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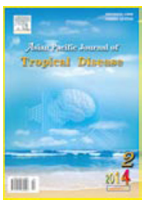

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Brain cystogenesis capacity of *Toxoplasma gondii*, avirulent Tehran strain in mice

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ABSTRACT

Objective: To investigate the brain cystogenesis capacity of Tehran strain of *Toxoplasma gondii* (*T. gondii*) that had been isolated from a patient with lymphadenitis in 1973.

Methods: A volume of 0.5 mL mice brain suspension containing 20 tissue cysts of Tehran strain of *T. gondii* was inoculated intraperitoneally to each of 25 male BALB/c mice. The number of brain cysts was counted in unstained squash-smears for 10 mice during weeks 7-9 and for 15 mice during weeks 13-14 post-infection. Nonparametric test of Mann-Whitney was used to demonstrate means differences.

Results: There was a significant difference in the means for the number of brain cysts between weeks 7-9 (228.3±144.8) and weeks 13-14 (1 239.8±429.3) post-infection ($P<0.05$). The minimum and the maximum of cysts were 70 and 1 531 during weeks 7-9 post-infection, and 12 and 5 170 during weeks 13-14 post-infection, respectively. The mean number of brain cysts in the right cerebral hemisphere was insignificantly higher than that of the left cerebral hemisphere. Furthermore, the number of cysts counted in the right or the left hemispheres was significantly higher than those enumerated for cerebellum+brain stem altogether.

Conclusions: It is concluded that the brain cystogenesis capacity of *T. gondii*, Tehran strain shows enormous variation in mice regarding the duration of infection. In addition, the cystogenesis observed in cerebellum+brain stem is lower than the right and left cerebral hemispheres.

1. Introduction

Toxoplasma gondii (*T. gondii*) is one of the most common protozoan parasites in humans and warm-blooded animals worldwide, so that, at least 20% of world populations are seropositive for this infection in most of developing[1-3] and developed countries[4-6].

Tissue cystogenesis is a part of developmental process of *T. gondii* occurring in both definitive and intermediate hosts. Tissue cysts are formed in many organs of the hosts, however, the frequency and distribution of cysts are partly controlled by the host and the strain of *T. gondii* involved[7]. In rats, higher number of tissue cysts is found in brain rather than in other organs[8], and therefore, the brain is considered as a selective organ for the *in vivo* cystogenesis of this parasite[9,10].

It has been shown that the tissue cysts form as early as six days post-infection in mice brain. They grow regularly and their size is stopped within less than 4 months. Young brain cysts may be measured as small as 5 μm in diameter containing only 2 bradyzoites, and older cysts reach up to 50-70 μm in diameter containing hundreds of bradyzoites[7]. The size of cysts may exceed 100 μm, as described in a report by Hooshyar *et al.* in which a cyst of about 125 μm was observed in the brain of a mouse, experimentally infected with local isolates of *T. gondii*[11].

Brain cystogenesis capacity as a biological characteristic shows significant diversities among avirulent strains of *T. gondii*[12]. Awareness of this capability of *T. gondii* strains will be helpful in using this parasite in chronic infections studies in mice. The Tehran strain of *T. gondii* is an

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avirulent strain isolated by Ghorbani *et al.* from a patient with lymphadenitis in Tehran, the capital of Iran, in 1973[13]. Since the initial isolation, it is maintained at the Department of Parasitology, Tehran University of Medical Sciences through intraperitoneal passages of brain homogenate containing the tissue cysts of this strain in mice. This strain has been used in a molecular study[14], however, this is the first report concerning the cystogenesis capacity of this strain in the brain of mice.

2. Materials and methods

2.1. Experiment

Brain suspension in saline was prepared from the mice infected with tissue cysts of *T. gondii*, Tehran strain three months earlier. A volume of 0.5 mL brain suspension containing 20 tissue cysts of Tehran strain was inoculated intraperitoneally to each of 25 male BALB/c mice (animals were seronegative for anti-*T. gondii* antibodies by Sabin-Feldman dye test)[15]. Mice were purchased from Razi Vaccine and Serum Research Institute and housed in plastic cages with food and water available *ad libitum*. Ten mice at weeks 7-9 and fifteen mice at weeks 13-14 were deeply anesthetized by intraperitoneal injection of ketamine (150 mg/kg) and xylazine (15 mg/kg), followed by removal of their brains from the skulls. Each brain was divided into 3 sections including, right cerebral hemisphere, left cerebral hemisphere, and the cerebellum+brain stem. Unstained squash-smears were prepared from the whole brains and the numbers of tissue cysts were counted at two magnifications of 100× and 400× using light microscopy.

2.2 Ethical considerations

The study protocol was approved by the Ethical Review Board of Qazvin University of Medical Sciences, Qazvin, Iran.

2.3. Statistical analysis

The data were analyzed by SPSS version 13. Nonparametric test of Mann-Whitney was used to demonstrate means differences. A *P*-value less than 0.05 was considered as significant.

3. Results

The number of tissue cysts in the brain of mice showed remarkable variations. The minimum and maximum number of cysts were 70 and 1531 at weeks 7-9 post-infection, and 12 and 5170 at weeks 13-14 post-infection, respectively. The frequency distribution of brain cysts in mice with experimental infection to *T. gondii*, Tehran strain is shown in Table 1.

Table 1

Frequency of tissue cysts in brain of 25 BALB/c mice inoculated intraperitoneally with 20 tissue cysts of *T. gondii*, Tehran strain at weeks 7-9 and 13-14 post-infection.

Weeks 7-9 (Group 1)		Weeks 13-14 (Group 2)			
Mice number	Number of tissue cysts	Mice number	Number of tissue cysts	Mice number	Number of tissue cysts
1	1531	1	112	11	476
2	117	2	208	12	153
3	72	3	312	13	59
4	87	4	302	14	12
5	89	5	2749	15	5170
6	70	6	549		
7	86	7	660		
8	95	8	2992		
9	55	9	4089		
10	81	10	754		

There was a significant difference in the means observed for the number of brain cysts between Group 1 and 2 ($P<0.05$). The mean±SEM numbers of brain cysts in Group 1 and 2 were 228.3±144.8 and 1239.8±429.3, respectively.

Overall, the mean number of brain cysts in the right cerebral hemisphere was higher than the left cerebral hemisphere; however the difference was not significant, statistically. On the other hand, the number of tissue cysts in the cerebellum+brain stem was significantly lower than that observed in the right and left cerebral hemispheres ($P<0.05$) (Table 2).

Table 2

Frequency of tissue cysts in the right cerebral hemisphere, left cerebral hemisphere, and cerebellum+brain stem of 25 BALB/c mice inoculated intraperitoneally with 20 tissue cysts of *T. gondii* Tehran strain at weeks 7-9 and 13-14 post-infection (mean±SEM).

Mice brain	Weeks 7-9 (Group 1)	Weeks 13-14 (Group 2)
Right cerebral hemisphere	78.30±40.79	625.60±221.34
left cerebral hemisphere	123.10±88.73	427.40±144.83
cerebellum+brain stem	26.90±15.37	186.80±68.59
Total	228.30±144.80	1239.80±429.30

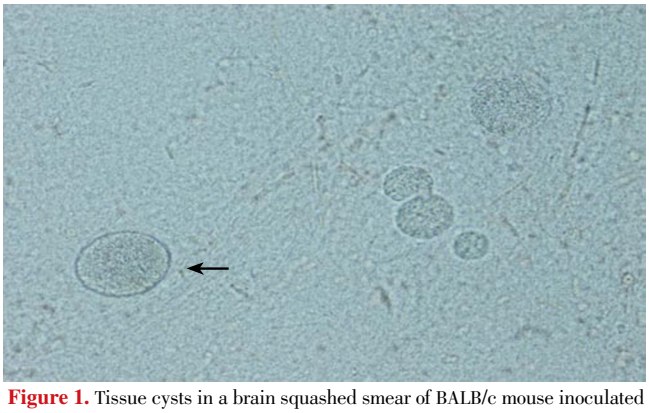

Figure 1. Tissue cysts in a brain squashed smear of BALB/c mouse inoculated intraperitoneally with tissue cysts of *T. gondii* at week 14 post-infection. Note the larger size (3-4 folds) of brain cysts at magnification 200×.