日本財団補助金による
２０００年度日中医学学術交流促進事業

⑤．在留中国人研究者研究助成

(2) 尿ＥＬＩＳＡを用いた法肺吸虫症の診断

尿 ELISA 法を用いた肺吸虫症の診断

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肺吸虫症の診断には糞便検査あるいは喀痰検査で虫卵を検出するのが困難なため、血清中に肺吸虫に特異的な抗体を検出する診断法が主に使われている。しかし流行地での集団検診には血液採取は困難な場合が多い。そこで、採取が簡単でかつ安全な尿を検体として用いた肺吸虫症の診断法の開発を目的として本研究を行なった。本研究では肺吸虫症患者の尿でウェステルマン肺吸虫成虫抗原に対する IgG、IgG4、IgM、IgA 抗体を測定した。その結果、患者尿中には抗ウェステルマン肺吸虫抗体が検出できた。IgG では感度８５％、特異性９４％であり、IgG4 ではそれよりやや高い感度特異性が得られ、疫学調査に有用な方法と考えられた。

Materials and Methods

1. Urine and serum samples :Totally 38 serum samples and 40 urine samples were obtained from 20 paragonimiasis westermani- patients in Japan. These patients were confirmed as paragonimiasis clinically and serologically. from patients with schistosomiasis japonicum(39), opisthorchiasis (24 samples), ascariasis (23 samples) and other parasitic infections(28 samples)were also used. Urine samples collected from 39 healthy Japanese students were used as controls.

2. Preparation of crude adult worm Antigen. P.westermani adult worm were recovered from dogs infected withmetacercariae collected from E.japonicus. The worms were washed repeatedly and homogenized in phosphate-buffered saline (PBS),pH7.2.The homogenate was centrifuged at 5,000×g for 20 minutes at 4℃ ,and the supernatant was used as crude AW antigen. After adjusting to 10mg/ml ,the antigens were kept at -20℃ until used.

3. ELISA for antibody detection .Ninety-six well polystyrene flat-bottom microtiter plate was coated with 10ug/ml of the AW crude antigen in a coating buffer(pH) at 4℃ overnight. After washing twice with washing buffer(0.05%Tween 20 in PBS).the plate was blocked with casein buffer 200ul/well at room temperature for 2h. then urine samples, four times diluted with PBS were applied and the plate was incubated at 37℃ for 2h.After washing four times, 4000 times diluted anti-human immunoglobulins conjugatedwith peroxidase; IgG or IgM or IgA was added and incubated at 37℃ for 2h.while IgG4 was used with 1000 times dilution and incubated at 25℃ overnight. After washing, ABTS was used as a substrate, and the absorbency at 415nm was measured using a microplate autoreader. The value Mean titer of healthy Japanese donors +3SD was used as a cut-off point. Three times serially diluted pooled positive sera were used in each plate to make a standard curve, antibody unit of each samples were calculated from the curve.

Results

Pretreatment of urine samples. On the prep-experiment for dilution of urine samples by using PBS solution,the result showed that the antibody titer of 4 times dilution increase significantly in most cases.(Fig,1).so it was decided using 4 times diluted urine samples at all experiments lately.

The AW-specific antibodies in urine form patients with parasitic diseases. The paragonimus westermani adult worm specific antibodies were examined, in parallel, in sera of patients, and urine of patients with schistosomiasis Japonicum(39), Opisthorchiasis(24 samples),Ascariasis(23 samples) and some different parasitic infections(28 samples) by ELISA. The data demonstrated that IgG antibodies to paragonimus westermani AW were detected in 85.0% (17/20)of paragonimiasis patients ,and 7 positive appeared in 114 patients infected with parasites other than paragonimus westermani such as schistosomiasis, Opisthorchiasis.(Fig,2).For case of IgG4, it seemed better than IgG in either sensitivity or specificity with 93.7% and 96.6%.The data was shown in Fig,3.Conversely,AW-specific IgM and IgA antibodies were not so good neither sensitivity nor specificity(The data were not shown).

In order to reduce the cross-reaction, A ELISA method using cysteine proteinase in ES instead of crude antigen was also be tried in the study.(The method has been reported by Ikeda.etc,1996).However, not better result was obtained in our condition.(Fig,4).

Follow up antibody detection for patients' urine samples. Using the urine samples from 11 patients collected before and after treatment, the IgG and IgG4 antibodies were detected, and changes were observed. In almost cases, the titer was going to down generally due to treatment (Fig5,6).This may be usefully in evaluation of efficacy for treatment.

Discussion

Enzyme-linked immunosorbent assays (ELISA) are widely used to immunodiagnosis infectious diseases including parasite infections. There have been many studies of paragonimiasis . Using fractionated antigens or characterized antigens with serum, already have given higher sensitivity and specificity of ELISA. However , for mass screening at the endemic area in the developing countries. it ask low cost, painless, rapid, and safety. There have been none using urine samples as examining individuals that collected easily and safely. Recently, urine samples have been used in some study at filariasis, schistosomiasis and more. For rapid diagnosis (7,8).In this study, the specimens-specific antibodies were detected with the same level reactivity of serum although the titers were lower than serum one.

About diluted treatment of urine samples, almost cases reached the highest titer at 4 times diluted point, and reduced orderly after 8 times dilution, but in some cases, those couldn't be detected appeared in specific antibodies detection without dilution and warming. The reason isn't known, but we may think that antibodies bind to the deposit and release into solution after dilution or warming for liberation of cold agglutination.

The ELISA using urine samples for antibodies detection showed higher sensitivity and specificity can compare with those of serum. This finding suggested that The URINE-ELISA may be a valuable method of immunodiagnosis in mass investigation helping us to master epidemiological information at endemic fields. Base on the above information, we propose that the specific antibodies detection like most usefully in both IgG detection and IgG4 detection. The IgG detection indicate much better than IgG4 with its less time need and similar specificity to IgG4.

After treatment, the antibody titer in urine samples from patients decreased orderly among a long period. This show that the specific antibody detection can help to judgment of treatment, but cannot be used to monitor the outcome of drug treatment or differentiate between past exposure to the parasite and the presence of an active infection.

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Fig1

Effect of dilution of urine on anti-*P.westermani* IgG titers

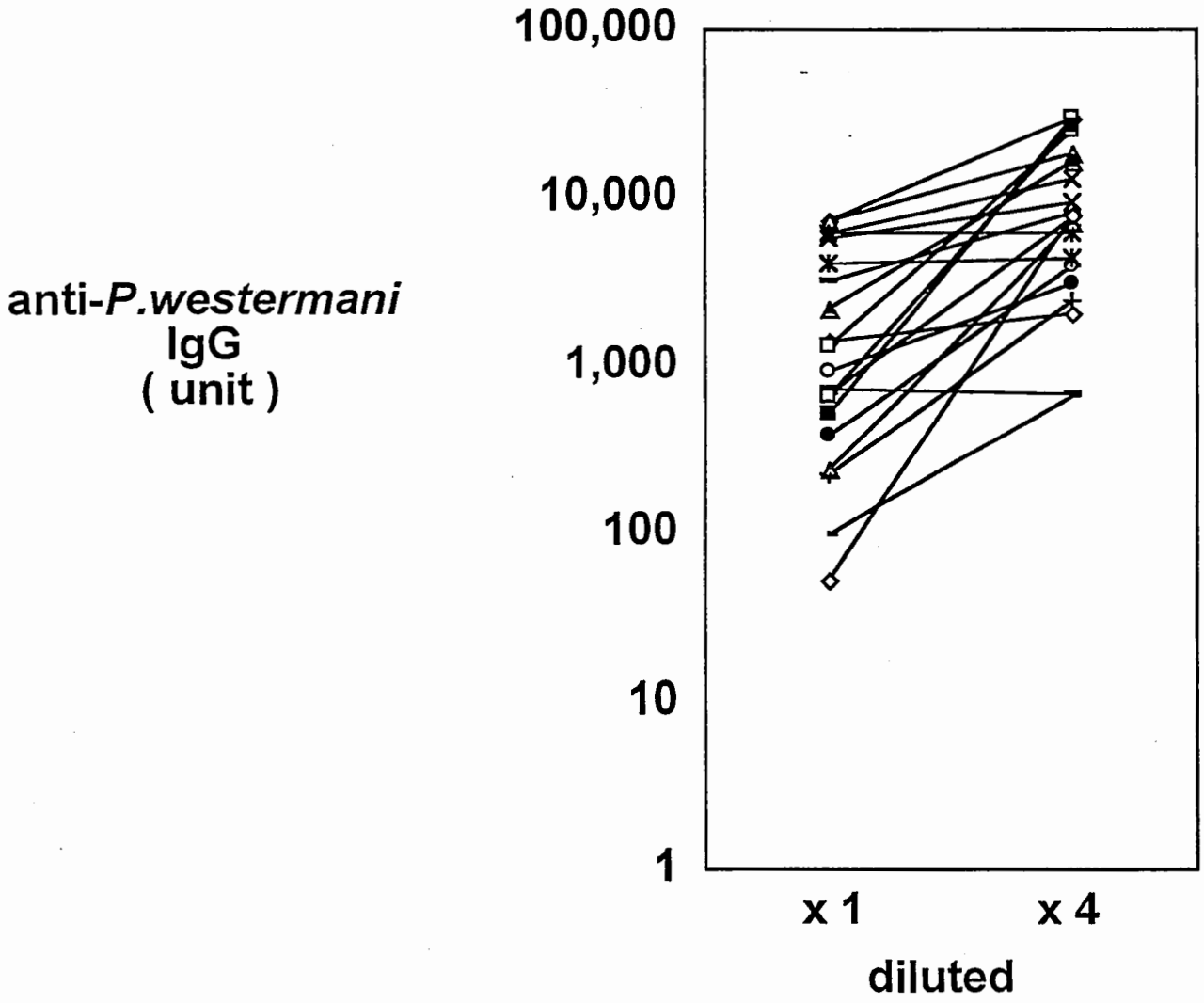


Fig2

IgG antibody units to *P.westermanii* adult worm according to different species of parasites

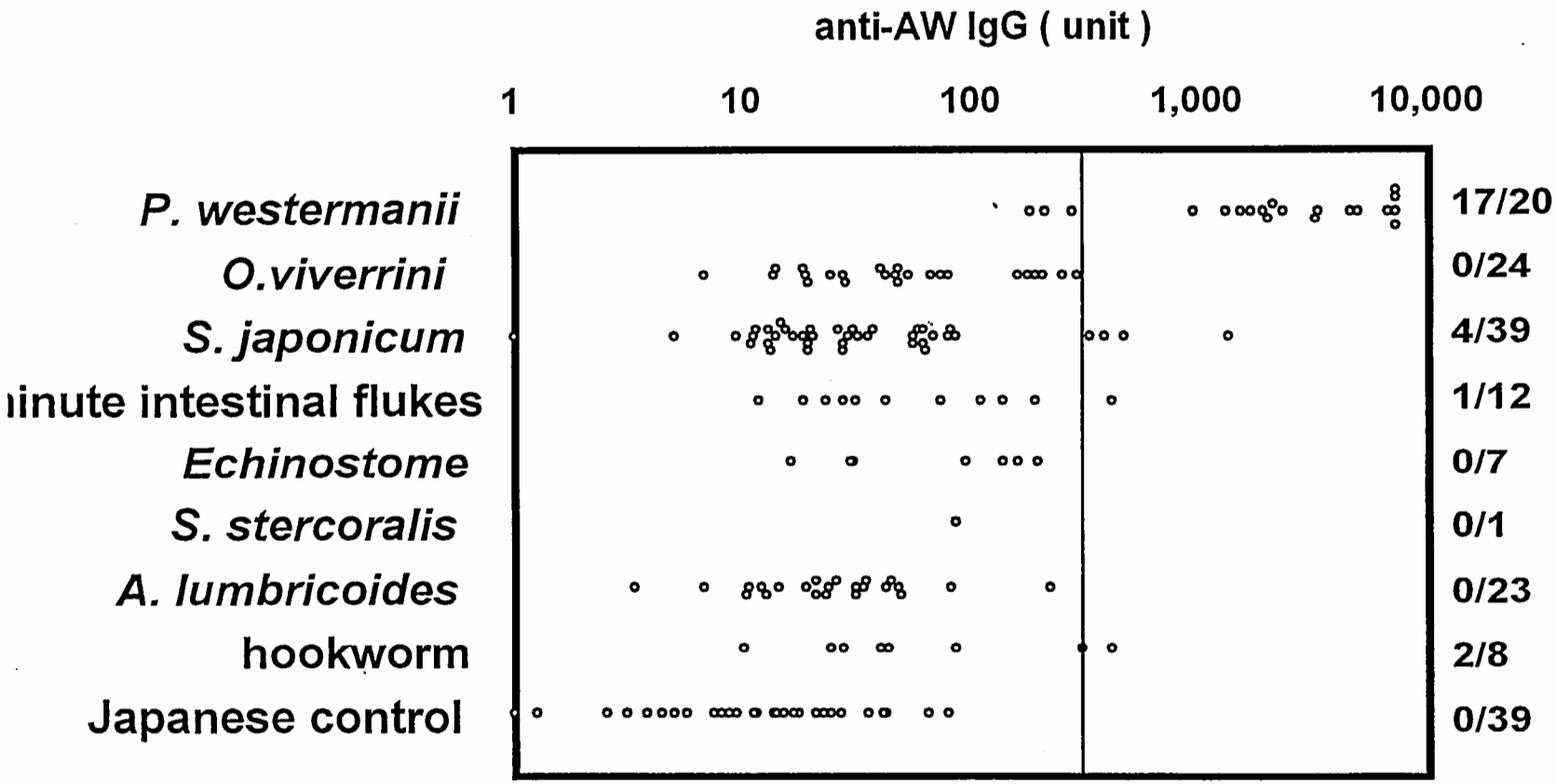


Fig3

IgG4 antibody units to *P.westerr...nii* adult worm according to different species of parasites

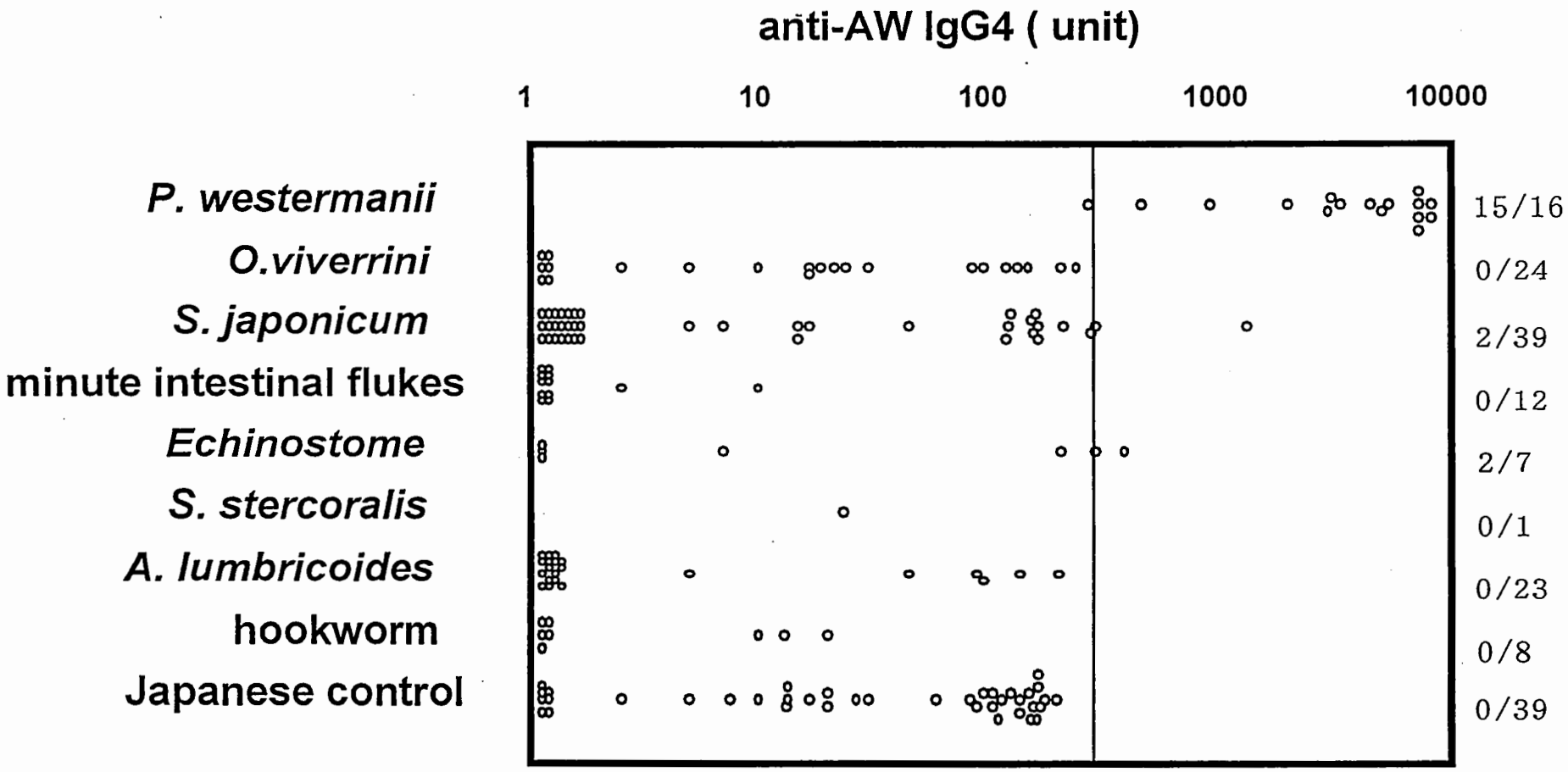


Fig4

Antibody units to *P.westermanii* cysteine proteinase according to different species of parasites

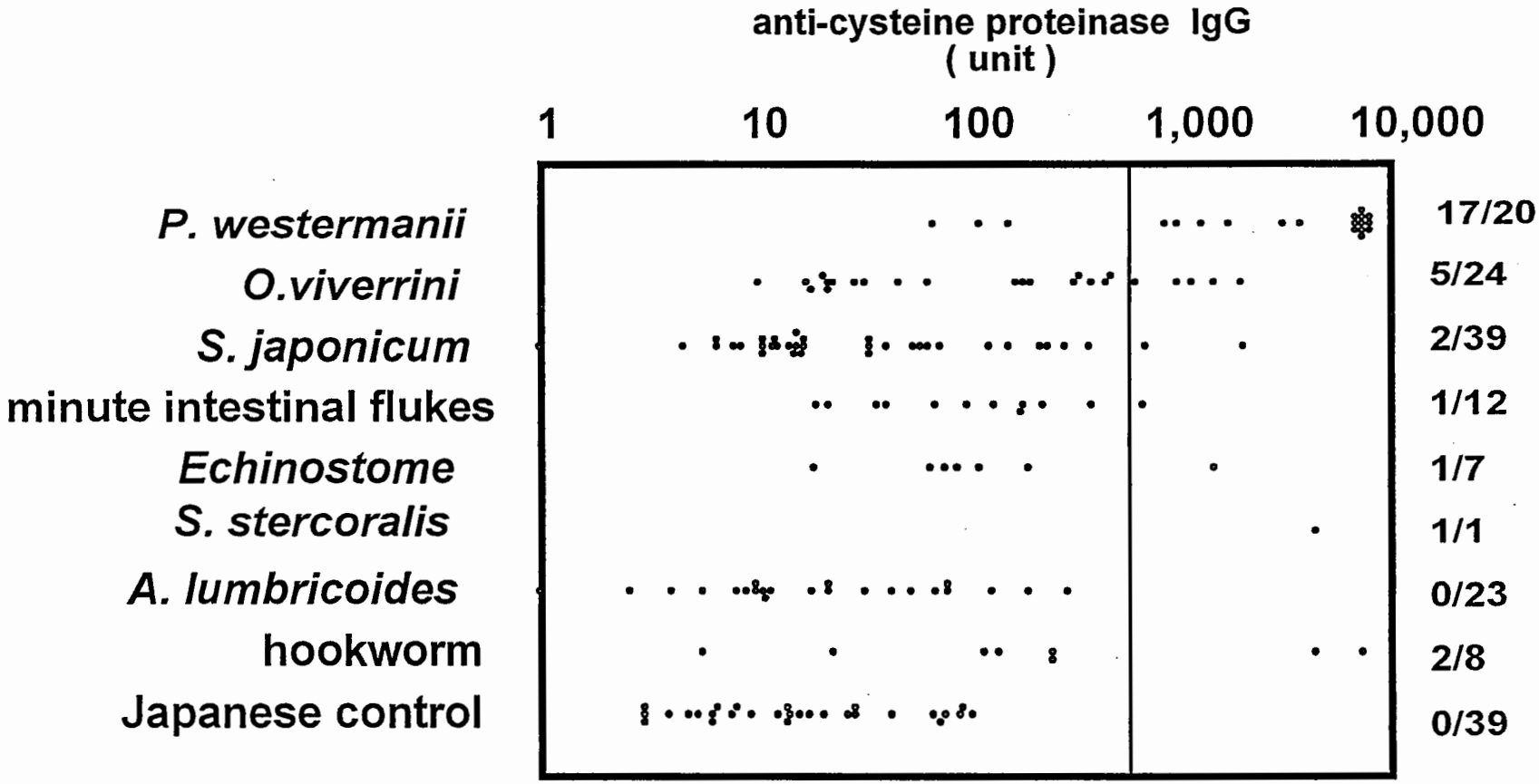


Fig5

Changes of anti-*P.westermanii* IgG in urine after treatment

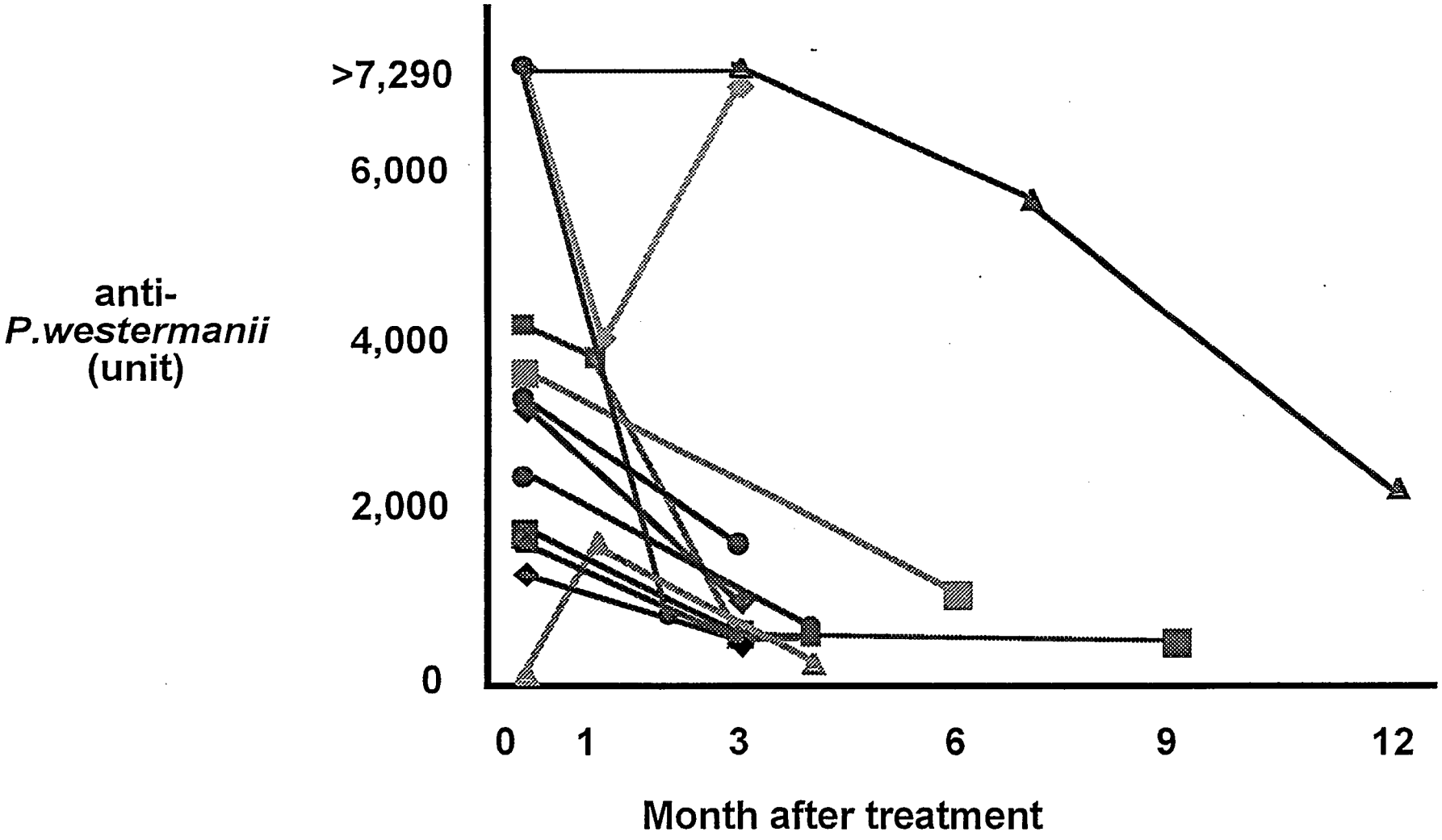
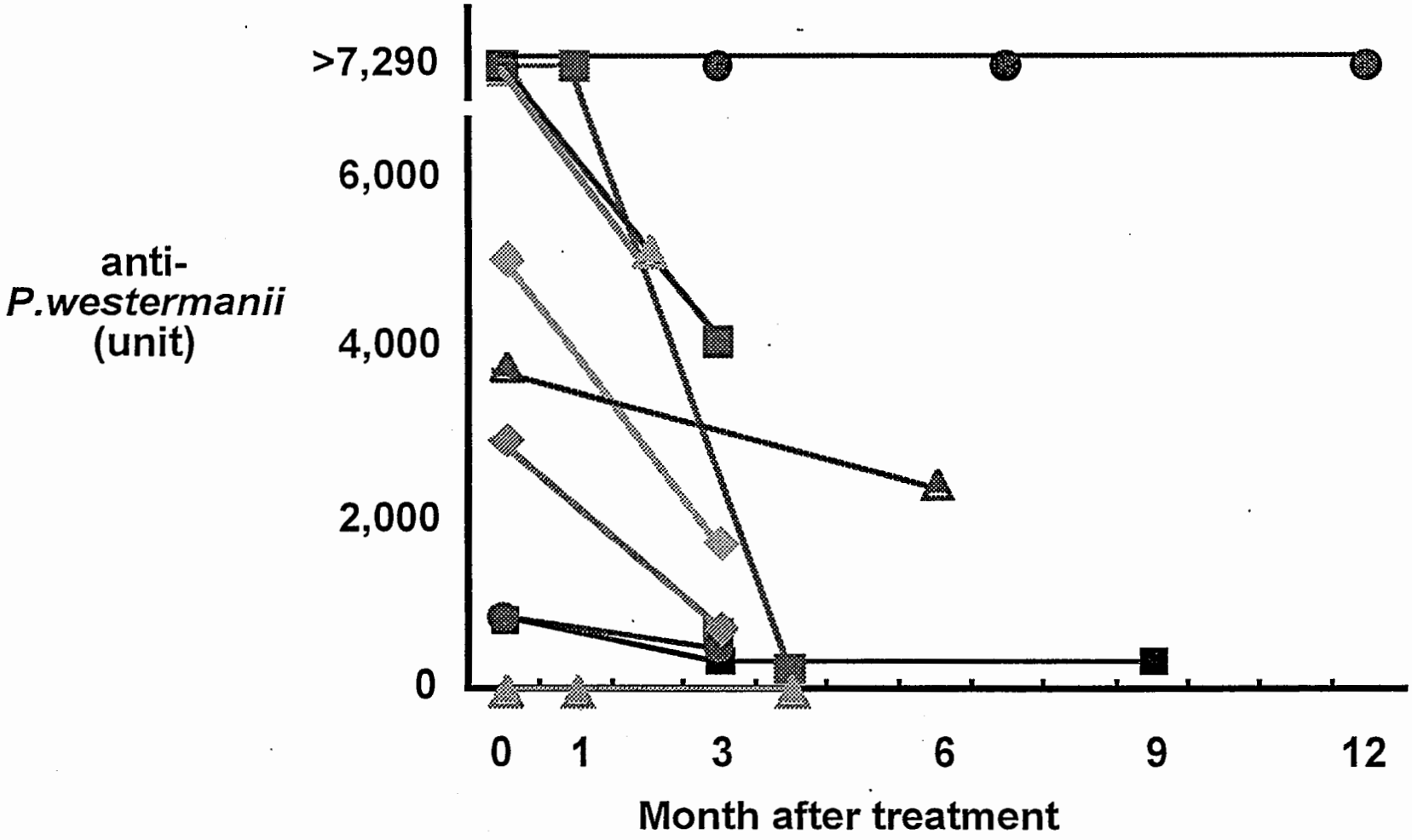


Fig6

Changes of anti-*P.westermanii* IgG4 in urine after treatment



Diagnosis for paragonimiasis by using URINE-ELISA

採集が簡単でかつ安全な尿を検体として用いた URINE-ELISA という方法で肺吸虫症患者の尿中でウェステルマン肺吸虫成虫抗原に対する IgG,IgG4,IgM,IgA 抗体を測定下。その結果、患者尿中には抗ウェステルマン肺吸虫抗体が検出できた。IgG では感度８５％、特異性９４％であり、IgG４ではそれよりやや高い感度、特異性が得られ、疫学調査に有用な方法だと考えられた。

（第３４回東海寄生虫談話会、名古屋市立大学）

第34回　東海寄生虫談話会

日時：　2000年11月25日（土）　午後2時より

会場：　名古屋市立大学医学部構内　　（同窓会館２Ｆ、寄生虫学会西日本支部大会会場の隣の２階建ての建物）

演題：

"-5 enhances intestinal immunity against adult Strongyloides nezuelensis.
Mohamed El-Marky　先生　（名市大・医学部・医動物）

Diagnosis of paragonimiasis by URINE-ELISA.
邱　旭光　先生（愛知医大・寄生虫）

Diagnosis of visceral leishmaniasis (Kala-azar) by ELISA using urine samples.
Mohammad Zahidul Islam　先生　（愛知医大・寄生虫）

ネズミマラリア原虫（P. berghei）オオキネートの蚊中腸壁への侵入に関係するCTRPタンパク質の免疫電顕観察
Wutipong Limviroj 先生（三重大・医学部・医動物）

旋毛虫のシスト形成における筋肉細胞の変異機序
呉　志良先生　（岐阜大・医学部・寄生虫）

会費：　1,500円