20 °C) until dissection. In voles, tissue samples from the lungs (right cranial and medial lobes), heart, liver, spleen, kidneys, stomach, intestines (jejunum and colon), and brain were fixed in 10% neutral buffered formalin. In shrews, tissue samples from the same major organs were fixed in formalin, but no samples of the gastrointestinal tract were obtained in shrews, due to the often fast deterioration of intestines after death. Standard histological sections (5 μm) were prepared and stained with Grocott's modification of Gomori's Methenamine Silver (GMS) stain (Grocott, 1955). Sections were studied by microscope at ×200 and ×400 magnification. Infection is used here to indicate the finding of at least 5 cysts per section in any one sample, but this term carries no implication of a disease state in the host. Intensity of the infection was recorded according to Laakkonen & Soveri (1995). Parasitological terms used were those used by Bush *et al*. (1997).

Statistics

Multiway contingency tables (log linear models) (Fienberg, 1970), or Pearson's Chi-square tests were used to analyse the dependence of various parameters (sex, age) and occurrence of *P. carinii*.

RESULTS

At each study locality (Evo and Luhanka) the seasonal dynamics of the occurrence of *P. carinii* in *M. agrestis* were similar, with their peak occurring in late fall (Fig. 1A and B). During the increasing host-density year of 1993 at Luhanka, no *P. carinii* cysts were detected in *M. agrestis*. During the next peak-density year, 1994, only 2 infected voles were caught. According to the best log-linear model (sex*age, PC; $\chi^2 = 4{\cdot}94$, D.F. = 3, $P = 0{\cdot}18$; with * indicating interaction, pooled data), there was no dependence between sex and age of *M. agrestis* and occurrence of *P. carinii*.

In *S. araneus*, the prevalence peaks also occurred in November but prevalences remained relatively stable during other months (between 20 and 30%, Fig. 1C and D). In *S. araneus*, *P. carinii* infection was more common in males (33% infected) than in females (20% infected; $P < 0{\cdot}01$, pooled data of Evo and Luhanka). When the localities were analysed separately, the difference between sexes was significant at the Evo study site ($P = 0{\cdot}02$) but not at Luhanka ($P = 0{\cdot}18$).

All infections both in voles and in shrews were mild. In each month, only 1–3 more heavily infected (> 20 cysts/tissue section) animals were caught of each host species. No extrapulmonary dissemination of *P. carinii* was observed either in voles or in shrews.

As for other fungal parasites, adiaspores of *Chrysosporium* sp. were found in *M. agrestis* at both study localities but their prevalences were usually low, with no seasonal pattern evident (voles caught at Luhanka in 1994 were not examined for these parasites). One adiaspore of *Chrysosporium* sp. was found in the lungs of 1 *S. araneus* in January 1992.

DISCUSSION

Interestingly, *P. carinii* showed a similar seasonal peak in prevalence in *M. agrestis* and in *S. araneus* in each locality in November. In *M. agrestis*, the seasonal dynamics of *P. carinii* was very similar to those observed in the same study localities in our previous study (Laakkonen *et al*. 1995). In contrast, in *S. araneus* prevalences remained relatively stable, excluding the November peak, during all months in

https://doi.org/10.1017/S0031182098003497 Published online by Cambridge University Press

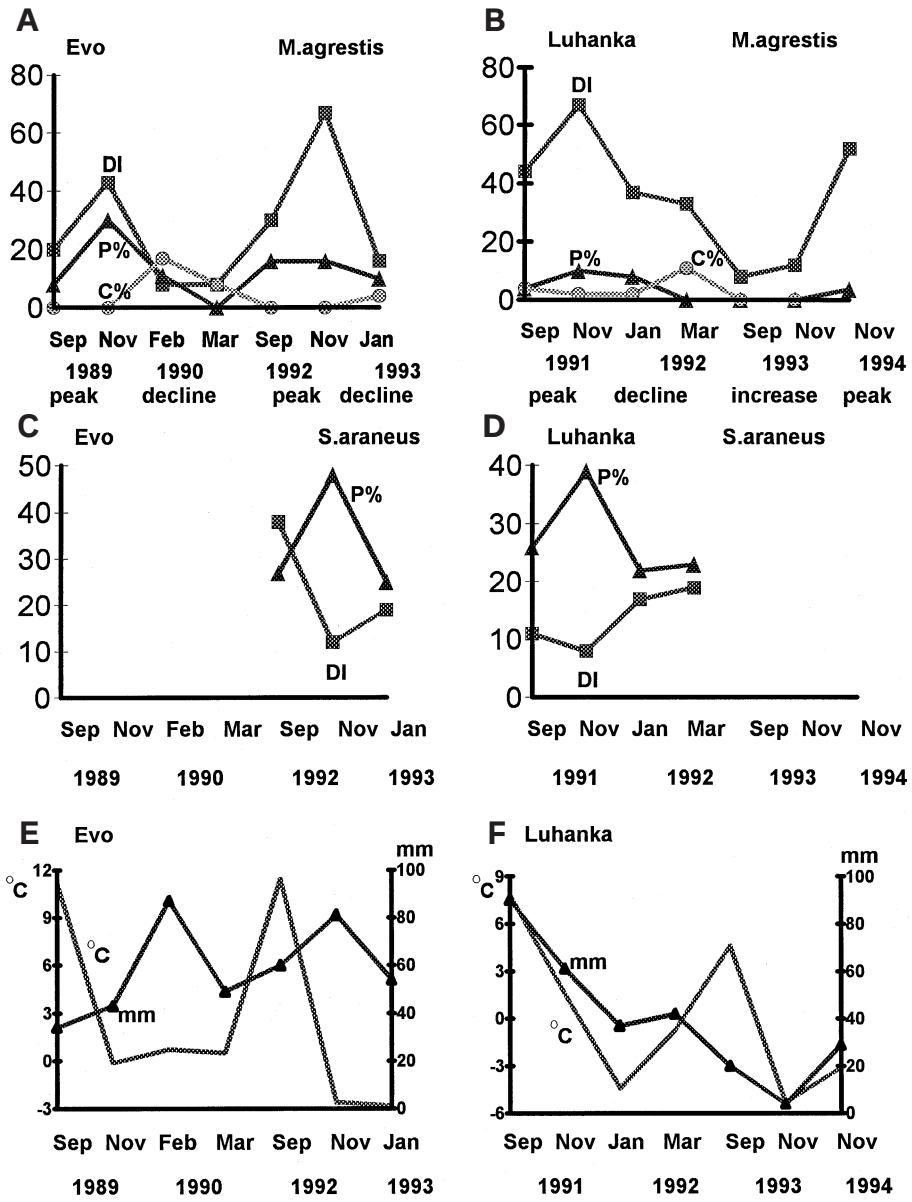

Fig. 1. Dynamics of *Pneumocystis carinii* in *Microtus agrestis* at Evo (A) and at Luhanka (B), and in *Sorex araneus* at Evo (C) and at Luhanka (D). The dynamics of *Chrysosporium* sp. in *Microtus agrestis* is also shown in A and B. Mean temperatures (°C) and precipitation (mm) of the trapping months are shown in E (Evo) and F (Luhanka). DI = host density, P % = prevalence of *P. carinii*, C % = prevalence of *Chrysosporium* sp.

both localities, and were surprisingly low compared to previous prevalences found in this host species in other localities in Finland (Laakkonen, 1998).

Results of other animal studies do not provide evidence for any clear seasonal patterns. Šebek & Rosický (1967) found *P. carinii* in samples of *S. araneus* in spring but not in autumn. In hares, *Lepus europaeus*, *P. carinii* was particularly common during the period from September to December (Poelma & Broekhuizen, 1972). In the mouse, *Apodemus speciosus*, the prevalence of *P. carinii* was somewhat higher in winter and spring than in summer or autumn (Shiota, Kurimoto & Yoshida, 1986). Experiments with laboratory rats indicate that seasonal variation of airborne *P. carinii* does not occur (Hughes, 1982).

In humans, the seasonal variation among *P. carinii* pneumonia cases has been evaluated in a few studies (see Cushion, 1994), but no clear pattern of temporal distribution has become evident and the influence of climatic factors is likely to be modified by prophylaxis. Generally, the occurrence of upper respiratory tract illnesses has been shown to be greater in colder climates (Hoover, 1996). Similarly, in small mammals, a high occurrence of other diseases predisposing to subsequent *P. carinii* infection may contribute to the peak prevalence of *P. carinii* in late autumn.

Pneumocystis carinii infection in animals is often associated with infancy or young age (Soulez *et al.* 1989). No comparative data are available for juveniles (nestlings) of wild mammals. Our study showed

https://doi.org/10.1017/S0031182098003497 Published online by Cambridge University Press

no difference in occurrence of *P. carinii* in *M. agrestis* between age groups. The low number of mature shrews present in the populations after summer prevented such comparisons in *S. araneus*. A previous study (Laakkonen 1996) on *S. araneus* populations sampled during summer months indicated that adult shrews were more often infected than juveniles. Because adult over-wintered shrews invest heavily in reproduction, they are in poor condition at the end of summer, and may thus be more susceptible to opportunistic parasites such as *P. carinii*.

Some of our findings indicated that males of *S. araneus* were more often infected than females. Such a difference between sexes has not been observed in previous studies of *P. carinii* in *S. araneus* (Laakkonen, 1996) or in other wild small mammals (Shiota *et al*. 1986).

Results of this and previous studies (Laakkonen, 1995) indicate that in *S. araneus*, host density is not related to the occurrence of *P. carinii*. The low number of trapping periods (2 cycles at each site including only 1 increase phase) does not allow the construction of proper statistical models to separate the effects of host density and climatic factors related to seasonality. Besides low host density, the absence of the parasite in *M. agrestis* during the fall of the increase year (Luhanka, 1993) could be due to the very cold and dry weather during that autumn, which is the factor most likely to hinder the development of the possible environmental form and the survival of the infectious forms (see discussion in Dei-Cas *et al*. 1992). Once animals are infected with *P. carinii*, however, their clinical manifestations may be more severe during harsh environmental conditions, as has been reported with regards to some dimorphous fungi (Mackinnon, 1968). Relatively cold and dry weather may explain the very low prevalence of this organism in *M. agrestis* also during the next-host density peak phase (Luhanka, 1994). In some cases, it is the combined effect of climatic factors and host density that explains the long-term dynamics of parasites (Haukisalmi & Henttonen, 1990). Interestingly, the other fungal organism examined in this study, *Chrysosporium*, did not seem to show any seasonal pattern, but the generally low prevalence of this fungus does not allow any of us to draw any definitive conclusions.

The interpretation of meteorological factors and occurrence of *P. carinii* is impeded by the fact that meteorological data collected from weather stations provide little information about microclimatic differences between habitats. Some fungi are assumed also to be cleared from the environment by the action of sunlight and other microorganisms (Ellis & Pfeiffer, 1990) as well as harsh weather conditions. Because the life-cycle of *P. carinii* is poorly understood, it is impossible to fully evaluate the effect of seasonality on the occurrence of *P. carinii*.

In this study, as in previous studies (see Laakkonen, 1998), the number of *P. carinii* cyst forms both in *S. araneus* and in *M. agrestis* lung samples was small, and no extrapulmonary dissemination was evident. The extra-pulmonary spread of *P. carinii* is thought to be infrequent or rare (Telzak & Armstrong, 1994), but the prevalence of bloodstream invasion and dissemination is poorly known. As a recent study by Oz, Hughes & Vargas (1996) indicates, extra-pulmonary dissemination may be more frequent for some *P. carinii* isolates (from ferrets) than for others (from rats). Furthermore, recent studies (Laakkonen & Sukura, 1997; Mazars *et al*. 1997) show that *P. carinii* may be much more common than indicated by histochemical examination of cyst forms.

Since the persistence of latent organisms is known to be limited (although it is about 1 year in rat *P. carinii* pneumonia; Vargas *et al*. (1995)) and the highest prevalences of the organism were always found in November, it appears that *M. agrestis* and *S. araneus* are, for unknown reasons, during high population densities more susceptible to *P. carinii* in late autumn which, incidentally, is the time when vole densities start to decline. Immunological competency, and seasonal density-dependent changes in it, may be factors contributing to the regular density variations in natural vole populations (Dobrowolska & Adamczewska-Andrzejewska, 1991, see also Lochmiller, Vestey & McMurry, 1994). Alternatively, environmental conditions may simply be favourable for parasite transmission at this time of year without any indication of actual change in disease resistance. Furthermore, low-protein diet experiments (Laakkonen *et al*. 1995) did not indicate that *P. carinii* is an important mortality factor in *M. agrestis* during any season.

Since in each host species the peak in prevalence occurred at the same time in late autumn, it would be important from the epidemiological point of view to know whether *P. carinii* organisms from different host species differ in infectivity and pathogenicity. *Pneumocystis carinii* of *S. araneus* is known to be antigenically (Laakkonen & Sukura, 1997) and genetically (Peters *et al*. 1994) distinct from isolates of other host species examined thus far. Recent results (Bishop *et al*. 1997, Mazars *et al*. 1997) also indicate that *P. carinii* of *M. agrestis* differs from those of other animals including *S. araneus*.

The high susceptibility of *S. araneus* to *P. carinii* may be related to the exceptionally high metabolic rate of *S. araneus* (Vogel, 1980). Another physiological property of shrews which could contribute to the general susceptibility of these hosts to opportunistic microorganisms may be their low number of leucocytes (Wolk, 1981). The numbers of leucocytes in shrews also show considerable seasonal variation falling from the maximum values in summer to a minimum during winter, and increasing

again slightly in spring (Wolk, 1981). Further studies are warranted to examine whether the high prevalence of *P. carinii* in *S. araneus* during late autumn is due to the decreasing number of leucocytes in their blood at that time.

REFERENCES

BISHOP, R., GURNELL, J., LAAKKONEN, J., WHITWELL, K. & PETERS, S. (1997). Detection of *Pneumocystis* DNA in the lungs of several species of wild mammal. *Journal of Eukaryotic Microbiology* **44**, 57S.

BUSH, A. O., LAFFERTY, K. D., LOTZ, J. M. & SHOSTAK, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**, 575–583.

CUSHION, M. T. (1994). Transmission and epidemiology. In *Pneumocystis carinii Pneumonia* (ed. Walzer, P. D.), pp. 123–140. Marcel Dekker, New York.

DEI-CAS, E., CAILLIEZ, J. C., PALLUAULT, F., ALIOUAT, E. M., MAZARS, E., SOULEZ, B., SUPPIN, J. & CAMUS, D. (1992). Is *Pneumocystis carinii* a deep mycosis-like agent? *European Journal of Epidemiology* **8**, 460–470.

DOBROWOLSKA, A. & ADAMCZEWSKA-ANDRZEJEWSKA, K. A. (1991). Seasonal and long-term changes in serum gamma-globulin levels in comparing the physiology and population density of the common vole, *Microtus arvalis* Pall. 1779. *Journal of Interdisciplinary Cycle Research* **22**, 1–19.

ELLIS, D. H. & PFEIFFER, T. J. (1990). Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. *Lancet* **336**, 923–925.

FIENBERG, S. E. (1970). The analysis of multidimensional contingency tables. *Ecology* **51**, 419–433.

GROCOTT, R. G. (1955). A stain for fungi in tissue sections and smears. *American Journal of Clinical Pathology* **25**, 975–979.

HAUKISALMI, V. & HENTTONEN, H. (1990). The impact of climatic factors and host density on the long-term population dynamics of vole helminths. *Oecologia* **83**, 309–315.

HOOVER, D. R. (1996). Factors associated with the development of *Pneumocystis carinii* pneumonia. *Clinical Infectious Diseases* **22**, 738.

HUGHES, W. T. (1982). Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. *Journal of Infectious Diseases* **145**, 842–848.

LAAKKONEN, J. (1995). Characterization of *Pneumocystis carinii* infection in *Sorex araneus* – a review. *Mammalia* **59**, 623–627.

LAAKKONEN, J. (1996). Characterization and ecology of *Pneumocystis carinii* infection in wild small mammals. Ph.D. thesis, University of Helsinki.

LAAKKONEN, J. (1998). *Pneumocystis carinii* in wildlife. *International Journal of Parasitology* **28**, 241–252.

LAAKKONEN, J. & SOVERI, T. (1995). Characterization of *Pneumocystis carinii* infection in *Sorex araneus* from southern Finland. *Journal of Wildlife Diseases* **31**, 228–232.

LAAKKONEN, J. & SUKURA, A. (1997). *Pneumocystis carinii* of the common shrew, *Sorex araneus*, shows a discrete phenotype. *Journal of Eukaryotic Microbiology* **44**, 117–121.

LAAKKONEN, J., HENTTONEN, H., SOVERI, T. & NIEMIMAA, J. (1995). *Pneumocystis carinii* in arvicoline rodents: seasonal, interspecific, and geographic differences. *Canadian Journal of Zoology* **73**, 961–966.

LOCHMILLER, R. L., VESTEY, M. R. & MCMURRY, S. T. (1994). Temporal variation in humoral and cell-mediated immune response in a *Sigmodon hispidus* population. *Ecology* **75**, 236–245.

MACKINNON, J. E. (1968). The effect of temperature on the deep mycoses. In *Systemic Mycoses* (ed. Wolstenholme, G. E. W. & Porte, R.), pp. 164–178. Churchill Livingstone, London.

MAZARS, E., GUYOT, K., FOURMAINTRAUX, S., RENAUD, F., PETAVY, F., CAMUS, D. & DEI-CAS, E. (1997). Detection of *Pneumocystis* in European wild animals. *Journal of Eukaryotic Microbiology* **44**, 39S.

OZ, H. S., HUGHES, W. T. & VARGAS, S. L. (1996). Search for extrapulmonary *Pneumocystis carinii* in an animal model. *Journal of Parasitology* **82**, 357–359.

PETERS, S. E., ENGLISH, K., LAAKKONEN, J. & GURNELL, J. (1994). DNA analysis of *Pneumocystis carinii* infecting Finnish and English shrews. *Journal of Eukaryotic Microbiology* **41**, 108S.

POELMA, F. G. & BROEKHUIZEN, S. (1972). *Pneumocystis carinii* in hares, *Lepus europaeus* Pallas in the Netherlands. *Zeitschrift für Parasitenkunde* **40**, 195–202.

ŠEBEK, Z. & ROSICKÝ, B. (1967). The finding of *Pneumocystis carinii* in shrews (Insectivora: Soricidae). *Folia Parasitologia* **14**, 263–267.

SHIOTA, T., KURIMOTO, H. & YOSHIDA, Y. (1986). Prevalence of *Pneumocystis carinii* in wild rodents in Japan. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene A***261**, 381–389.

SOULEZ, B., DEI-CAS, E., CHARET, P., MOUGENOT, G., CAILLAUX, M. & CAMUS, D. (1989). The young rabbit: A nonimmunosuppressed model for *Pneumocystis carinii* pneumonia. *Journal of Infectious Diseases* **160**, 355–356.

STRINGER, J. R. (1996). *Pneumocystis carinii*: What is it, exactly? *Clinical Microbiology Reviews* **9**, 489–498.

TELZAK, E. E. & ARMSTRONG, D. (1994). Extrapulmonary infection and other unusual manifestations of *Pneumocystis carinii*. In *Pneumocystis carinii Pneumonia* (ed. Walzer, P. D.), pp. 361–378. Marcel Dekker, New York.

VARGAS, S. L., HUGHES, W. T., WAKEFIELD, A. E. & OZ, H. S. (1995). Limited persistence in and subsequent elimination of *Pneumocystis carinii* from the lungs after *P. carinii* pneumonia. *Journal of Infectious Diseases* **172**, 506–510.

VOGEL, P. (1980). Metabolic levels and biological strategies in shrews. In *Comparative Physiology : Primitive Mammals* (ed. Schmidt-Nielsen, K., Bolis, L. & Taylor, C. R.), pp. 170–180. Cambridge University Press, New York.

WOLK, E. (1981). Seasonal and age changes in leukocyte indices in shrews. *Acta Theriologica* **26**, 219–229.