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Prevalence of *Cryptosporidium* Infection among House Rats, *Rattus rattus* and *R. norvegicus*, in Tokyo, Japan and Experimental Cryptosporidiosis in Roof Rats

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Abstract

An epidemiological survey for *Cryptosporidium* infection was carried out on house rats, *Rattus rattus* (roof rats) and *R. norvegicus* (brown rats), captured in the Tokyo Metropolitan District. Of a total of 231 house rats, consisting of 175 roof rats, 48 brown rats, and 8 species-indeterminate rats, 32 (13.9%) were found to be positive for *Cryptosporidium* oocysts in their feces. The incidence of the infection was 17.7% for roof rats and 2.1% for brown rats, respectively. In roof rat groups classified by body weight, the incidence was consistently high (13.8%～25.0%). There was no significant difference between the incidence of infection and the sexes. The size of the oocysts of the isolates from roof rats measured $3.7 \pm 0.22 \times 4.8 \pm 0.33$ μ m. Roof rats experimentally inoculated with *Cryptosporidium* oocysts from naturally infected rats began to shed oocysts on days 2 to 3 post inoculation (PI). The number of oocysts in feces peaked on days 5 to 8 PI, declined rapidly, and thereafter markedly small numbers of oocysts were detected intermittently until day 60 PI. None of the roof rats showed any apparent clinical symptoms such as diarrhea in experimental infection.

Key words: *Cryptosporidium, Rattus rattus, Rattus norvegicus,* natural infection, experimental infection, oocyst shedding

Introduction

Cryptosporidium is a coccidian parasite inhabiting primarily the brush border of the intestinal epithelia of a wide variety of vertebrates including fish, reptiles, birds and mammals distributed all over the world. *Cryptosporidium* sp., which has a small type of oocysts, is known to cause symptoms such as diarrhea, vomiting, anorexia and abdominal pain lasting from days to weeks in immunocompetent persons and to cause an overwhelming and life-threatening illness in immunocompromised patients such as those with AIDS or cancer and those receiving organ transplantation surgery (Tzipori, 1983; Fayer and Unger, 1986). Although the transmission and source of infection have not been well understood, the possible involvement of domestic and wild animals has been strongly suggested in the transmission to humans (Koch *et al.*, 1983; Hart *et al.*, 1984; Lewis *et al.*, 1985).

Rattus rattus (roof rats) and *R. norvegicus* (brown rats) are significant wild animals that are common both in urban and rural environments. For epidemiological studies on cryptosporidiosis among house rats have recently been carried out in several areas in Japan, and have shown high incidence of the *Cryptosporidium* infection (Miyaji *et al.*, 1989).

This paper presents the results of a survey for *Cryptosporidium* infection among roof rats and

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brown rats captured in Tokyo, Japan. The pattern of oocyst shedding in roof rats experimentally inoculated with *Cryptosporidium* oocysts isolated from naturally infected roof rats was also described.

Materials and Methods

A total of 231 house rats, consisting of 175 roof rats, 48 brown rats and 8 species-indeterminate rats were obtained from two areas in Tokyo, Japan, from November 1989 to January 1990. They were mainly captured in buildings housing restaurants (area A) and grocery stores (area B). Fecal contents were taken from the large intestine of the house rats at necropsy and examined for *Cryptosporidium* oocysts by the sugar-centrifugal flotation technique (McNabb *et al.*, 1985). Three loopfuls of the floating layer were placed on a glass slide with a metal loop which was 4 mm in diameter and bent at an angle of 90° at the point of contact, and observed under a light microscope at a magnification of ×400. The size of the oocyst was measured on fresh specimens with an ocular micrometer at a magnification of ×1,000. More than 30 oocysts were measured for each isolate from the rats.

In experimental infection, each of three young roof rats weighing 50–70g and previously free from *Cryptosporidium* infection was inoculated orally with 0.8, 1.4, and 8.0×10^5 of *Cryptosporidium* oocysts isolated from naturally infected roof rats, respectively. Oocysts for the inoculation were concentrated by the sugar-centrifugal flotation technique, washed three times, and suspended in sterile saline. After the inoculation, 1g of fecal specimens from each rat was collected daily and examined for *Cryptosporidium* infection by the method described above. Oocysts were counted in 30 microscopic fields under a magnification of ×400. Two uninoculated roof rats served as controls.

Results

The incidence of *Cryptosporidium* infection among house rats is shown in Table 1. Of a total of 231 house rats, 32 (13.9%) were found to be infected. The incidence of the infection in roof rats was 31/175 (17.7%), ranging from 12.0% to 25.5% by areas, and in brown rats 1/48 (2.1%), ranging from 0% to 5.9%. None of the 8 species-indeterminate rats were positive for the oocyst. The incidence of *Cryptosporidium* infection in roof rats was much higher than that in brown rats.

Table 2 shows the relationship between the incidence of infection and the body weight of the

Table 1 Incidence of *Cryptosporidium* infection among *R. rattus* and *R. norvegicus*

Species	Area	Month and year	Number infected/ Number examined (%)	
R. rattus	A	Nov.1989	13/51 (25.5)	
	A	Dec. 1989	3/25 (12.0)	
	B	Dec. 1989	9/64 (14.1)	31/175(17.7)
	B	Jan. 1990	6/35 (17.1)	
R. norvegicus	A	Nov.1989	0/31 (0.0)	
	A	Dec. 1989	1/17 (5.9)	1/48 (2.1)
Species-indeterminate rats	A	Nov.1989	0/ 8 (0.0)	
Total			32/231(13.9)	

A: A department store housing restaurants
B: A block of 12 grocery stores located at basement

Table 2 Incidence of *Cryptosporidium* infection in *R. rattus* by body weight

Body weight (g)	Number infected/ Number examined (%)
<50	13/ 94 (13.8)
50~99	12/ 48 (25.0)
≧100	6/ 33 (18.2)
Total	31/175 (17.7)

Table 3 Incidence of *Cryptosporidium* infection in *R. rattus* by sex

Sex	Number infected/ Number examined (%)
♂	17/ 97 (17.5)
♀	14/ 78 (17.9)
Total	31/175 (17.7)

roof rats. No statistically significant difference in the incidence of *Cryptosporidium* infection was found among the three groups (less than 50g, 50~99g, and 100g or more in body weight).

The infected roof rats consisted of 17 males and 14 females, as shown in Table 3. No statistically significant difference in the prevalence of the infection was shown between the sexes ($p>0.01$).

The oocysts discharged from the roof rats were spherical to ovoid measuring $3.7\pm0.22\times 4.8\pm0.33\,\mu$m, and there were no morphological differences between oocysts from the roof rats and brown rat. No large oocysts of *C. muris* (more than 7 μm in length) were found in this study.

The pattern of oocyst shedding in experimentally infected roof rats is shown in Fig. 1. All

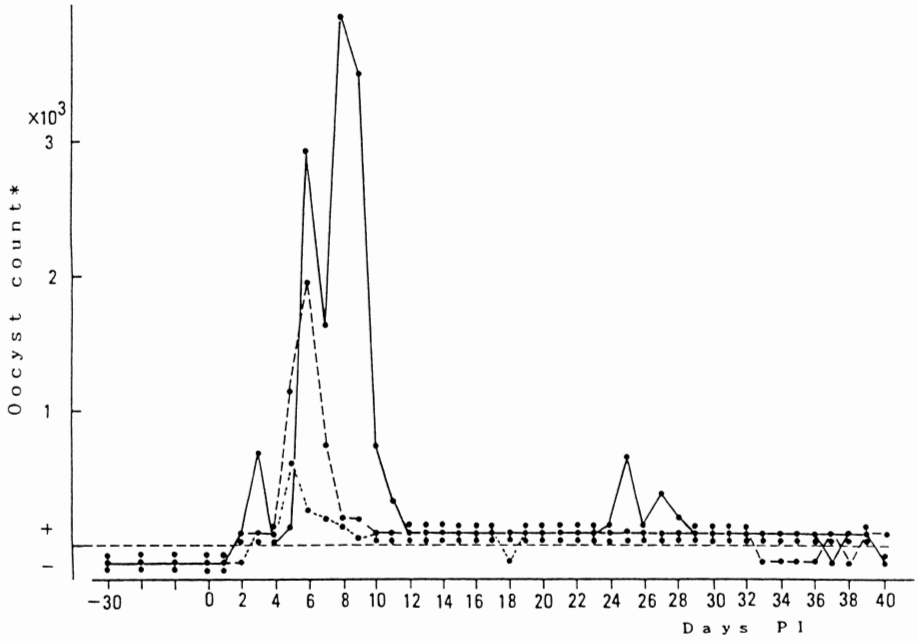

Fig. 1. Shedding of *Cryptosporidium* oocysts from roof rats experimentally inoculated with 8.0 (———), 1.4 (– – – –), and 0.8 (-- -- --)$\times 10^5$ oocysts. Each point represents the mean of duplicated countings. The oocysts were detected in none of uninoculated control rats. *The number of oocysts found in 30 microscopic fields under a magnification of ×400.

three rats began to shed oocysts on days 2 to 3 PI. The intensity of oocyst shedding of each roof rat reached a peak on days 5 to 8 PI. Thereafter, numbers of oocysts declined rapidly and markedly small numbers of oocysts were detected intermittently through the observation period of 60 days PI. In one roof rat, the first peak was followed by a second lower peak on day 25. None of the roof rats experimentally infected showed any apparent clinical symptoms such as diarrhea. No *Cryptosporidium* oocysts could be demonstrated in feces of uninoculated control rats.

Discussion

In this study, the incidence of *Cryptosporidium* infection was shown to be 17.7% for roof rats and 2.1% for brown rats on average. Higher incidence rates (48.5% for roof rats and 21.3% for brown rats) have been recently described in house rats captured in Tokyo, Osaka and Chiba, Japan (Miyaji *et al.*, 1989). Furthermore, Iseki (1986), in a survey of 61 brown rats in Osaka, Japan, detected *Cryptosporidium* oocysts of both small form (in 6, or 9.8% of the rats) and large form (in 3, or 4.9% of the rats). These findings indicate that *Cryptosporidium* infection is common among house rats in Japan.

Experimental studies have shown that young animals are more susceptible than older ones to *Cryptosporidium* infection (Sherwood *et al.*, 1982; Tzipori *et al.*, 1983). Our epidemiological study showed that the infection was detected in 31 roof rats and one brown rat, and that the incidence of the infection was consistently high in roof rat groups classified by body weight. On the other hand, Miyaji *et al.* (1989) have shown that the incidence of *Cryptosporidium* infection in roof rats decreased according to the body weight of rats.

Although it is known that diarrhea is the most prominent symptom in cryptosporidiosis in humans and other domestic animals (Current *et al.*, 1983; Fayer and Unger, 1986; Stehr-Green *et al.*, 1987), none of the roof rats experimentally infected in the present study showed any apparent clinical symptoms such as diarrhea. This is consistent with the earlier finding that no clinical symptoms have been observed in brown rats with *Cryptosporidium* (Iseki, 1986). Cats with *Cryptosporidium* have also been described as showing no appreciable clinical symptoms (Arai *et al.*, 1990). It is not known why there are differences in the degree of clinical symptoms exhibited by different host animal species.

The number of valid species in genus *Cryptosporidium* is still under debate. At least two species of *Cryptosporidium* are known to cause mammalian cryptosporidiosis. One is *C. muris,* which has a large oocyst measuring 7.0–8.4 μm in length, and the other is *C. parvum,* which has a small one measuring 4.0–5.3 μm (Upton and Current, 1985; Iseki, 1986). The latter has appeared in most of the previously well-documented cases of cryptosporidiosis in humans and domestic animals. The size of the oocysts found in roof rats in this study measured 3.7×4.8 μm on the average, which represents those of small type.

Earlier investigations have reported that the experimental transmission of oocysts from calf was possible to calves, lambs and laboratory mice during the suckling period (Tzipori *et al.*, 1981, 1983; Sherwood *et al.*, 1982). It has also been shown that adult rats (Brasseur *et al.*, 1988) and hamsters (Rossi *et al.*, 1990) must be put in an immunosuppressed state for them to become infected with the *Cryptosporidium* oocyst from calves. Our present data indicated that juvenile or nearly adult roof rats were highly susceptible to oocysts isolated from naturally infected roof rats, and that any immunosuppressants were not necessary for establishment of the infection. Immunocompetent adult humans are also known to be susceptible to *Cryptosporidium* infection (Fayer and Unger, 1986). In addition, adult guinea pigs (Chrisp *et al.*, 1990) and wild mice (Klesius *et al.*, 1986) have been reported to be easily infected with *Cryptosporidium* derived from guinea pigs and wild mice, respectively. Clarification of differences in susceptibility between homologous and heterologous transmission must await further information on the biological nature of the parasite.

Roof rats experimentally inoculated with

Cryptosporidium oocysts were found to begin shedding oocysts as early as days 2–3 PI, and to reach peaks on days 5–8, followed by intermittent shedding of markedly small numbers of oocysts. This prepatent period is similar to that found with experimentally infected rats (Brasseur *et al.*, 1988) and hamsters (Rossi *et al.*, 1990). In the studies on immunocompetent human and animal cases, estimates of the duration of oocyst shedding have widely varied. Several recent observations of roof rats (Miyaji *et al.*, 1989), mice (Klesius *et al.*, 1986), and humans (Shepherd *et al.*, 1988) have shown recurrence of and long-lasting shedding of oocysts. Although the precise mechanisms responsible for recurrence of the oocyst shedding in several animal species with *Cryptosporidium* are not yet known, this may be explained by autoinfection (Fayer and Unger, 1986).

From this study, it is conceivable that roof rats and brown rats may involve in human cryptosporidiosis in urban areas as a serious source of the infection, when *Cryptosporidium* sp. derived from house rats has little or no host specificity as is the case for those from calves and humans (Fayer and Unger, 1986). Further studies are presently being undertaken on the infectivity and the pathogenicity of *Cryptosporidium* sp. from roof rats for other animal species.

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