We recently surveyed parasitic infections in 334 foreigners living as permanent residents in Japan. The survey results are presented herein. Our results highlight open issues in Japan, with reference to measures taken in Western countries where immigrants have long been accepted. In addition, we present our epidemiological method for investigating parasitic infection, making use of simple and valid large-scale screening. Among the foreigners participating in this survey, parasitic infections other than those in toxoplasmosis antibody positive individuals were rare. However, in view of the recent trend for increased total numbers of foreigners living in Japan, the onset of parasitic infections is anticipated to increase in Japan henceforth.

Key word: Parasitic Infections, Foreigners

Introduction

As of the end of 2009, the number of foreigners living in Japan (excluding those with special permanent residence permits) exceeded 1.78 million. Many of them are coming from areas where parasitic infections are still endemic. Several reports have described surveys of tuberculosis or human immunodeficiency virus (HIV) infection in foreigners living in Japan, but there have been no surveys on parasitic infections in this group. According to past reports on parasitic infections in Japan, food-borne zoonosis (a parasitic infection associated with dietary habits) is more likely to develop than vector-borne zoonosis (a parasitic infection associated with environmental factors), because of a well-developed infrastructure (drinking water supply, sewage system, etc.). It is plausible that chronic parasitic infections remain occult in some foreigners who recently arrived, from countries where these infections remain common. Unlike fatal infections such as tropical malaria, which follows an acute course after onset, chronic parasitic infection manifests its pathogenic features when host immune function is compromised (e.g., toxoplasmosis). Some types of parasitic infection persist long after onset and induce symptoms such as fever and malaise, regardless of host immune function. Infection with these parasites can reduce the learning capabilities of children and the productivity of workers, thereby serving as an obstacle to economic development of poor countries. For this reason, chronic parasitic infections can be neither ignored nor overlooked. Here, we conducted an investigation of parasitic infections in foreigners living in Japan and analyzed the results to obtain findings useful for planning future control measures against these infections.

Materials and Methods

Participating facilities: Our facility (Section of Environmental Parasitology, Department of International Health Development, Division of Public Health, Graduate School, Tokyo Medical and Dental University) and facilities involved in parasitology research at other universities in the Kanto District of Japan constitute the study group (organized under the auspices of the Ministry of Health, Labour and Welfare, Japan)
conducting this investigation. All participants of this study were enrolled by their voluntary agreement, regardless of their age or sex.

Subjects: The study period was from 2007 to 2009. We informed foreigners living in Kanagawa and Gunma Prefectures of the significance and necessity of this study. We enrolled 334 of these foreigners who gave consent at their own discretion. To explore a simplified screening method, dot-ELISA assay was additionally performed for 202 subjects with serum samples collected between 2007 and January 2008. On the basis of the dot-ELISA data from these samples, we evaluated the usefulness of this method as a means of screening for parasitic infection. On the survey of 202 cases in the first year, dot-ELISA method we performed showed less sensitive than that of the rapid tests. Therefore, in the second year of the study we did not conduct the dot-ELISA test.

Sampling: Each blood sample, collected on the day of examination, was centrifuged and stored refrigerated until the serum was tested at our facility. Fecal and urine samples were brought by individual subjects to the facility on the appointed day. Thus, it was not possible to collect samples from all subjects.

Prior to the study, we prepared a leaflet explaining the study in four languages (a health management booklet written in Japanese, Chinese, English and Spanish) and delivered it to all candidate subjects. Abdominal ultrasonography and electrocardiography were carried out on each subject to check for liver and heart diseases. We informed all subjects that these tests would be free of charge, in an attempt to motivate them to undergo examination. We endeavoured to increase the number of participants with the cooperation of Catholic churches, a medical facility trusted by foreigners living in Japan (Kobayashi International Clinic Kanagawa) and community leaders. Most study subjects were from Central/South America and Southeast Asia. No Chinese nationals participated.

Prior to the study, we confirmed that we would comply with Japanese statutes. Our intention of precisely reporting any illegally working foreigner detected among the subjects to the regulatory authority was approved by the institutional review board.

This study was approved by the ethical committee of Tokyo Medical and Dental University. Written informed consent was obtained from all the subjects.

Testing methods: Serum antibody tests were used to detect chronic parasitic infections. As our goal was to develop a simple test using serum, we first evaluated the dot-ELISA method (Fig 1). In a preliminary survey evaluating the accuracy of dot-ELISA, we performed both dot-ELISA and a conventional serological test on blood samples collected from 202 subjects between 2007 and January 2008. Dot-ELISA is designed to allow a qualitative judgment (positive or negative). In practice, however, the intensity of reactions to dot-ELISA varied among cases (semi-quantitative judgment). We recorded cases showing reactions comparable to the positive control as "strongly positive" and those showing faint spots as "weakly positive."

With this dot-ELISA method, the number of antigens which can be tested at one time is limited to 6 (the number of antigens which can be dotted onto the sheet at a single time). For this reason, we selected the following antigens: (1) excreting-secretory products on Toxocara canis larva, (2) Gnathostoma hispidum larva, (3) muscle stage larvae of Trichinella spiralis, (4) Paragonimus westermani adult, (5) Cysticercus cellulosae, and (6) Entamoeba histolytica HM-1.

Anti-Toxocara antibody and anti-amaeba antibody were checked with rapid diagnosis kits developed by our group (ToxocaraCHEK and InstantAmoebeaCHEK). Antibodies to visceral leishmaniasis, Chagas disease, was measured with a commercial kit, Chagas-StatPack (CHEMBIO, NY) and Instant-ChagasCheck (EY-Labo, HK). Toxoplasma antibody was measured with a commercial kit, Toxotest-MT (EIKEN, Tokyo).

Results

Of the 334 individuals participating in the survey,
Parasitic Infections in Foreigners Living in Japan

Table 1: Results of dot-ELISA, the simultaneous test with the instant diagnosis kit and the serological test (Total subjects: 202)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Dot-ELISA</th>
<th>Instant diagnosis kit + Serological test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Weakly-positive</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Paragonimus westermani</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Gnathostoma hispidum</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cystocercus cellulosae</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Echinococcus multilocularis</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Because dot-ELISA is a method for semi-quantitative testing, with the intensity of reactions varying among cases, we rated those showing reactions comparable to the positive control as strongly positive and those with faint spots as weakly positive.

Table 2: Foreigners living in Japan immunoserologically positive for chronic parasitic infections and their origin of country

<table>
<thead>
<tr>
<th>Antibody against</th>
<th>n</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoeba</td>
<td>1</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Toxoplasma</td>
<td>61</td>
<td>20 (Brazil), 20 (Bolivia), 9 (Peru), 1 (Columbia), 1 (Argentina), 4 (Vietnam), 2 (Philippines), 1 (Japan), 2 (Nigeria), 1 (Unknown)</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>1</td>
<td>Bolivia</td>
</tr>
<tr>
<td>Gnathostoma</td>
<td>5</td>
<td>3 (Bolivia), 1 (Philippines), 1 (Unknown)</td>
</tr>
<tr>
<td>Schistosoma</td>
<td>1</td>
<td>Nigeria</td>
</tr>
<tr>
<td>Toxocara</td>
<td>17</td>
<td>6 (Vietnam), 5 (Philippines), 3 (Thailand), 2 (Bolivia), 1 (Korea)</td>
</tr>
<tr>
<td>Trichinella</td>
<td>1</td>
<td>Philippines</td>
</tr>
</tbody>
</table>

Immunoserologically positive: 87 individuals in total (among 306 serum samples)

Note: Anti-amaeba antibody and anti-Toxocara antibody were checked with rapid diagnosis kits developed by our group (InstantAmoeba CHEK and ToxocaraCHEK). Toxoplasma antibody was measured with a commercial kit, Toxotest-MT (EIKEN). Antibodies to visceral leishmaniasis, Chagas disease, was measured with commercial kits, Chagas-Stat-Pack (CHEMBIO) and Instant-ChagasCheck (EY-Labo).

306 consented to blood donation and 147 supplied feces. Table 1 shows the individuals rated as positive or weakly positive in the dot-ELISA test conducted on samples from 202 subjects between 2007 and January 2008 for the purpose of evaluating the accuracy of dot-ELISA. When checked for *Entamoeba histolytica* infection, 23 (11.4%) of the 202 subjects were rated as positive by dot-ELISA (including weakly positive cases). *Table 1* shows the individuals rated as positive with the higher accuracy instant diagnosis kit employed simultaneously. When the instant diagnosis kit was used, only one (0.5%) of the 202 subjects was rated as *Entamoeba histolytica* antibody positive. Thus, the *Entamoeba histolytica* judgment by dot-ELISA was false positive in 22 subjects (10.9%). Some subjects were rated as positive by dot-ELISA for *Paragonimus*, *Gnathostoma* and *Trichinella*, but these subjects were rated as negative when tested by instant diagnosis kit (*Table 1*). Cystocercus and Echinococcus were negative in all subjects when tested by dot-ELISA.

Of the subjects rated by dot-ELISA as positive or weakly positive, only one was rated as positive with instant diagnosis kit. The *Entamoeba histolytica* judgment by dot-ELISA (one strongly positive case) was consistent with that by the rapid diagnosis kit. All subjects rated as negative by dot-ELISA were also rated as negative by the rapid diagnosis kit. Thus, dot-ELISA yielded no false negatives.

*Table 2* shows the total of 87 foreigners living in Japan suspected of having chronic parasitic infections...
and their countries of origin. The number was strikingly high for anti-Toxoplasma antibody positive individuals (61 cases), and there was also one case rated as anti-Chagas disease antibody positive.

No individuals had hepatic lesions (hepatic cyst, etc.), with the network pattern characteristic of Schistosoma infection, on abdominal ultrasonography. Electrocardiography revealed no signs of cardiac disease in the anti-Chagas disease antibody positive case.

The test results were directly delivered to individual subjects by in writing, by mail, etc. Some subjects were lost to follow-up, making reporting of their results impossible. Individuals rated as positive were referred to other medical facilities, but they were not followed as to treatment, etc.

**Discussion**

Most cases of parasitoses encountered in Japan, including foreigners living in Japan, have food-borne parasitoses or imported parasitoses. These types of parasitoses often assume the form of larva migrans, and have the following features: (1) parasitism targets organs other than the digestive tract; (2) eggs are laid in the peritoneal or thoracic cavity; (3) the parasite cannot advance from the larval stage to mature worms; and/or (4) paucity of subjective symptoms. With fecal testing, direct isolation of worms or eggs is often impossible in these cases. As a method for rapid testing useful in such cases, the anti-parasite IgG antibody test based on multiple dot-ELISA has been introduced. In addition to the antibody test results, information as to the patient’s lifestyle, medical history and dietary habits is also helpful in making the diagnosis.

The conventional diagnostic method using feces and urine is, of course, reliable to some extent as a means of checking for intestinal parasitic infections among food-borne infection, and clinical symptoms such as diarrhea often trigger detection of such infections. As a rule, gastrointestinal parasitoses, which induces diarrhea, should be definitively diagnosed by directly examining feces for pathogens (eggs, larva, cyst, etc.). In cases of extra-intestinal parasitic infections, worms or eggs are seldom detected in feces. Intestinal parasitosis is unlikely to induce antibody production, while immunological diagnosis is useful for extra-intestinal parasitosis.

Herein, we evaluated the significance and necessity of antibody test using serum. This was based on our view that screening of donated blood should include serum antibody testing for chronic parasitic infections producing few clinical symptoms.

Dot-ELISA is a semi-quantitative test, and therefore, its sensitivity and specificity can vary depending on the type of antigen adsorbed onto the test sheet and the extent of serum dilution. We prepared the test sheet, adopting the antigens and the serum dilution ratio conventionally used to check for parasitic infections in Japanese people. However, with some parasites, only subspecies have conventionally been detected in Japan, and foreigners routinely exposed to parasites in their daily lives are reported to have lower IgE levels than Japanese people. We therefore consider it necessary to review the types of antigens and the serum dilution ratio used for parasite screening in foreigners by means of dot-ELISA. In this study, we employed partially purified proteins of Toxocara or Entamoeba antigens to prepare a rapid diagnostic test kit. On the contrary, unpurified crude antigens were used in dot-ELISA. We believe that this is the reason why the dot-ELISA showed high false positive rate than the rapid diagnostic kit, Toxocara CHEK and instant Amoeba CHEK. It seems probable that the accuracy of this method would be improved by modifications of the technique and other features. In this study, all individuals rated as negative by dot-ELISA were also negative with the rapid diagnosis kit. This means that there were no false negative cases by dot-ELISA, suggesting the potential of this method for initial screening.

A problem with ELISA lies in that its specificity is low despite high sensitivity. Multiple dot-ELISA is a qualitative testing method designed to detect IgG class antibodies and to make a judgment by naked eye. On the other hand, microplate-ELISA is a semi-quantitative testing method, capable of quantification through measurement of absorbance and enabling analysis of antibody titer time courses. In cases where neither worms nor eggs are found, microplate-ELISA may facilitate evaluation of disease activity. However, multiple dot-ELISA basically involves manual operation and does not require any complex equipments, thus having the advantage that it can be used even in developing countries. The results obtained with multiple dot-ELISA are likely to be affected by the skill of the examiner since it is operated manually. This is a shortcoming of multiple dot-ELISA. A simple kit for this method would be useful as a means of global screening.

Low specificity is not always a problem. For example, Paragonimus, lung fluke of Chinese origin, are known. Identification with homologous antigen
is difficult for these species of *Paragonimus*, and ELISA method is likely to undergo cross-reactions with other fluke antigens, occasionally making it impossible to identify the species of *Paragonimus*. However, immunological diagnosis of *Paragonimus* is sufficient with multiple dot-ELISA. Because the treatment is basically the same for all types of paragonimiasis, inability to identify its type does not pose clinical problems. Identification of the type using homologous antigens is indispensable for elucidating the route of transmission and epidemiological survey of *Paragonimus* infection. In cases with protozoan infections, such as Chagas disease and leishmaniasis, detection of the protozoa is difficult but immunological diagnosis is useful and identification is possible by checking for bands with Western blotting (WB). In cases of amoebiasis as well, detection of protozoa is not always successful, and immunodiagnosis with ELISA, the Ouchterlony method, indirect fluorescent antibody assay, etc. are useful. Rapid judgment is possible with the commercially available indirect hemagglutination kit, but it is desirable to perform this kind of test and make the judgment based on adequate understanding of the characteristics of individual testing methods. For protozan infections, the use of ELISA for screening is recommended and the judgment can be confirmed using WB. If samples and testing methods are selected which correspond to the target, these methods may be valid for screening.

According to one report in Japan, requests for parasite testing were placed for 208 cases during a 16-year period, and 109 cases of infection with 31 types of parasites were detected. This detection rate is not particularly high, in view of the fact that the purpose of this type of request is often to rule out parasitic infection. The route of transmission was oral in 79 of the 109 cases and 23 cases had imported parasitic infections. According to the report, transmission often occurred via food contaminated with parasites (even fresh food), and there were many cases of opportunistic infection following frequent use of drugs for the control of AIDS or immunosuppressants which resulted in severe courses of these parasitic infections.

In the present study, we initially considered collection of feces and urine by means of mailing. However, it is forbidden by Japanese law to mail samples with the potential to transmit pathogens. We therefore asked each participant to carry their fecal and urine specimens to the examination site. With this study design, collection of feces from all participants was not possible. Of the 334 participants, 306 consented to blood sampling and 147 submitted feces. Among 147 stool samples, 14 were positive for intestinal parasites, such as *Entamoeba* cyst, *Giardia* cyst, *Cryptosporidium* sp oocyst and hookworm egg (data not shown). Thus, fecal samples were collected from about half of the participants who consented to blood sampling. If mailing of feces and urine had been possible, samples might have been collected from a higher percentage of participants. Mailing seems to be a valid means of enhancing the accuracy of this type of survey, but it is difficult to implement at present because relevant laws, etc. must first be revised. We hope that the present findings will stimulate renewed recognition and awareness of parasitic infections by those involved in administration and healthcare and encourage actions such as system improvement.

**Competing interests**

The authors have no conflicts of interest to declare.

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Contributions
Keisuke Nakayama and Nobuaki Akao performed a portion of the laboratory examinations, and Keisuke Nakayama wrote the main draft of the manuscript. Nobuaki Akao participated in the medical check-ups for foreign residents in Japan and writing this manuscript. Nobuo Ohta coordinated the study and contributed to writing this manuscript.

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