FOCUS OF HUMAN TRICHINELLOSIS IN PAPUA NEW GUINEA

IFOR L. OWEN, EDOARDO POZIO, ALESSANDRA TAMBURRINI, ROBERT T. DANAYA, FABRIZIO BRUSCHI, AND MARIA ANGELES GOMEZ MORALES

Department of Agriculture and Livestock, Boroko, Papua New Guinea; Istituto Superiore di Sanità, Rome, Italy; Port Moresby General Hospital, Port Moresby, Papua New Guinea; University of Pisa, Pisa, Italy

Abstract. Human trichinellosis and teniasis (Taenia solium) are meat-borne helminthic infections with a wide distribution throughout the world. However, there is little information on the prevalence of these infections in Papua New Guinea. In 1999, serum samples were collected from 97 people in 6 villages in the remote Bensbach area of Papua New Guinea. Enzyme-linked immunosorbent assay and Western blot analyses were used to detect anti-Trichinella immunoglobulin (Ig) G and anti-cysticercus IgG in this population. The prevalence of Trichinella antibodies among inhabitants of the Bensbach area was 28.9% (28 of 97; 67.8% in men), suggesting a high consumption of poorly cooked meat. The higher prevalence of infection for Trichinella in men compared with women may be explained by the inclination of men to eat undercooked pork while hunting. All serum samples were negative for cysticercus antibodies. This is to our knowledge the first serosurvey showing anti-Trichinella antibodies in a human population living in Papua New Guinea (Australian region).

INTRODUCTION

Meat-borne infections with Trichinella spp. and Taenia solium have a worldwide distribution and are present in both industrialized and nonindustrialized countries, although prevalence is higher in the latter. Infection with Trichinella has not been reported in humans in any part of New Guinea, although infections in sylvatic swine have been reported in Bensbach, a remote area of Papua New Guinea (PNG). The parasites associated with these infections have nonencapsulated larvae in host muscles and have been identified as a new species, Trichinella papuae. In contrast, Taenia solium is found in humans and pigs in New Guinea, but it is present only in the central highlands of West Papua (Irian Jaya), the Indonesian western half of the island of New Guinea, although serological evidence of cysticercosis has been documented once in PNG, in a West Papuan refugee.

The objective of the present study was to estimate the seroprevalence of Trichinella antibodies in humans living in the Bensbach area and to determine whether T. solium has spread from West Papua into the local population of PNG.

PATIENTS AND METHODS

Area of investigation. Bensbach is a remote, undeveloped, rural, and flat area, with distinct wet and dry seasons. The region is traversed by the Bensbach River (Figure 1). The population of this region is sparse, with ~ 1 inhabitant per square kilometer. The people live in villages, not in isolated households, and they are traditionally gardeners, hunters, and fishermen. Although the soil is not very fertile, the area has a rich fauna. Food supplies are therefore abundant, except during droughts, when crops fail and access to drinking water becomes a problem.

The people of the Bensbach area are Melanesians and linguistically are non-Austronesians, who were the original colonizers of New Guinea, probably arriving from Southeast Asia 40–50,000 years ago. Government authority and Christian missions were established in the Bensbach area early in the 20th century.

Collection of biological samples. In November 1999, blood samples were collected from people living in 6 small villages in the Bensbach area, Balamuk, Bondaber, Bula, Komboro, Torwaia, and Wando. The population of these 6 villages numbered 455 in 1990. The purpose of the study was explained to the people gathering in each village. Collection of samples followed informed consent of voluntary participants. Approval for the study was given by the Medical Research Advisory Committee of Papua New Guinea. Serum samples were collected from whole blood with a manual centrifuge (there is no electricity in the investigated area) to shorten the sedimentation time, and samples were preserved with 0.1% merthiolate at room temperature. Two blood spots were taken from each person, fixed on Whatman paper, dried, and preserved in a plastic bag with silica gel. Blood smears were also prepared to determine eosinophilia. All relevant signs and symptoms (fever, diarrhea, facial or diffuse edema, myalgia) in participants were documented. Blood spots, serum samples, or both were collected from 97 people (21.3% of people living in the 6 villages) and 13 dogs from the Bensbach area. Blood smears were prepared for 56 people from the Bensbach area. All serum and blood spot samples were sent by courier to the Laboratory of Parasitology, Istituto Superiore di Sanità (Rome, Italy), within 2 weeks.

Elution of serum samples from blood spots. The individual dried blood spots were cut out from the Whatman paper in circles 12 mm in diameter (~ 113 mm2 area) and placed in a 24-well plate (Falcon 3047). Each blood spot was eluted in 1.6 mL of phosphate-buffered saline (pH 7.2), 0.4% bovine serum albumin (BSA) and 4% Tween 20 at room temperature on an orbital shaker for 1.5 hr. Because a spot of 113 mm2 absorbs ~ 20 μL, each blood sample was diluted 1:80. Consequently, the final estimated dilution of serum samples was 1:160. Blood spot samples were used both for enzyme-linked immunosorbent assay (ELISA) and Western blot assay without further dilutions.

Control serum samples. Control samples for all serodiagnostic tests comprised serum samples, which were stored at the International Trichinella Reference Center (Rome, Italy), from uninfected people and from those known to be infected with Trichinella and/or T. solium cysticerci. Similarly, serum samples from uninfected dogs and those infected with Trichinella were used as controls. The number of control serum samples used in each test is reported below.

Serodiagnosis for Trichinella. Trichinella spiralis excre-
Figure 1. Map of the island of New Guinea showing the Bensbach area in Papua New Guinea.

Tory/secretory (E/S) antigen was obtained from muscle larvae as previously described, and protein concentration was determined by the Bradford method. To confirm the results obtained with the E/S antigen and to exclude the possibility of cross-reactivity with other antigens, a 3,6-dideoxyhexose sugar (tyvelose), one of the major immunodominant highly specific epitopes of *Trichinella*, coupled with BSA (i.e., synthetic tyvelose glycan-BSA [stg-BSA; Heska, Fort Collins, CO]), was used as antigen in an ELISA. By use of this synthetic antigen, the ELISA shows a sensitivity of 93.4% after 3 weeks of infection and a specificity of 99.9% (data not shown).

An indirect ELISA method was used to detect *Trichinella*-specific immunoglobulin (Ig) G. Briefly, *T. spiralis* E/S antigen was diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6) and used at a concentration of 5.0 μg/mL. We used stg-BSA antigen at 1.25 μg/mL. Serum and blood samples were investigated at 1:200 dilutions. Peroxidase-labeled anti-human IgG conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used at 1:10,000 dilution. Serum and blood samples were incubated with human sera diluted 1:100 and with the blood spot samples. The peroxidase-labeled anti-human IgG conjugate was used at 1:3,000 dilution, and the reaction was developed with 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical, St. Louis, MO).

**Serodiagnosis for *C. cellulosae***. Commercially available cysticercosis diagnostic strips for the detection of antibodies against *T. solium* cysticerci (Yerkes Primate Research Center, Atlanta, GA) were used. Strips were incubated with serum samples diluted 1:100 in phosphate-buffered saline, 0.3% Tween 20, and 2% gelatin and with undiluted samples recovered from blood spots. Anti-human IgG conjugate (Yerkes Primate Research Center) was used at 1:2,000 dilutions. The reaction was developed with DAB (Sigma). Five positive and 5 negative serum samples were used as controls.

**Statistical analysis.** To evaluate associations between *Trichinella* infection, sex, and age, seroprevalence was calculated separately for men and women and for 4 age groups (15–24, 25–34, 35–44, and ≥45 years). Associations were expressed as odds ratio, and statistical significance was determined by the chi-square test. For the separate age groups, the chi-square test for linear trend was used. Furthermore, to evaluate sex and age differences, data were adjusted for each of them, and a logistic regression analysis for the binary outcome of *Trichinella* serostatus (i.e., positive and negative) was performed with age and sex as covariates. The effect of age was evaluated both as a categorical variable, as reported above, and as a continuous variable.
RESULTS

In the Bensbach area, 28.9% (28 of 97) of those tested were positive for *Trichinella* by ELISA (Table 1) by E/S and stg-BSA antigens. The prevalence of infection among the 6 villages ranged from 16% (4 of 25 people from Korombo) to 39.1% (9 of 23 people from Bula) (Table 2). All ELISA-positive samples reacted with a set of bands in the range of 45 kDa; no reactivity was observed with ELISA-negative samples. Bivariate logistic regression analysis showed that women were less likely than men to be positive for *Trichinella* (adjusted odds ratio, 0.38, 95% confidence interval, 0.15–0.98, *P* = 0.12) (Table 1). Of the 56 blood smears analyzed, eosinophilia was detected in 43 people from the Bensbach area (11–20% eosinophilia in 17 people, 21–30% in 7 people, and 31–90% in 19 people). Of 21 *Trichinella*-positive people, 3 (14%) did not show eosinophilia, 5 (24%) showed 11–20% eosinophilia, 4 (19%) showed 21–30% eosinophilia, and 9 (43%) showed 31–90% eosinophilia. The prevalence of eosinophilia in *Trichinella*-positive people (18 of 21, 85.7%) was higher than in *Trichinella*-negative people (25 of 35, 71.4%); however, because of the low sample number, this difference was not statistically significant. None of the serologically positive people from the Bensbach area showed clinical signs or symptoms pathognomonic for *Trichinella* infection.

The spot and serum samples from the dogs of the Bensbach area were all negative for *Trichinella* by ELISA. All 97 human samples from the Bensbach area were negative for cysticercosis by Western blot analysis.

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
<th>Positive for <em>Trichinella</em> n (%)</th>
<th>Crude odds ratio</th>
<th>95% confidence interval</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects examined</td>
<td>97 (100.0)</td>
<td>28 (28.9)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51 (52.6)</td>
<td>19 (67.8)</td>
<td>1.00</td>
<td>–</td>
<td>0.06</td>
</tr>
<tr>
<td>Female</td>
<td>46 (47.4)</td>
<td>9 (32.1)</td>
<td>0.41</td>
<td>0.16–1.03</td>
<td>–</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–24</td>
<td>24 (24.7)</td>
<td>9 (37.5)</td>
<td>1.00</td>
<td>–</td>
<td>0.15*</td>
</tr>
<tr>
<td>25–34</td>
<td>29 (29.9)</td>
<td>9 (31.0)</td>
<td>0.75</td>
<td>0.24–2.35</td>
<td>–</td>
</tr>
<tr>
<td>35–44</td>
<td>27 (27.9)</td>
<td>7 (25.9)</td>
<td>0.58</td>
<td>0.18–1.92</td>
<td>–</td>
</tr>
<tr>
<td>≥45</td>
<td>17 (17.5)</td>
<td>3 (17.6)</td>
<td>0.36</td>
<td>0.08–1.59</td>
<td>–</td>
</tr>
</tbody>
</table>

* Chi-square test for linear trend.

### TABLE 2

<table>
<thead>
<tr>
<th>Village</th>
<th>Seroprevalence (positive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balamuk</td>
<td>37.5 (3/8)</td>
</tr>
<tr>
<td>Bondaber</td>
<td>31.6 (6/19)</td>
</tr>
<tr>
<td>Bula</td>
<td>39.1 (9/23)</td>
</tr>
<tr>
<td>Korombo</td>
<td>16.0 (4/25)</td>
</tr>
<tr>
<td>Torwaia</td>
<td>25.0 (4/16)</td>
</tr>
<tr>
<td>Wando</td>
<td>33.3 (2/6)</td>
</tr>
</tbody>
</table>

### DISCUSSION

A new species of nonencapsulated *Trichinella* larvae, *Trichinella papuae*, was identified in 8.8% of wild pigs from the Bula Plain between 1988 and 1998. This is the main hunting ground for people living in the villages participating in the present survey, where pig hunting occurs regularly. The serological results from our study indicate that *T. papuae* is transmitted frequently to humans in this area and that this parasite can be considered to be a human pathogen. The high risk for human infection is believed to be associated with the local methods of cooking meat. The most common method is the use of earth ovens (*mumu*), where meat is cooked with heated stones in a shallow pit. However, because the area is devoid of stones, which are essential for heat retention and adequate cooking, the people use substitute materials that do not adequately retain heat, such as pieces of termite mounds (locally called *konzo* and gene) and dry clay (*ngant*), resulting in undercooking.

The high risk of infection may also be associated with hunting practices. After a successful pig hunt, the carcass, which is too heavy to be carried back to the village, is butchered in the field. Small portions of meat, often cooked only briefly over a fire, are then eaten. Traditionally, the hunter who kills a pig receives the head and a strip of the ventral abdominal wall. To lighten the load, the lower jaw is frequently separated from the head, and the tongue, masseter muscles, and other tissues, such as neck muscles and diaphragm, may be consumed at the hunting site. These customs would explain the higher prevalence of infection in men (67.8%, versus 32.1% for women). Impatience of youth and the widespread belief that eating bloody meat provides strength and is “macho” could account for the higher prevalence in the age group 15–24 years (37.5%) compared with other age groups (range, 17.6–31.0%) (Table 1).

Limits and possible biases of our study include the small sample size (21.3% of the total population living in the area), which limits the capacity to detect true differences in prevalence (apparent differences in age and sex were not statistically significant). Bias may also be introduced by the study enrollment process being on a voluntary base.

The results obtained with E/S and stg-BSA were identical, demonstrating that the stg-BSA antigen, which is known to have a high specificity (99.9%), has a level of sensitivity comparable to that of the E/S antigen. These results are con-
sistent with those of a recent study and strengthen the validity of the current study.

The lack of clinical symptoms and signs pathognomonic for *Trichinella* in serologically positive people suggests the following: (1) infections involve a low number of larvae; (2) the infecting strain of *T. papuae* has a low virulence in humans; (3) the infection did not occur recently; and (4) clinical manifestations characteristic of trichinellosis are not specific. The high prevalence of eosinophilia (71.4%) in people with sera negative for *Trichinella* may be due to the presence of other helminthic infections such as hookworm, which is common in this population. The lower seroprevalence for *Trichinella* in older people can be explained both by differences in eating habits, as described above, or may be due to low-grade reinfections that cannot be detected by the tests used.

The serological pattern detected in people living in the 6 small villages of the Bensbach area is similar to that of Inuits in Greenland, where 22% of the examined people (135 of 604) had anti-*Trichinella*-specific antibodies. Infection in that population was associated with frequent consumption of raw walrus meat; there were no clinical signs or symptoms of trichinellosis, and men were more likely to be infected than women. As in our study, these findings suggest an association with hunting practices.

This is to our knowledge the first autochthonous focus of human trichinellosis detected in the Australian region. In a previous survey on trichinellosis in the swine population of PNG, *Trichinella* infection was detected only in the Bensbach area. In this region, infection of animals with *T. pseudospiralis* has been documented in Tasmanian marsupials and birds. *Trichinella spiralis* infection, imported from Europe, has been reported in synanthropic rats and sporadically in domestic pigs of New Zealand. Only one infection with *T. pseudospiralis* has been documented in humans. Although most people in Indonesia are Muslims and do not eat pork, the majority of people in the island of Bali are Hindus. Trichinellosis has been documented in 19.5% of young people from Bali by serology. It is also found in domestic swine from Tapanuli, the northern region of the island of Sumatra; however, local customs of cooking or roasting meat hinder transmission to humans. The etiological agent of trichinellosis in domestic pigs from Bali and Sumatra has not been identified, although it has been reported to be *T. spiralis*. In Malaysia, an outbreak of trichinellosis occurred in Singapore among 84 students and teachers who had visited a neighboring Malaysian island in 1998. In southeast Asia, >6,000 infections were documented in Thailand, and several infections were reported in Laos. The present findings demonstrate the presence of another tropical zone where trichinellosis is transmitted to humans.

Wild pigs in PNG were probably introduced into New Guinea by human migrants 6,000–10,000 years ago. The source of the nonencapsulated species *T. papuae* is not known, but may be southeast Asia. It is surprising that none of the village dogs tested positive for trichinellosis. It is possible that they were nonhunting animals and thus did not eat wild pig carcasses after a kill.

Cysticercosis has never been reported in humans in PNG, although the infection has been documented several times in humans and pigs in parts of the central highlands of West Papua. The absence of *T. solium* infection in the Bensbach area of PNG indicates that the infection in West Papua has not spread from the highlands to the southern lowlands, west of the international border, where the villages in the current investigation are located. The small number of domestic pigs that are usually kept penned would hinder spread of cysticercosis in the Bensbach area, as would the low population density (~1 person per square kilometer). The presence of *T. solium* in West Papua is due to introduction of infected pigs from Bali in the early 1970s. This suggests that *Trichinella* infections in wild pigs of PNG and *T. solium* infections in domestic pigs of West Papua have different temporal and geographical origins.

Finally, we mention a technical consideration: serum samples eluted from blood spots have been used in both ELISA and Western blot analyses with similar and reproducible results, suggesting that this method of blood collection under adverse conditions due to the climate (tropical zone) and poor facilities (lack of electricity) is very useful not only for ELISA but also for Western blot analysis.

Acknowledgments: We thank the Heska Corporation, which provided us with the ss-g-BSA antigen; we also thank Dr. P. Pezzotti (Istituto Superiore di Sanità, Rome, Italy) for statistical analysis, Dr. Evelyn Lavu (Medical School, University of Papua New Guinea) for hematological analysis, Dr. Budi Tapari (Department of Geography, University of Papua New Guinea); and Thomas Malaisa (National Agriculture Quarantine and Inspection Authority, Papua New Guinea) for supplying field information, and Asmo Pisau (Department of Health Clinic, Morehead, Western Province, Papua New Guinea), for assistance in field work. Access to lines of communication was provided by the National Agriculture Quarantine and Inspection Authority, Papua New Guinea.

Financial support: This epidemiological survey was supported by the Surveillance Project on Emerging and Reemerging Infectious Diseases, Istituto Superiore di Sanità art. 502/12, Ministry of Health of Italy. Funds for air fares within Papua New Guinea for 2 of the authors (I.L.O. and R.T.D.) were supplied by the North Australia Quarantine Strategy.

Authors’ addresses: Ior L. Owen, % National Veterinary Laboratory, Department of Agriculture and Livestock, P.O. Box 6372, Bo-roko, Papua New Guinea. Edoardo Pozio, Alessandra Tamburrini, and Maria Angeles Gomez Morales, Laboratory of Parasitology, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy. Robert T. Danaya, Department of Child Health, Port Moresby General Hospital, Port Moresby, Papua New Guinea. Fabrizio Bruschi, Department of Experimental Pathology, University of Pisa, via Roma 55, 56100 Pisa, Italy.

Reprint requests: Dr. Edoardo Pozio, Laboratory of Parasitology, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy. Telephone: +39-06-4990-2304, Fax: +39-06-4938-7065 (e-mail: pozio@iss.it).

REFERENCES


