**TOXOPLASMA GONDII INFECTION IN RATS**

**BY THE RH STRAIN: INOCULUM AND AGE EFFECTS**

DE CHAMPS C.*, PELLOUX H.**, DECHELOTTE P.***, GIRAUD J.C.*, BALLY N.* & AMBROISE-THOMAS P.**

**Summary:**

Toxoplasma gondii strains are classified according to their virulence in mice. Rats are considered to be resistant to the infection, depending on the age. Newborn rats are fully susceptible but weaned rats are resistant. However the effect of inoculum has not been examined. Using RH strain inocula of $10^2$, $10^4$, $5 \times 10^7$ and $10^8$ tachyzoites intraperitoneally inoculated into Wistar and Fischer rats of 7, 11, 21, 24 and 46 days old, the authors show that inoculum and not the age of the host had a statistically significant effect ($p < 0.01$) on the survival curve.

**KEY WORDS:** Toxoplasma gondii, RH strain, virulence, rat, liver lesion.

**INTRODUCTION**

Toxoplasma gondii strains are classified as virulent or non-virulent based on infectivity in mice. The RH strain of *T. gondii* is the most virulent strain for mice. It was isolated from the brain of a six-year old child (Sabin, 1941). Genetically, it is one of the type I strains (Howe & Sibley, 1995) which are extremely virulent in mice; one infective tachyzoite is lethal to mice (Dubey, 1998). Virulence of types II and III strains of *T. gondii* is variable (Howe & Sibley, 1995). Rats are considered to be one of the most resistant hosts for RH strain *T. gondii* but opinions differ with respect to age of resistance acquisition, lethal dose, and formation of tissue cysts (Lainson, 1955; Lewis & Markell, 1958; Kulasiri, 1962; De Meuter, 1972; Chinchilla *et al.*, 1981; Dubey & Frenkel, 1998). There is general agreement, except for the conflicting report of Remington *et al.* (1958), that natural resistance starts at an early age and that inoculums $< 10^7$ do not kill the animal. The aim of this study was to determine the effect of dose on morbidity and mortality in rats inoculated intraperitoneally (i.p.) with RH strain.

**MATERIAL AND METHODS**

Tachyzoites of the RH strain of *T. gondii* were obtained from mouse peritoneal fluid three days after i.p. inoculation and washed twice in physiological saline (0.85 % NaCl). The strain was subinoculated twice weekly into Swiss OF1 mice (IFFA-CREDO, L’Arbresle, France) in our laboratory. In total 30 rats were used in the present study (Table I). 24 male and female Wistar rats from three litters were assigned to 12 pairs. Three different pairs were i.p. inoculated at the age of 7, 11, 24 and 46 days with either $10^2$, $10^4$, $5 \times 10^7$ or $10^8$ tachyzoites of the *T. gondii* RH strain (De Champs *et al.*, 1997). In each pair, one was killed two weeks and the other four weeks after inoculation by barbiturate overdose. They were weighed at the time of inoculation and at sacrifice. A quarter of each rat brain was homogenized in 1 ml of phosphate buffer saline (PBS) and 500 µl inoculated into a Swiss mouse and another quarter was used for histological examination. The remaining half was...
The specimens were observed by epifluorescence microscopy. The survival curves were compared using the Krushall-Wallis rank-test (Woolson, 1987). Among the surviving rats, one rat inoculated with $10^2$ tachyzoites at seven days lost its hair. With the inoculum $\geq 10^4$ a delay in growth was observed, and with $5 \times 10^7$ and $10^8$ the rats lost almost three standard deviations in weight (Table II).

On histological examination, the rats that died during the acute infection had no lesions in the lungs or spleen. In the liver, the Glisson capsule was thicker and infiltrated by lymphocyte cells and numerous tachyzoites were seen. Tachyzoites were observed in the brain of two rats, and inflammatory lesions in their heart were associated with tachyzoites-shaped organisms. A total amount of $10^2$ to $10^6$ tachyzoites was harvested from the ascites.

Tissue cysts were observed in the homogenate brain of nine killed rats (Table III) and none at the histological examination of the brain or the liver (De Champs et al., 1997). Eleven of the 17 mice inoculated i.p. with the brain of these rats died.

### RESULTS

The mortality rate according to age and inoculum are summarized in Table I. All deaths occurred within the first eight days after inoculation. No rat died after inoculation with $10^2$ tachyzoites. The rat that died after an inoculation with $10^4$ tachyzoites was seven days old when inoculated. The statistical analysis of the survival curve showed a significant difference for the inoculum ($p < 0.01$) but not for age.

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No tissue cyst was observed in the homogenate brain of the four rats inoculated with 5.10^7 tachyzoites and killed seven months later. The mice subinoculated with the brain of two rats survived, and those inoculated with the brain of two other rats died 87 and 130 days later. T. gondii were observed in ascites from one of the mice. The proportion of mice that died after i.p. sub inoculation with the brain of rats decreased when the rats were killed later and when the rats were older at inoculation (Table IV). The association between the age of rats at inoculation and the number of sub inoculated mice that died was statistically significant (p < 0.01).

<table>
<thead>
<tr>
<th>Age at injection (days)</th>
<th>No of rats killed</th>
<th>No of rats with tissue cyst in brain homogenate</th>
<th>No of infected rats*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>46</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* By bioassay in mice inoculated i.p. with rat brains.

Table IV. - Persistence of RH strain T. gondii in brains of rats according to the date at inoculation.

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