STUDY ON THE DIAGNOSIS OF *GNATHOSTOMA* INFECTION IN CATS BY RADIOIMMUNOASSAY

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INTRODUCTION

In general practice, diagnosis of gnathostomiasis is largely based on clinical symptoms of migratory swellings which can not be differentiated from other parasitic infections. Skin test and precipitin tests have been tried (Yamaguchi, 1952; Egashira, 1953; Ando, 1957; Furuno, 1959) but the results were unsatisfactory due to insensitivity and false positive results or cross reaction with other parasitic diseases (Tada *et al.*, 1966; Morisita *et al.*, 1969). Skin test gave a false positive of 30% (Daengsvang, pers. comm.). In the present study, radioimmunoassay (RIA) technique was attempted for the diagnosis of this disease by means of measuring antibody activity in cat serum against *Gnathostoma spinigerum* antigen (Gn-Ag).

MATERIALS AND METHODS

The study was performed on 4 groups of domestic cats : 4 non-infected cats (control), 4 cats with natural infection of *Toxocara cati*, 4 cats with mixed infection of *Opisthorchis sp.* and hookworm or tape worm, and 8 cats experimentally infected with *Gnathostoma spinigerum* as described by Daengsvang (1968) and Daengsvang *et al.*, (1970).

Gnathostoma antigen (Gn-Ag) was prepared by water extraction of *G. spinigerum* third stage larva according to the method described by Sawada *et al.*, (1965). Anti cat IgG antibody (Ab_2) was isolated on an immunoadsorbent (Miles and Hales, 1968) and was iodinated by the modificated chloramine-T method of Greenwood *et al.*, (1963) as described by Beck and Hales (1975).

Gnathostoma antigen at the concentration of 5 ng/ml was coated on the inner surface of polyethylene tube (Catt and Tregear, 1967). After washing out the excess antigen, the cat serum at the dilution of 1:200, 1:400, 1:800, 1:1,600, 1:5,000 and 1:10,000 in 0.01 M, pH 7.4 phosphate buffer containing 5% bovine serum albumin were allowed to react with Gn-Ag. The tube was washed after the first incubation followed by the binding of ^{125}I-Ab_2 to cat antibody which was already bound to Gn-Ag on the tube surface. After the second incubation the tube was again washed and counted in an automatic gamma counter (Packard Auto-Gamma Scintillation Spectrometer). The optimum incubation time and temperature during the first and second incubation was also studied.

RESULTS

The effects of the incubation time and temperature on the binding of antibody in the cat serum to Gn-Ag (first incubation) are shown in Fig. 1. The binding expressed as count per minute (cpm) at 37°C was better than at 4°C. At these temperatures, the binding varied directly to the incubation period up to 4-6 hours then became stable. The optimum time for the first incubation was found to be 4 hours at 37°C. The result of the second incubation for the binding of ^{125}I-Ab_2 to cat antibody is shown in Fig. 2. The binding at 37°C was also better than at 4°C and the optimum incubation time was 16 hours.

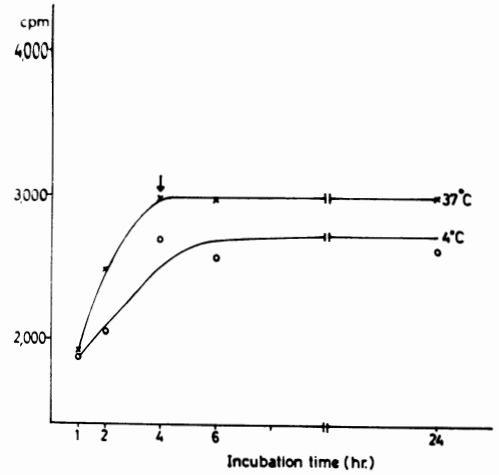

Fig. 1—First incubation time showing the binding of antibody in cat serum to *Gnathostoma* antigen at 4°C (o——o) and 37°C (x——x).

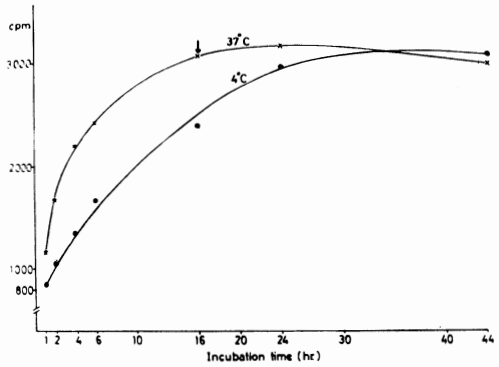

Fig. 2—Second incubation time showing the binding of ^{125}I-labelled anti cat IgG antibody to antibody in cat serum at 4°C (•——•) and 37°C (x——x).

The results of antibody activity detected in sera of four groups of cats are shown in Fig. 3. The mean value and standard error of the results, expressed as cpm, of each group were plotted against serum dilution. The results showed that even when the serum was diluted to 1:10,000, its antibody was still detectable by this method. Antibody activity of *Gnathostoma* infected group was significantly higher ($p < 0.01$) than those of control group and of *Toxocara* infected group at every dilution of sample. In comparison with *Opisthorchis* infected group, the antibody activity of *Gnathostoma* infected group was also statistically higher at 1:200 and 1:400 but not at the dilution greater than 1:400.

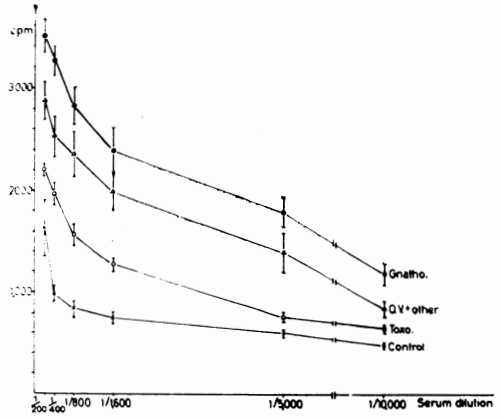

Fig.—3 Antibody activity against *Gnathostoma* antigen of *Gnathostoma spinigerum* infected cats (•——•). *Toxocara sp.* infected (o——o), *Opisthorchis sp.* infected (△——△) and control cats (x——x) expressed as mean cpm ± S.E.

DISCUSSION

The sensitivity of the technique of using solid phase RIA and ^{125}I-labelled anti cat IgG antibody is high but the specificity is not satisfactory. The cross reaction between Gn-Ag and *Opisthorchis* infection was observed in the present study.

^{125}I-labelled anti cat IgG antibody was used instead of the direct radio-iodinated *Gnathostoma* antigen since Gn-Ag labelled with ^{125}Iodine gave a very low yield in iodination and was denatured within a very short time within 24 hours. It has been shown that the iodination process could damage some proteins of the labelled antigen causing a decrease in the sensitivity of the assay system (Bolton and Hunter, 1973). Labelled antibody was found to be very stable. It can be used. over two months with no significant loss of assay properties (Woodhead *et al.*, 1974).

SUMMARY

A diagnostic method for gnathostomiasis by radioimmunoassay was carried out. Antibody activity produced in cat serum against *Gnathostoma* antigen was measured by using solid phase method and ^{125}I-labelled anti cat IgG antibody. The result showed that the antibody activity in cats infected with *Gnathostoma spinigerum* was significantly higher than those of the control group and the *Toxocara* infected cats even at the dilution of 1:10,000, except in the *Opisthorchis* infected group at the dilution higher than 1:400. The results in the present study seem to be promising for the diagnosis of gnathostomiasis. Cross reaction with other parasitic infections and the development for the higher specificity by this method is in progress.

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