Jpn. J. Med. Sci. Biol., 43, 7 - 14, 1990.

**PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION AMONG
DOMESTIC CATS IN THE TOKYO METROPOLITAN DISTRICT,
JAPAN**

Hideto ARAI, Yutaka FUKUDA, Tatsuko HARA, Yasuyuki FUNAKOSHI,
Satoko KANEKO, Takeo YOSHIDA, Hiroko ASAHI¹*, Mitsuyoshi KUMADA¹,
Keiko KATO¹ and Tsutomu KOYAMA¹

*Tokyo Metropolitan Animal Protection Center, 1-3-Saki, Jyonanjima, Ohta-ku,
Tokyo 103 and ¹Department of Parasitology, National Institute of Health,
Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received March 6, 1990. Accepted March 23, 1990)

SUMMARY: From February, 1988 to February, 1989, an epidemiological survey for feline cryptosporidiosis was carried out on domestic cats that had been committed to the Tokyo Metropolitan Animal Protection Center by owners residing in the Tokyo Metropolitan District. Of a total of 608 cats, 23 (3.8%) were found to have *Cryptosporidium* oocysts. The percentage of cats positive for the infection ranged from 0 to 13.6% by month. The infection was found to be more common in "baby" and "young" cats than in "middle" and "old" ones. No significant difference in incidence was shown between the sexes. In each positive cat, no apparent clinical symptom such as diarrhea was found, and the size of the oocysts measured from 4.3 to 4.7 μ m in diameter (small type).

新井英人・福田 豊・原 樹子・船越康之・金子哲子・吉田武雄 (東京都動物管理事務
所動物愛護センター 東京都大田区城南島1-3先)

朝日博子・熊田三由・加藤桂子・小山 力 (国立予防衛生研究所寄生虫部)

*Address reprint requests to: Hiroko Asahi, Department of Parasitology,
National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141.

INTRODUCTION

Cryptosporidia are coccidian parasites belonging to genus *Cryptosporidium*, and it is known that at least one species of them (*C. parvum*) can cause in immunocompetent persons symptoms such as diarrhea, vomiting, anorexia and abdominal pain lasting from days to weeks (1,2). The disease, cryptosporidiosis, often can become an overwhelming and life-threatening illness in immunocompromised patients such as those with AIDS or cancer and those receiving organ transplantation surgery (1,2).

The protozoon inhabits primarily the brush border of the intestinal epithelia of a wide variety of vertebrates including fish, reptiles, birds and mammals (1,2). Little is known, however, about the transmission, source of infection, or biological nature of the parasite. In the transmission to human, the possible involvement of pet cats has been strongly suggested (3-5), but little is known of the epidemiology of *Cryptosporidium* infection among cats.

This paper presents the results of a survey of domestic cats for *Cryptosporidium* infection. In addition, several techniques were evaluated for their sensitivity for the detection of *Cryptosporidium* oocysts.

MATERIALS AND METHODS

From February, 1988 to February, 1989, this epidemiological survey was carried out on domestic cats that had been committed to the Tokyo Metropolitan Animal Protection Center by the owners residing in the Tokyo Metropolitan District. The age of each cat was estimated by observing its tooth condition and general appearance. The cats were classified into four age groups: "baby", consisting of all those whose milk teeth had not yet been replaced with permanent teeth; "young", all those whose milk teeth had been replaced and in whom abrasion of incisors was not marked; "middle", those in whom abrasion of incisors was marked; and "old", those whose permanent teeth were severely abrasive. About 3 g of the colon and rectum contents were taken at necropsy of the cats and were examined for *Cryptosporidium* oocysts by the sugar-centrifugal flotation techniques (SF) (6). Namely, three loopfuls of the floating layer were observed under a light microscope at a magnification of $\times 400$. SF combined with a modification of modified cold type Kinyoun's acid-fast staining (SMMCK) and SF combined with a modification of modified Kohn's one-step staining technique (SMMK) (7) were also used.

In SMMCK, the materials in the floating layer obtained by SF were collected with a metal loop which was 4 mm in diameter and was bent at an angle of 90° at a point of contact with a handle. More than 15 loopfuls of the materials were diluted with more than 10 ml of distilled water and centrifuged at 750 ×g (2,500 rpm) for 10 min to remove sugar. The sediment was smeared on glass slides and stained by a modification of modified cold-type Kinyoun's acid-fast staining (MMCK). In MMCK, an additional washing with 50% ethanol was inserted between staining with carbol-fuchsin and decolorization with sulfuric acid in Kinyoun's acid-fast staining (MCK) (8).

Ileal smears were stained by MMCK to detect *Cryptosporidium* oocysts.

Pathological changes of cats were inspected with the naked eye.

Size of oocyst was measured on specimens stained by SMMK with an ocular micrometer at a magnification of ×1,000. More than 20 oocysts were used for each measurement.

RESULTS

For comparison of the sensitivity for detecting *Cryptosporidium* oocysts, the three methods, SF, SMMCK and SMMK, were applied on fecal samples, and MMCK was applied on ileal smears. Of 19 cats confirmed to be positive for the oocysts through the combination of SMMCK and SMMK, only 13 (68.4%) and 8 (42.1%) were detected to be positive by SF on fecal samples and MMCK on ileal smears, respectively. Therefore, SF or MMCK alone was not considered to be fully sensitive for detecting *Cryptosporidium* oocysts in cats. Feces of cats, thus, were subsequently screened for the presence of the oocysts by the combination of SF and SMMCK. In this procedure, when a specimen was suspected to be positive for the oocysts, the oocyst's identity was confirmed by examining smears stained with SMMK.

The incidence of the *Cryptosporidium* infection among cats is shown in Table I. Of a total of 608 cats tested, 23 (3.8%) were found to be infected. The percentage of cats positive for the infection by month ranged from 0 to 13.6%. The highest incidence rate was observed in September.

Table II shows the relationship between the body weight of the cats and percentage of positivity and between the estimated age of the cats and percentage of positivity.

The highest incidence rate was found in cats less than 2.5 kg and in the "baby" group, followed by the "young" group. No oocyst was found in the feces of

Table I. Monthly incidence of *Cryptosporidium* infection among cats

Month and year	Number infected/ Number examined (%)	Month and year	Number infected/ Number examined (%)
Feb., 1988	1/16 (6.3)	Sept., 1988	6/44 (13.6)
Mar.	0/49 (0.0)	Oct.	3/37 (8.1)
Apr.	2/65 (3.1)	Nov.	1/46 (2.2)
May	0/70 (0.0)	Dec.	0/25 (0.0)
Jun.	1/72 (1.4)	Jan., 1989	2/30 (6.7)
Jul.	2/60 (3.3)	Feb.	4/54 (7.4)
Aug.	1/40 (2.5)		
		Total	23/608 (3.8)

Table II. Incidence of *Cryptosporidium* infection in cats by body weight or age

Body weight (kg)	Number infected/ Number examined (%)	Estimated age	Number infected/ Number examined (%)
-1.5	6/108 (5.6)	Baby	7/92 (7.6)
1.6-2.5	11/198 (5.6)	Young	10/167 (6.0)
2.6-3.5	5/203 (2.5)	Middle	6/319 (1.9)
3.6-	1/99 (1.0)	Old	0/30 (0.0)
Total	23/608	Total	23/608

the "old" group. This suggests that *Cryptosporidium* infection is more common in younger cats ("baby" and "young") than in older ones ("middle" and "old").

The infected cats consisted of 13 males and 10 females as shown in Table III. No significant difference in positivity was shown between the sexes ($p > 0.05$).

Table III. Incidence of *Cryptosporidium* infection in cats by sex

Sex	Number infected / Number examined (%)
♂	13 / 248 (5.2)
♀	10 / 360 (2.8)
Total	23 / 608

Table IV. Size of oocysts found in 10 positive cats

Cat No.	Sex	Body weight (kg)	Size of oocyst (µm)
V6	♀	2.0	4.6 ± 0.35*
317	♀	2.7	4.5 ± 0.27
365	♂	1.7	4.3 ± 0.38
419	♂	4.6	4.4 ± 0.37
430	♂	3.1	4.4 ± 0.25
448	♂	1.8	4.5 ± 0.27
536	♀	0.6	4.5 ± 0.40
560	♂	1.6	4.7 ± 0.42
561	♂	1.4	4.5 ± 0.49
602	♂	3.2	4.5 ± 0.37

* Mean ± S. D. (n=more than 20 oocysts)

Of the 23 positive cats, the size of oocysts found in 10 are given in Table IV. The oocysts discharged from each cat were spherical to ovoid and measured from 4.3 to 4.7 µm in diameter. In the 10 infected cats, no apparent clinical symptom such as diarrhea was found. The other positive cats also showed no apparent clinical symptoms and the size of oocysts was almost the same as those shown in Table IV. No large oocysts (more than 7 µm in large diameter) were found in the positive cats in this study.

DISCUSSION

In this study, the incidence of *Cryptosporidium* infection was investigated in domestic cats living in the Tokyo Metropolitan District. The incidence was shown to be 3.8% on average. A similar incidence rate (3.9%) has recently been found in cats in Hyogo Prefecture, Japan (9). Furthermore, Iseki (10) has detected *Cryptosporidium* oocysts in five of 13 (38.5%) domestic cats in Osaka. These findings may indicate that *Cryptosporidium* infection among cats in Japan is very common. In fact, in our survey, the incidence varied in each month and reached 13.6% in September, 1988.

Epidemiological studies on human cryptosporidiosis have recently been carried out in various regions of the world, and have revealed that *Cryptosporidium* may be a common cause of diarrhea among infants and children, particularly in developing countries (11-14). Experimental studies have shown that young animals are more susceptible than older ones to *Cryptosporidium* infection (15,16). Our study also showed that the infection was more common among younger cats, although it was detected also in older ones.

Although it is known that diarrhea is the most prominent symptom in cryptosporidiosis in humans and other domestic animals (1,17,18), no cat in the present survey exhibited any clinical symptoms. This is consistent with the case described by Koch et al. (4), who stated that no appreciable clinical symptoms were observed in cats infected with *Cryptosporidium*. Furthermore, no clinical symptoms have been observed in murine cryptosporidiosis (19). It is not known why there are differences in the degree of clinical symptoms exhibited by different host animal species.

At present, the number of valid species in genus *Cryptosporidium* is still under debate. Most medical protozoologists today believe that at least two species of *Cryptosporidium* are known to cause of mammalian cryptosporidiosis: *C. muris*, which has large oocysts measuring 7.0-8.4 μm in diameter, and *C. parvum*, which has small ones measuring 4.0-5.3 μm (19,20). 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 cats in this study also measured from 4.3 to 4.7 μm , suggesting that the *Cryptosporidium* sp. with small oocysts may be responsible for most feline cryptosporidiosis. On the other hand, possible occurrence of natural infection with the *Cryptosporidium* sp. with large oocysts among cats was considered, since Iseki et al. (21) succeeded in

experimental infection of cats with *Cryptosporidium* sp. with large oocysts originated from house rats.

REFERENCES

1. Fayer, R. and Ungar, B. L. P. (1986): *Cryptosporidium* spp. and cryptosporidiosis. Microbiol. Rev., 50, 458-483.
2. Tzipori, S. (1983): Cryptosporidiosis in animals and humans. Microbiol. Rev., 47, 84-96.
3. Hart, C. A., Baxby, D. and Blundell, N. (1984): Gastro-enteritis due to *Cryptosporidium*: a prospective survey in a children's hospital. J. Infect., 9, 264-270.
4. Koch, K. L., Shankey, T. V., Weinstein, G. S., Dye, R. E., Abt, A. B., Current, W. L. and Eyster, M. E. (1983): Cryptosporidiosis in a patient with hemophilia, common variable hypogammaglobulinemia, and the acquired immunodeficiency syndrome. Ann. Intern. Med., 99, 337-340.
5. Lewis, I. J., Hart, C. A. and Baxby, D. (1985): Diarrhoea due to *Cryptosporidium* in acute lymphoblastic leukaemia. Arch. Dis. Childhood, 60, 60-62.
6. McNabb, S. J. N., Hensel, D. M., Welch, D. F., Heijbel, H., McKee, G. L. and Istre, G. R. (1985): Comparison of sedimentation and flotation techniques for identification of *Cryptosporidium* sp. oocysts in a large outbreak of human diarrhea. J. Clin. Microbiol., 22, 587-589.
7. Asahi, H., Kumada, M., Kato, K. and Koyama, T. (1988): A simple staining method for cryptosporidian oocysts and sporozoites. Japan. J. Med. Sci. Biol., 41, 117-121.
8. Ma, P. and Soave, R. (1983): Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea. J. Infect. Dis., 147, 824-828.
9. Uga, S., Matsumura, T., Ishibashi, K., Yoda, Y., Yatomi, K. and Takaoka, N. (1989): Cryptosporidiosis in dogs and cats in Hyogo Prefecture, Japan. Japan. J. Parasitol., 38, 139-143.
10. Iseki, M. (1979): *Cryptosporidium felis* sp. n. (Protozoa: Eimeriorina) from the domestic cat. Japan. J. Parasitol., 28, 285-307.
11. Bogaerts, J., Lepage, P., Rouvroy, D. and Vandepitte, J. (1984): *Cryptosporidium* spp., a frequent cause of diarrhea in Central Africa. J. Clin. Microbiol., 20, 874-876.
12. Mata, L., Bolanós, H., Pizarro, D. and Vives, M. (1984): Cryptosporidiosis in children from some highland Costa Rican rural and urban areas. Amer. J. Trop. Med. Hyg., 33, 24-29.

13. Pape, J. W., Levine, E., Beaulieu, M. E., Marshall, F., Verdier, R. and Johnson, W. D., Jr. (1987): Cryptosporidiosis in Haitian children. *Amer. J. Trop. Med. Hyg.*, 36, 333-337.
14. Weitz, J. C. V., Tassara, R. O. and Mercado, R. (1988): Cryptosporidiosis in Chilean children. *Trans. Roy. Soc. Trop. Med. Hyg.*, 82, 335.
15. Sherwood, D., Angus, K. W., Snodgrass, D. R. and Tzipori, S. (1982): Experimental cryptosporidiosis in laboratory mice. *Infect. Immun.*, 38, 471-475.
16. Tzipori, S., Smith, M., Halpin, C., Angus, K. W., Sherwood, D. and Campbell, I. (1983): Experimental cryptosporidiosis in calves: Clinical manifestations and pathological findings. *Vet. Rec.*, 112, 116-120.
17. Current, W. L., Reese, N. C., Ernst, J. V., Bailey, W. S., Heyman, M. B. and Weinstein, W. M. (1983): Human cryptosporidiosis in immunocompetent and immunodeficient persons. *New Engl. J. Med.*, 308, 1252-1257.
18. Stehr-Green, J. K., McCaig, L., Remsen, H. M., Rains, C. S., Fox, M. and Juranek, D. D. (1987): Shedding of oocysts in immunocompetent individuals infected with *Cryptosporidium*. *Amer. J. Trop. Med. Hyg.*, 36, 338-342.
19. Iseki, M. (1986): Two species of *Cryptosporidium* naturally infecting house rats, *Rattus norvegicus*. *Japan. J. Parasitol.*, 35, 521-526.
20. Upton, S. J. and Current, W. L. (1985): The species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) infecting mammals. *J. Parasitol.*, 71, 625-629.
21. Iseki, M., Maekawa, T., Moriya, K., Uni, S. and Takada, S. (1989): Infectivity of *Cryptosporidium muris* (strain RN66) in various laboratory animals. *Parasitol. Res.*, 75, 218-222.