

## ISOLATION OF TOXOPLASMA GONDII FROM HUMAN TISSUES

M. Ghorbani \*  
A. Hafizi \*  
A. Sanati \*  
H. Modarres-Musavi \*\*

### ABSTRACT

Nineteen lymph node biopsies, 3 curettings, 15 blood samples obtained from patients with lymphadenitis and high titres of Toxoplasma antibodies, and 27 tonsillar tissues removed from patients because of chronic tonsillitis were inoculated intraperitoneally into mice for isolation of Toxoplasma gondii. Toxoplasms were isolated from 5 lymph node biopsies, one curettage material, 2 tonsillar tissues and none of the blood samples.

### INTRODUCTION

Toxoplasmic lymphadenitis or glandular toxoplasmosis is the most common clinical form of acquired toxoplasmosis (1,2,3). The importance of glandular toxoplasmosis lies in its differentiation from much more serious conditions, specially Hodgkin's disease and lymphosarcoma for which it may be mistaken in both clinical and pathological grounds (4).

Laboratory diagnosis of toxoplasmosis can be satisfactorily made by isolation of the causal organism or by demonstration of a significant rise of antibody titres in the

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\* School of Public Health and Institute of Public Health Research, University of Teheran, Iran.

\*\* Amir-Aalam Hospital, Saadi Street, Teheran, Iran.

Serum (1). However, isolation of the parasite is the most convincing proof of the infection. Inoculation of suspected materials into susceptible laboratory animals is the method of choice for the isolation (5).

Isolation of Toxoplasma gondii has been reported from human lymph node biopsies (1,6,7), uterine curetting and placenta (8,9), tonsillar tissues (10,11) and blood specimens (12, 13, 14, 15, 16) in different areas. In Iran, this organism has also been isolated from human lymph node and congenitally infected infant (17, 18).

The present paper reports on isolation of Toxoplasma from human tissues removed from patients with lymphadenitis or tonsillitis by mouse inoculation during 1973 to 1978 in Iran.

## MATERIALS AND METHODS

### Specimens:

Lymph nodes: 19 lymph node biopsies removed from patients (aged 7 to 55 years, 10 males and 9 females) in several hospitals in Tehran, Iran. The patients had lymphadenopathy and Toxoplasma antibody titres ranging from 1:800 to 1:12800 as measured by indirect fluorescent antibody (IFA) technique.

Curettings: 3 curettage materials obtained from women (aged 22 to 32 years, all in the first trimester of pregnancy) with lymph node enlargement and IFA Toxoplasma antibody titres ranging from 1:6400 to 1:25600. In these patients curettage was carried out because of clinical manifestation and serological evidence of acute toxoplasmosis during pregnancy.

Tonsils: 27 tonsillar tissues removed from patients (aged 4 to 22 years, 22 females and 5 males) with chronic tonsillitis, admitted to Amir-Aalam Hospital in Teheran for tonsillectomy. Thirteen of them had Toxoplasma antibody titres of 1:2 to 1:64 as measured by latex agglutination slide test (LAST) and the rest were sero-negative. In physical examinations cervical lymph node enlargement were found in 5 of the patients.

Blood: 15 blood samples collected in heparinized test tubes from patients (aged 10 to 42 years, 11 males and 4 females) with lymphadenitis and Toxoplasma antibody titres ranging from 1: 1600 to 1:102400 as measured by IFA technique.

### Serological examination:

Latex agglutination slide test and indirect fluorescent antibody technique were performed for determination of Toxoplasma antibodies according to the procedures used by Ghorbani and Hafizi (19) and Ghorbani et al. (20) respectively.

### Isolation of the parasite:

For isolation of parasite, materials were inoculated intraperitoneally into albino mice according to the Beverley technique (5). Penicillin was added only to the prepared tonsillar tissues suspensions to give a final concentration of 2000 units per ml. Each specimen was injected into 4 to 10 mice and each mouse was given 0.5 to 1 ml of the inoculum. Normal mice were used as control. The animals were under observation for a period of 4 to 6 weeks. In the case of mice dying during this period wet mount smears from peritoneal exudate (if present) and brain tissues as well as impression smears from spleen, liver, kidneys and lungs were prepared, stained with Giemsa, and microscopically examined for toxoplasms. After the observation period, LAST were performed on the blood obtained from tails of the surviving mice. Mice with sero-positive reactions were killed and their brain tissues were examined for Toxoplasma cysts.

## RESULTS

Lymph nodes: Toxoplasms were isolated from 5 out of 19 lymph-node materials (26.3%). The parasitologically positive nodes were removed from cervical regions of patients (aged 18 to 28 years, 3 males and 2 females) with Toxoplasma antibody titres of 1:6400 or higher as measured by IFA technique.

Curettings: The parasite was isolated only from one of the curettage material obtained from a 32-years old woman with IFA Toxoplasma antibody titre of 1:6400.

Tonsils: Toxoplasms were isolated from 2 of the tonsillar tissues removed from 2 girls, 7 and 14 years old, with a history of chronic tonsillitis. They had Toxoplasma antibody titres of 1:32 and 1:64 as measured by LAST. In one patient cervical lymph node enlargement was present.

All of the isolated strains of Toxoplasma were recovered in the forms of tissue cysts from the brains of

inoculated mice 4 to 6 weeks after inoculation and they were all avirulent to mice.

## DISCUSSION

Glandular toxoplasmosis diagnosed on the basis of isolation of the parasite or on serological evidence or a histological picture of the lymph node has been reported from various countries (10, 17, 21). In the present work among the 8 cases of parasitologically proved toxoplasmosis, 7 patients had lymphadenopathy. The high *Toxoplasma* antibody titres in all of the parasitologically positive cases may indicate acute or recent infections, although we could not follow up the patients to determine the changes of the antibody titres. Most of the lymph node biopsies from which Toxoplasma was not isolated were also removed from highly sero-positive patients. Possibly the enlargement of the lymph nodes in such patients may not be a direct result of infection in the nodes but an immunological response to the infection, mainly at the sites other than lymph nodes, as it has been mentioned by Beverley et al. (21).

Despite clinical manifestations and serological evidence of acute toxoplasmosis in 3 pregnant women Toxoplasma was isolated only from one of them. This finding may be interpreted by the statement of Desmonts and Couvreur (9) that maternal toxoplasmosis acquired during pregnancy does not necessarily result in congenital infection.

Tonsillitis in 2 parasitologically positive patients, one with cervical lymphadenitis, and both with high Toxoplasma antibody titres, may have been due to acute stage of toxoplasmosis. Further investigation is required to explain the probable relationship between tonsillitis and toxoplasmosis.

Isolation of Toxoplasma gondii from blood specimens have been reported by different investigators (12, 13, 14, 15, 16). However, we failed to isolate the organism from blood samples collected from patients that were diagnosed as having toxoplasmosis on the basis of clinical manifestations and the results of serological tests. It is not clear that parasitaemia was absent in the patients or passive transferred antibodies suppressed the reproduction of the organism in mice.

The results of this study and the previous reports on isolation of Toxoplasma from man and animals in Iran (17,

18, 19, 22) indicates that toxoplasmosis is common in this country and lymphadenopathy with toxoplasmic etiology is considerably important.

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