Detection and Confirmation of *Toxoplasma* Oocysts in the Soil

Shingo ITO and Kiyoshi TSUNODA

National Institute of Animal Health, Kodaira-shi, Tokyo 187

Yoshiatsu TSUTSUMI

Daiichi Seiyaku Co., Ltd., Nihombashi 3, Chuo-ku, Tokyo 103

Toshihiro MATSUI

Department of Parasitology, School of Medicine, Kyorin University, Mitaka-shi, Tokyo 181

Hiroaki NISHIKAWA

Department of Parasitology, The Jikei University School of Medicine, Minato-ku, Tokyo 105

Tatsuo IIDA and Yoshihide SASAKI

*Shizuoka Prefectural Institute of Animal Health,
1-2-45 Aoba-cho, Fujieda-shi, Shizuoka 426*

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Abstract. An outbreak of swine toxoplasmosis occurred in a hoggerly in Shizuoka Prefecture. In the hoggerly, pigs had been fed soil with pig-feed for 3 consecutive days from 1 week before the outbreak of the disease. *Toxoplasma* oocysts were detected directly from the soil in the hoggerly.

A combination (Method III) of ultrasonic treatment and sugar floatation method gave satisfactory results. Moreover, the isolation of *Toxoplasma* was established by the inoculation test on mice with the suspension of oocysts collected by Method III. Therefore, the causal agent of swine toxoplasmosis in the hoggerly was the *Toxoplasma* oocyst found in the soil examined. It was indicated that *Toxoplasma* oocysts played an important role in the transmission of toxoplasmosis in natural conditions.

Since the coccidial stage of *Toxoplasma* was clarified [2], *Toxoplasma* oocysts have been assumed to play an important role for the spread of toxoplasmosis. However, it has been considered difficult to clarify the relationship between the natural outbreak of swine toxoplasmosis and *Toxoplasma* oocysts. Therefore, the authors have had an intention to ascertain that a natural outbreak of swine toxoplasmosis is caused by *Toxoplasma* oocyst infection. Recently, they

had a chance to examine a natural outbreak of swine toxoplasmosis in a hoggerly in Shizuoka Prefecture. A detailed report on this outbreak will be published in near future by Sasaki, one of the authors, and his associates. In this hoggerly soil had been fed to pigs with a diet for 3 consecutive days beginning at 7 days before the first case of the disease appeared. Therefore, it was presumed that a causal agent of this disease might have been derived from the soil.

土からの *Toxoplasma* オオシストの検出およびその確認: 伊藤進午・角田 清 (農林省家畜衛生試験場), 堤可厚 (第一製薬株式会社), 松井利博 (杏林大学医学部寄生虫学教室), 西川洋昭 (東京慈恵会医科大学寄生虫学教室), 飯田辰夫・佐々木栄英 (静岡県家畜衛生研究所)

The present report deals with the microscopic detection of *Toxoplasma* oocysts from the soil in the hogger and the identification of the detected oocysts by the mouse inoculation test.

Materials and Methods

Soil: The specimen of soil used for detection and isolation of *Toxoplasma* oocysts was obtained from the original soil which had been fed to pigs with a diet for 3 consecutive days beginning at 7 days before the natural outbreak of toxoplasmosis in the hogger.

Mice: CFW mice weighing about 15 g were used in the present studies. They were inoculated with a suspension of oocysts collected from the soil to isolate *Toxoplasma*. They were also used for the passage of the isolated *Toxoplasma*.

Kittens: Kittens weighing less than 500 g free from *Toxoplasma* and/or other coccidia were used

for the inoculation test with each visceral organ of mice at the 1st and 2nd *Toxoplasma* passage level.

Detection of *Toxoplasma* oocysts from soil: Several different techniques were employed comparatively. To the soil specimen was added a sufficient volume of water and mixed well to make a homogeneous suspension. The suspension was allowed to stand for a given period of time and centrifuged at 3,000 rpm for 5 minutes. The resulting supernatant was discarded and the remaining sediment used as an original sample of soil. Then the following four methods were carried out.

Method I—Sugar floatation method: Ten tubes 13 mm in diameter and 100 mm in length of specimen were prepared separately from each sample of sediment, A (0.5 g), B (1 g) or C (2 g), and subjected to the sugar floatation method (specific gravity: 1.266).

Method II—RD method: This method was originally devised by Dubey et al. [1] and applied by Ruiz et al. [8] to the isolation of *Toxoplasma* from soil. In the present studies, it was somewhat modified in

Fig. 1. Procedure of Method III for detecting oocysts from soil

the procedure following the 2nd centrifugation for the microscopic examination of oocysts. In the modified procedure, 0.5 ml of the surface layer of the resultant supernatant was mixed with more than 5 ml of water, instead of 5 ml of 2% sulfuric acid used in the original procedure, and the mixture centrifuged at 3,000 rpm for 20 minutes. The resultant supernatant was discarded and the sediment resuspended in a small amount of water in order to make a suspension for the microscopic examination. Eight tubes were used for each specimen.

Method III—Combined method of ultrasonic treatment and sugar floatation (US method): This method was unique, since ultrasonic treatment by the ultrasonic cleaner of Branson (Bransonic 220; frequency: 50 KHz) was applied prior to the sugar floatation method, as shown in Fig. 1.

Method IV—Stirring method: This method was the same as Method III, except the 3rd line in Fig. 1. Stirring by a magnetic stirrer of the 2nd line was applied for 20–40 minutes, instead of ultrasonic treatment.

In the 1st experiment, Methods I, II and III were compared with regard to merits and demerits for the detection of oocysts from one drop of material covered with a coverglass 18 mm square in size by means of 20 x objective and 10 x eyepiece.

In the 2nd experiment, Methods III and IV were compared with regard to the number of oocysts detected from 1/3 volume of each final suspension.

Isolation of Toxoplasma from oocyst suspension originated from soil on test: To confirm the detected oocyst to be that of Toxoplasma, 0.2 ml of each oocyst suspension was inoculated into five mice orally.

The isolated Toxoplasma was maintained by inoculating with the suspension of the mesenteric lymph node of the 1st passage mouse at first, and then with the peritoneal fluid of the 2nd passage mouse.

Inoculation test of the isolated Toxoplasma with kittens: Seven kittens were employed to examine the infectivity of the isolated Toxoplasma or the oocyst discharged in their feces.

Five of the seven kittens were inoculated orally with organ suspensions originated from two mice at the 1st passage level. Kitten No. 1 was inoculated with 6.0 ml of pooled suspension of liver, spleen, and lung from these mice. Kitten No. 2 was injected with 4.5 ml of the mesenteric lymph node suspension and No. 3 with 5.0 ml of the suspension of the ileum. Kitten Nos. 4 and 5 were inoculated with 3.0 ml of the pooled suspension of the brain and

heart muscle.

The remaining two kittens, Nos. 6 and 7, were inoculated with the brain suspension derived from two mice at the 2nd passage level which were killed 44 days after infection. Each kitten was given such amount of the brain suspension as derived from the organ of one mouse.

Sabin-Feldman dye test: The sera of two mice killed 10 days after inoculation with the oocyst suspension originated from the soil were examined by the Sabin-Feldman dye test. The sera of four kittens, Nos. 4 to 7, were subjected to the same test. All the sera used for the test were collected before inoculation and at the time of sacrifice after inoculation.

Results

1. Detection of Toxoplasma oocysts from soil

In the 1st experiment, three methods, I to III, were applied to 48 tubes of the specimen for the detection of Toxoplasma oocysts. As a result, suspected Toxoplasma oocysts were detected by every method, although Method III gave the best results, as shown in Table 1.

In the 2nd experiment, Methods III and IV were compared to confirm the usefulness of ultrasonic treatment in Method III. Method III gave better results than Method IV, as shown in Table 2, although oocysts were detected by both methods.

In addition, oocysts of *Isospora felis* were also detected from the soil by Methods I, III and IV.

2. Isolation of Toxoplasma from the soil

Five mice were inoculated orally with a suspension of oocysts obtained by Method III. Of them, three died 8, 9 and 10 days after inoculation, showing characteristic pathological changes. Toxoplasma organisms were demonstrated from the mesenteric lymph nodes and other organs by direct microscopic examination. The remaining two mice were moribund and killed 10 days after inoculation. Organs were collected from them and inoculated into five kittens,

Table 1. Detection of *Toxoplasma* oocysts from soil (1)

Method for detection of oocysts	Amount of soil in each tube (g)		Number of tubes examined	Presence or absence of oocysts									
	A	0.5		+	F	F	F	—	—	—	—	—	—
Method I (Sugar floatation method)	B	1	10	F	F	—	—	—	—	—	—	—	—
	C	2	10	—	—	—	—	—	—	—	—	—	—
Method II (RD method)		5	8	+	—	—	—	—	—	—	—	—	—
Method III (US method)		5	10	+	+	+	+	+	+	F	—	—	—

Remarks.

+: *Toxoplasma* oocysts were detected.F: *I. felis* oocysts were detected.

—: No oocysts were detected.

Table 2. Detection of *Toxoplasma* oocysts from soil (2)

Method for detection of oocysts	Number of oocysts detected*			
	Toxoplasma		<i>I. felis</i>	
	Unsporulated oocysts	Sporulated oocysts	Unsporulated oocysts	Sporulated oocysts
Method III (US method)	10	23	11	15
Total**	33		26	
Method IV (Stirring method)	0	1	8	8
Total**	1		16	

Remarks.

*: The number of oocysts detected from 1/3 volume of each final suspension.

**: The total mean number of unsporulated and sporulated oocysts.

Nos. 1 to 5.

The saline suspension of the mesenteric lymph node of a mouse which died 8 days after inoculation was inoculated intraperitoneally into five mice for the 2nd passage.

All the five mice at the 2nd passage level began to show such clinical symptoms as observed usually in experimental toxoplasmosis 6 or 7 days after inoculation, although all of them recovered from the disease. The peritoneal fluid was obtained from one of these mice 9 days after inoculation and used for the next mouse passage. The isolate was named the SS strain to distinguish it from any other *Toxoplasma* strain. The sub-

sequent passage of this strain was conducted at intervals of 5 to 9 days. Two of five surviving mice at the 2nd passage level were killed 44 days after inoculation and their brains were inoculated to kittens.

3. Inoculation test of kittens with isolated *Toxoplasma*

Two of five kittens (Nos. 4 and 5) inoculated with the organ suspensions of two mice at the 1st passage level began to discharge *Toxoplasma* oocysts 28 and 29 days after inoculation, respectively. The discharge of oocysts continued until they were sacrificed 41 days after inoculation. No other kittens (Nos. 1, 2 and 3) discharged Toxo-

plasma oocysts.

Two kittens inoculated with the brain suspension of two mice at the 2nd passage level (Nos. 6 and 7) began to discharge Toxoplasma oocysts 5 days after inoculation. The discharge of oocysts continued until they were sacrificed 17 days after inoculation.

The number of oocysts per gram of feces (OPG) reached a maximum of approximately 10^7 in four kittens, Nos. 4, 5, 6 and 7. Such a large OPG as 10^7 continued for 4 to 8 days, and then a level of 10^6 for 5 to 10 days. These four kittens were killed at the termination of oocyst discharge, and examined for endogenous Toxoplasma and pathological changes in the visceral organs. As a result, Toxoplasma was demonstrated in almost all the organs examined, but no pathological changes were observed in any organ.

4. Sabin-Feldman dye test

In the two mice at the 1st passage level, the dye test titer increased from negative to 1 : 16 after the oocyst inoculation. In the four kittens positive for Toxoplasma oocysts, it was 1 : 256 for Nos. 4 and 5, 1 : 1024 for No. 6, and 1 : 4096 for No. 7, although it was negative before infection. Thus the isolated organisms were also confirmed serologically to belong to Toxoplasma.

Discussion

Toxoplasma oocyst has been suspected to be a very important causal agent of toxoplasmosis, because of its high resistance [5] to various physical and chemical influences and changes in environmental conditions, except heating [6] and drying. It was confirmed experimentally that Toxoplasma oocysts play a very important role in the spread of toxoplasmosis [3, 4, 9]. Toxoplasma oocyst, however, had not yet been demonstrated as one of the most important causal agents of swine toxoplasmosis in nat-

ural conditions until the present studies were completed.

Toxoplasma has been demonstrated in soil. Ruiz et al. [8] isolated Toxoplasma by mouse inoculation from soil specimens collected in a yard which many cats inhabited in Costa Rica. On the other hand, Plant et al. [7] reported an outbreak of ovine toxoplasmosis with abortion, which was suspected to have been induced by the contamination of grains for ewes by cat feces containing Toxoplasma oocysts.

In the present studies, the authors succeeded in detecting Toxoplasma oocysts by direct microscopic examination in soil specimens collected from a hogger, in which a natural outbreak of swine toxoplasmosis occurred. The soil was considered to have been contaminated with cat feces, because *Isospora felis* oocysts were detected from the soil specimens. Moreover, the oocysts isolated from the soil were identified as those of Toxoplasma by the mouse inoculation test, although this test was conducted 217 days after the outbreak of swine toxoplasmosis in the hogger. These results had been anticipated easily from the authors' unpublished data that Toxoplasma oocysts of the Beverley strain were infective to mice even after storage at 0–4°C for 3 years.

Method III used in the present studies was considered to be a useful technique for the detection of Toxoplasma oocysts in the soil. It may not always give highly positive results when applied to soil specimens, especially when such specimens contain a very small number of oocysts.

From the results obtained, it was found that the present outbreak of the disease had been caused by Toxoplasma oocysts contained in the soil. Toxoplasma oocysts really play an important role in the spread of swine toxoplasmosis in natural conditions.

References

- [1] Dubey, J. P., Swan, G. V., and Frenkel, J. K. (1972). A simplified method for the isolation of *Toxoplasma gondii* from the feces of cats. *J. Parasitol.*, **58**, 1005-1006.
- [2] Frenkel, J. K. (1973). Toxoplasma in and around us. *Bio-Science*, **23**, 343-352.
- [3] Ito, S., Tsunoda, K., Fujita, J., and Nishikawa, H. (1971). Successful oral infection with fecal form of Toxoplasma to specific-pathogen-free piglets. *Jap. J. vet. Sci.*, **33** (Suppl.), 247.
- [4] Ito, S., Tsunoda, K., Nishikawa, H., and Matsui, T. (1974). Pathogenicity for piglets of Toxoplasma oocysts originated from naturally infected cat. *Nat. Inst. Anim. Hlth Quart.*, **14**, 182-187.
- [5] Ito, S., Tsunoda, K., Shimada, K., Taki, T., and Matsui, T. (1975). Disinfectant effects of several chemicals against Toxoplasma oocysts. *Jap. J. vet. Sci.*, **37**, 229-234.
- [6] Ito, S., Tsunoda, K., Taki, T., Nishikawa, H., and Matsui, T. (1975). Destructive effect of heating against Toxoplasma oocysts. *Nat. Inst. Anim. Hlth Quart.*, **15**, 128-130.
- [7] Plant, J. W., Richardson, N., and Moyle, G. G. (1974). Toxoplasma infection and abortion in sheep associated with feeding of grain contaminated with cat feces. *Aust. vet. J.*, **50**, 19-21.
- [8] Ruiz, A., Frenkel, J. K., and Cerdas, L. (1973). Isolation of Toxoplasma from soil. *J. Parasitol.*, **59**, 204-206.
- [9] Sasaki, Y., Iida, T., Oomura, K., Tsutsumi, Y., Tsunoda, K., Ito, S., and Nishikawa, H. (1974). Experimental Toxoplasma infection of pigs with oocysts of *Isospora bigemina* of feline origin. *Jap. J. vet. Sci.*, **36**, 459-465.