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# ASCARIASIS AND TOXOCARIASIS. II — DIFFICULTIES IN THE DIFFERENTIAL SEROLOGICAL DIAGNOSIS EMPLOYING A *TOXOCARA CANIS* ANTIGEN

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## SUMMARY

The Author presents the results of complement fixation test, passive haemagglutination and immunodiffusion in sera from rabbits and Rhesus monkeys infected with *Ascaris suum* and *Toxocara canis*. The antigen used was *T. canis* extract, and extensive serological cross-reactivity was observed throughout the experiment. Some comments are made as to the possible explanations of this phenomenon and the approaches to a solution.

## INTRODUCTION

The antigenic cross-reactivity between *Ascaris* and *Toxocara* genera has already been studied by several researchers [4, 6, 9, 11, 15, 16]. This phenomenon has occasionally included other nematodes, such as *Trichinella* [5] and *Necator* [1], and it is quite important in tropical countries, since it may impair the specificity of diagnostic tests for toxocariasis. The skin test for toxocariasis yielded consistently different percentages of positive results in random "normal" populations when performed in Great Britain [19], Malaysia [2] and Uganda [18], varying between 2.1% to 25%, and "background" infection with ascarids was advanced as one of the possible explanations for these data, which was reinforced by experimental infections [20].

The present work shows that cross-reactivity between these genera is also observed in other immunodiagnostic tests for toxocariasis.

## MATERIALS AND METHODS

Three Rhesus monkeys and three white rabbits were employed, one animal of each species being infected with *T. canis* embryonated eggs, another with *A. suum* and the third serving as a non-infected control. The monkeys received 15,000 eggs and the rabbits 10,000, and reinfection was carried out 90 days after infection with the same number of eggs. The details of these infections are described elsewhere [20].

Serological tests were performed at weekly intervals during the first six weeks and fortnightly until the 20th week, when the experiment was ended. The antigen used in these tests was the *T. canis* extract obtained according to the description of JESKA [6], the concentration of which was adjusted to the optimum point in each test, using a "checkerboard" system with rabbit hyperimmune homologous antiserum. Protein concentration of the antigens was determined by optical density at 210 nm [17]. Micro-complement fixation (MCF) was performed according to SEVER [14], passive haemagglutination (PHA) according to the method of Boyden, adapted by JUNG & PACHECO [9], and double immunodiffusion (ID) was done as described by OUCHTERLONY [13].

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TABLE I

Micro-complement fixation results in experimental animals (Reciprocal titres). R = rabbit; M = monkey; 1 = infected with *T. canis;* 2 = infected with *A. suum;* 3 = non-infected control; — = negative; ... = not performed.

| Animal \ Day | C | 7 | 14 | 21 | 28 | 35 | 42 | 56 | 70 | 84 | 98 | 112 | 126 | 140 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | — | — | 5 | 20 | 40 | 20 | — | 5 | — | — | 20 | ... | ... | ... |
| $R_2$ | — | 160 | 640 | 160 | ... | ... | ... | 5 | — | — | 20 | ... | ... | ... |
| $R_3$ | — | — | — | — | — | — | — | — | — | — | — | — | 10 | 10 |
| $M_1$ | 5 | 10 | 40 | 80 | 40 | 40 | — | — | — | — | 160 | 80 | 80 | 40 |
| $M_2$ | — | 20 | 160 | 640 | 160 | ... | 160 | 20 | — | — | 160 | 40 | 40 | 40 |
| $M_3$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

TABLE II

Passive haemagglutination results in experimental animals (Reciprocal titres). R = rabbit; M = monkey; 1 = infected with *T. canis;* 2 = infected with *A. suum;* 3 = non-infected control; — = negative; ... = not performed

| Animal \ Day | C | 7 | 14 | 21 | 28 | 35 | 42 | 56 | 70 | 84 | 98 | 112 | 126 | 140 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | — | 4 | 8 | 64 | 512 | — | — | — | — | — | — | — | 64 | 256 |
| $R_2$ | — | 32 | 512 | 512 | ... | ... | 32 | 4 | — | — | ... | ... | ... | ... |
| $R_3$ | — | — | 16 | — | — | ... | ... | 32 | 32 | 16 | 8 | 4 | 4 | 4 |
| $M_1$ | 16 | 2048 | 512 | 512 | 512 | 128 | 256 | 128 | 16 | 16 | 64 | 128 | 16 | 64 |
| $M_2$ | ... | 2048 | 512 | 512 | 256 | 128 | 256 | 64 | 16 | 16 | 16 | 128 | 16 | 64 |
| $M_3$ | ... | 16 | 16 | 16 | 32 | 32 | 16 | 16 | 16 | 32 | 16 | 16 | — | — |

TABLE III

Immunodiffusion results in experimental animals. R = rabbit; M = monkey; 1 = infected with *T. canis;* 2 = infected with *A. suum;* 3 = non-infected control; + = positive; — = negative; ... = not performed.

| Animal \ Day | C | 7 | 14 | 21 | 28 | 35 | 42 | 56 | 70 | 84 | 98 | 112 | 126 | 140 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | — | — | + | + | + | ... | ... | — | — | — | — | — | + | + |
| $R_2$ | — | — | + | ... | ... | + | ... | + | — | — | + | ... | ... | ... |
| $R_3$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| $M_1$ | — | — | + | + | + | — | — | + | — | — | — | + | + | + |
| $M_2$ | — | — | — | + | + | — | + | + | + | + | — | + | + | + |
| $M_3$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

## RESULTS

**MCF** — The antigen was used in the final protein concentration of 0.16 mg/ml. Table I shows the results obtained. Titres below 1:10 were considered negative.

**PHA** — The tanned cells were coated with antigen at a concentration of 0.6 ug protein per ml. Titres below 1:32 were considered negative. The results are displayed in Table II.

**ID** — The protein concentration of antigen employed was 10.8 mg/ml. Although some sera gave two or more precipitin bands, they were simply recorded as positive. These results are in Table III.

## DISCUSSION

Our results show two outstanding facts. First, consistent results were obtained with all the tests employed. There was a wide variation as to the titre of each serum, which can be understood as reflecting the variation of the sensitivity of the tests [10]. Reinfection caused a second rise in titres.

Secondly, all three tests gave extensive positive reactions in *Ascaris* infected animals. This finding presents a serious limitation to the serological diagnosis of visceral larva migrans, especially in countries where ascariasis is endemic. It has not been established so far whether this serological cross-reactivity is limited to the tissue-migrating phase of the *Ascaris*-larvae or if it extends to all stages of infection. The reason for such cross-reactivity probably lies in the antigen employed, which was found to contain up to 41 immunogenic components, when properly fractionated and studied [6]. This richness in components, while being very important for the establishment of the antigenic composition of the worm, can be very disturbing when applied to serology, as in the present work, since common group antigens are introduced in the reaction.

There are two possibilities of obviating these difficulties. One of them consists in purifying the antigen until a genus-specific component is obtained. Although this has already been accomplished [7, 8], it is out of the reach of a routine diagnostic laboratory. Other (and simpler) alternatives for antigen fractionation have been tried, with apparently good results [12]. A small percentage of false-positive results, however, could not be excluded.

Another possibility consists in the absorption of the test sera with *Ascaris* extracts. This is easier to perform in routine tests and has already given good results in the fluorescent antibody technique [3]. Its value should be acessed in the other tests as well.

## RESUMO

*Ascaríase e toxocaríase. II — Dificuldades encontradas no imunodiagnóstico diferencial quando do emprego de um antígeno de* Toxocara canis.

O Autor apresenta os resultados de provas de micro-fixação do complemento, hemaglutinação passiva e gel-difusão em coelhos e macacos *Rhesus* infectados com *Toxocara canis* e *Ascaris suum*. O antígeno utilizado foi um extrato de *T. canis*, e os testes revelaram intensa reatividade cruzada entre os soros dos animais com ambas as infestações. Este fenômeno é comentado, sendo discutidas as possibilidades de sua eliminação.

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