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Survey on *Toxoplasma* Infection in Stray Cats in Western Area of Japan During a Two-year Period

HIROSHI OIKAWA[1], YOSHITAKA OMATA[2], MASANORI KANDA[1], KOUJI MIKAZUKI[1], KEN'ICHI YANO[3] AND TOSHIO NAKABAYASHI[4]

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Abstract

The incidence of *Toxoplasma gondii* oocysts in feces and antibodies in sera of 335 stray adult cats in the western area of Japan was studied over a two-year period. Oocysts were detected in only one (0.3%) cat, while antibody to *T. gondii* was positive in 86 (25.7%) cats tested by indirect immuno-fluorescent assay (IFA). In one case of natural infection in which oocysts were detected, antibody was negative during oocyst shedding and then turned positive. Based on the changing patterns of oocyst shedding and antibody titers in the experimental infection, the infection in this natural case may be due to ingestion of cysts developing in the paratenic host and not to oocysts in soils.

Key words: *Toxoplasma gondii,* stray cat, antibody titers, oocyst shedding, incidence

Introduction

Among domestic, pet and laboratory animals, the cat is the only known to be definite host of *Toxoplasma gondii* to shed oocysts. Many other warm-blooded animals including humans are recognized as intermediate or paratenic host of *T. gondii.* Stray cats as predators often come into contact with various animals and may contaminate soils with oocysts excreted in the feces. Thus *T. gondii* can be transmitted to humans directly via soils and/or indirectly via livestock (reviewed by Dubey and Beattie, 1988). Therefore the prevalence of *T. gondii* in stray cats is critically important in view of zoonotic parasites.

A large number of surveys have been conducted on the incidence of *T. gondii* oocysts in feces and antibodies to *T. gondii* in sera of stray or domiciled cats in urban and rural areas (reviewed by Dubey, 1986; Dubey and Beattie, 1988; and Jackson and Hutchson, 1989). Usually oocyst shedding cases were rare with near 1% detection rate except for a few cases of high rate 41.3%. On the other hand, the rate of antibody positive cases were reported up to 96% with a wide range of variation. Such variation may be due to many factors such as years and seasons, countries and districts, urban or rural areas, stray or domiciled life styles, sanitary circumstances and also differences in examination techniques, especially for antibody titers.

In the present study, simultaneous examinations on *T. gondii* oocysts in feces and antibody titers in sera were carried out in stray cats obtained from the western area of Japan over a two-year period.

[1] *Aburahi Laboratories, Shionogi Research Laboratories, Shionogi & Co., Ltd., Koka-cho, Shiga 520-34, Japan*

[2] *Department of Veterinary Physiology and Protozoan Immunology, Obihiro University, School of Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080, Japan*

[3] *Department of Protozoology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan*

[4] *Department of Parasitology, Fujita Health University, School of Medicine, Toyoake, Aichi 470-11, Japan*

及川　弘　神田政典　三日月幸治（塩野義製薬油日ラボラトリーズ）
小俣吉孝（帯広畜産大学獣医学部）
矢野健一（大阪大学微生物病研究所）
中林敏夫（藤田学園保健衛生大学医学部）

Materials and Methods

Cats

Three hundred and thirty-five stray or feral adult cats of both sexes (304 males and 31 females) of unknown age, weighing from 2.5 to 4.0 kg, and six stray kittens after weaning were obtained by animal dealers from the western area of Japan including 15 prefectures in Kinki, Chugoku and Shikoku area over the two-year period from April 1988 to March 1990.

The cats were taken to Aburahi Laboratories and housed individually in aluminum cages in isolated boxes to prevent airborne or contagious diseases. The animal room was kept conditioned at $21 \pm 2°C$ and 30–60% relative humidity. The animals were fed daily with commercial dry cat food and canned fish meat. Water was available *ad libitum*.

Fecal examination for oocysts

Feces were collected daily, but some cats did not defecate for the first few days in the new surroundings. The examination was done at least 3 times per cat for the first two weeks.

Feces suspended in a 33% zinc sulfate solution (S.G. 1.18) were filtrated into a tube using a sheet of gauze and centrifuged at 2,000 rpm for 5 min. Flots were taken onto slide glass and examined under a microscope ($\times 200$ and $\times 400$). Daily oocyst count was done by using hemocytometor.

Oocysts collected from the cats' feces were kept in 2% (v/v) sulfuric acid at 20°C for 4 days for sporulation and then stored in a refrigerator at 4°C.

Serological test

Cats newly brought to the laboratory were weighed and bled by venous puncture under anesthesia with ketamine hydrochloride. Sera were stored at –20°C until used.

For serological testing, endozoites of the RH strain of *T. gondii* were harvested from *in vitro* cultures of infected murine embryonic cells. The parasites were fixed with 1% paraformaldehyde in phosphate buffered saline (PBS) at 4°C for 15 min, mounted onto slide glasses and dried at room temperature, and then stored at –70°C until used. Titration of antitoxoplasma IgG antibodies was performed by indirect immunofluorescence assay (IFA) (Omata et al., 1989, Omata et al., 1990).

Serial 4 fold dilutions of the test serum were prepared in PBS. The diluted samples were mounted onto the antigen coated slide glass and incubated at 4°C for 15 min. The slides were washed in PBS 3 times for 5 min each, and the incubated with FITC anti-cat IgG at 37°C for 5 min. The slides were washed in PBS 3 times. Specific fluorescence of the sample was observed by a fluorescence microscope. The IFA titers at 1:4 or higher were judged as positive.

Identification of the parasite

Sporulated oocysts obtained from a stray cat were inoculated orally into DS-mice bred in a parasite-free colony at Aburahi laboratories. The mice were sacrificed 30 days after inoculation. The stumped smears of the brains were observed under a microscope to detect cysts.

Experimental infection in kittens

Kittens bred free of Toxoplasma infection and kept in an isolated box were used for experimental infection. The rearing conditions were about the same as those described above for stray cats.

Sporulated oocysts from a stray cat (No. 88-093) were inoculated orally into kittens weighing 540–840 g at 47–74 days of age. Daily oocyst examination and once or twice weekly antibody titration were done before and up to 30 days after inoculation.

Cysts from mouse brains and oocysts from kitten infected with a laboratory strain (S-273) of *T. gondii* were inoculated orally into kittens at 10^2 cysts/kitten and 5×10^4 oocysts/kitten, respectively. Oocyst in feces and serum antibody production in those kittens were traced for 30 days after inoculation.

Results

Survey of stray adult cats

Of 335 adult cats tested, oocysts were detected

in one cat (0.3%) and antibody was positive in 86 cats (25.7%) (Table 1).

The rate of antibody positives was rather high as 53.8% (70/130) in the first year, and low as 7.8% (16/205) in the second year. The difference between the two years was significant at $P<0.005$ by the χ^2 test. The difference among four seasons during two-year period was significant at $P<0.005$. Seasonal changes of positive rates was as Jul.-Sep. > Oct.-Dec. > Jan.-Mar. > Apr.-Jun. in order.

A case of natural infection

One adult male cat (No. 88-093) weighing 3 kg began to shed oocysts at 4 days after being brought to this laboratory. Oocysts were shed for 3 days with the total number of oocysts over 1×10^6. Antibodies to *T. gondii* was negative during oocyst shedding and turned to positive thereafter (Table 2).

By the inoculation of the sporulated oocysts from a stray cat (No. 88-093) into mice, a large number of cysts were recovered from the brains of the mice 30 days after inoculation. Thus the oocysts of the strain was identified as *T. gondii*.

Survey of stray kittens

Six weaned orphan kittens aged about 6 weeks old were brought to the laboratory. No oocysts were detected from all the kittens but antibodies were positive in three stray kittens and negative in three domiciled kittens (Table 3). It is not known wheather the antibody found was due to congenital or post-natal infection.

Table 1. Incidence of *Toxoplasma gondii* oocysts in feces and antibody in sera of stray adult cats in the western area of Japan

Year	Month	No. of cats examined	No. of cats with oocysts	No. of cats with antibody (%)	
1988	Apr.–Jun.	46	1*	15 (32.6)	
	Jul.–Sep.	29	0	29 (100.0)	(53.8)
	Oct.–Dec.	26	0	14 (53.8)	
1989	Jan.–Mar.	29	0	12 (41.4)	
	Apr.–Jun.	69	0	3 (4.3)	
	Jul.–Sep.	52	0	6 (11.5)	(7.8)
	Oct.–Dec.	41	0	3 (7.3)	
1990	Jan.–Mar.	43	0	4 (9.3)	
Total		335**	1(0.3%)	86 (25.7)	

* Positive for oocysts but negative for antibody

** Male: 304, female: 31

Table 2. A case of natural infection of *Toxoplasma gondii* in a stray tom-cat

	Date of observation (1988)										
	13/ Jun.	14/ Jun.	15/ Jun.	16/ Jun.	17/ Jun.	18/ Jun.	19/ Jun.	22/ Jun.	4/ Jul.	13/ Jul.	1/ Aug.
Clin. sign	–	–	–	–	–	–	–	–	–	–	–
Oocyst shedding	X	X	–	++	+++	++	–	–	–	–	–
Antibody titer	<4							<4	4^4	4^7	$4^7<$

X: No defecation

++: Moderate, +++: Heavy

Table 3. Observation for oocyst shedding and antibody titers to *Toxoplasma gondii* in stray and domiciled kittens

		Period after introduction into the laboratory						
		0			1 week	2 weeks		3 weeks
Kitten No.	Sex	Age	Oocysts	Antibody titer	Oocysts	Oocysts	Antibody titer	Oocysts
1[a]	F	ca.6W[b]	–	4^6	–	–	4^4	–
2[a]	F	ca.6W	–	4^1	–	–	4^4	–
3	F	ca.6W	–	4^4	–	–	4^3	–
4–6	F&M	ca.6W	–	<4	–	–	<4	–

a) Litter mates b) W = weeks of age
No. 1–3: Stray kittens No. 4–6: Domiciled kittens

Experimental infection in kittens

The characteristics of a field strain (No. 88-093) of *T. gondii* were compared with those of a laboratory strain (S-273). The results are shown in Table 4. The prepatent and patent periods in regard to oocyst shedding were 20 days and 7 days, respectively, and the figure were almost same as those of the laboratory strain.

Typical changes in oocyst shedding and antibody production of the cat infected with *T. gondii* by oral inoculation of cysts or oocysts of the laboratory strain (S-273) and oocysts of the field strain (88-093) are shown in Fig. 1. The field case which is shown in Table 2 was similar to the case by cyst-induced situation which is shown in the top panel of Fig. 1, but not to the oocyst-induced one.

Discussion

With Japanese stray cats, a low isolation rate of *T. gondii* oocysts in feces, but a high positive rate of antibodies to *T. gondii* in sera have commonly been observed. In 1972, in a survey of 90 cats of all ages belonging to U.S. Forces families in Japan, fecal oocysts were isolated from one (1.1%) adult cat and serum antibodies to *T. gondii* were found in 44% of the cats tested by indirect fluorescent antibody test (IFAT) (Werner and Walton, 1972). A study of 100 feral cats in the Osaka area in 1974, oocysts was not isolated but a high positive rate (60.2%) was found for antibodies to *T. gondii* by the HA test (Iseki et al., 1974). In 1974 survey in the Tokyo area, Toxoplasma oocysts were isolated from 4

Table 4. Some characteristics of a field strain (88-093) compared with the laboratory strain (S-273) of *Toxoplasma gondii* in cats

Strain	Infection	Host	Prepatent period	Patent period	Total number of oocysts shedded	Antibody titers at oocyst shedding
88-093	Spontaneous	1 tom-cat	unknown	3 days	1×10^6	–
(field)	Oocysts 5×10^4po*	1 kitten	20 days	7 days	190×10^6	4^7
S-273	Cysts po	9 kittens	4–9 days	6–7 days	$2–280\times10^6$	–
(laboratory)	Oocysts 5×10^4po	1 kitten	23 days	4 days	9×10^6	4^6
	Oocysts 10×10^4po	2 kittens	18&20 days	7&9 days	$170\&920\times10^6$	$4^4\&4^7$

* Per os

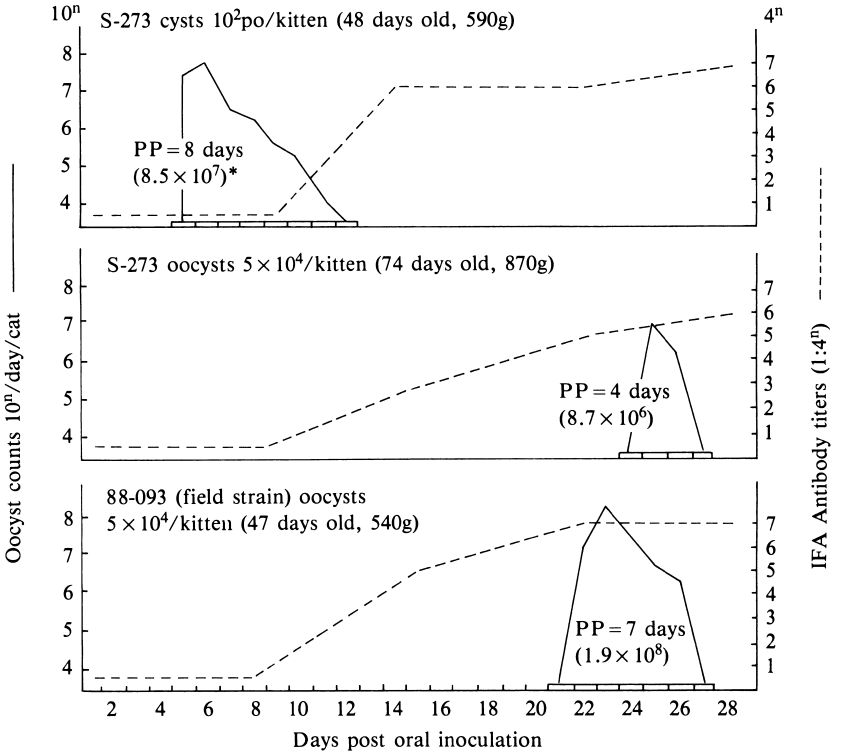

Fig. 1. Changes in oocyst shedding and antibody titers to *Toxoplasma gondii* post oral inoculation of S-273 cysts, S-273 oocysts and 88-093 (field strain) oocysts in kittens.

(0.9%) out of 446 young kittens weighing less than 500 g. No oocysts were isolated from kittens over 500 g and adult cats. (Ito et al., 1974). Over a five year period from 1973, 14 (0.9%) of 1515 stray kittens in the Tokyo area exhibited oocysts in feces. (Shimura et al., 1978). In 1985, of 151 stray adult cats in Tokyo and its suburbs, 33% were positive for antibodies to *T. gondii* when tested by the Latex Agglutination test, but no oocysts were isolated (Tanaka et al., 1985).

In the present survey, oocysts were isolated from one stray adult cat corresponding to 0.3% of 335 cats. The oocysts were identified as *T. gondii* by their morphological features and by infection tests with mice and kittens. Antibodies were found in 25.7% of the case tested during a period of two years, which was less than the rate in the references cited.

The low rate of oocyst isolation in feces may have been due to the short duration of the patent period in the individual cats. Repeated examination of the fecal samples may increase the isolation rate.

Three infectious stages is known in *T. gondii* 1: Tachyzoites or endozoites, 2: bradyzoites or cysts, 3: oocysts. The last two are important in natural infection. In experimental infection with cysts or oocysts by oral administration, the changing patterns of antibody titers were almost similar but the prepatent period for oocyst shedding was quite different, being about 21 days in a oocyst-infected case and within 7 days in cyst-infected one, as shown in Fig. 1. The reason for the shorter prepatent period in cyst-induced than oocyst-induced has not been testified experimentally as is discussed by Freyre et al. (1989).

Thus one case of natural infection (cat No. 88-093) in this report is thought to have been cyst-induced and not oocyst-induced.

Since most cats are thought to be infected with *T. gondii,* as is proved by the high incidence of antibodies, and the shedding as many as 10^8 oocysts in their feces for several days during their infection, and the oocysts can survive many months in soils (reviewed by Jackson and Hutchson, 1989), public hygiene for soil contamination with oocysts excreted by cats in public places and private gardens is to be seriously considered.

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