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GROWTH OF TOXOPLASMA GONDII (RH STRAIN) IN TWO DIFFERENT SPECIFIC-PATHOGEN-FREE STRAIN OF MICE

S.Z. Jawdat, L.KH. Rifaat and Z.M. Kamel

Department of Microbiology, Biological Research Center, Scientific Research Council, Jadiriyah, Baghdad, Iraq.

#### SUMMARY

Specific-pathogen-free males Balb/c and C57 Black mice were inoculated intraperitoneally with different doses of Toxoplasma tachyzoites.

The results showed that, mice inoculated with smaller dose gave maximum yields of tachyzoites at the time of death, and survived longer time than mice inoculated with larger dose.

#### INTRODUCTION

<u>Toxoplasma</u> gondii is one of the commonest protozoan parasites of human and animals, and occurs worldwide. Most human infections are subclinical, and those infectons which are recognised usually mild (1). However some infected persons may suffer from mild symptoms. Cat and other felines were found to be the only known specific host for <u>T. gondii</u> (1,2,3, and 4). Because <u>Toxoplasma</u> is an obligate intracellular parasite and multiplies only in living cells (4), cultures such as embryonated eggs are difficult to maintain and tissue cultures are expensive, this has led researchers to use mice for maintainance and propagation of <u>Toxoplasma</u> parasites (3).

It has been shown that, there is a relationship between the size of <u>Toxoplasma</u> inoculum and the time of death in mice (5). Johnson <u>et al</u> (3) described a method for obtaining maximum yield of <u>Toxoplasma</u> tachyzoites using LACA strain of mice. The present work was designed using 2 different specific-pathogen-free (SPF) strains of mice to study the relationship between the dose of  $\underline{T.gondii}$  and the survival time of the infected mice and the parasite yields at the time of their death.

# MATERIALS AND METHODS

Mice

Six to eight week-old SPF males of outbred Balb/c mice and C57 Black mice with a body weight ranged between 20-25 g were used for all experiments. Parasite

The RH strain of <u>T.gondii</u> was routinely maintained intraperitoneally in Balb/c mice twice weekly, the pooled exudate washigs of infected mice were passed through Nylon wool for filtration. After filtration, the viable cells were counted using haemocytometer to obtain the desired number for inoculation.

Ten groups of Balb/c and ten groups of C57 Black mice of 4 mice each were inoculated intraperitoneally with suspensions containing  $10^{\circ}$ ,  $10^{\circ}$ ,  $10^{\circ}$ ,  $10^{\circ}$  and  $10^{\circ}$ extracellular <u>Toxoplasma</u> tachyzoites in PBS. Each group was kept in seperate cage. The two strains of mice were kept under observation. First trial, mice were left until death, but in the second trial, mice were sacrificed when they lost appetites and appeared to be dying.

#### RESULTS

The recovery volume of harvested tachyzoites from peritoneal exudate was nearly similar in both infected strains of mice used. Table 1 shows the mean values + S.D. of parasite numbers harvested from both infected strains of mice. The maximum yields of tachyzoites were obtained from the peritoneal exudate of mice inoculated with smaller doses and survived longer time.

Contamination of peritoneal exudate with bacteria occurred at postmortem, in order to obtain uncontaminated parasites, experiment have been repeated.

Table 1. Groups of Balb/c mice and groups of Black C57 micc inoculated with different doses of <u>T. gondii</u>. The values represent the mean numbers of tachyzoties at the time of death.

| Size of<br>inoculum<br>Per/ml |   | Mean values + S.D. of <u>T</u><br>Mean Groups of Balb/c<br>days mice | Mea | ondii /m1<br>an Groups of Black C57<br>ys mice |
|-------------------------------|---|--|-----|--|
| 105                           | 6 | 5.8x10 <sup>7</sup> +3.2x10 <sup>7</sup>                             | 6   | 5.4x10 <sup>7</sup> +3.5x10 <sup>7</sup>       |
| 105                           | 7 | 4.1x10 <sup>7</sup> +1.4x10 <sup>7</sup>                             | 6   | 5.1x10 <sup>7</sup> +1.9x10 <sup>7</sup>       |
| 104                           | 8 | 1.5x10 <sup>6</sup> +0.7x10 <sup>6</sup>                             | 7   | 8.4x10 <sup>7</sup> +1.2x10 <sup>7</sup>       |
| 103                           | 8 | 1.2x10 <sup>5</sup> +0.7x10 <sup>5</sup>                             | 8   | 2.9x10"+0.6x10"                                |
| 102                           | 9 | 1.4x10 <sup>a</sup> +1.2x10 <sup>a</sup>                             | 9   | 3.5x10 <sup>n</sup> +0.8x10 <sup>n</sup>       |

Table 2. Groups of Balb/c mie and groups of Black C57 mice inoculated with different doses of <u>T. gondii</u>. The values represent the mean numbers of tachyzoites at time required.

|                 | Mean | values + S.D. of T.<br>Groups of Balb/c<br>mice | gondii /m1<br>Mean Groups of Black C57<br>days mice |  |
|-----------------|------|---|---|--|
| 105             | 6    | 1.1x10 <sup>7</sup> +0.3x10 <sup>7</sup>        | 4   | 6.4x10°+2.3x10°                          |
| 10              | 6    | 2.7x10 <sup>7</sup> +0.7x10 <sup>7</sup>        | 5   | 2.5x10 <sup>7</sup> -0.7x10 <sup>7</sup> |
| 104             | 7    | 3.2x10 <sup>7</sup> +0.7x10 <sup>7</sup>        | 6   | 4.7x10 <sup>7</sup> +2.4x10 <sup>7</sup> |
| 103             | 7    | 6.0x10 <sup>7</sup> +0.2x10 <sup>7</sup>        | 7   | 2.4x10 <sup>7</sup> +0.8x10 <sup>7</sup> |
| 10 <sup>2</sup> | 8    | 1.1x10 <sup>n</sup> +0.6x10 <sup>n</sup>        | 8   | 6.4x10 <sup>7</sup> +1.3x10 <sup>7</sup> |

-33-

Mice under observation have sacrificed when they lose appetites and appeared dead. Table 2 shows the mean values + S.D. of parasite numbers for infected mice inoculated with different doses of tachyzoites. Maximum yields of parasites were obtained from inoculated mice with smaller doses of tachyzoites and longer survival time. During the present study cannibalism did not occur among the two strains of used mice.

#### DISCUSSION

The overall results of the present study indicated that, both strains of mice which received small inoculum of tachyzoties survived for longer time than those injected with larger dose. These results came in egreement with previous studies (3,5 and 6). However, the final numbers of <u>Toxoplasma</u> parasite present in the peritoneal cavity at the time of death have not been reported for Balb/c and Black mice by Olise <u>et. al.</u> (60). While in the present work we confirmed that mice infected with smaller dose of tachyzoites contained more <u>Toxoplasma</u> parasites in the peritoneal cavity at the time of death than mice infected with larger number of parasites.

Cannibalism was not observed among all groups of mice, on the other hand cannibalism occured among all strains used by Oilsa (6).

The results reported here can be applied for cultivating parasite in large number for antigen preparation for research work and serological diagnosis.

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### REFERENCES

- John, A and Chase, T.M. (1987). Toxoplasmosis, Veterinary Clinics of North America. Small animals Practice, 17(1): 55-71.
- 2. Johnson, A.M. (1985). the antigenic structure of <u>Toxoplasma gondii</u>: Areview. Pathology, 17:9-19.
- Johnson, A.M; McDonald, P.J. and Neoh, S.H. (1979) Kinetics of the growth of <u>Toxoplasma gondii</u> (RH strain) in mice. International J. for Parasitology, 9: 55-65.
- Richard, O'Connor G. (1985) Host-parasite interaction. International Opthalmology Clinics, 25(2): 63-69.
- 5. Eyles, D. and Coleman, N. (1956). Relationship of size inoculum to time to death in mice infected with <u>Toxoplasma gondii</u>, J. Parasitol. 42:272-276.

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 Olisa, E.G.; Herson, J.; Beadings, V.E. and Poindexter, H.A. (1977) <u>Toxoplasma gondii</u>: Survival time and variability in mouse host stratins. Experimantal parasitology. 41:307-313.

# نمو الطفيلي توكسوبلازما كوندي نوع (RH) في مربين من الفئران الحساسة

سعاد زكي جودت، ليلى خليل رفعت وزينب مولود كامل

قسم الاحياء المجهرية مركز بحوث علوم الحياة مجلس البحث العملي، الجادرية، بغداد

الذلام

تم حقق نوعين من ذكور الفئران الحساسة Balb/c and C57 بتراكيز منت ذكور الفئران الحساسة Balb/c and C57 بتراكيز منتلفة من العترة توكسوبلازما كوندي (PH). الفئران التي حقنت بأقل جرعة من الطفيلي عاشت فترة أطبول من الفئران التي حقنت باعلى جرعة وكذلك تم حمد اعلى تركيز من تاكي زويد التوكسوبلازما من الحيوانات التي حقنت بأقل جرعة من الطفيلي.