

Case Report

A Case Report of a Middle-Aged Married Couple with Eosinophilia Who were Simultaneously Diagnosed Immunoserologically as Ectopic Ascariasis

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Ascariasis was one of the major problems in public health in Japan, when human manure was used for fertilizers. Over 80% of Japanese people were suffered from this disease (Yamaguchi, 1988). However, like other parasitic diseases, the incidence of ascariasis drastically decreased along with the improvement of public health conditions. Extensive mass screening and treatment also had contributed to reduce the epidemics of this disease. Still, a few cases, especially those with ectopic migration in bile ducts or other sites than digestive tracts, have been sporadically found all over the country (Kisu *et al.*, 1989). In addition, rare cases of severe infection were also reported from the rural areas (Katada *et al.*, 1991; Nishiyama *et al.*, 1991; Oshikawa *et al.*, 1992). Because the majority of ascariasis cases were easily diagnosed by direct detection of worms and/or parasite eggs, the diagnostic value of immunoserological tests for this disease or other intestinal helminthiasis is considered to be relatively low except for ectopic cases (Tsuji, 1991). In this report we describe a case of a middle-aged married couple with eosinophilia who were simultaneously diagnosed immunoserologically as ectopic ascariasis.

Case Report

The patients were a 58 year-old man and his 57 year-old wife who were living together in the mountainous area of Kagoshima Prefecture. They have never been to abroad nor had an experience of serious illness. Once every year they go to a regional hospital to take periodical health check-up for over 5 years. Although they had never been pointed out any abnormalities until last year, both of them were pointed out marked eosinophilia (the husband's total white blood cell count was 19100 with 62% eosinophils and those of his wife was 8600 and 62%, respectively) by the haemogram in a series of laboratory examinations on May 31 this year. Although they did not have any subjective complaints, further detailed evaluation was carried out at the Department of Dermatology, Miyazaki Medical College, because parasitic diseases were strongly suspected. No abnormalities were detected by physical, radiological, and ultrasonographic examinations. By laboratory examinations, the husband was found to have eosinophilia and elevation of total IgE level, whereas the wife had eosinophilia without elevation of total IgE. Slight liver dysfunction (GOT: 67 IU/dl, GPT: 44 IU/dl, γ GTP: 150 IU/dl) and elevations of CRP (+) was noted in the husband and elevations of alkaline phosphatase (12.1 K-A U) and total γ -globulin (total protein 8.4 g/dl, γ -globulin 27.9%, A/G ratio 1.1) were noted in the wife. When their sera were subjected for immunoserological diagnosis for parasitic diseases, both samples showed strong positive reaction against *Ascaris lumbricoides suum*

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antigen with some cross reactivities against other nematode antigens by multiple-dot enzyme-linked immunosorbent assay (dot-ELISA) (Fig. 1). By an Ouchterlony's double diffusion test in agar, both samples produced strong specific precipitin bands against *Ascaris* antigen without remarkable cross reactions against other nematode antigens (Fig. 2). The precipitin bands produced by the patients' sera

against *Ascaris* antigen were completely fused to that produced by the positive control serum from a confirmed ascariasis patient (Fig. 3). Subsequently, inhibition of the binding of the patients' sera to *Ascaris* antigen by homologous and heterologous antigens (*Toxocara canis*, *Anisakis simplex*, *Gnathostoma doloresi*, *Paragonimus westermani*) was tested by microplate ELISA to confirm the specificity

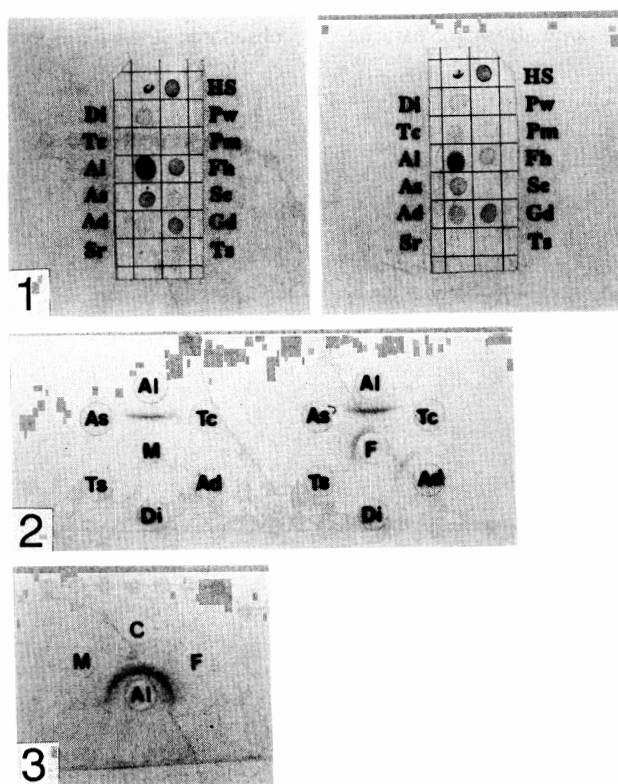


Fig. 1 The multiple dot-ELISA test showing strong positive reaction against *Ascaris lumbricoides* antigen and some cross reactions against other nematode antigens.

left: the husband's serum, right: the wife's serum.

HS: Normal human serum, Di: *Dirofilaria immitis*, Pw: *Paragonimus westermani*, Tc: *Toxocara canis*, Pm: *Paragonimus miyazakii*, Al: *Ascaris lumbricoides*, Fh: *Fasciola hepatica*, As: *Anisakis simplex*, Se: *Spirometra erinacei*, Ad: *Ancylostoma duodenale*, Gd: *Gnathostoma doloresi*, Sr: *Strongyloides ratti*, Ts: *Trichinella spiralis*.

Fig. 2 Ouchterlony's double diffusion test in agar showing precipitin band against *Ascaris* antigen. The patients' sera (M: the husband, F: the wife) were tested against an array of parasite antigens. Abbreviations were the same as those in the legend to Fig. 1.

Fig. 3 Specificity of precipitin reaction by an Ouchterlony's test.

The patients' sera (M: the husband, F: the wife) and positive control serum (C) were tested against *Ascaris* antigen (Al).

of the binding to the antigen. As shown in Fig. 4, almost complete inhibition was observed by homologous antigen at low concentrations (approx. 6 $\mu\text{g/ml}$). Although *T. canis* antigen almost completely inhibited the binding of the patients' sera to *Ascaris* antigen, the amount required to give 50% inhibition was about 30–50 times higher than that of homologous antigen. Two other heterologous nematoda antigens or *Paragonimus* antigen did not inhibit the binding of the antibodies to *Ascaris* antigen. Although stool examinations for parasite

eggs (direct wet mount and AMS-III sedimentation method) were repeatedly carried out, neither *Ascaris* eggs nor other parasite eggs were detected in the stool specimens. The patients have been, as outpatients, followed up for haematological and immunoparasitological changes for over 4 months. As is summarized in Fig. 5, eosinophilia was persisting in both patients for the period examined. In addition, total IgE level of the husband also elevated persistently. Elevation of total IgE level was never observed in the wife. Specific IgG antibody titre for

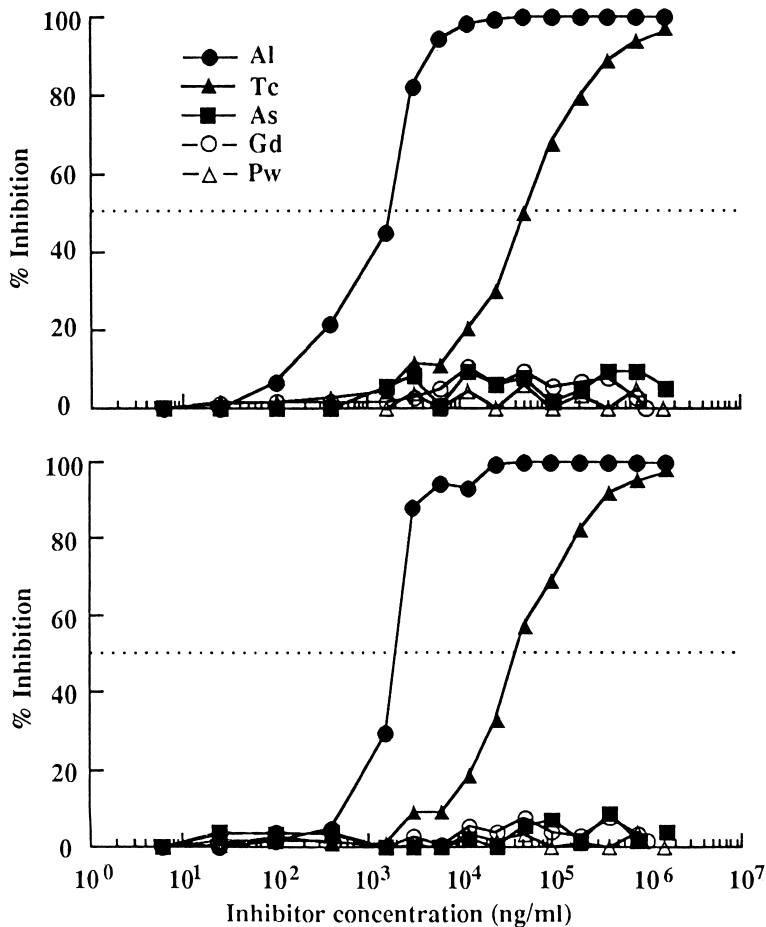


Fig. 4 ELISA inhibition test.

Sera from the patients diluted 1:10000 were incubated at 4°C overnight with various concentrations of *Ascaris lumbricoides*, *Toxocara canis*, *Anisakis simplex*, *Gnathostoma doloresi*, and *Paragonimus westermani* antigens, and then added to microtitre wells coated with *A. lumbricoides* antigen (4 $\mu\text{g/ml}$). Inhibition of the binding of the patients' sera to the antigen was calculated relative to buffer control.

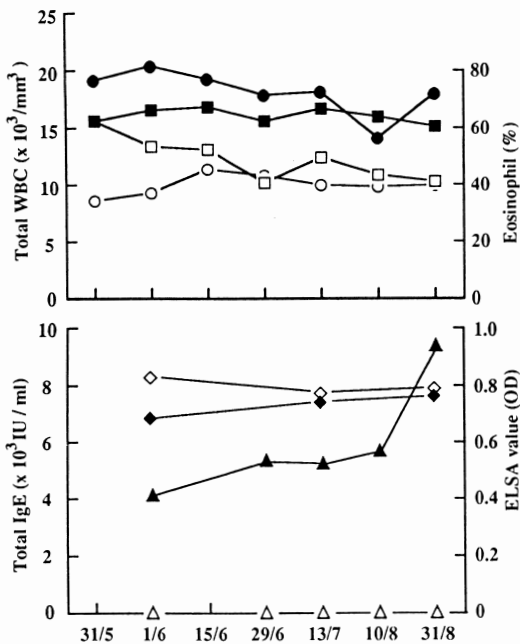


Fig. 5 Kinetics of total white blood cell count (●, ○), eosinophilia (■, □), total IgE (▲, △), and ELISA values (◆, ◇) of the patients. closed symbols: the husband, open symbols: the wife.

Ascaris antigen in their serial serum samples remained extremely high for the period examined. Although we made an effort of treating them with anthelmintic drug (Combantrin®), neither the worms were purged in their stool nor was their eosinophilia improved. From these results, the patients were diagnosed as ectopic ascariasis of unknown site. Because they were asymptomatic except for eosinophilia with or without elevation of total IgE level, further follow-up study without treatment is still going on in our departments.

Discussion

We describe herewith two cases of ectopic ascariasis which were diagnosed only by immunoserological study while the presence of the parasite was not confirmed. Although *A. lumbricoides* is naturally an intestinal helminth of human beings, this parasite occasionally causes ectopic infections in various sites (Maki, 1961). In addition, *Ascaris* larvae often cause *larva migrans* in the lung known as pulmonary infiltration with eosinophilia (PIE

syndrome) or Löffler's syndrome (Yoshida, 1991). In the present cases, neither parasite eggs nor worms were detected in spite of repeated stool examinations. In parallel with persistent eosinophilia, *Ascaris*-specific IgG antibody titres measured by microplate-ELISA tests were consistently high in their serial serum samples throughout the period examined. Therefore, both of them were diagnosed as ectopic ascariasis without identification of the site of infection. Concerning the possible site of infection, peritoneal cavity is most likely because no abnormalities were found in lungs, liver or digestive tract by radiologic and ultrasonographic examinations. Since the patients were a married couple living together and had no abnormalities by the medical examinations one year ago, they seem to be infected simultaneously by eating the same foods contaminated with *Ascaris* eggs within one year.

In the present study, the only evidence to support ectopic infection with *Ascaris* worms was the continuous detection of the specific antibody in the patients' sera. Immunoserological examinations have been widely used for the diagnosis of parasitic diseases and are particularly useful for ectopic parasitosis (Araki, 1991). In ascariasis, however, applicability of immunodiagnosis such as an Ouchterlony's double diffusion test or ELISA has not been extensively studied because the majority of cases were easily diagnosed by stool examination for parasite eggs or direct demonstration of the presence of the parasite. In addition, Tsuji (1986) pointed out that some caution should be paid for the immunodiagnosis of ascariasis because sera from patients with ulcerative colitis, Crohn's disease, and some other autoimmune diseases often showed false-positive reaction against *Ascaris* antigen. This possibility is unlikely for the present case because the patients did not show any signs or symptoms related to these diseases.

The patients showed persistent eosinophilia and high antibody titre against *Ascaris* antigen and were assumed to be infected with *Ascaris* simultaneously. Nevertheless, elevation of total IgE was only observed in the husband but never seen in his wife. Eosinophilia is primarily dependent on IL-5 (Yamaguchi *et al.*, 1988) and IgE production on IL-4 (Kühn *et al.*, 1991), and both IL-4 and IL-5 are produced by Th2 subsets of T cells (Mosmann and

Coffman, 1989). Since parallel regulation of IL-4 and IL-5 in human helminth infections has been reported (Mahanty *et al.*, 1992), the dissociation of IgE production observed in present cases provide an interesting clue as to the cytokine producing activity of T cell subsets in response to specific antigen.

In conclusion, two cases reported here add a value on the importance of immunodiagnosis for ectopic ascariasis.

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