SEROLOGICAL EVIDENCE FOR RECENT EXPOSURE TO TAENIA SOLIUM IN VENEZUELAN AMERINDIANS


Centro de Investigaciones Biomedicas BIOMED Universidad de Carabobo, Maracay, Venezuela; Instituto de Medicina Tropical, Universidad Central de Venezuela (UCV) Caracas, Venezuela; Departamento de Parasitología Universidad de Carabobo, Maracay, Venezuela; Centro de Microbiología y Biología Celular IVIC Caracas Venezuela; University of Edinburgh, Department of Tropical Animal Health, Sir Alexander Robertson Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian, Scotland, United Kingdom; Institute for Animal Health, Pirbright laboratory, Ash Road, Woking, Surrey, England, United Kingdom; Gulbenkian Institute for Science, Oeiras, Portugal

Abstract. This study examined the seroprevalence and serum antibody isotype profile for Taenia solium cysticercosis in an Amerindian community in the Amazonas state of Venezuela. An antigen-trapping enzyme-linked immunosorbent assay (Ag-ELISA) was used to detect viable cysticercosis. Indirect ELISA (Ab-ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) was performed by using antigens prepared from T. solium metacestodes to detect anti-parasite antibodies. The Ag-ELISA and Ab-ELISAs revealed 64.7% and 79.0% seropositivity, respectively, in the Amerindian population. Immunoglobulin (Ig) M was the predominant antibody class, suggesting recent infection. Ag-ELISA, compared with 86–92% seropositivity by Ab-ELISA, and IgG was the predominant antibody subclass detected. The EITB antigen recognition patterns of the hospitalized patients were very similar to that of the Amerindians, confirming exposure to the parasite. These results, combined with the predominance of IgM antibody responses and the marked detection of secreted products of viable parasites, strongly suggest that recent exposure to T. solium had occurred in the Amerindian population.

INTRODUCTION

Taenia solium cysticercosis and neurocysticercosis in humans is an important public health problem in Latin America.1–3 The disease is found in areas with poor sanitary conditions where pigs are raised freely. Transmission of cysticercosis is by ingestion of T. solium eggs from the environment and through contaminated hands, water, or food, and autoinfection also occurs.4 The occurrence of the infection is linked to social, economic, and environmental characteristics and is found predominantly in rural areas of endemic countries. Many epidemiological studies have been carried out in such rural populations of Latin American countries.5–9 In Venezuela, however, there is a paucity of information on the prevalence and socioeconomic impact of cysticercosis. Most available information is derived from urban clinical centers.10 Health Ministry data for 1996 revealed only 4 fatal cases of cysticercosis,11 but these data are incomplete and do not reflect disease prevalence or associated morbidity in the communities at risk.2,4,12 The prevalence of cysticercosis and neurocysticercosis in Amerindian communities is largely unknown.

The aim of this study was to conduct a systematic serological study in such a community and to compare the results with a group of clinically verified cases of neurocysticercosis. Thus, in these 2 different population groups, the prevalence of viable parasite infection was evaluated through an antigen-detection enzyme-linked immunosorbent assay (Ag-ELISA). The value of this assay in the diagnosis treatment and follow-up of patients with neurocysticercosis has been evaluated.13,14 Antibody and antigen recognition profiles and antibody isotype responses were examined by enzyme-linked immunoelectrotransfer blot (EITB) and indirect ELISA (Ab-ELISA) assays.

MATERIALS AND METHODS

Serum samples. Informed consent was obtained from all the adults participating in the study and from the parents or legal guardians of minors. The project was approved by the institutional review board (Dirreccion de Investigacion de Universidad de Carabobo Nucleo Aragua).

Amerindian test sera. The blood samples were taken during a vaccination campaign against yellow fever, measles, poliomyelitis, and diphtheria-tetanus-pertussis carried out by the Venezuelan Ministry of Health. They were obtained from Amerindians living in the Venezuelan state of Amazonas. All the study subjects lived in fixed locations near the riverbank, far from non-Amerindian communities and urban areas. This population makes few or no visits to urban centers. There is a general lack of sanitary and hygiene facilities in these communities. The people live communally, with no access to piped water or latrines. Although pork is not considered an important part of the Amerindian diet, it is eaten occasionally; in addition, contact with other ethnic groups or environmental contamination with T. solium eggs cannot be ruled out. As far as can be determined, there are no known wild-game reservoirs of T. solium, and certainly there are none locally.

For reasons of medical ethics, the serum sampling was restricted to the participants in the vaccination program. It was not possible to obtain samples of feces because the beliefs of the Amerindians would not permit such sampling. The 68 samples were randomly chosen for analysis in the study. The main criterion was availability of serum sufficient to carry out the analysis. All the Amerindians were asymptomatic at the time of serum sampling.

The Amerindians were inhabitants of the following zones: San Juan de Manapiare, Morrocoy, Macuruco, Laja Pelú, Laguna de Tígré, Magua, and Maroeta y la Venturosa. They belonged to various Amerindian ethnic groups, as follows:
Piaroa (n = 15), Guahibo (n = 15), Curripacos (n = 5), Cunare (n = 1), Creoles (n = 3), and unknown (n = 29). The numbers and ages of the Amerindians were as follows: 20 (0–10 years), 16 (11–20 years), 8 (21–30 years), 8 (31–40 years), and 2 (> 41 years). The age of 14 of these patients was unknown, but we were able to place them within one of the age ranges indicated.

**Control positive sera.** Sera were obtained from 26 patients with confirmed cystercerosis from the Tropical Medicine Institute (Central University of Venezuela, Caracas). Diagnosis was confirmed for clinical, laboratory, computed axial tomography, or magnetic resonance imaging and immunodiagnosis. The patients were aged 4–40 years, except 2 older patients, one of whom was 65 and one of whom was 95.

All the patients were recently referred to the hospital and presented with convulsions for which they had not previously sought medical help. They had clinical histories of seizures going back ~1 year. None of them had yet entered a treatment program. All had central nervous system lesions, as determined by computed axial tomography or magnetic resonance imaging scans.

**Control negative sera.** In order to establish appropriate cutoff values for the assays, we sampled 28 healthy people and 20 people with other infections, including hepatitis (n = 5), leishmaniasis (n = 5), onchocerciasis (n = 5), hymenolepiasis (n = 2), and ascaris (n = 3). These sera were used to calculate appropriate cutoff values for the assays so that a direct comparison could be made between the Amerindian and hospitalized patient groups. All sera were kept frozen at −70°C until use.

**Antigens.** Three different *T. solium* extracts were used as antigens in the Ab-ELISA and EITB assays. Cyst fluid, from *T. solium* cysticerci, was obtained as previously described, and a surface-enriched antigen, N-octyl-β-D-glucopyranoside extract (NOG) of *T. solium* cysticerci and a cystercercus surface glycoproteins (GP) antigen were prepared accordingly to previously described methods.

**Detection of circulating antigens secreted by viable parasites (Ag-ELISA).** We performed Ag-ELISA with slight modifications from the original report. Briefly, the following steps were taken. Microplate wells (Immulon 1, flat bottom; Dynatech Laboratory) were coated overnight with 100 µL of a 5-µg/mL solution of monoclonal antibody HP10 diluted in carbonate buffer pH 9.6. This antibody recognizes a glycoprotein secreted by viable parasites and was purified by precipitation with 50% ammonium sulfate. After washing and blocking with phosphate-buffered saline (PBS; pH 7.3) containing 0.05% Tween 20 and 1% bovine serum albumin (BSA), 100 µL of undiluted serum sample was added and incubated for 30 min at 37°C. The plates were washed, and then biotin-conjugated HP10 monoclonal antibody was added at the optimal dilution. The reaction was visualized by the addition of streptavidin–peroxidase conjugate (Pierce Chemical, Rockford, IL) at optimal dilution. After the final plate wash, the color was developed with the substrate tetramethylbenzidine (liquid substrate system; Pierce). Plates were read at 450 nm on a Microplate reader (Cambridge Technology). A sample was considered positive if the specific optical density (OD) value was greater than the cutoff values. The cutoff value was calculated with the mean value of negative serum samples plus 3 standard deviations.

**Detection of anti-*T. solium* antibodies (Ab-ELISA).** Microplate wells (Immulon 2, flat bottom; Dynatech Laboratory) were coated with 100 µL (2 µg/mL) of the antigens (cyst fluid, NOG, or GP), and left overnight at 4°C. After washing and blocking the plates with PBS pH 7.3 containing 0.05% Tween 20 and 1% BSA, experimental and control sera (diluted 1/500) were added. After washing, goat anti-human immunoglobulin (Ig) G conjugated with peroxidase (H and L chains; Bio-Rad, Hercules, CA) was added. Finally, substrate (10 µL H₂O₂ and 4 mg O-phenylenediamine in 10 mL citrate buffer) was added, and plates were read at 490 nm on a microplate reader (Cambridge Technology). A sample was considered positive if the specific OD value was greater than the cutoff values. Cutoff value was obtained with the mean value of negative serum samples plus 3 standard deviations.

**Isotype-specific anti-*T. solium* antibody detection.** Microplate wells (Immulon 2, flat bottom; Dynatech Laboratory) were coated with 100 µL (2 µg/mL) of the different antigens (cyst fluid, NOG, and GP). After washing and blocking with PBS pH 7.3 containing 0.05% (w/v) Tween 20 and 1% (w/v) BSA, serum (diluted 1/1,000) was added. After washing again, 100 µL per well of isotype-specific mouse antihuman IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA, or IgE (Bio-Rad monoclonal antibodies) was added. Then, after a further incubation and wash step, 100 µL per well of goat anti-mouse IgG (H and L chains [Bio-Rad] absorbed on human IgG-Sepharose) conjugated to peroxidase was added. After a final wash, substrate (10 µL H₂O₂ and 4 mg O-phenylenediamine per 10 mL citrate buffer, pH 6.0) was added, and the reaction was stopped with 2 M H₂SO₄. The absorption of the ELISA plates were read at 490 nm on a Microplate reader (Cambridge Technology). A sample was considered positive when the specific OD value was greater than the cutoff values. The cutoff value was calculated as the mean value of negative sera plus 3 standard deviations.

**Performing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and EITB.** We carried out SDS-PAGE analysis of the different antigens (cyst fluid, NOG, and GP) with 10% polyacrylamide gels. The separated proteins were electrophoretically transferred to Immobilon PVDF transfer membranes (Millipore, Bedford, MA) with a semidry blotting apparatus (Sartoblot II), then blocked in 3% BSA-PBS overnight at 4°C. Incubation with diluted experimental and control human sera was carried out for 1 hr at 37°C. Finally, goat anti-human IgG conjugated to peroxidase (H and L chains; Bio-Rad) was added and incubated for 1 hr at 37°C; the reaction was developed in 3,3-diaminobenzidine–H₂O₂ substrate solution. The color reaction was stopped by placing the EITB strips in water. The presence of stained reaction bands on the nitrocellulose strips was taken to indicate exposure to infection.

**RESULTS**

**Detection of circulating parasite antigens and antiparasite antibodies.** *Taenia solium* parasite antigen, indicating infection with viable parasites, was detected in 64.7% of the Amerindian sera, as opposed to only 27% of the hospitalized
The antigen recognition patterns obtained by EITB (immunoblotting). The antigen recognition patterns obtained with sera from confirmed neurocysticercosis patients were highly variable. However, in general, a complex of 5 components with a relative molecular mass (Mr) of 200–100 kDa and other immunodominant antigens of approximately 60, 45, 40, and 32 kDa were recognized in cyst fluid. In the NOG extract, antigens of Mr approximately 120, 90, 60, and 37 kDa were recognized; in the GP fraction, we found antigens of approximately Mr 100 and 70, plus ~37 kDa were recognized; in the GP fraction, we found antigens of Mr 60–30 kDa. Sera from the Amerindians were similar to antigens found in the hospitalized patients with cysticercosis (Figure 1).

All 54 (79.4%) of 67 of the Amerindian patients who were positive by Ab-ELISA were also positive by EITB, and a similar pattern was observed with the hospitalized patients with cysticercosis; of these, 23 (88.5%) of 26 experienced a positive reaction to cyst fluid and NOG extract, and 24 (92.3%) of 26 reacted positively to the glycoprotein preparation.

**DISCUSSION**

To our knowledge, the majority of epidemiological studies on the prevalence of cysticercosis in Latin America have been conducted in rural populations, where sociological and other factors favor transmission.3,8 High seroprevalence of cysticercosis has been observed in these rural Latin American communities. For example, EITB seroprevalences of 10% and 17% were reported in 2 Guatemalan rural communities.4 By use of the same technique, seroprevalences of 10.8% in Morelos, Mexico, and 23.8% in the Peruvian highlands were also observed.2,14 Higher seroprevalences of 34% were found in a rural community in Honduras by EITB,8 and seroprevalences of 9%, 4.5%, and 2% were recorded in 3 rural communities in Bolivia.19

**FIGURE 1.** Representative enzyme immunoelectrotransfer blot (EITB) antigen recognition patterns to *Taenia solium* cyst fluid (CF), an N-octyl-β-D-glucopyranoside metacestode extract (NOG) or an extract of *T. solium* metacestode glycoproteins (GP) preparations (first, second, and third strips, respectively) obtained by use of sera from confirmed hospital neurocysticercosis patients (A), Amerindians (B), or healthy controls (C). Relative molecular mass (Mr; kDa) is indicated on the right-hand side.
The prevalence of cysticercosis in Amerindian communities is largely unknown. This report, which documents high circulating 
T. solium metacestode antigen (64.7%) and anti-parasite antibody seroprevalence of 79.4% is to our
knowledge the first systematic investigation into the prevalence of T. solium cysticercosis in such a community. Thus,
the seroprevalence detected by antigen and antibodies in our
assays in this Amerindian community is considerably higher
than that of previous reports describing endemic areas of
Latin America.

Considering that all the Amerindians in our study popula-
tion were asymptomatic at the time of sampling, these
results are concerning because they indicate not only that this
population is exposed to infection, but also that active in-
fec tion with viable parasites is present. These findings are
consistent with the premise that the Amerindian population
were a naive population before challenge. If they had never
before been exposed to challenge with T. solium eggs
through environmental or other contamination, they would
have been highly susceptible to such challenge.

The antigen recognition patterns obtained in EITB with
sera from the Amerindians was similar to that obtained with
sera from patients with confirmed cysticercosis. This can be
taken as indirect confirmation for the presence of the same
infection in both of the study groups.

Evidence for recent infection in the Amerindian commu-
nity stems from 2 sources. First is the predominance of the
IgM antibody class response. In comparison, the results from
the hospitalized patients indicate that a shift in the predom-
inant antibody-class response from IgM to IgG and a wid-
ening of the IgG subclass response had already occurred.
Such a pattern is indicative of chronic, longer-term infection,
which was the case with these hospitalized patients (de Noya
A, personal communication). The second indication is the
high percentage of the Amerindian sera positive for circu-
lating parasite antigen, a finding indicative of active viable—
that is, recent—infestation with cysts, as opposed to the lower
number of hospitalized patients with cysticercosis who had
active, viable infections (only 27%). This is indicative of a
longer-term infection in which cysts have started to degen-
erate or die and form foci for epileptic seizures.

Although the Amerindians do not raise pigs themselves,
they do consume pork. If either raw or undercooked pork is
eaten, there is a risk of exposure to the parasite and of the
infection of the intestine adult tapeworm infection. Tape-
worm infection would increase fecal-oral exposure to T. so-
lium eggs either directly by external autoinfection or to peo-
ple in close contact.

A second possible explanation for this infection in the
Amerindian community would be that an immigrant T. so-
lium carrier could have recently acted as a source of contam-
nation of food or water. All of the Amerindians lived near
the riverside, and environmental contamination can occur
through water-borne T. solium eggs. It has been demonstrat-
ed that exposure to immigrants from endemic Central Amer-
ican countries was statistically associated to seropositivity in
a low-prevalence population (an urban Orthodox Jewish
community).20

The results from this study give an indication of the sit-
atuation as it applies in one area of Venezuela. The concern
is therefore that this community and possibly other Amer-

indian communities are at potential risk of T. solium infec-
tion. Further studies and possibly a control program in the
Amerindian community we studied is clearly indicated and
may be appropriate for other such communities. However,
before the implementation of control programs in this or any
other community, sociological studies are required to deter-
mine the best methods to disseminate the necessary infor-
mation and control strategies to the population. In order to
succeed, the recommended control measures must be adapt-
ed for local conditions and must be suited to the Amerindian
lifestyle.

Acknowledgments: We are grateful to María Lares for her useful
technical assistance and to Licensiada Carmen Bracho for the collec-
tion of serum samples.

Financial support: This work was supported by grants CT95–0002
from the INCO-DC program of the European Union.

Authors’ addresses: E. Ferrer, M. M. Cortez, and Z. Cabrera, Centro
de Investigaciones Biomedicas BIOMED, Universidad de Carabobo,
Maracay, Venezuela. H. Perez and M. De la Rosa, Centro de Mi-
crobiología y Biología Celular IVIC, Caracas, Venezuela. B. Alarcon
de Noya, Instituto de Medicina Tropical, Universidad Central de
Venezuela, Caracas, Venezuela. I. Dávila, Departamento de Parasi-
ología Universidad de Carabobo, Maracay, Venezuela. L. J. S. Har-
rison, University of Edinburgh, Department of Tropical Animal
Health, Centre for Tropical Veterinary Medicine, Easter Bush, Ros-
lin, Midlothian, Scotland, United Kingdom EH25 9RG. M. Foster-
Cuevas, Institute for Animal Health, Pirbright Laboratories, Ash
Road, Pirbright, Woking, Surrey, England, United Kingdom, GU24
0NF. R. M. E. Parkhouse, Gulbenkian Institute for Science, Rua
Quinta Grande 6, Oeiras, 2780–155, Portugal.

Reprint requests: L. J. S. Harrison, University of Edinburgh, Centre
for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian,
Scotland, UK EH25 9RG. M. Foster-Cuevas, Institute for Animal Health, Pirbright Laboratories, Ash Road, Pirbright, Woking, Surrey, England, United Kingdom, GU24 0NF. R. M. E. Parkhouse, Gulbenkian Institute for Science, Rua
Quinta Grande 6, Oeiras, 2780–155, Portugal.

REFERENCES

2. Sarti E, Schantz PM, Plancarte A, Wilson M, Gutierrez IO, Lopez
Taenia solium taeniasis and cysticercosis in humans and pigs
in a village in Morelos, Mexico. Am J Trop Med Hyg 46:
677–685.
3. Garcia HH, Gilman RH, Martinez M, Tsang VCW, Pilcher JB,
Risk factors for human cysticercosis morbidity: a popula-
tion-based case-control study. Epidemiol Infect 119: 231–
235.
5. Garcia HH, Martinez M, Gilman R, Herrera G, Tsang VCW,
Pilcher JB, Diaz F, Verastegui M, Gallo C, Porras M, Alva-
rado M, Naranjo J, Miranda E, the cysticercosis working
6. Garcia HH, Gilman RH, Tsang VCW, Gonzalez AE, the cyti-
cercosis working group in Peru, 1997. Clinical significance of
neurocysticercosis in endemic villages. Trans R Soc Trop Med
Hyg 91: 176–178.
J, Flisser A, 1994. Community-based epidemiological inves-
tigations of cysticercosis due to Taenia solium: comparison of
serological screening test and clinical findings in two popu-
8. Garcia-Noval J, Allan JC, Fletes C, Moreno E, De Mata F, Tor-


