

# STUDIES ON THE USE OF COBALT-60 IRRADIATION TO CONTROL INFECTIVITY OF *TOXOPLASMA GONDII* CYSTS

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**Abstract.** Mouse brains harboring the Chinese NT strain of *Toxoplasma gondii* cysts were homogenized with normal saline and irradiated with cobalt-60 gamma rays at various doses. The homogenate was introduced intraperitoneally into NIH mice or per os into kittens. Loss of infectivity was confirmed according to the following criteria: no cyst found in mouse brain impression smears on the 50th day after inoculation; no oocyst found in feces of kittens 3–15 days after inoculation; subinoculation in mice and a negative IHA test. All bioassays, parasitological examinations and serological tests in the control group gave positive results. Activity of radioactive source: 10 KCi; uniform dosage: 1238 rad/min; dose range of irradiation: 0.1–1.0 KGy. Minimal effective dose of gamma rays to control infectivity of *T. gondii* cysts was 0.55 KGy. Infectivity of bradyzoites irradiated with gamma rays at a dose of 0.45 KGy decreased by 10,000 times. Minimal effective dose of gamma rays to control infectivity of American ME-49 and Ts-2 strain, is slightly higher (0.6KGy) than that of NT strain. These studies present useful data for practical use of cobalt-60 to control infectivity of *T. gondii* in meat products.

## INTRODUCTION

Toxoplasmosis is a cosmopolitan zoonosis which can be contacted congenitally or by ingestion of the infected host or oocysts. It accounts for numerous deaths in AIDS patients and is a serious problem in animal husbandry. The disease is present in 28 provinces (municipalities, autonomous regions) in China (Yu and Xu, 1988). The average seropositive rate of antibody against *Toxoplasma gondii* in human populations is approximately 10%, with the highest over 30%. It is 21.0% in Hangzhou region, where 63.37% of the butchers (Fu *et al*, 1989) and 52.79% of the swine are seropositive (Fu *et al*, 1987). Cysts in porcine muscle are a major source of infection. Gamma rays are effective in killing certain species of parasites in meat products (Brake *et al*, 1985; Dubey *et al*, 1986). For this reason, studies on the effect of cobalt-60 gamma rays to control infectivity of the Chinese NT strain *T. gondii* were conducted during 1988–1989. Comparative studies were also carried out between Chinese and American strains isolated from different geographical origins.

## MATERIALS AND METHODS

### Parasite

The NT strain of *T. gondii* was isolated from infected pigs by the Nantong Animal Husbandry and Veterinary Station in Nantong County, Jiangsu Province. ME-49 and Ts-2 strains of American origin were provided by Dr JP Dubey of the US Department of Agriculture. These strains were maintained in mice in our laboratory.

### Animals

Female NIH mice, 20–25 g body weight, were provided by Zhejiang Center for Experimental Animals. Kittens, 1–2 months of age with negative IHA reactions for toxoplasmosis, were also used.

### Preparation of specimens

Mouse brains containing cysts of *T. gondii* were triturated in two volumes of normal saline and passed through a 100 mesh copper wire sieve to make homogenate. This was put into 10 ml plastic vials (2.5×5.0 cm) and kept for irradiation experiments.

In the studies on the effect of gamma rays to control the infectivity of the bradyzoites, the irradiated homogenate was added to equal parts of digestive juice (0.4% trypsinase solution) and incubated at 37° C for 20 min. Normal saline was added and centrifuged to remove the digestive juice. Normal saline was added to the sediment of released bradyzoites to make a 10<sup>-1</sup> suspension. Five successive 10 fold dilutions were made to obtain 10<sup>-2</sup>-10<sup>-6</sup> suspensions. The whole procedure of digestion, washing and inoculation of mice was completed within 2 hours.

In the comparative study on different geographic strains, the procedure was the same except the digestion and ten-fold dilutions were omitted. The density of cysts in the homogenate was about 12 cysts/ml. The homogenates of ME-49 and Ts-2 strains were mixed in equal volumes.

#### Radioactive source and dosage

Cobalt-60 gamma rays were applied with an activity of 10 KCy, providing a uniform dosage of 1,238 rad/min. Doses of irradiation at different spots where the specimens were located was measured with a Frick Ferrous-Sulfate Dosimeter. The dose range in the experiment was 0.1-1.0 KGy.

#### Determination of infectivity

The infectivity of *T. gondii* was confirmed by bioassay in mice or kittens. One ml of homogenate or suspension irradiated at various doses (including that of the control group) was injected intraperitoneally into each mouse. The mice were necropsied on the 50th day after inoculation and impression smears of the cerebral cortex were made and examined for *Toxoplasma* cysts. IHA test was carried out with carotid blood. A titer higher than or equal to 1:64 was judged as positive. If kittens were used in the bioassay, 4 ml of homogenate were fed per os. Examination of kitten feces for oocysts started on the third day after inoculation, and continuing for 15 consecutive days. IHA test was conducted on the 7th day after inoculation. Blind subinoculation was done if the above mentioned results were all negative.

Loss of infectivity was confirmed if the bioassay also showed negative results and the results for the control group were all positive. The minimal

effective dose to control infectivity of the *T. gondii* was determined.

## RESULTS

**Experiment 1.** Effective dose of gamma rays to control infectivity of NT strain *T. gondii* cysts: After the mouse brain homogenates containing cysts of NT strain *T. gondii* (not treated with digestive juice or diluted) were irradiated at 0.10, 0.25, 0.50, 0.75 and 1.00 KGy, they were inoculated intraperitoneally into mice or fed orally to kittens. No cysts were found in the 1.00 and 0.75 KGy groups at the 50th day after inoculation, and the IHA test and blind subinoculation also showed negative results. Cysts were found in all other groups, and the IHA test also showed positive reactions. The number of cysts per mouse detected and the number of positive mice were both related to the doses applied. The geometric mean titers of IHA for doses of 0 (control group), 0.10, 0.25 and 0.50 KGy were 203, 161, 90 and 64 respectively. Results of experiments on kittens were consistent with that of mice, no oocysts were found in kittens of the 1.00 or 0.75 KGy dose groups and the IHA tests were also negative. In the control and other groups all the results were positive (Table 1).

**Experiment 2.** Minimal effective dose (MED) of gamma rays to control infectivity of NT strain *T. gondii* cysts: On the basis of the former experiments gamma ray doses at 0.50, 0.55, 0.60 and 0.65 KGy were applied to mouse brain homogenates, and then inoculated into healthy NIH mice. Mice of the control group were inoculated with non-irradiated cysts. On the 50th day post-infection cysts were detected in every mouse of the control group and the IHA test were positive. Cysts were found in one out of 7 mice in the 0.50 KGy group, and the IHA test was also positive, consistent with the former experiment. No cysts were found in mice of the 0.55 KGy or stronger dose groups. IHA tests in these groups were all negative (Table 2). These results show that the MED of gamma rays to control infectivity of NT strain *T. gondii* cysts is close to 0.55 KGy.

**Experiment 3:** Effect of gamma rays at various doses on infectivity of NT strain *T. gondii* bradyzoites: Mice of different groups were inoculated with bradyzoite-containing suspensions irradiated at various doses of gamma rays

Table 1

Examination for cysts in mouse brains on the 50th day after infection with NT strain *Toxoplasma gondii* cysts irradiated at various doses of r-rays.

Dose (KGy)	No. mice infected	Cyst-positive		
		No. mice	Positive rate (%)	Mean density*
0	8	8	100	3.13
0.10	8	6	75	1.73
0.25	8	4	50	0.92
0.50	8	2	25	0.83
0.75	8	0	0	0
1.00	8	0	0	0

\* denotes the number of cysts detected on an area of 400 mm<sup>2</sup> of mouse brain impression smear.

Table 2

Examination for cyst in mouse brain and detection of antibody by IHA in sera of mice on the 50th day after inoculation with one NT strain *T. gondii* cysts irradiated at various doses of r-rays.

Dose (KGy)	No. mice inoculated	Cyst-positive		IHA-positive	
		No. mice	Percentage	No. mice	Percentage
0	8	8	100	8	100
0.50	7	1	14	1	14
0.55	8	0	0	0	0
0.60	8	0	0	0	0
0.65	8	0	0	0	0

(bradyzoite-containing suspension prepared with cyst-containing mouse brain homogenate irradiated with gamma rays at various doses, treated with digestive juice and diluted at different multiples). Results showed that mice in the control group were infected when inoculated with non-irradiated bradyzoite suspension diluted up to 10<sup>-5</sup>. The multiple dilutions decreased along with the increase of gamma ray doses. When the gamma ray doses increased from 0.10, 0.25, 0.35 up to 0.45 KGy, the maximal dilutions of bradyzoite-containing suspension capable of infecting mice were in the following order: 10<sup>-5</sup>, 10<sup>-3</sup>, 10<sup>-2</sup> and 10<sup>-1</sup> (Table 3). The ratio of the number of bradyzoites needed to infect the mice between the 0.1 and 0.45 KGy dose groups was 10 suggesting that

the infectivity of bradyzoites irradiated at a dose of 0.45 KGy decreased by 10,000 times.

**Experiment 4:** *Toxoplasma gondii* from China and ME-49 Ts-2 strains from USA were irradiated with gamma rays at 0.4, 0.5, 0.6, 0.7 and 1.0 KGy and inoculated into mice or kittens. Results of the first experiment showed that no cyst or oocyst were found in mouse brain tissue or in feces of kittens when the dose of gamma rays was equal to or higher than 0.6 KGy, whereas large number of cysts or oocysts were found in every mouse of the control group. Although cysts or oocysts were also found in animals of the 0.5 and 0.4 KGy groups, their numbers were relatively low (Table 4). A second experiment was carried out

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Table 3

Infection of mice inoculated with *T. gondii* bradyzoite-containing suspension irradiated with r-rays and diluted at different multiples.

Dose (KGy)	Dilutions of cyst suspension					
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
0	5 (7.5)	5 (2.6)	5 (0.7)	4 (0.5)	2 (0.4)	0
0.10	5 (3.2)	5 (2.0)	5 (0.3)	2 (0.2)	1 (0.2)	0
0.25	3 (2.4)	2 (1.0)	2 (1.0)	0	0	0
0.35	2 (2.0)	1 (1.0)	0	0	0	0
0.45	1 (1.0)	0	0	0	0	0
0.55	0	0	0	0	0	0

5 mice in each group

Data are expressed as No. mice positive for *T. gondii* and its density showing in the ( )

Table 4

Examination of mouse brain for cyst on the 50th day after inoculation with three strains of *T. gondii* cysts irradiated at various doses of r-rays.

Dose (KGy)	No. mice inoculated	Cyst-positive					
		No. mice		Percentage		Mean density	
		ME-49/Ts-2	NT	ME-49/Ts-2	NT	ME-49/Ts-2	NT
0	10	10	10	100	100	290	33
0.40	10	6	2	60	20	2	2
I 0.50	10	2	1	20	10	1	1
0.60	10	0	0	0	0	0	0
0.70	10	0	0	0	0	0	0
0	8	8	8	100	100	-	-
0.50	8	2	1	25	13	-	-
II 0.55	8	1	0	13	0	-	-
0.60	8	0	0	0	0	-	-
0.65	8	0	0	0	0	-	-

"-" indicates no observation

Density denotes the number of cysts found in an area of 400 mm<sup>2</sup> of mouse brain impression smear.

aimed at seeking the MED. Results demonstrated that the MED of gamma rays to control infectivity of NT strain of *T. gondii* cysts was 0.55 KGy, while that for ME-49 and Ts-2 strains was 0.60 KGy (Table 4). This suggests that although there is a difference in radiosensitivity to cobalt-60 gamma rays between NT strain from China and ME-49 and Ts-2 strains from USA, the difference was not significant.

DISCUSSION

Dubey *et al* (1986) reported that a dose of 0.5 KGy, using Cs-137 and Co-60 as radioactive source, had been able to eliminate the infectivity of *T. gondii* cysts in a mixture of 8 strains (GT-1, Ts-1, ME-49, 740-2 and C<sub>1,2,3,4</sub>). Wikerhauser *et al* (1988) reported that r-irradiation of *T. gondii* tissue cysts with doses up to 0.5 KGy might

not be sufficient to control their infectivity. Our study indicates that the minimal effective dose to control infectivity of NT strain in mouse brain was 0.55 KGy when irradiated with cobalt-60. In our experiment serological assays as well as bioassay was used to determine the infectivity of cysts. Mice and kittens were used as experimental animals because they are more sensitive to *Toxoplasma* infection. Treatment with digestive juice removed the cyst membrane, liberated the bradyzoites into suspension, thus enhanced the opportunity of infecting their host and as a result improved the reliability of the experiment.

NT strain from China and ME-49, Ts-2 strains from USA are different geographic strains and exhibit some distinct differences in biological characteristics. Wikerhauser *et al* (1988) also reported that different isolates show different radiosensitivity. There were differences between the densities of cysts in mouse brain (number of cysts found in an area of 400 mm on a mouse brain impression smear) despite the same positivity rate (10/10) of inoculated mice. That is to say, the density of NT strain was 33 while that of ME-49 and Ts-2 strains was 290, implying a difference in intensity of infection. There were also some differences in tolerance toward gamma rays, namely, when the dose was 0.4 KGy, the positive rate of cyst in NT strain group was 20%, clearly lower than that of ME-49 and Ts-2 strains (Table 4). Meanwhile the minimal effective dose to control infectivity of NT strain *T. gondii* was 0.55 KGy, but that for ME-49 and Ts-2 strains was 0.60 KGy.

Cysts in mouse brain tissue were used as experimental material in this study. As for the radiosensitivity of cysts in porcine tissue, the work is just underway. On the basis of these experiments, it is evident that gamma rays are a powerful agent in killing cysts, 0.6 KGy can be

considered as an effective dose, which is far lower than 10 KGy, believed by FAO/IAEA/WHO as a safety dose.

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