

TOXOCARIASIS AND ASCARIASIS: A COMPARISON

I — Description of infection, haematological response, serum proteins and skin test with *Toxocara canis* antigen

F. R. ZYNGIER (1)

SUMMARY

The Author reports the experimental infection of rabbits and monkeys with *Toxocara canis* and *Ascaris suum*. A peak of leukocytosis and eosinophilia was observed after infection and reinfection with *T. canis*. *Ascaris* infections were followed by eosinophilia and leukocytosis, but reinfection elicited eosinophilia only. Plasma protein electrophoresis evidenced discrete hypergammaglobulinemia in the *T. canis*-infected animals. The skin test showed positive results in both groups of infected animals.

INTRODUCTION

Several Authors have tried to emphasize the similarities between the *Ascaris* and *Toxocara* genera. The approaches employed have varied from antigenic analyses^{9, 11, 12, 13, 16} to cross-reactivity in immunological tests^{2, 6, 8}. The differential morphology of the larvae requires careful study for correct distinction^{19, 20}. Although these helminth infections behave differently from the clinical point of view, they may overlap in several aspects.

This work will describe some comparative aspects of *Ascaris suum* and *Toxocara canis* infections in experimental animals.

MATERIALS AND METHODS

Infective eggs

T. canis and *A. suum* female worms were recovered from naturally infected dogs and pigs respectively. Each species was dealt with separately. The worms were dissected

and the vagina and proximal third of uterus were removed and chopped in small pieces in a Petri dish containing a small amount of formalin (1 per cent v/v) in water. After mixing thoroughly, the suspension was filtered through a sieve and the eggs were placed into a 100 ml Erlenmeyer flask. The egg suspension was aerated using an aquarium air pump and incubation was allowed to proceed for at least one month at room temperature. The eggs were checked for motility of the larvae and stored at 4°C until used.

Skin test

T. canis antigen for skin testing was obtained according to the method of WOODRUFF & THACKER²⁷. All animals were shaved periodically in the abdomen, and 0.1 ml of antigen (1:1,000 dilution) was injected intradermally. A saline control injection was done at the same occasion. The test was read after 20 minutes, as follows: initial induration doubled or more = positive; ini-

This work was submitted in partial fulfillment of requirements for the M.Sc. degree at the University of Liverpool

(1) Department of Immunology, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco I, Cidade Universitária, ZC-32, 20.000, Rio de Janeiro, RJ, Brasil

tial induration increased but not doubled = doubtful positive; initial induration unchanged or smaller = negative.

Blood cell counts, protein determinations and electrophoresis were performed according to standard procedures^{17, 23}.

Experimental infections

One rabbit (R1) and one *Rhesus* monkey (M1) were infected with 10,000 and 15,000 embryonated eggs of *T. canis* respectively. Another rabbit and monkey were given a similar number of *A. suum* embryonated eggs (R2 and M2), and the third animal

of each species remained as a non-infected control (R3 and M3). Reinfection with the same number of eggs was performed 90 days after the first infection. The infections were carried out through a stomach tube, the monkeys being sedated with Sernylan. The animals were bled weekly until the sixth week after infection and then fortnightly until the end of the experiment (20th week).

RESULTS

The haematological counts are depicted in Figs. 1 to 4. Serum protein determinations are shown in Table I. The skin test results are displayed in Table II.

TABLE I

Serum protein determination in the *T. canis* infected animals. R₁ = *T. canis* infected rabbit; M₁ = *T. canis* infected monkey; R₃ and M₃ = non-infected control animals

Week	Animal	Total protein	Albumin	Globulins			
				alpha-1	alpha-2	beta	gamma
0	R ₁	6.37	4.12	0.73	0.61	0.43	0.43
4		—	—	—	—	—	—
8		6.76	4.16	0.57	0.50	0.55	0.96
12		6.49	2.73	1.66	0.77	0.31	0.96
16		6.40	2.09	1.03	1.10	0.54	1.62
20		9.50	2.83	2.83	1.29	0.77	1.75
0	R ₃	—	—	—	—	—	—
4		—	—	—	—	—	—
8		6.10	4.62	0.31	0.18	0.41	0.55
12		5.30	3.54	0.46	0.30	0.40	0.57
16		5.71	3.87	0.36	0.38	0.52	0.56
20		5.40	3.70	0.30	0.30	0.60	0.50
0	M ₁	—	—	—	—	—	—
4		5.35	2.85	0.41	0.61	0.63	0.83
8		5.00	3.07	0.94	0.40	0.23	0.83
12		5.40	2.92	0.55	0.56	0.53	0.82
16		5.33	3.15	0.21	0.32	0.68	0.96
20		5.14	3.25	0.17	0.27	0.23	1.21
0	M ₃	—	—	—	—	—	—
4		7.95	4.63	0.84	0.98	0.71	0.76
8		7.00	4.97	0.85	0.48	0.42	0.70
12		7.06	4.88	0.34	0.88	0.41	0.53
16		7.02	5.17	0.28	0.43	0.53	0.58
20		6.12	4.39	0.19	0.51	0.46	0.55

TABLE II

Skin test results. + = positive; - = negative; D = doubtful; ... = not done;
 M = monkey; R = rabbit; 1 = infected with *T. canis*; 2 = infected with *A. suum*;
 3 = non-infected control

Animal	Day	7	14	21	28	35	42	56	70	84	98	112	126	140
R ₁	C	-	D	D	D	+	+	+	D	D	D	D	+	+
R ₂		-	-	D	D	D	D	-	-	-
R ₃		-	-	-	-	-	-	-	-	-	-	-	-	-
M ₁		-	-	D	+	D	+	+	+	D	D	D	+	+
M ₂		-	-	D	+	D	D	+	D	D	+	D	D	+
M ₃		-	-	-	-	-	+	-	-	-	-	-	-	-

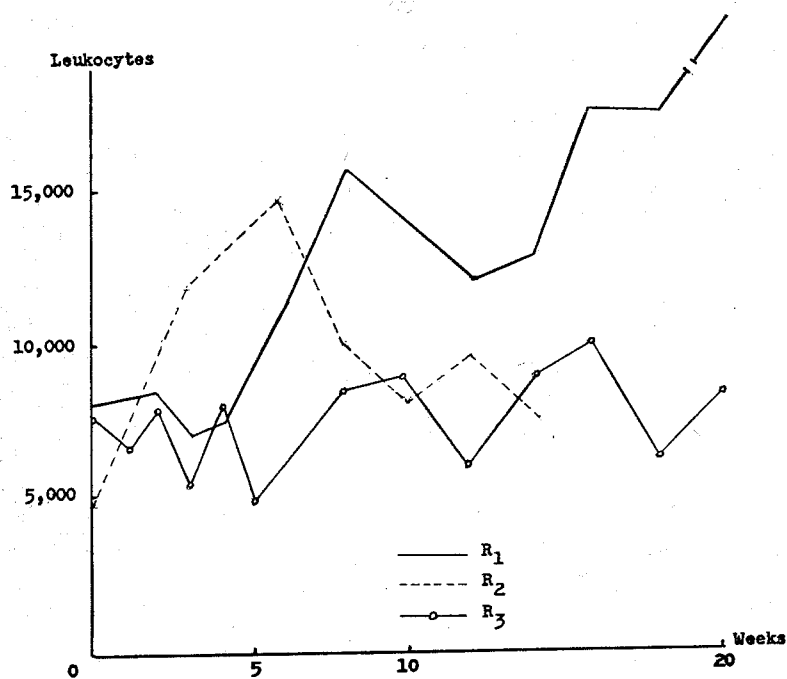


Fig. 1 — Total leukocyte counts on the *T. canis* (R₁), *A. suum* (R₂) and non-infected (R₃) rabbits. The animals were infected with 10,000 embryonated eggs on days 0 and 90.

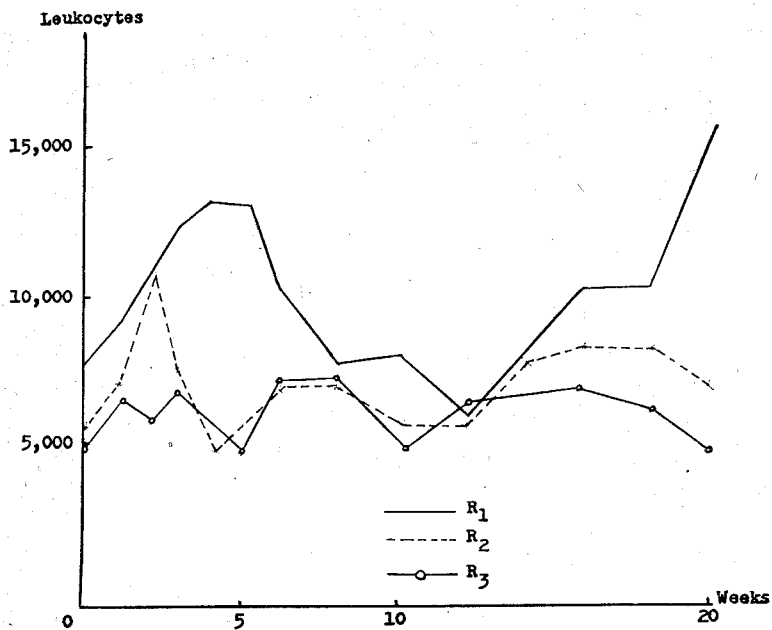


Fig. 2 — Total leukocyte counts on the *T. canis* (M₁), *A. suum* (M₂) and non-infected (M₃) Rhesus monkeys. The monkeys were infected with 15,000 eggs on days 0 and 90.

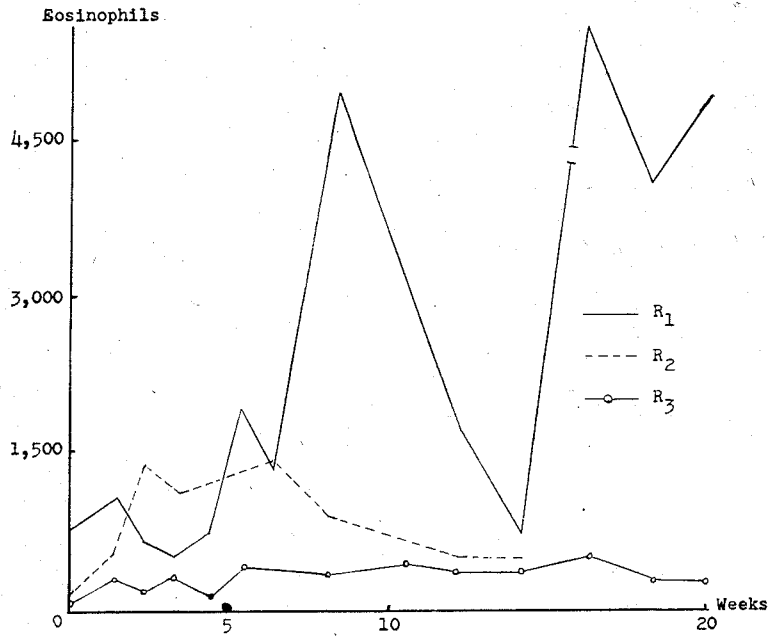


Fig. 3 — Eosinophil counts on the animals depicted in Fig. 1.

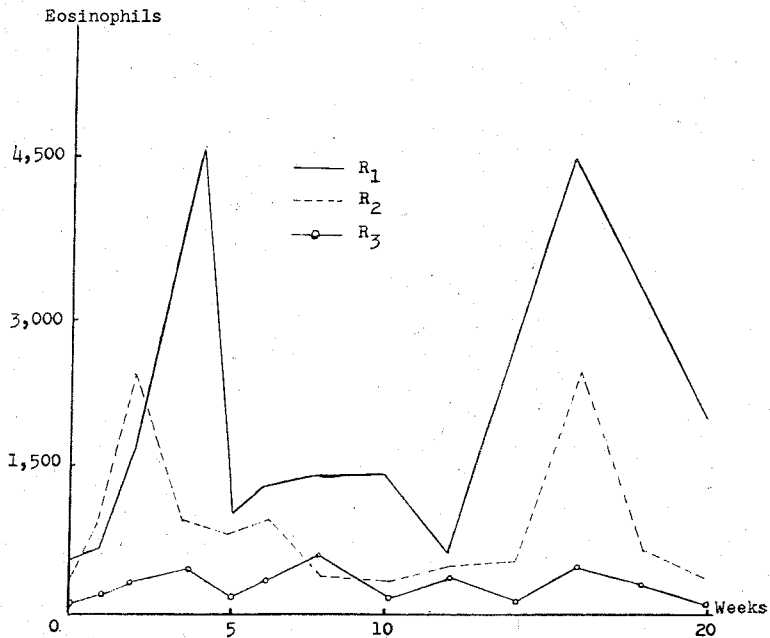


Fig. 4 — Eosinophil counts on the animals depicted in Fig. 2.

DISCUSSION

The leukocyte and eosinophil levels of the infected animals were comparable to those previously reported^{1, 3, 4, 10, 24}. In the *Toxocara*-infected monkey the eosinophils never returned to normal, although leukocytosis was limited to the first six weeks. The *Ascaris*-infected monkey showed leukocytosis and eosinophilia of four weeks duration. After reinfection only the eosinophils showed a second peak. These results were comparable in the infected rabbits. These results may be explained on the basis of the different migratory patterns of *A. suum* and *T. canis*^{5, 21, 26}, which favour a more prolonged contact — and possibly antigenic stimulation — of *T. canis* larvae with the host tissues.

Other factors may interfere in the genesis of leukocytosis and eosinophilia. The dose of infective eggs was reported to be roughly proportional to the degree of haematological response in guinea-pigs²². WISEMAN et al.²⁵ confirm this finding by giving low infective doses of *T. canis* eggs to four monkeys and observing no leukocytosis. Previous contacts with the nematode might be another variable¹⁰.

The increase of gamma globulin observed in the infected monkey is similar to previous observations on visceral larva migrans^{1, 18}. Although the immunoglobulin class was not identified, the elevation of IgE in the serum of *Ascaris*¹⁴ and *Toxocara* infected⁷ patients or animals is described.

The skin test in this experiment showed some cross-reactivity between the two infections in monkeys and rabbits, although the reactions tended to be somehow more positive in the *Toxocara*-infected animals. This is consistent with the studies of JUNG & PACHECO¹⁵ and BALL, VOLLER & TAFFS² showing similar cross-reactivity, and might eventually explain the difference in prevalence of positive *T. canis* skin test in "normal" people in different countries^{3, 26}. The different "background rate of infection of these populations with *Ascaris lumbricoides* might be the reason. Although the skin test has been demonstrated to be quite sensitive in controlled tests²⁵, it is our opinion that fur-

ther studies would be necessary to establish its specificity. These studies should be done parallel to a parasitological stool examination in order to disclose the presence of ascariasis.

RESUMO

Ascariase e toxocaríase: Uma comparação. I — Descrição da infecção, resposta hematológica, proteínas séricas e intradermorreação com antígeno de T. canis

O Autor compara infestações experimentais de coelhos e macacos *Rhesus* com *Toxocara canis* e *Ascaris suum*. A infestação e reinfestação por *T. canis* provocou grandes elevações nos leucócitos e eosinófilos sanguíneos. A infestação por *A. suum* provocou leucocitose e eosinofilia, porém a reinfestação provocou apenas eosinofilia. A eletroforese de proteínas séricas revelou hipergamaglobulinemia discreta apenas nos animais infestados por *T. canis*. A intradermorreação com antígeno de *T. canis* apresentou resultados positivos em ambas infestações.

REFERENCES

1. ALJEBOORI, T.I. & IVEY, M.H. — *Toxocara canis* infections in baboons. I. Antibody, white cell and serum protein responses following infection. *Amer. J. Trop. Med. Hyg.* 19:249-254, 1970.
2. BALL, P.A.J.; VOLLER, A. & TAFFS, L. F. — Hypersensitivity to some nematode antigens. *Brit. Med. J.* 1:210-211, 1971.
3. BISSERU, B. — Skin test suggesting human toxocariasis in West Malaysia. *Med. J. Malaya* 23:35-40, 1968.
4. DHAR, D.N.; PALLANYAK, S. & BASU, P.C. — Prevalence of *Toxocara canis* (Werner, 1782) Johnston 1916 and other intestinal infection in dogs in Delhi and experimental studies on *T. canis* infection in laboratory animals. *Bull. Ind. Soc. Mal. Commun. Dis.* 3:303-315, 1966.
5. DOUVRES, F.W. & TROMBA, F.G. — Comparative development of *Ascaris suum* in rabbits, guinea pigs, mice and swine in eleven days. *Proc. Helminth Soc. (Washington)* 38:246-252, 1971.
6. FERNANDO, S.T. & VASUDEVAN, B. — Precipitin reactions in monkeys (*Macaca sinica*) experimentally infected with *Toxo-*

ZYNGIER, F. R. — Toxocariasis and ascariasis: A comparison. I — Description of infection, haematological response, serum proteins and skin test with *Toxocara canis* antigen. *Rev. Inst. Med. trop. São Paulo* 18:251-257, 1976.

- caracanis* and in children with visceral larva migrans syndrome. *J. Compt. Path.* 80:407-414, 1970.
7. HOGARTH-SCOTT, R.S.; JOHANSSON, S. G.O. & BENNICH, H. — Antibodies to *Toxocara canis* in the sera of visceral larva migrans patients: the significance of raised levels of IgE. *Clin. Exp. Immunol.* 5:619-625, 1969.
 8. HUNTLEY, C.C.; COSTAS, M.C. & LYERLY, A. — Visceral larva migrans syndrome: clinical characteristics and immunological studies in 51 patients. *Paediatrics* 36:523-536, 1965.
 9. HUNTLEY, C.C. & MORELAND, A. — Gel diffusion studies with *Toxocara* and *Ascaris* extracts. *Amer. J. Trop. Med. Hyg.* 12:204-208, 1963.
 10. JALAYER, T. — *Studies on Toxocara canis and visceral larva migrans*. Ph.D. [Thesis]. University of Liverpool, 1969.
 11. JESKA, E.L. — Antigenic analysis of a metazoan parasite, *Toxocara canis*. I. Extraction and assay of antigens. *Exp. Parasit.* 20:38-50, 1967.
 12. JESKA, E.L. — Antigenic analysis of a metazoan parasite, *Toxocara canis*. II. Purification and analysis of two antigenic components. *J. Immunol.* 98:1290-1300, 1967.
 13. JESKA, E.L. — Purification and immunochemical analysis of a genus specific cuticular antigen of *Toxocara canis*. *J. Parasit.* 55:465-471, 1969.
 14. JOHANSSON, S.G.O.; MELBIN, T. & VAHLQUIST, B. — Immunoglobulin levels in Ethiopian preschool children with special reference to high concentration of immunoglobulin E (Ig ND). *Lancet* 1:1118-1122, 1968.
 15. JUNG, R.C. & PACHECO, G. — Use of a hemagglutination test in visceral larva migrans. *Amer. J. Trop. Med. Hyg.* 7:185-191, 1958.
 16. KAGAN, I. — Serum-agar double diffusion studies with *Ascaris* antigens. *J. Infect. Dis.* 101:11-19, 1957.
 17. KOHN, J. — *Small scale and micro-membrane filter-electrophoresis and immunoelectrophoresis in "Protides of the Biological Fluids"*. Amsterdam, Elsevier Publ. Co., 1959.
 18. MOK, C.H. — Visceral larva migrans. A discussion based on review of the literature. *Clin. Pediatrics* 7:565-572, 1968.
 19. NICHOLS, R.L. — The etiology of visceral larva migrans. I — Diagnostic morphology of infectious second stage *Toxocara* larvae. *J. Parasit.* 42:349-362, 1956.
 20. NICHOLS, R.L. — The etiology of visceral larva migrans. II — Comparative larval morphology of *Ascaris lumbricoides*, *Necator americanus*, *Strongyloides stercoralis* and *Ancylostoma caninum*. *J. Parasit.* 42:363-399, 1956.
 21. OLSON, L.J. — Organ distribution of *Toxocara canis* larvae in normal mice and in mice previously infected with *Toxocara*, *Ascaris* or *Trichinella*. *Texas Rep. Biol. Med.* 20:651-657, 1962.
 22. OLSON, L.J. & SCHULTZ, C.W. — Nematode induced hypersensitivity reaction in guinea-pigs: onset of eosinophilia and positive Schultz-Dale reactions following graded infections with *Toxocara canis*. *Ann. New York Acad. Sc.* 113:440-455, 1963.
 23. TOMBS, M.P.; SOULTER, F. & MACLAGAN, N.F. — The spectrophotometric determination of protein at 210 mu. *Biochem. J.* 73:167-171, 1959.
 24. WISEMAN, R.A. & WOODRUFF, A.W. — *Toxocara* skin sensitivity tests and other observations in animals experimentally infected with *Toxocara canis*. *Trans. Roy. Soc. Trop. Med. Hyg.* 63:827-834, 1969.
 25. WISEMAN, R.A.; WOODRUFF, A.W. & PETTIT, L.E. — Further *Toxocara* skin sensitivity tests in animals experimentally infected with *Toxocara canis*. *Trans. Roy. Soc. Trop. Med. Hyg.* 63:246-250, 1969.
 26. WOODRUFF, A.W. — Toxocariasis. *Brit. Med. J.* 3:663-666, 1970.
 27. WOODRUFF, A.W. & THACKER, C.K. — Infection with animal helminths. *Brit. Med. J.* 1:1001-1005, 1964.

Recebido para publicação em 26/6/1975.