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# Seroepidemiology of Urogenital Trichomoniasis in Japan

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## Abstract

An enzyme-linked immunosorbent assay (ELISA) for detecting antibody against *Trichomonas vaginalis* was used to survey trichomonal infection in Japan. Serum samples from randomly chosen, symptom-free, 451 volunteers (227 males and 224 females) were examined; 19 females (8.5%) tested positive for IgG antibody. The incidence of seropositivity is much higher than that obtained by vaginal smear. The absence of seropositivity in the samples from the male volunteers suggests that the organisms may be non-invasive in males, most of whom likely serve only as vectors of the parasite. The incidence of seropositivity was high in the 25—29, 45—49, and 55—59 year old females. The IgM, IgA and IgG antibody fractions showed a logarithmic normal distribution. As to specificity, no cross-reactivity was found against two other protozoan parasites, *Entamoeba histolytica* and *Giardia lamblia*.


**Key words:** *Trichomonas vaginalis*, Epidemiology, Enzyme-linked immunosorbent assay (ELISA)

## Introduction

*Trichomonas vaginalis* is a very common parasite of the urogenital tract, especially in the female. Trichomoniasis is recognized as one of the sexually transmitted diseases. The expression of the disease varies widely; generally asymptomatic or only a genital discharge, but it can be severe. Diagnosis is by demonstration of the parasite, most commonly in wet film preparations of vaginal and urethral discharges and prostatic secretions, less frequently when it is recognized in Papanicolaou smears by astute cytotechnologists. Although culture of the organisms can improve diagnostic accuracy, the method is complex and time-consuming. In the search for an accurate and rapid diagnostic test, numerous immunologic procedures have been studied, including gel diffusion (Mathews and Healy, 1983), indirect hemagglutination (Kuberski, 1978), direct immunofluorescence (Krieger *et al.*, 1988) and enzyme immunoassay (Alderete, 1984; Street *et al.*, 1982; Yule *et al.*, 1987). The enzyme-linked immunosorbent assay (ELISA) is the most sensitive, compared with the other immunological methods (Cogne, 1985). In this study, antibodies to *Trichomonas vaginalis* in symptom-free, 451 volunteers were sought by ELISA. Of 224 females, 19 (8.5%) tested positive; of 227 males, surprisingly, none were positive.

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## Materials and Methods

Source of sera. Serum samples from symptom-free, 451 volunteers (227 males and 224 females), who underwent health screening, were collected and frozen at —20°C until used. The ages of the volunteers ranged from 19 to 63 (average age, 33 years).

Antigen. *T. vaginalis* organisms were maintained axenically in Diamond medium (Diamond, 1957) at 37°C and subcultured at 48 and 72 hr intervals. Organisms were harvested for antigenic preparation by pooling 48 hr cultures and centrifuged at 1,000 × g for 5 min. The supernatant was removed by aspiration, and the sedimented organisms were washed 3 times in 0.01M phosphate buffered saline (pH 7.2, PBS). The sedimented parasites were resuspended in approximately 1 ml of 0.01M carbonate buffer (pH 9.6), then sonicated. The sonicate was centri-

fuged at 10,000 × g for 20 min at 4°C and the supernatant was used as antigen. To test for cross-reactivity, *Entamoeba histolytica* and *Giardia lamblia* were used. These organisms were subcultured axenically in TYI-S-33 medium (Diamond, 1968; Keister, 1983) and the procedure followed for the preparation of antigen was the same as that outlined above for *T. vaginalis*. The protein concentrations of the three antigenic preparations were determined by the method of Lowry *et al.* (1951). The final concentration of all antigenic solution was adjusted to 0.01 mg/ml in 0.01M carbonate buffer (pH 9.6).

ELISA. ELISA multiplates (Sumitomo Bakelite Corp., Tokyo), with 96 flat-bottomed wells, were sensitized with 0.1 ml (0.01 mg/ml) of antigen for 2 hrs at 37°C. The plates were then washed 3 times with 0.05M PBS supplemented with 0.05% Tween 20 (PBS-T). 0.1 ml of serum, diluted with PBS-T containing 1% bovine serum albumin (BSA-T), was added to the wells for IgG determinations, the serum was diluted 1:400; for IgM and IgA, 1:100. After incubation for 1 hr at 37°C, the plates were washed again and 0.1 ml aliquots of anti-human IgG (heavy and light chains specific), anti-human IgM ( $\mu$ chain specific), or anti-human IgA ( $\alpha$ chain specific), conjugated with horseradish peroxidase (Cappel) were diluted 1:1,000 in BSA-T and added to each well. The plates were incubated for another 1 hr at 37°C, then washed 3 times with PBS-T. Next, 0.1 ml of a 0.03% 2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid) diammonium salt (Sigma) containing 0.003% hydrogen peroxide, prepared in 0.1 M citric acid-0.2 M disodium hydrogen phosphate buffer (pH 4.8), was added to each well. After a final incubation of 1 hr at room temperature, 0.025 ml of 1.25% sodium fluoride solution was added to each well to stop the reaction. The results were expressed as absorbance at 405 nm ($OD_{405}$). All determinations were performed in triplicate.

## Results

Anti-*T. vaginalis* IgG (ELISA) in the serum samples of 227 males showed a logarithmic normal distribution. The mean and one standard deviation in logarithm were −1.28 (absorbance = 0.052) and −2.793, respectively. The upper limit of the 99.7% confidence interval (mean + 3SD) was 0.218. The distribution of anti-*T. vaginalis* IgG in serum samples of 224 females yielded 2 peaks, i.e., a bigger peak in the low ELISA range and a smaller peak in the high range (Fig. 1). The absorbance of the bigger peak was 0.051, the same as the mean for the males, and that of the smaller peak was 0.816. Nineteen of the 224 females (8.5%) showed ELISA values higher than the upper limit of 99.7% confidence interval among males. To examine the relation between

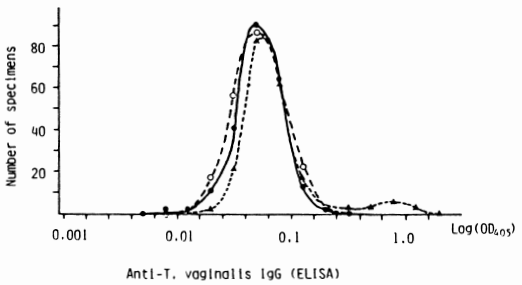


Fig. 1. Distribution of anti-*T. vaginalis* IgG (ELISA) in randomly chosen, symptom-free, 227 males and 224 females. Males (●); females (▲); calculated normal distribution for males (○).

Table 1 Age distribution of seropositive females (ELISA)

| Age group | Samples (No.) | Positive* (No.) | Positive (%) |
|---|---|---|---|
| 15—19 | 10 | 0 | 0 |
| 20—24 | 81 | 2 | 2 |
| 25—29 | 61 | 9 | 15 |
| 30—34 | 26 | 2 | 8 |
| 35—39 | 14 | 1 | 7 |
| 40—44 | 9 | 1 | 11 |
| 45—49 | 7 | 2 | 29 |
| 50—54 | 10 | 1 | 10 |
| 55—59 | 3 | 1 | 33 |
| Unknown | 3 | 0 | 0 |
| Total | 224 | 19 | 8.5 |

* Values greater than 3 standard deviations from the mean of the calculated normal distribution of the males.

infection and age, the seropositive individuals were grouped according to age. The results are shown in Table 1, and indicate three peaks, at 25—29, 45—49 and 55—59 years of age. No positives were found in females under 19 years of age.

To detect acute infections with *T. vaginalis,* the specific IgM of all 224 female and 47 male sera was measured by ELISA. As the levels of IgA also be useful in the diagnosis of trichomoniasis, it was also measured. The logarithmic distributions of both the anti-*T. vaginalis* IgM and IgA were similar to that of IgG antibody. The mean and one standard deviation of the IgM antibody were —2.05 ± —2.71 (M+3SD= 0.865), and those of the IgA antibody were —1.08 ± —2.66 (M+3SD=0.920). All sera that tested positive for IgG had IgM and IgA values within normal ranges. There was no correlation between IgG antibody and IgM ($p>0.05$) and IgA ($p>0.05$) (Figs. 2, 3).

Cross-reactivity with the other protozoan antigens, i.e., *E. histolytica* and *G. lamblia,* was not observed (Figs. 4, 5). The ELISA readings were consistently negative for anti-*E. histolytica* IgG ($p>0.05$) and for anti-*G. lamblia* ($p>0.05$).

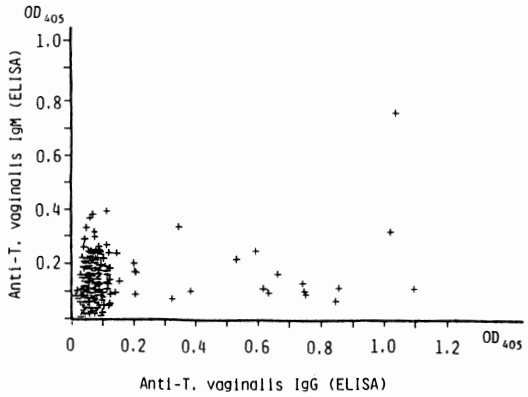


Fig. 2. Correlation between anti-*T. vaginalis* IgG and IgM (ELISA). $r=0.240$ ($p>0.05$).

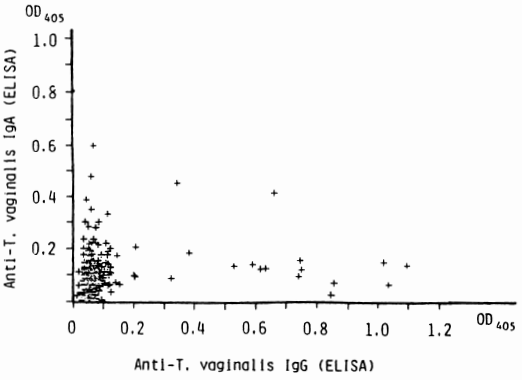


Fig. 3. Correlation between anti-*T. vaginalis* IgG and IgA (ELISA). $r=0.107$ ($p>0.05$).

## Discussion

*Trichomonas vaginalis* is a very common

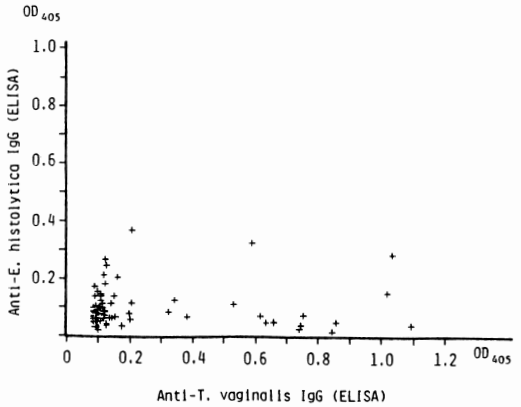


Fig. 4. Lack of cross-reactivity (ELISA) between anti-*T. vaginalis* IgG and anti-*E. histolytica* IgG. $r=0.068$ ($p>0.05$).

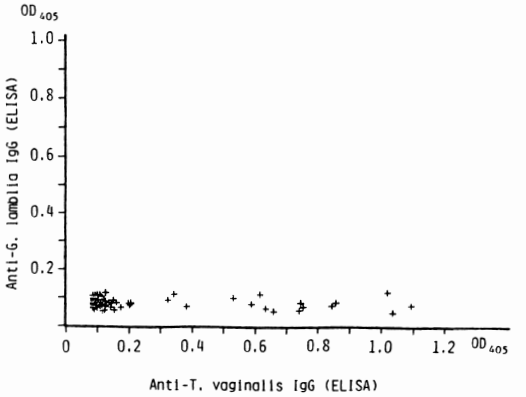


Fig. 5. Lack of cross-reactivity (ELISA) between anti-*T. vaginalis* IgG and anti-*G. lamblia* IgG. $r=0.034$ ($p>0.05$).

human parasite, especially of the female genital tract, where it may cause vaginitis. Melvin and Healy (1985) estimated that 5 million females and 1 million males in the United States are infected. The prevalence of trichomonal infection in Japanese females was determined to be 0.65% by vaginal smear (Uekusa *et al.*, 1987). However, the diagnosis of trichomoniasis is frequently difficult. The routine procedure of microscopic examination of vaginal exudate often fails to detect the organism. Culture methods may be employed and will sometimes increase the percentage of positive identifications. Bland *et al.* (1931) have shown that trichomonas infection is exceedingly common in pregnant women, but Kimura (1986) also indicated that the detection of the organism is more difficult in pregnant women. A simple and accurate method for diagnosis is therefore greatly needed now. Immunological methods, especially ELISA, are very effective for that purpose. Street *et al.* (1982) state that 80.4% of females with vaginal trichomoniasis and 13.7% of supposedly negative females, examined by the microscopic method, were detected by ELISA. In this study, we measured anti-*T. vaginalis* IgG among symptom-free people by ELISA to determine the prevalence rate of trichomoniasis. Specific IgG antibody in the sera of 227 males was not detected by ELISA. This indicates no infected males in this group. On the other hand, the IgG antibody distribution in the sera of 224 females showed two peaks. The number of seropositive females is much higher than that determined by microscopic examination of vaginal smears (Uekusa *et al.*, 1987). This demonstrates that the serodiagnosis by ELISA is more sensitive than the examination of urethral discharge. Patients with symptoms and individuals with a history of trichomoniasis can be included in the group of seropositive females. In the males, nobody tested positive. This finding is inexplicable because the infection is commonly associated with sexual intercourse. It therefore suggests that the organisms may be non-invasive in males, most of whom likely serve only as vector of the parasite.

To examine for the presence of active infection in the seropositive females, specific IgM and IgA antibodies were also measured. The levels of both immunoglobulins were within the normal range, thus denying recently acquired active infections in the 19 IgG positive females. This interpretation is based on the data from experimental animals, where IgM and IgA antibodies appear and increase in parallel soon after infection, then begin to decrease 2 to 3 weeks later. Concomitantly, IgG levels begin to rise (Alderete, 1984).

The possibility that an accurate diagnosis may be handicapped by cross-reactivity in serological examinations is well known in parasite infections (Kagan, 1963). However, anti-*T. vaginalis* antibodies did not cross-react with either *E. histolytica* or *G. lamblia* antigens.

The specimens tested by ELISA were obtained from apparently symptom-free men and women. In this study, we detected 19 seropositive individuals (all female), suggesting that the incidence of trichomonal infection is quite high in Japanese women. The age distribution of the seropositive females shows that the prevalence rate was low in females under 24 years of age, and most common during the age period of 30 to 40 years. These findings are in good agreement with previous observations that the maximum rate of infection coincides with the time of greatest sexual activity (Beaver *et al.*, 1984; Mascall, 1954). Jirovec and Petru (1968) proposed that symptoms of trichomoniasis during the late luteal and early estrogen phase of the menstrual cycle. These investigations also found that in most infected women, inflammatory manifestations worsened during menses and pregnancy, presumably because of the alkaline shift in the pH of the vagina that accompanies these events. This physiologic hypothesis is logical and serves to explain the lack of symptoms among the seropositive females reported here.

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