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# Karyotypes of *Pneumocystis carinii* derived from several mammals

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**Abstract:** *Pneumocystis carinii* is the most important opportunistic pathogen of humans in the world. *Pneumocystis carinii* is experimentally detected in the lungs of rats, mice, rabbits, and monkeys, however, the organisms from different mammals are identical in microscopic morphology. The present study tried to find out more mammalian hosts of *P. carinii* and also to differentiate the organisms from different mammals by karyotyping. Rats, mice, hamsters, rabbits, cats, and dogs were successfully infected by *P. carinii*, but guinea pigs and pigs were not. Karyotype of *P. carinii* from rabbits showed similar size range of chromosomes with that of the prototype, but in different pattern. The patterns from cats and dogs were also different from that of rats. The present study confirms that cats and dogs are infected by *P. carinii* and at least total three karyotype strains of *P. carinii* are proven in Korea.

**Key words:** *Pneumocystis carinii*, rat, dog, cat, rabbit, karyotype

## INTRODUCTION

*Pneumocystis carinii* is a pathogenic protist which causes opportunistic pneumonia in immunocompromised hosts. It can infect not only humans but also other mammals. The organisms from different animals are morphologically identical, therefore, it is impossible to differentiate the organisms from different species of hosts by microscopical findings. The hosts which are known to be infected by *P. carinii* except for human are rats, mice, guinea pigs, hamsters, ferrets, rabbits and monkeys (Matsumoto et al., 1987; Walzer et al., 1989; Bauer et al., 1993). Other animals were not proved yet as its host. Hong et al. (1992a) recorded that they failed to verify the organism from guinea pigs, hamsters, rabbits, cats, dogs, and pigs. However, the mammals still have the possibility of *P. carinii* infection because immunosuppression in that study was insufficient.

No concrete data have shown the organisms from different animals are one species or not. Since the organisms derived from rats or different hosts are antigenically and genetically complexed (Hong et al. 1990, 1995; Bauer et al., 1993; Weinberg and Durant, 1994;

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**Table 1.** Status of *Pneumocystis carinii* infection in different animals

| Animals | No. of exam | No. of infected | Infection rate (%) | Mean No. of cysts |
|---|---|---|---|---|
| Rats | | | | |
| (Wistar) | 59 | 57 | 96.6 | ND[a] |
| (Sprague-Dawley) | 57 | 57 | 100 | ND |
| (Fisher) | 26 | 26 | 100 | ND |
| Subtotal | 142 | 140 | 98.6 | ND |
| Mice | 29 | 10 | 34.5 | 16.3 |
| Hamsters | 21 | 2 | 9.5 | 0.8 |
| Guinea pigs | 13 | 0 | 0 | 0 |
| Rabbits | 14 | 9 | 64.3 | 12.0 |
| Cats | 10 | 5 | 50.0 | 65.0 |
| Dogs | 10 | 8 | 80.0 | 7.4 |
| Pigs | 2 | 0 | 0 | 0 |

[a]Not done.

Vasquez et al., 1996), they cautiously agreed that *P. carinii* is the only one valid species and variants are subspecies (Stringer et al., 1997).

Since many species of mammals are infected by *P. carinii*, it is necessary to determine whether *P. carinii* from different species of mammals are genetically same or not. The present study has two objectives. The first one is to verify whether cats and dogs are infected by *P. carinii*. The other is to observe how many karyotype strains are found from different mammals in Korea. Eight different mammals were immunosuppressed by steroid injection and the isolates of *P. carinii* were purified and analyzed by molecular karyotyping.

## MATERIALS AND METHODS

### Immunosuppression of mammals for *P. carinii* infection

The rats, mice, hamsters, guinea pigs, rabbits, cats, dogs, and pigs were weekly injected with methylprednisolone 10 mg/kg for 5 to 10 weeks (Table 1). They were fed with commercial diet and tap water. After the experiment, their lungs were smeared on glass slides by impression. The slides were stained in Diff-Quik solution (Fisher Scientific, USA) and were microscopically observed under immersion oil lens magnification.

### Preparation of *P. carinii* from the lungs of mammals

The lungs were dissected after ether anesthesia and chopped into pieces as small as possible. The chopped materials were homogenized in a blender (Stomacher, Seaward Medical, UK). The procedures for purification and gel blocks were same as described previously (Hong et al., 1992b). The gel blocks were stored in 0.5 M EDTA (pH 8.0) at 4°C until use.

### Molecular karyotyping by CHEF (contour clamped homogeneous electric field gel electrophoresis) and FIGE (field inversion gel electrophoresis)

*Pneumocystis carinii* nuclei of $10^9$ were prepared in one block of low melting point agarose. Each block was loaded into the trough in 1% agarose gels, and the electrophoresis was run. The conditions for gel electrophoresis by CHEF and FIGE were individually modified. The gels were stained in ethidium bromide solution and observed through UV-illumination.

## RESULTS

### Infection of *P. carinii* in different mammals

The rats, mice, hamsters, rabbits, cats and dogs were proven of *P. carinii* infection in their

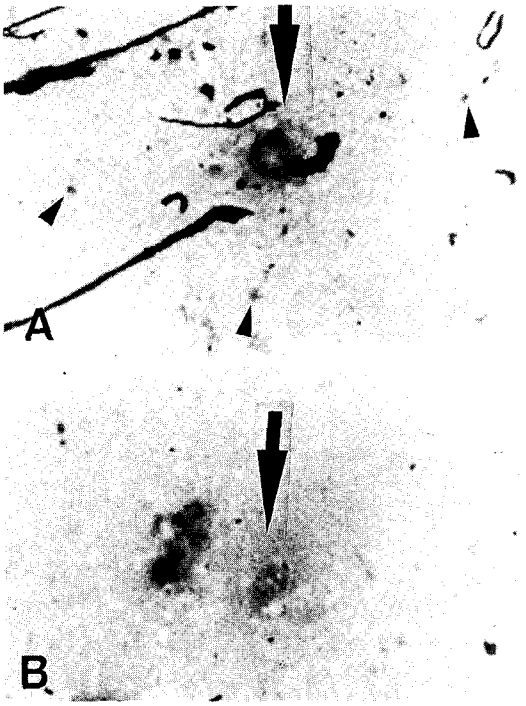


**Fig. 1.** *Pneumocystis carinii* discovered from the lungs of an infected cat, Diff-Quik stained, original magnification x1,500. **A.** One octanucleate cystic form (arrow) and a few trophic forms (arrow heads) are shown. **B.** One developing cystic form (arrow) began to divide the nucleus (arrow).

lungs (Fig. 1). The mammals used in this study were summarized in Table 1. The infected mammals showed big differences between the intensity of infection. However, no cystic forms were confirmed on the lung smears of guinea pigs and pigs.

### Karyotypes of *P. carinii* from different mammals by FIGE and CHEF

Two karyotype patterns of *P. carinii* were found from rats (Fig. 2). One was 16 bands from 275 to 695 bp, and the other is 15 bands from 275 to 695 bp. The two patterns were not determined by strains of rats but by the source of the rats. The rat colony in different vendors showed different karyotype patterns. Density of the organisms in mice was too low to identify the karyotype pattern. The karyotype of *P. carinii* from rabbits was found different from that of rats (Figs. 2, 3). Total 14

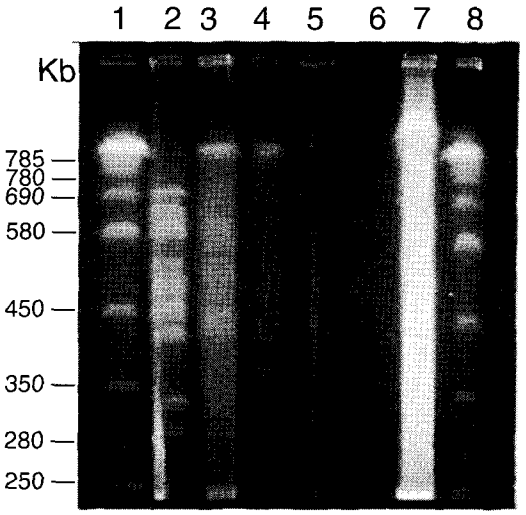


**Fig. 2.** Karyotype patterns of *Pneumocystis carinii* from rats, cats, dogs and rabbits in a 1% agarose CHEF gel in 0.5X TBE buffer. Running conditions were 50 sec initial and 200 sec final, 1:1 A/B ratio, and 6 V/cm for 40 hr. Lane 1, size marker of *Saccharomyces cerevisiae* AB 972; 2, W37, *P. carinii* from Wistar rats, 3, C2-3 *P. carinii* from cats; 4, D2-2 *P. carinii* from dogs; 5-7, *P. carinii* from rabbits; 8, same size marker of lane 1.

chromosomal bands were recognized between 300 and 700 bp. The karyotypes from cats and dogs were too faint to exactly identify the band patterns but the largest chromosomal band from cats measured 730 bp, which was the first band of *P. carinii* over 700 bp (Figs. 2, 3). However exact size estimation of individual band was difficult.

## DISCUSSION

The present study succeeded to demonstrate that rabbits, cats and dogs are infected by *P. carinii*. Both trophic forms and cystic forms of *P. carinii* were found on the impression smears of the three animals (Fig. 1). Of course they were quite same in microscopic features with those from rats. The present success suggests that previous unsuccessful findings with dogs and cats (Farrow et al., 1972; Hong et al., 1992a) may be due to improper immunosuppression. The present data also strongly suggest that almost all kind of mammals may be the host of *P. carinii*.

Although the organisms from cats and dogs

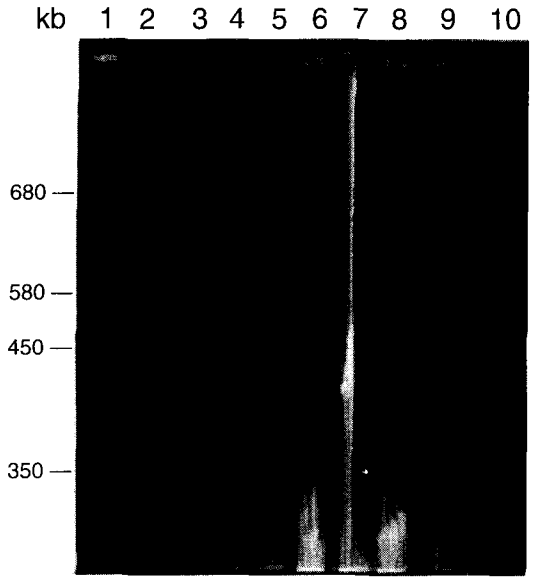


**Fig. 3.** Karyotype patterns of *Pneumocystis carinii* from different animals in a 1% agarose gel of FIGE. Running parameters were 50 sec forward and 25 sec backwards, and 105 V for 120 hr. 1, size marker from *Saccharomyces cerevisiae* AB 972; 2, W26 *P. carinii* from wistar rats; 3, C2-3 *P. carinii* from cats; 4-6, D2-1, D2-2, D4 *P. carinii* from dogs; 7-9, Rb2, Rb9, Rb6-1 *P. carinii* from rabbits; 10, size marker from *S. cerevisiae* AB 972.

are morphologically identical, the organisms were not same with those from rats in the karyotype pattern. The taxonomical confusion of *P. carinii* had long been a hot topic, but the molecular karyotype shows that the organisms which we handle as *P. carinii* are a complex of genetically variant protists (Hong et al., 1990; Lundgren et al., 1990; Cushion et al., 1993; Stringer et al., 1997).

It is evident that *P. carinii hominis* which infect humans are genetically different from those infecting rats (Sinclair et al., 1991; Stringer et al., 1993; Pariset et al., 1997). Some organisms transmitted among Americans were found not including the repeat sequence which is common in all chromosomes of the prototype *P. carinii carinii* from rats (Stringer et al., 1993), and this fact must be very important. Since the human isolate from Koreans was insufficient, karyotype of human *P. carinii* has not been observed yet. The karyotype pattern is a future topic of great interest, because the basic karyotype pattern of rat *P. carinii* is very similar over the world.

Infection of *P. carinii* has been noticed in many species of mammals. Among them, the rat is the host of prototype organism which is used as a standard experimental model for this research, because most of the organisms are obtained in vivo from rats. This is one of rare protists which can not be supplied by in vitro cultivation. Cultivation of *P. carinii* is still unsuccessful by too many knotty requirements which are unclarified. All of its researches should supply the organisms from rats.

Though the guinea pig is a known host of *P. carinii*, we failed to find infected guinea pigs in this study and also in the previous trial (Hong et al., 1992a). The same was in pigs. Goats and foals were reportedly unsuccessful (McConnel et al., 1971; Shively et al., 1973). Reasons of this failure may be that the animals were exposed to many kinds of saprophytous microorganisms and they died by acute infections after immunosuppression or other complications much earlier than development of pneumocystosis.

The karyotype of *P. carinii* from cats showed different pattern from that of rat *P. carinii*. The largest band of cat *P. carinii* was 730 bp and this is the largest band ever recorded. Although the karyotypes from rabbits and dogs were not clear enough for definite analysis, we found at least 3 different patterns of *P. carinii* karyotype from rats or cats in Korea (Hong et al., 1990, 1992b). Of course additional karyotypes may be added by further studies on *P. carinii* from cats, dogs, and humans. In this context, karyotyping and gene mapping is one of useful methods to probe a specific strain or to differentiate mixed strains.

In conclusion, this study proved the dogs and cats can be hosts of *P. carinii*. More specification is necessary for different nomenclature of subspecies. Also three karyotype strains of *P. carinii* are confirmed among mammals in Korea.

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