Assessment of Pneumocystis species carriage in captive primates

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standing. This surgical technique may also allow dyspnoea to be relieved while extended antibiotic or alternative therapies are administered.

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Veterinary Record (2003) **152,** 811-813

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Assessment of *Pneumocystis* species carriage in captive primates

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Pneumocystis species are recognised as major opportunistic fungal pathogens which cause life-threatening pneumonia in severely ill or malnourished mammals, especially primates. In human beings, *Pneumocystis* pneumonia remains the most common initial manifestation of acquired immunodeficiency syndrome (AIDS) (Roux and others 1998). Cancer patients and transplant recipients may also be affected. In other primate species, cases of pneumocystosis have regularly been reported in animals with naturally occurring or experimentally induced immunodeficiency syndromes. Most cases have concerned macaque species infected with either simian immunodeficiency virus (Baskerville and others 1991, Furuta and others 1993, Vogel and others 1993, Yanai and others 1999) or chimaeric simian-human immunodeficiency virus (Shibata and others 1997, Durand-Joly and others 2000). New World primates may also be affected (Long and others 1975, Poelma 1975, Richter and others 1978, Kobayashi and others 1999). In most cases, the presence of *Pneumocystis* species was detected in lung sections after postmortem examination. To the authors' knowledge, an epidemiological survey concerning the carriage of *Pneumocystis* species in captive primates has not been attempted. This short communication describes a study to determine the frequency of *Pneumocystis* species in captive primates in France.

Lung tissue samples from non-human primates were obtained at four French zoological parks (La Palmyre, Mulhouse, Vincennes and Jardin des Plantes de Paris). The lungs were frozen after postmortem examination and stored at –20°C before DNA extraction. For each animal, data, including the animal's specific identification, age, sex, date of death and postmortem examination results, were retrieved from medical records and reported on a standardised form. The number and the age of conspecific primates which had been living with the animal were also recorded.

The lungs were finely minced, homogenised by crushing and subjected to sequential membrane filtration (Ceré and others 1997). The final filtrates were used for direct examination and DNA extraction. Cystic forms of *Pneumocystis* species were detected in 2·5 µl air-dried smears stained with toluidine blue O (Chalvardjian and Grave 1973). A volume of 100 µl from the final filtrates of lung extracts was then frozen at –20°C and digested with proteinase K (1 mg/ml). A phenol-chloroform extraction was then performed, with a final precipitation in ethanol. The presence of *Pneumocystis* DNA in the lung was assessed by nested PCR at the mitochondrial large subunit (mtLSU) ribosomal RNA gene (Wakefield 1996). The primer sets pAZ102-H/pAZ102-E (5'-GATGGGTGTTTCCAAGCCCA-3'/5'-GTGTACGTTGCAAAGTACTC-3') and pAZ102-X/R1/pAZ102-Y/R1 (5'-GGGAATTCGTGAAATACAAATCGGACTAGG-3'/5'-GGGAATTCT CACTTAATATTAATTGGGGAGC-3') were used. Primers pAZ102-H and pAZ102-E are routinely used for the diagnosis of pneumocystosis in human beings. Primers pAZ102-X/R1 and pAZ102-Y/R1 were initially designed for the detection of *Pneumocystis* species DNA in environmental samples, but they were shown to be appropriate for the detection of *Pneumocystis* species in lung tissue samples from non-human primates as well (Demanche and others 2001). Negative controls were included in both the DNA extraction and PCR amplification stages to monitor for possible contamination. The PCR products were electrophoresed in 2 per cent agarose gels and visualised by ethidium bromide staining.

Fisher's exact probability tests were carried out using Epi Info 2000 software (version 1.1.2) to compare the prevalences of *Pneumocystis* species carriage in the simian populations. $P<0{\cdot}05$ was considered to be significant.

Lung tissue samples were collected from 83 primates representing 26 different species; 15 New World species (63 animals) and 11 Old World species (20 animals). Forty-one of the primates were female, 34 were male and eight were too young for their sex to be determined. Twenty-five samples were collected from unweaned primates, 12 from subadult animals and 46 from sexually mature adults. Serological analyses proved that none of the Old World primates was infected by an immunosuppressive retrovirus. There was no history of the use of immunosuppressive or cytotoxic medication.

Pneumocystis cysts were observed in lung samples from seven New World primates (one squirrel monkey, two Geoffroy's marmosets, one red-handed tamarin, one Goeldi's monkey and two Weddell's tamarins). However, there was no clinical or pathological evidence of pneumocystosis in these

animals. Cysts were not found in any of the 20 lung tissue samples from the Old World primates. A positive *Pneumocystis* mtLSU rRNA gene amplification was obtained with all lung samples in which *Pneumocystis* cysts were detected. A positive amplification was obtained with 15 other lung samples.

Overall, the prevalence of *Pneumocystis* species carriage was 26·5 per cent. The prevalence of carriage was similar in New World (28·6 per cent) and Old World (20·0 per cent) primates. As shown in Table 1, the age, sex, date of death and the size of the group of origin did not influence the frequency of *Pneumocystis* DNA detection.

The various causes of death of the 83 primates included in the study are listed in Table 2. The frequency of *Pneumocystis* species DNA detection was significantly higher in primates with an underlying illness (33·9 per cent) than in animals that had died from trauma or been euthanased because of aggressive behaviour (4·8 per cent) ($P<0·01$). Pulmonary diseases and anaemia were the most frequent underlying diseases in primates for which a positive PCR amplification was obtained (Table 2).

Although *Pneumocystis* pneumonia continues to be a common AIDS-defining illness associated with significant mortality in human beings, many questions about the epidemiology of the infection and the transmission of *Pneumocystis* species remain unanswered. In the absence of standardised methods of in vitro cultivation of *Pneumocystis* organisms, the use of an animal model is still required. Because *Pneumocystis* organisms derived from different mammalian hosts exhibit striking molecular and antigenic variation at a number of genetic loci, it has become clear that the genus contains a large number of distinct species (Stringer and others 2001). These *Pneumocystis* species have undergone a prolonged process of genetic and functional adaptation to each mammalian host species (Guillot and others 2001). A recent study demonstrated that specific mitochondrial and genomic DNA sequences of *Pneumocystis* species could be attributed to each primate species (Demanche and others 2001). The genetic divergence among primate-derived *Pneumocystis* biotypes varied in proportion to the degree of the phylogenetic divergence among the corresponding host species, suggesting a process of co-evolution. Non-human primates may thus constitute a valuable population for the analysis of *Pneumocystis* species biology and the epidemiology of corresponding infections.

In the present study, no pneumocystosis was diagnosed, but the presence of *Pneumocystis* DNA was demonstrated in 22 of the 83 primates studied. However, the positivity of nested PCR only, with negative microscopical examination in 15 of the 22 lung samples, suggests a low burden of *Pneumocystis* organisms in the lungs of the primates. These results are in accordance with previous studies concerning HIV-negative human beings with no apparent pneumocystosis. Probst and others (2000) obtained positive nested PCRs in 36 of 163 respiratory samples (22·1 per cent) from patients with chronic pulmonary diseases. Patients with chronic obstructive pulmonary disease or lung cancer had the highest prevalence of *Pneumocystis* species carriage (40·5 per cent and 35·0 per cent, respectively). When postmortem lung tissue samples from individuals without predisposing diseases were investigated, no evidence, or only a very low rate of prevalence, of *Pneumocystis* carriage was detected (Peters and others 1992).

Recent studies have demonstrated that *Pneumocystis* DNA can frequently be detected in nasopharyngeal aspirates from healthy infants (Nevez and others 2001, Vargas and others 2001). These studies raised the hypothesis that they may be an infectious reservoir of *Pneumocystis* species in the human community. Similar observations have been made in some other mammalian species. In wild rabbits, a positive amplification was systematically obtained from samples collected from animals less than one month old (Guillot and others 1999). A large retrospective study concerning *Pneumocystis* infection in pigs indicated that animals from herds in which adult and young pigs shared the same airspace were more heavily infected than those from herds in which adults and weaners were reared separately (Kondo and others 2000). The results obtained in the present study did not confirm the possible role of young animals as reservoirs of *Pneumocystis* species, because the sizes of the groups of origin and the presence of unweaned animals did not influence the prevalence of *Pneumocystis* carriage in lung tissue samples (Table 1). However, the presence of *Pneumocystis* species DNA in twin stillborn marmosets (*Callithrix jacchus*) suggested that vertical transmission of *Pneumocystis* species may occur in non-human primates. This mode of transmission was ruled out in mice (Ito and others 1991) but is suspected in human beings (Mortier and others 1995) and has been proved in rabbits (Ceré and others 1997).

Another reservoir of *Pneumocystis* species could be the environment. This possibility would account for the seasonal variations in *Pneumocystis* carriage observed in wild rodents and insectivores in Finland (Laakkonen and others 1999). As shown in Table 1, the prevalence of *Pneumocystis* species was higher in primates which had died in the spring or summer (33·3 per cent) than in the colder seasons (19·5 per cent). However, these differences were not significant. Only a few studies have indicated seasonal variations in the occurrence of human cases of pneumocystosis (Miller and others 1992, Vanhems and others 1992).

The presence of an underlying disease seemed to be the only factor that influenced the prevalence of *Pneumocystis*

TABLE 1: Results of *Pneumocystis* species mtLSU rRNA gene amplification using 83 lung tissue samples from captive non-human primates

Characteristics of animals	Number of animals	Number (%) of positive results in nested PCR
Sex		
Male	34	11 (32·4)
Female	41	10 (24·4)
Not determined	8	1 (12·5)
Age		
Unweaned	25	6 (24·0)
Subadult	12	3 (25·0)
Adult	46	13 (28·3)
Size of the social group of origin		
Five animals or fewer	50	13 (26·0)
More than five animals	33	9 (27·3)
Presence of unweaned primates in the social group of origin		
Yes	38	8 (21·1)
No	45	14 (31·1)
Season of death		
Spring	13	4 (30·8)
Summer	29	10 (34·5)
Autumn	16	4 (25·0)
Winter	25	4 (16·0)

TABLE 2: Correlation of *Pneumocystis* species mtLSU rRNA gene amplification with underlying diseases

Cause of death	Number of animals	Number (%) of positive results in nested PCR
Trauma or euthanasia	21	1 (4·8)
Neonatal pathology	8	2 (25·0)
Anaemia (infectious or autoimmune)	8	4 (50·0)
Respiratory infectious diseases	6	4 (66·7)
Digestive infectious diseases	21	8 (38·1)
Other diseases	19	3 (15·8)

species in lung tissue samples from captive primates. One-third of the study group, which was composed of sick non-human primates, had detectable amounts of *Pneumocystis* DNA in their lungs. However, it is not known whether these animals were only transient carriers or truly colonised animals and reservoirs of the infection.

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ABSTRACT

Morphological changes induced by oxfendazole in early stage *Taenia solium* cysticerci

PIGS infected with early stage *Taenia solium* cysticerci were treated with a single dose of 30 mg/kg oxfendazole. One day later there were no obvious changes in the general appearance of the larvae, but under the electron microscope there was an apparent reduction in the number of microtriches and the tegument had been completely disrupted and its cells had accumulated many granules, some of which were electron-lucent and others had an electron-dense core. By the second day only the fibrous skeleton and muscle cells were preserved, and by the fifth day the cysticerci were all in an advanced stage of degeneration. By 45 days all the cysts were calcified.

YONG-JIE, L., QING-ZHANG, L. & YAN-HONG, H. (2003) Morphological changes to early stage *Taenia solium* cysticerci following oxfendazole treatment. *Veterinary Journal* **165,** 73-77

Correction

Abstract (*VR*, May 31, p 692). Efficacy of magnets for the treatment of traumatic reticuloperitonitis in cows. The reference details given for this abstract were incorrect. The correct reference should have been as follows: Braun, U., Gansohr, B. & Fluckiger, M. (2003) Radiographic findings before and after oral administration of a magnet in cows with traumatic reticuloperitonitis. *American Journal of Veterinary Research* **64,** 115-120. The error is regretted.

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Veterinary Record 2003 152: 811-813
doi: 10.1136/vr.152.26.811