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Division of Disease Control

PN-ABW-375 7.208

June 14,1995

Dr. J. Daly
USAID
Bureau for Global Programs , Field support and Research
Center for Human Capacity Development
Office of Policy and Programs (Research)
Room 608 SA-18
Washington DC 20523-1814

Dear Dr. Daly;

I am sending to you by DHL our final report on the PSTC PROJECT no. 7,208 Cysticercosis : New Opportunities for Targeting Detection and Treatment Using ELISA Technology.

Yours truly,

Robert H. Gilman
Professor

mh.

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JUN 20 1995

CC: BOSTID

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Executive Summary-

Cysticercosis - New Opportunities for Targeting Detection and Treatment Using ELISA Technology

This project enabled Peru to determine the burden of cysticercosis disease. The study demonstrated that 10% of acute care beds were occupied by patients infected with T. solium. The study also demonstrated that over 40% of pigs and 10% of individuals living in an endemic area were infected by T. solium.

The study established a testing service for the serologic demonstration of T. solium infection in the laboratory of parasitology at the UPCH which has processed over 1500 specimens. This laboratory is self financing and stable.

The project has also emphasized the importance of control of cysticercosis in Latin America. Control projects are now being undertaken in the sierra of Peru under the sponsership of the NIH of the USA.

Subsequent to this grant further projects on cysticercosis were obtained from the IDRC of Canada, the NIH of USA and from private industry from Smith Kline Beecham and Syntex.

Management Final Report

Final Report - Cysticercosis-New Opportunities for Targeting Detection and Treatment Using ELISA Technology.

PI- R. Gilman
Universidad Peruana Cayetano Heredia and
John Hopkins University

Collaborator - E. Miranda
Universidad Peruana Cayetano Heredia
Project number -7,208

REPORT TO AID JUNE 1988 TO June 1990
ROBERT H. GILMAN M.D.

In the study on cysticercosis our objectives were the following:

a) To isolate and define antigens of Taenia solium using serum free, in vitro cultures of T. solium worms.

b) To use these antigens to prepare diagnostic reagents for the detection of cerebral cysticercosis.

c) To determine using Elisa assay the sensitivity and specificity of these reagents for the detection of verified cases of cerebral cysticercosis and compare them with whole worm antigens currently in use.
d) To compare a new latex bead assay the Centrifugation - Augmented Solid Phase Immunoassay (CASPIA) with the standard ELISA method for the diagnosis of cerebral cysticercosis.

We divided the project into the initial exploratory phase and the second productive phase. While working on the ELISA and CASPIA assay for the detection of cerebral cysticercosis we were introduced to the enzyme linked immune transfer technique or Western blot developed by Dr. Victor Tsang at the CDC. Dr. Tsang agreed to collaborate with us in this project and sent Joy Pilcher to the Universidad Peruana Cayetano Heredia to train five individuals in the laboratory of parasitology to enable them to perform the western blot for cysticercosis.

This test was then used for all subsequent studies because of its high sensitivity and specificity (92% and 98% found in 38 patients with confirmed disease).

We have considerably extended the role of serology in cysticercosis through the use of a new Western blot assay developed by the CDC. This assay was used in Peru to aid in the diagnosis of clinical cysticercosis, to help define the epidemiology of the disease and to determine the proteins excreted as exoantigens.

EXOANTIGEN IN THE ELISA ASSAY

Our original objective to assay the use of an exoantigen in the ELISA assay for use as a diagnostic test was completed. The antigen was assayed for sensitivity and specificity in comparison with a crude

antigen preparation. The use of an exoantigen improves both the sensitivity and specificity of the ELISA assay but requires a great deal of time and effort to produce relatively small lots of antigen. We compared the use of this antigen with that of another antigen made at CDC. The CDC antigen in combination with an immunoblot assay gave superior sensitivity and specificity. We have therefore elected to continue working with the CDC antigen. We are one of the few laboratories outside of the CDC that is routinely isolating the antigen and performing the Western blot assay for the diagnosis of cysticercosis.

CASPIA TEST -

We have also worked extensively with the CASPIA assay. This assay appears to be slightly more sensitive than latex agglutination but is less sensitive than either the ELISA or the Western blot assay.

STANDARDIZATION OF THE ELISA FOR CYSTICERCOSIS -

Because of wide variations in results of the ELISA assay obtained between different laboratories we have developed a method for standardizing the ELISA assay using corrected O.D. This method permits different laboratories to use the assay when a standard antigen and antisera are available. The use of sensitivity, specificity response curves permits us to obtain cut off points in the ELISA assay that permit an enhancement of sensitivity and specificity compared to values obtained when standard methods for determining cutoff points are utilized.

WESTERN BLOT ASSAY FOR CYSTICERCOSIS -

This assay uses a purified preparation of glycoproteins as the source of antigen. It is still not pure enough to use as a diagnostic reagent in the ELISA but is highly reliable in the Western blot assay. The Western blot assay is over 92% sensitive and 98% specific when tested against confirmed negatives and positive patients. We have therefore directed most of our recent efforts to use this test as a gold standard for the diagnosis of cysticercosis.

CLINICAL AND EPIDEMIOLOGICAL STUDIES -

We have instituted a series of studies involving

the use of the Western Blot assay in order to better define the epidemiolcgy and clinical significance of cysticercosis in a developing country like Peru.

These studies demonstrate the importance of T. solium infection in Peru. All details are contained in the appended publications at the end of this report.

INFORMATION DISSEMINATION -

We have utilized the information obtained by Western blot to aid in a recent Pan American Health Organization publication on Zoological diseases. In addition we have presented our data on cysticercosis at local meetings of parasitology and microbiology. Three of our abstracts were presented in the Am. Meeting of Tropical Medicine in December 1989.

MANUSCRIPTS Published -

The following five manuscripts were published as part of this study.

Gonzalez A.E., V.Cama' R.H. Gilman' V. Tsang, et.al. Prevalence and Comparison of Serologic Assays, Necropsy, and Tongue Examination for the Diagnosis of Porcine Cysticercosis in Peru. Am. J. of Trop Med and Hygiene. 1990. 43: 194-199.

Garcia H., Martinez M. Gilman R. et. al. Diagnosis of cysticercosis in endemic regions. Lancet. 1991. 338: 549-551.

Tsang V., Pilcher J., Zhou W., Boyer A., Kamango E., Rhoads M., Murrell K. W. Schantz P. M., and R. H. Gilman . Efficacy of the immunoblot assay for cysticercosis in pigs and modulated expression of distinct IgM/IgG activities to Taenia solium antigens in experimental infections. Veterinary . Immunology and Immunopathology. 1991. 29 : 69-78.

Diaz F., Verastegui M., Gilman R., Tsang V., Pilcher J. Gallo C., Garcia H., Torres P., Montenegro T., Miranda E. and the Cysticercosis Working Group in Peru(CWG)

Immunodiagnosis of Human Cysticercosis (Taenia Solium): A Field Comparison of an Antibody Enzyme - Linked Immunosorbent Assay (ELISA) An Antigen -ELISA and an Enzyme Linked Immunoelectrotransfer Blot (EITB) Assay in Peru. American J. of Tropical Medicine and Hygiene. 1992. Vol 46 ,pg 610 -615.

Diaz F., Garcia H.H., Gilman R. H., Gonzales A.E., Castro M. Tsang V.C., Pilcher J. B., Vasquez L.E., Lescano M., Carcamo C., Madico G., Miranda E. and the Cysticercosis Working Group in Peru. Epidemiology of Taeniasis and Cysticercosis in a Peruvian Village. American J. of Epidemiology. 1992. 135: 875-882.

DEVELOPMENT OF CYSTICERCOSIS WORKING GROUP IN PERU -

In order to obtain cooperation from a wide variety of hospitals and groups we organized a Working Group for the study of cysticercosis. This was highly successful and we were able to obtain access to patients from nearly all major hospitals in Lima. The patients that were tissue confirmed cases were utilized to form a serum bank both at the CDC and in Peru. This is especially important since banks of sera from confirmed positive and negative cases are not easily available.

Our data and subsequent publications demonstrate that 30% of adult onset epilepsy in individuals born outside of Lima were associated with serological evidence of infection.

Methods and Results - See appended articles.

The results presented in these articles demonstrate that cysticercosis is highly prevalent in Peru and further programs to control this important disease need to be started.

Impact, Relevance and Technology Transfer - We have now set up the western blot as a methodology in the UPCH laboratory of parasitology. This facility obtains specimens from all surrounding hospitals. In addition we have demonstrated that a large portion of pigs in endemic areas are infected and that 10% of a rural population is infected with T. solium. In the process of this study Dr. Victor Tsang of the CDC transferred the western blot technology to Peru and began a collaboration that is still ongoing. Further grants from the IDRC and the NIH were made possible by this grant. In addition multiple scientists were trained in the western blot technology. This training was so successful that Dr. Verastigue and others published their own findings on the Western blot for hydatidosis in the J. Clin Micro. Dr. E. Gonzalez and

H Garcia were both incorporated into the cysticercosis team and now are experts in their own right. Dra. Verastigue went to the CDC as part of this project and trained at the CDC for a two month period in antigen purification.

The Cysticercosis Working Group in Peru has over 15 papers on this subject either published or in press. This group was initiated as part of the PSTC grant. The group is internationally known and various members have been invited to speak at meetings which include those of international agencies such as PAHO.

We think this PSTC grant embodies the ideals that were envisoned when this grant program was initiated. It was obviously a failure of both USAID and the US government that the PSTC program was cast aside in favor of other programs that were far more costly, accomplished far less, but which had a far more powerful and wealthy political constituency.

7. Project Activities/Outputs - Am J of Trop Med - 1987
2. J. Pilcher - Peru training
3. M. Verastigue - USA training
4. Publications - listed above.

8. Project Productivity - The goal of being able to target effectively using serological methodologies was accomplished in spades.

9. Future - Yes, We are currently involved in control programs for cysticercosis with funds provided by the NIH.

10) Literature cited- See above list.

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PREVALENCE AND COMPARISON OF SEROLOGIC ASSAYS, NECROPSY, AND TONGUE EXAMINATION FOR THE DIAGNOSIS OF PORCINE CYSTICERCOSIS IN PERU

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PRISMA, Lima, Peru; Universidad Peruana Cayetano Heredia, Lima, Peru; Johns Hopkins University, Baltimore, Maryland; Centers for Disease Control, Atlanta, Georgia; Universidad Nacional Mayor de San Marcos, Lima, Peru

Abstract. Swine cysticercosis, a severe zoonotic disease which is part of the *Taenia solium* life cycle, causes major economic losses in pig husbandry. Throughout South America, farmers diagnose cysticercosis by examining the tongues of their pigs for cysticercus nodules. Farmers do not bring pigs believed to be infected to the slaughterhouse for fear of confiscation. Therefore, reliable statistics on porcine cysticercosis can only be acquired at the household level. We examined the utility of the tongue test as a diagnostic tool for porcine cysticercosis. The results of the tongue test was compared with 2 serologic methods for the detection of cysticercosis, the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked immunoelectrotransfer blot assay (EITB), and with necropsy results. We examined 11 animals from an endemic area (Huancayo) and 42 animals from an area free of cysticercosis (Lima). The tongue test has a sensitivity of 70% and a specificity of 100%, the EITB a sensitivity and specificity of 100%, and the ELISA a sensitivity of 79% and a specificity of 75%. Thus, the tongue examination, being a test essentially without cost and having fair sensitivity and high specificity, can be useful in epidemiological surveys. Prevalence for porcine cysticercosis in Huancayo is 23.4% by tongue examination, 31.2% by necropsy, 37.7% by ELISA, and 51.9% by EITB.

Cysticercosis is an important disease in Peru and other countries of Latin America. In a neurology hospital in Mexico, over 9% of all autopsies were positive for cysticercosis.[1] In Peru, 10%–15% of neurology ward beds are devoted to the care of cysticercosis patients (H. Garcia, Universidad Peruana Cayetano Heredia, Lima, Peru, personal communication). This high rate of neurocysticercosis may be due, indirectly, to the large reservoir of porcine cysticercosis present in these countries. Unless this reservoir is decreased, the rate of human taeniasis and neurocysticercosis cannot be decreased.

Future control and eradication programs will require accurate data about rates of infection in pigs.[2] *Taenia solium* causes not only severe zoonotic disease,[3] but also great economic losses to pig husbandry.[4] In Peru, diagnosis of pig cysticercosis is generally made upon meat inspection during slaughter.[5–7] At Peruvian slaughterhouses, the prevalence of cysticercosis in pigs is 1–9%[8–10] when assessed by veterinary inspection of meat, and 12% when assessed by serologic testing.[11] Because 91% of the pigs are not registered and 55% are illicitly slaughtered,[12] control measures to prevent human consumption of infected meat are inadequate. In Latin America, pigs are usually bought and sold in an informal farmers' market. In some areas of the Peruvian sierra, villagers pay a small fee to a local examiner to detect cysticercus nodules in the tongue. Infected pigs are sold for less than price of uninfected pigs and are either used for home consumption or are sold and processed in the informal market. Local farmers only send pigs with negative tongue tests to the slaughterhouses, as these are most likely to pass inspection. In slaughterhouses where control and confiscation are not practiced, rates of infection among pigs may be as high as 30%.[13] Thus, slaughterhouse statistics may underestimate the percentage of pigs infected with cysticercosis.

No standardized method to estimate the prevalence of porcine cysticercosis under field conditions is available. A standardized field method permitting the easy sampling of living pigs could provide unbiased, quantitative epidemiologic data. To determine if clinical examination of the

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FIGURE 1. Tongue of an infected pig. Arrows point to clusters of T. solium *in cysts on the ventral surface.*

pig's tongue, the diagnostic method used by Peruvian peasants, is a suitable field surveillance method, we compared the results of this method with necropsy results. Concurrently, we also evaluated 2 recently developed serologic methods, the enzyme-linked immunosorbent assay (ELISA)[14–18] and the enzyme-linked immunoelectrotransfer blot assay (EITB).[19,20]

MATERIALS AND METHODS

Animals

Seventeen animals were obtained from a cysticercosis-endemic area in the Peruvian highlands (Coto-Coto Fair, Huancayo) and 42 from a cysticercosis-free zone on the coast (Lima). Animals were randomly selected.

Tongue examination

The tongue test consists of palpation of nodules and/or visual identification of cysticercus cysts. The pig is placed on its side, held by the neck behind the occipital protuberances with its forelimb pulled above its shoulder, and firmly restrained. A hard rubber or wooden rod is used to keep the mouth open. A veterinarian using a cloth gently pulls the tongue, examines, and palpates it throughout the base (Fig. 1). The pig is considered positive for cysticercosis if cyst-like nodules are either seen or felt.

In order to evaluate subjective, examiner-dependent variations, 3 examiners, 2 experienced and 1 trained only a day before the study, examined 52 pigs from Maceda, a village with endemic cysticercosis. All 3 examiners studied all pigs separately without knowledge of the others' results.

ELISA antigens

T. solium cysts were carefully dissected from 17 naturally infected pigs and washed extensively at 4°C in phosphate buffered saline (PBS; 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4) containing protease inhibitor (2 mM phenylmethylsulfonylfluoride [PMSF], Sigma Chemical Co., St. Louis, MO). The excised larvae were homogenized in PBS/PMSF at 4°C using a glass

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tissue homogenizer and sonicated on ice 6 times for 3 min at 20 kHz. The sonicated larvae were then centrifuged at 25,000 × *g* at 4°C for 1 hr; the supernatant was collected, divided into small portions, and stored at −70°C.[21] Protein content was determined by a protein-dye binding method.[22]

EITB antigens

T. solium cysts were dissected as described above. The cysts were immediately placed in 5 volumes (1/5 w/v) of sucrose/HEPES/PMSF buffer (0.05 M HEPES/NaOH, 0.25 M sucrose, 2.0 mM EDTA, 5 mM PMSF, pH 7.20) at 4°C, drained, blotted dry, and stored frozen at −70°C until used. The cysts were then shipped to the Centers for Disease Control (CDC), Atlanta, GA where the glycoprotein antigens were prepared.[19] After homogenization, the membrane fraction of the cysts was solubilized in 8 M urea. The lipids were extracted with Freon and the solution was desalted and passed through a lectin column. α-D-methyl-mannoside was used to elute lentil lectin-bound glycoprotein from the column. The purified glycoprotein antigens were stored in the presence of 77% glycerol at −70°C.

ELISA

ELISA antigens were diluted in carbonate buffer (pH 9.8) to a concentration of 100 ng/ml. One hundred μl was added to each well and the plate incubated overnight at 4°C. Control wells received only carbonate buffer. The serum was diluted 1:100 and 1:5 with PBS with 0.05% Tween 20. Following 3 washes at 5 min each with PBS-Tween, 100 μl of each serum dilution was added to antigen-coated and control wells. The plates were then incubated at room temperature for 2 hr. After 3 washes, 100 μl of diluted peroxidase-conjugated goat swine antibody (Kirkegaard and Perry, Gaithersburg, MD) was added to each well and the plates were incubated for 2 hr at room temperature. The plates were then washed 3 times and 100 μl of orthophenyldiamine (Sigma) peroxidase substrate was added to each well. After 15 min, 100 μl of 0.1 M sulfuric acid was added to stop the reaction. Optical density was determined by ELISA reader (Flow-Minitech, McLean, VA).[21]

EITB

The EITB was performed in Lima and Atlanta as previously described.[19] This assay used purified glycoprotein antigens (LL-GP) in an EITB format to detect infection-specific antibodies in serum. Peroxidase-labeled goat swine antibody (Kirkegaard and Perry) was used at 1:2. Bound antibodies were visualized using an H_2O_2/DAB substrate system.[23,24] A positive test was indicated by a reaction to any of 7 glycoprotein bands (GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13).

Necropsy

T. solium larvae were sought in the brain, viscera, and muscle in infected pigs. The following muscles were examined using at least 2 cuts per muscle: neck musculature, masseter, psoas, anconeus, frontal, dorsal, serratus, and gluteus. We also recorded diagnosis of hydatid disease, fascioliasis, and *T. hydatigena* infections.

Data analysis

For the purpose of test comparison, 66 pigs were selected: 24 necropsy-positive pigs (diseased animals) from Huancayo and 42 necropsy-negative pigs (animals without disease) from Lima. No necropsy-negative pigs from the endemic zone of Huancayo were included in this reference population because of a reasonable degree of uncertainty regarding the sensitivity of necropsy to detect low-level infections. The results of serology were compared with those of necropsy and [illegible]. Test sensitivity, specificity, predictive values, and efficiency were calculated as previously described.[19]

RESULTS

Results of all tests are listed in Table 1. Accuracy of the tongue examination was verified by the fact that there was no significant differences among the readings of the 3 observers, including the newly-trained observer, in the tongue test examiner variation trial ($P < 0.05$). The EITB data generated in Lima was compared to that generated at CDC with <1% discrepancy.

To calculate and compare the relative sensitivity, specificity, and efficiency of different diagnostic tests, a definitive disease state must first be defined and the test population divided into infected and uninfected groups. In this study of 119 pigs, necropsy-positive animals were clearly infected. Negative necropsy results, however, may

TABLE 1
Test and examination results of all pigs (n = 119)

Test results*	Huancayo (endemic) n = 77		Lima (non-endemic) n = 42	
	No.	Percent	No.	Percent
Necropsy/+	24	31.2	0	0
Necropsy/−	53	68.8	42	100
Tongue/+	18	23.4	0	0
Tongue/−	59	76.6	42	100
EITB/+	40	51.9	0	0
EITB/−	37	48.1	42	100
ELISA/+	29	37.7	10	23.8
ELISA/−	48	62.3	32	76.2

* Necropsy = parasitologic examination for *T. solium* cysts in various tissues; tongue = tongue examination; EITB = immunoblot assay with lectin purified antigens; ELISA = standard immunoassay with crude antigens.

TABLE 2
*Comparative test efficacies calculated with respect to the "reference standard" population of necropsy-positive and -negative pigs (n = 66)**

	Tests		
	Tongue	ELISA	EITB
Sensitivity	70.8%	79.2%	100%
Specificity	100%	76.2%	100%
Predictive value (+)	100%	65.5%	100%
Predictive value (−)	85.7%	86.5%	100%
Test efficiency	89.4%	77.3%	100%

* "Reference standard" population consisted of 42 necropsy-negative pigs from non-endemic Lima, herein defined as uninfected, and 24 necropsy-positive pigs from endemic Huancayo, defined as infected animals.

not have been adequate for the selection of uninfected animals from an endemic zone. Even with exhaustive searches during necropsy, there is still a possibility of missing low-level infections. For the true negative, therefore, we only counted those animals from a non-endemic area (Lima) with negative necropsy results. Our constraint for reference selection left us with 24 diseased animals from Huancayo and 42 uninfected ones from Lima, a total of 66 reference pigs. All calculations of test efficacies were against this population.

Table 2 shows the test efficacies of the ELISA, tongue examination, and EITB as calculated from these 66 pigs. In Table 3, test results were further partitioned according to necropsy data and geographical locations.

There was a high rate of false-positive reactions in the ELISA when pigs were infected with other helminths. In pigs negative by both necropsy and EITB, the ELISA was positive in 1 of 4 pigs infected with *T. hydatigena*, 1 of 7 pigs infected with *Echinococcus granulosus*, and 2 of 9 pigs infected with *Fasciola hepatica*.

DISCUSSION

In an endemic area of Peru (Huancayo), the prevalence for porcine cysticercosis ranged from 51.9% (EITB) to 31.2% (necropsy). Clinical examination of the tongue, a traditional Peruvian method for evaluating cysticercosis, was relatively sensitive and highly specific for detecting *T. solium*-infected animals. The test was easily learned and the inter-observer variation was low. When corroborated by necropsy, results obtained by this method compared favorably with those obtained by EITB.

The EITB is a highly sensitive and specific test for human cysticercosis[19] and for pigs experimentally infected with cysticercosis.[20] This test appeared to detect antibodies in a large number of necropsy-negative animals, but only from the endemic area of Huancayo. It is possible that the pigs had immature or occult cysts that were not easily visible at necropsy but were still able to produce a humoral response. In *T. saginata* infections, detection was augmented by increasing the number of slices at necropsy.[25,26] Cross-reactivity with other parasitic infections in this assay has not been observed since its invention almost 3 years ago.

The ELISA was not as sensitive or as specific as the EITB test. The sensitivity of the ELISA in pigs (79.2%) is similar to that found in human studies.[27] Thirty-one percent of necropsy-negative pigs sampled at a Lima slaughterhouse had a positive reaction by ELISA. In contrast, a study of the serology of porcine cysticercosis in India found higher levels of sensitivity (91%) and specificity (92%) when either a fractionated cyst or scolex antigen was used.[28] This study disregarded

TABLE 3
Breakdown of test results with respect to necropsy data (n = 119)

Test results	Necropsy positive		Necropsy negative	
	Huancayo	Lima	Huancayo	Lima
Tongue +	17	0	1	0
Tongue −	7	0	52	42
EITB/+	24	0	16	0
EITB/−	0	0	37	42
ELISA/+	19	0	10	10
ELISA/−	5	0	43	32

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sera from pigs infected with other parasites thereby increasing the specificity of their assay.[29,30] Cross-reactions with *T. hydatigena* larvae and other cestodes, including *Echinococcus* species, may be responsible for the high rate of false positives in the ELISA.[29,30] With better purified antigens, the specificity of the assay may be increased.

Some of the pigs used in this study, as in a previous study,[28] were heavily parasitized with *F. hepatica, E. granulosus,* and *Cysticercus tenuicolis (Hydatigena taeniformis).* Cross-reacting antibodies to these parasites were not found by the EITB, since *T. solium*-specific bands are used for the diagnosis of cysticercosis.

The tongue test for the diagnosis of swine cysticercosis was highly correlated with necropsy. The tongue test was also comparable to the 2 serologic tests used and resulted in a positive predictive value of 100% and a negative predictive value of 85.7%. Where porcine cysticercosis is endemic, the tongue test may be a useful and practical survey tool for estimating the prevalence of porcine cysticercosis.

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REFERENCES

1. Shenoe H, Ramirez R, Rojas A, 1973. Aspectos epidemiologicos de la neurocisticercosis en America Latina. *Bol Chile Paras 28:* 61–72.
2. Acha PN, Szyfres B, 1986. *Zoonoses and communicable diseases common to man and animals.* Scientific publication no. 354. Washington: Pan American Health Organization, 763–774. (ui) 8007037
3. Wiederholt WC, Grisolia JS, 1982. Cysticercosis. An old scourge revisited. *Arch Neurol 39:* 533. file m80; (ui) 82283422
4. Acevedo-Hernamdez A, 1982. Economic impact of porcine cysticercosis. Flisser A, Willms K, Laclette P, Larralde C, eds. *Cysticercosis, present state of knowledge and perspectives.* New York: Academic Press, 53–62. (ui) 8304133
5. Schnaas G, 1972. [Sanitary control of cysticercosis]. *Gac Med Mex 103:* 246–249. file m72; (ui) 72203350
6. Benson AJ, 1983. *El control de las enfermedades transmisibles.* OPS ed. Publicacion Scientifica, 442.
7. Matyas Z, Pawlowski Z, Soulsby EJ, Nelson GS, Larralde C, Rosick, 1983. Guidelines for surveillance, prevention, and control of taeniasis and cysticercosis. *WHO/VPH 83:* 207.
8. Escalante S, 1977. [Epidemiology of cysticercosis in Peru]. *Rev Neuropsiquiatr 40:* 29–39. file m77; (ui) 79013872
9. Inope L, Rojas M, Bullon F, 1977. [Cysticercosis in Peru. Various statistical aspects]. *Rev Neuropsiquiatr 40:* 40–52. file m77; (ui) 79013874
10. Rojas M, 1983. Cysticercosis, una Imponderada Zoonosis en el Peru. *Boletin Informativo del Colegio Medico-Veterinario del Peru 18:* 19–23
11. Montenegro T, Gonzalez AE, Torres P, and others, 1988. *Monitoreo serologico de la cisticercosis porcina en camales de chiclayo y Cajamarca por el metodo de ELISA.* Anales del XI Congreso Panamericano de Ciencias Veterinarias. Lima: Asociacion de Medicos Veterinarios del Peru.
12. Ministerio de Agricultura, 1986. *Unidad de servicio pecuario.* Peru: Ministerio de Agricultura.
13. Llontop R, Nolte C, Urbina R, and others, 1980. *Cysticercosis en cerdos beneficiados en el camal frigorifico No. 37 de Tarapoto.* Publicacion del Ministerio de Agricultura y Alimentacion RA XI, Laboratorio de Sanidad Animal y Zoonosis Tarapoto.
14. Diwan AR, Coker-Vann M, Brown P, Subianto DB, Yolken R, Desowitz R, Escobar A, Gibbs CJ Jr, Gajdusek DC, 1982. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to cysticerci of Taenia solium. *Am J Trop Med Hyg 31:* 364–369. file m80; (ui) 82179875
15. Tellez Giron E, Ramos MC, Dufour L, Montante M, 1984. [Use of the ELISA method in the diagnosis of cysticercosis]. *Bol Of Sanit Panam 97:* 8–13. file m83; (ui) 85000040
16. Mohammad IN, Heiner DC, Miller BL, Goldberg MA, Kagan IG, 1984. Enzyme-linked immunosorbent assay for the diagnosis of cerebral cysticercosis. *J Clin Microbiol 20:* 775–779. file m83; (ui) 85030969
17. Espinoza B, Ruiz-Palacios G, Tovar A, Sandoval MA, Plancarte A, Flisser A, 1986. Characterization by enzyme-linked immunosorbent assay of the humoral immune response in patients with neurocysticercosis and its application in im-

munodiagnosis. *J Clin Microbiol 24:* 536–541. file m86; (ui) 87034185

18. Rosas N, Sotelo J, Nieto D, 1986. ELISA in the diagnosis of neurocysticercosis. *Arch Neurol 43:* 353–356. file m86; (ui) 86158416
19. Tsang VC, Brand JA, Boyer AE, 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (Taenia solium). *J Infect Dis 159:* 50–59. file med; (ui) 89080354
20. Tsang VC, Brand JA, Zhou W, and others, 1990. Efficacy of the immunoblot assay for cysticercosis in pigs and modulated expression of distinct IgM/IgG activities to Taenia solium antigens in experimental infections. *Vet Immunol Immunopathol:* (in press).
21. Estrada JJ, Kuhn RE, 1985. Immunochemical detection of antigens of larval Taenia solium and anti-larval antibodies in the cerebrospinal fluid of patients with neurocysticercosis. *J Neurol Sci 71:* 39–48. file m83; (ui) 86114094
22. Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem 72:* 248–254. file m72; (ui) 76250790
23. Tsang VC, Peralta JM, Simons AR, 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. *Methods Enzymol 92:* 377–391. file m83; (ui) 83218475
24. Tsang VC, Hancock K, Wilson M, and others, 1986. *Enzyme-linked immunoelectrotransfer blot technique (western blot) for human t-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) antibodies.* Monograph: Immunology Series No. 15, Procedural Guide. Atlanta: USDHHS, PHS, CDC.
25. Walter M, 1980. Taenia saginata cysticercosis: a comparison of routine meat inspection and carcass dissection results in calves. *Vet Rec 106:* 401–402.
26. Smyth JD, 1984. Epidemiology and control of cysticercosis. *An Trop Med Parastiol 3:* 262–263.
27. Pammenter MD, Rossouw EJ, Epstein SR, 1987. Diagnosis of neurocysticercosis by enzyme-linked immunosorbent assay. *S Afr Med J 71:* 512–514. file m86; (ui) 87178533
28. Kumar D, Gaur SN, 1987. Serodiagnosis of porcine cysticercosis by enzyme-linked immunosorbent assay (ELISA) using fractionated antigens. *Vet Parasitol 24:* 195–202. file m86; (ui) 87293737
29. Gottstein B, Tsang VC, Schantz PM, 1986. Demonstration of species-specific and cross-reactive components of Taenia solium metacestode antigens. *Am J Trop Med Hyg 35:* 308–313. file m86; (ui) 86156462
30. Gottstein B, Zini D, Schantz PM, 1987. Species-specific immunodiagnosis of Taenia solium cysticercosis by ELISA and immunoblotting. *Trop Med Parasitol 38:* 299–303. file m86; (ui) 88159079

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Efficacy of the immunoblot assay for cysticercosis in pigs and modulated expression of distinct IgM/IgG activities to *Taenia solium* antigens in experimental infections*

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ABSTRACT

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A recently invented immunoblot assay for human cysticercosis was evaluated for efficacy in pigs. The test population consists of 45 pigs with parasitologically confirmed cysticercosis, 47 with heterologous infections, 45 SPF or concrete raised control animals. With this group of 137 animals the test performance was 100% sensitive and 100% specific.

The antigen-specific responses of immunoglobulin A (IgA), IgG and IgM in four pigs infected with *Taenia solium* eggs derived from a human were quantified by immunoblot. Antigen-specific activities were observed as early as 1 week postinfection. The first antigen-specific isotypic response was IgM antibodies directed against a glycoprotein at 97 KD (GP97). This activity generally disappeared between the sixth and ninth week postinfection. Between Weeks 5 and 8, IgG activity rose as IgM activity fell. The IgG activity, however, was directed mostly towards GP50 and GP42 antigens. If the same response occurs in people with cysticercosis, identifying specific isotype activity may help to distinguish new infection from old.

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ABBREVIATIONS

CSF, cerebrospinal fluid; DAB, 3,3′-diaminobenzidine; EITB, enzyme-linked immunoelectrotransfer blot, immunoblot or "Western blot"; LL-GP, lentil-lectin purified glycoprotein antigens; POD, horseradish peroxidase (donor: H_2O_2 oxidoreductase, EC 1.11.1.7); SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

INTRODUCTION

Human cysticercosis, the potentially fatal invasion of various tissues by the larval stage of *Taenia solium*, has increased dramatically as a medical problem in the U.S.A. since 1977 (Richards et al., 1985). A strong impetus to improve the diagnostics of cysticercosis was the development of praziquantel as an effective chemotherapeutic agent for the disease (Nash and Neva, 1984; Soltelo et al., 1985). Moreover, increased travel and immigration from Mexico and Central America where the disease is highly endemic, made recognition and treatment of cysticercosis imperative.

Recently we reported the development of an immunologic assay for human cysticercosis (Tsang et al., 1989) with 100% specificity and 98% sensitivity (based on a test population of 578 persons). This test is based on the EITB (Western blot or immunoblot) and depends on the recognition of any one of seven glycoprotein bands by patients' antibodies. Our data show that most cysticercosis patients recognize more than one of these antigens. We did not know if this test could distinguish between old and new infections. We also did not know if the test would perform in the pig system. It is highly desirable to have a specific and sensitive assay for pigs for epidemiologic surveys of areas where the disease is endemic.

We report here the application of the EITB for the diagnosis of cysticercosis in pigs and the apparent alternation of the IgM and IgG responses to different glycoprotein antigens during the course of experimental infection.

MATERIALS AND METHODS

Serum samples in study population

All cysticercosis serum samples with the exception of those from the four experimentally infected animals were obtained from Peruvian pigs. Natural infections of *T. solium* in these animals were confirmed by the presence of cysts at necropsy. Animals with heterologous infections of *Ascaris*, *Trichinella* and *Trichuris* were from experimentally infected animals. Natural infections of hydatidosis and facioliasis were from Peruvian pigs and confirmed at necropsy. Uninfected control animals were of U.S. origin and were either SPF or concrete raised.

Experimental infection in pigs

Four pigs (2 months old, weighing 10–15 kg) were dosed orally with 10 000–20 000 eggs (20% viable) from human-derived *T. solium* proglottids, as previously described (Rhoads et al., 1987). The infections were maintained for 13–15 weeks when the animals were killed and systematically examined for cysts. All four animals were lightly infected; the number of cysts found per pig ranged from 12 to 74 (Rhoads et al., 1987).

EITB

The EITB assay for antibodies specific for *T. solium* was performed as previously described (Tsang et al., 1989). Briefly, the assay utilizes a group of lentil–lectin purified glycoprotein antigens (LL-GP) in an immunoblot format to detect infection-specific antibodies in serum and/or CSF samples. The assay is performed in the Accutran™ system (Schleicher & Schuell, Inc., Keene, NH). There are seven major diagnostic antigens in LL-GP: GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13. The GP prefix indicates glycoprotein and the number is molecular weight as shown by SDS-PAGE. Antibody reactions against these glycoproteins in serum and/or CSF were visualized with the H_2O_2/DAB substrate system (Tsang et al., 1983, 1986). All EITB bands were quantified with the Visage 110 system (Bio Image®, Ann Arbor, MI). Quantitation was based on whole-band analysis with background subtraction.

Immunologic reagents

Immunoaffinity purified goat anti-pig IgG (heavy- and light-chain activity) labelled with POD was prepared in our laboratory; the ratio of IgG:POD was approximately 1:2.5 (Tsang et al., 1984). POD-labelled goat anti-pig isotype-specific reagents (i.e. anti-IgM, IgG and IgA conjugates) were purchased from [illegible] MD).

Antibody assays with pig serum samples

All pigs were bled weekly. Serum samples [illegible] were diluted [illegible] with PBS/Tween/milk (phosphate buffered saline (0.10 M NaCl, [illegible] M Na_2HPO_4/NaH_2PO_4, pH 7.20) containing 0.3% vol/vol Tween-20 and 5% wt/vol nonfat dried milk). Immunoblot strips containing all of the resolved LL-GP antigens were exposed to the diluted serum samples for 2 h. After washing with PBS/Tween to remove unbound serum proteins, the strips were probed with Ig-specific (H & L) or isotype-specific, POD-labelled conjugates. Bound POD activities were visualized with H_2O_2/DAB substrate (Tsang et al., 1989).

RESULTS

Test performance of the EITB in pigs

Table 1 contains the EITB results of all animals. In this control population the test performance of the EITB is 100% specific and 100% sensitive. This confirms the results of our earlier human trials (100% specific and 98% sensitive).

General Ig reactions (H&L)

All four experimental pigs were found to be infected when killed 13–15 weeks postinfection. Although the number of cysts recovered corresponded to eggs inoculated, the degree of calcification did not. All four pigs responded to more than one glycoprotein antigen from *T. solium* larval stages (Fig. 1). The antibody reactions shown in Fig. 1 were derived from a heavy- and light-chain specific conjugate; thus, all isotypes were detected but not differentiated. The most striking feature of the EITB reactions was the early appearance and the subsequent disappearance of anti-GP97 activity in all four. Pigs 1 and 2 produced anti-GP97 antibodies as early as 1 week postinfection. Anti-GP97 activity rapidly disappeared between Weeks 6 and 9. In contrast, antibody activities against the lower molecular weight glycoproteins (e.g. GP50, GP42-39 etc.) appeared later but were sustained until the end of the experiment.

IgM reactions

Differential probing of EITB strips exposed to serum samples with isotype-specific conjugate reagents resulted in the dissection of the general antibody

TABLE 1

EITB results of pig serum samples

Infections[1]	Immunoblot results	
	Positives	Negatives
Cysticercosis (*T. solium*)[1]	45	0
Ascariasis	0	12
Trichinosis	0	12
Trichuriasis	0	6
Hydatidosis (*E. granulosus*)	0	7
Fasioliasis (*F. hepatica*)	0	10
Uninfected control[2]	0	45

[1]Cysticercosis infection ($n=45$); heterologous infections ($n=47$); healthy controls ($n=45$); total samples tested ($n=137$). All infections were confirmed by the presence of organisms at necropsy.
[2]From healthy U.S. animals (SPF or concrete raised).

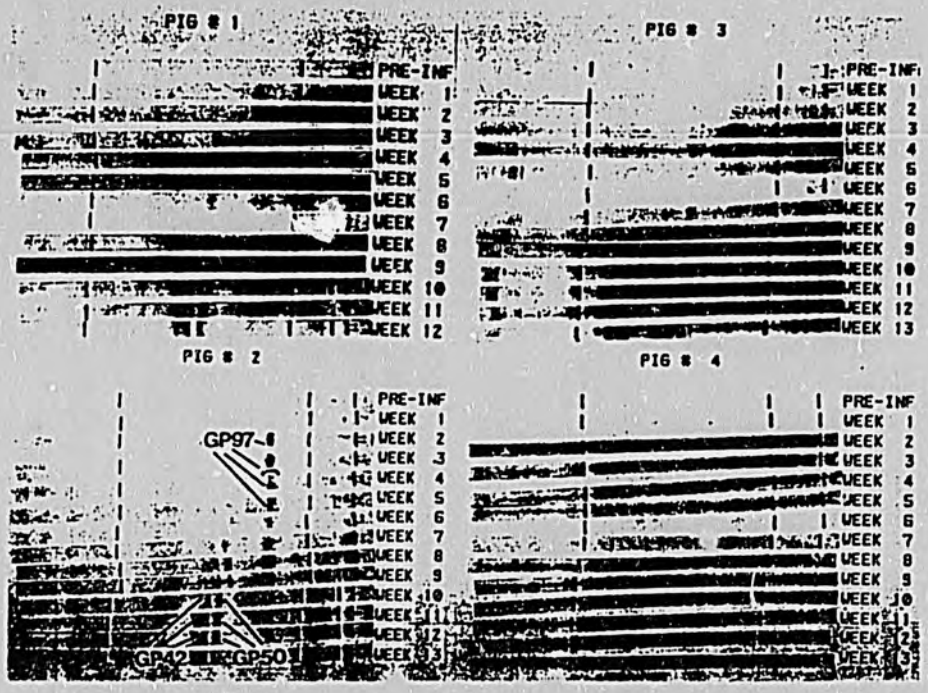


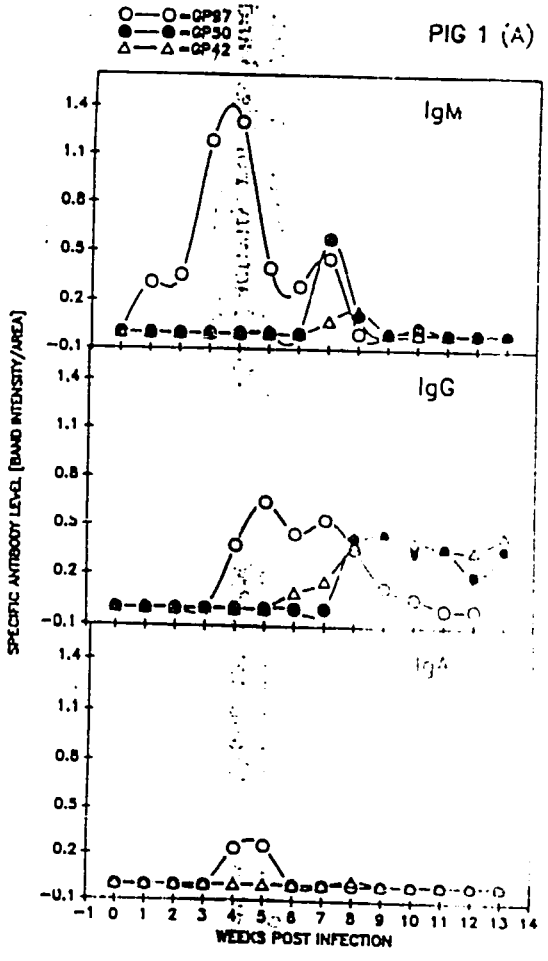
Fig. 1. Total immunoglobulin response (all isotypes) to LL-GP antigens from *T. solium* cysts. Total antigen (protein) load, 20 ng/mm of strip width, each strip, 3 mm. Serum concentration, 1:10 in PBS/Tween/milk; diluted serum volume, 0.5 ml; serum incubation time, 2 h. Second antibody conjugate, goat anti-pig Ig (heavy and light chain), activities detected thus include all isotypes. Week numbers equal weeks postinfection. Ball point pen lines on each strip are position references for use during scanning.

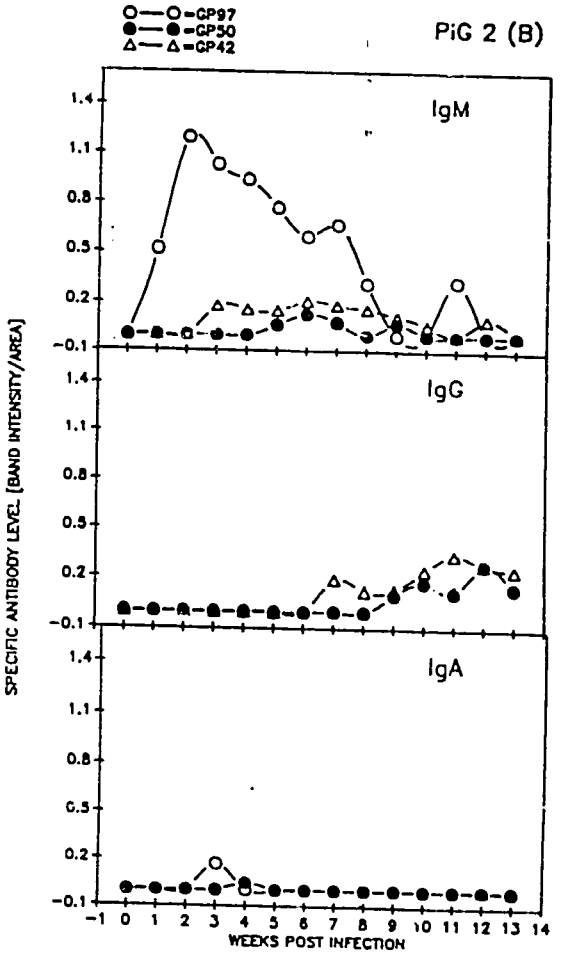
reactions into specific isotypes (Fig. 2A–2D). IgM activity for all four pigs appeared 2 weeks postinfection and seemed to be almost [illegible] associated with GP97. IgM activity was transient; by Week 8, all anti-GP97 activity became undetectable, except for that of Pig 2 (Fig. 2B). Two weeks later, Pig 2 also stopped producing anti-GP97 IgM.

IgG reactions

With the disappearance of IgM anti-GP97 activity, we observed a concomitant rise of IgG and anti-GP50 and IgG anti-GP42 activity. For Pigs 3 and 4, IgG activity was followed by the appearance of IgG antibodies directed against LL-GPs of even smaller molecular weight (see Fig. 1). Unlike IgM activity however, the IgG activity against GP50, GP42 and lower molecular weight LL-GPs persisted throughout the experiment.

Pigs 1 and 3 also exhibited transient IgG activity against GP97 (Fig. 2A,C). The IgG anti-GP97 activity for Pig 1 (Fig. 2A) appeared to peak 1 week (Week 5) after that of the IgM, which peaked between Week 3.5 and 4.


O—O=GP97
●—●=GP50
Δ—Δ=GP42
PIG 1 (A)
IgM
IgG
IgA
SPECIFIC ANTIBODY LEVEL [BAND INTENSITY/AREA]
WEEKS POST INFECTION


O—O=GP97
●—●=GP50
Δ—Δ=GP42
PiG 2 (B)
IgM
IgG
IgA
SPECIFIC ANTIBODY LEVEL [BAND INTENSITY/AREA]
WEEKS POST INFECTION

20

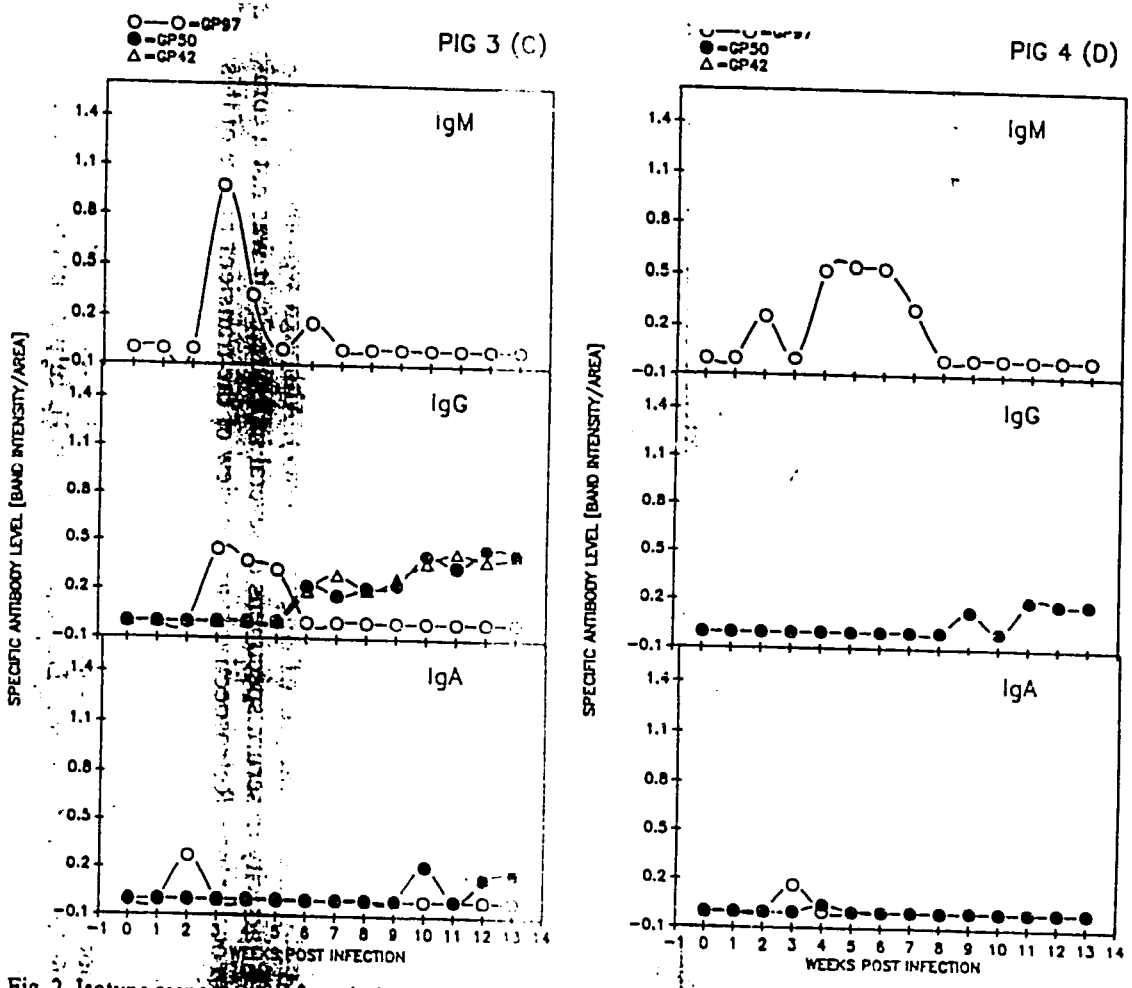


Fig. 2. Isotype response of infected pigs to LL-GP antigens from *T. solium* cysts. All conditions are the same as for Fig. 1, except for the second antibody conjugate. Isotype-specific conjugates were used to detect specific reaction of various isotypes. All strips were scanned by reflectance and bands quantified by whole band analysis. Specific antibody level (plotted on the Y axis) is an integrated function of band intensity (in optical density) and total band area (area under peak). Numbers indicate weeks postinfection, Week 0 is the pre-infection sample.

IgA reactions

The IgA reactivity for all three pigs was not remarkable, and except for Pig 3, what little IgA reaction there was seemed to be limited to anti-GP97. Pig 3 produced an additional low level of anti-GP50.

DISCUSSION

Our EITB assay for human cysticercosis is based on the recognition of any one of seven LL-GPs by infection associated antibodies (Tsang et al., 1989). Our previous data show that most parasitologically confirmed cysticercosis patients have antibodies against at least one of these glycoproteins, but no single antigen is recognized by all patients. We concluded that reaction to any one of the seven LL-GP bands constitutes a positive indication for cysticercosis, and that our assay is 100% specific and 98% sensitive. The EITB assay for human cysticercosis vastly improves diagnosis of the disease. If this assay could be proven successfully in the pig system, we would have an additional useful tool for monitoring the disease. In cysticercosis endemic areas, the domestic pig is considered an important reservoir for this parasite. A sensitive and specific assay for cysticercosis in pig populations will inevitably affect the epidemiology of and control efforts for this disease.

In evaluating the test performance of the EITB assay in pigs, we have purposefully adopted stringent criteria in the selection of serum samples. Only animals with parasitologically confirmed cysticercosis were included. We are also mindful of the fact that necropsy with visual examination is a crude means of establishing infection. While the presence of a single cyst is proof positive for cysticercosis, the absence of cyst even after an exhaustive search of a carcass, may not necessarily mean the animal is uninfected. For this reason, we have included only necropsy positive pigs from endemic areas. Uninfected control animals were all of U.S. origin, and raised on concrete or under SPF conditions. We detected antibodies specific for *T. solium* as early as 1 week postinfection even in lightly infected animals. Although our data are for only four pigs, the similarity in responses for all of them indicates that this assay may well be useful for monitoring cysticercosis infection in pigs.

For our initial EITB assay for human cysticercosis, GP97 was not considered a diagnostic antigen (Tsang et al., 1989). Although we observed anti-GP97 activity in serum specimens of several individuals, it was not a common occurrence. Retrospectively and in view of the transient nature of this antibody activity, at least in the pig, this is to be expected. IgM antibodies are known to be associated with early phases of infection. The most studied example of this phenomenon is probably that which occurs with toxoplasmosis (Naot et al., 1982; Sharma et al., 1983; Partanen et al., 1984; Potasman et al., 1986; Brooks et al., 1987; Decoster et al., 1988). In patients with toxoplasmosis, IgM, IgG and IgA isotypes have both unique and shared target anti-

gens, with shared specificity for target antigens being most common (Naot et al., 1982; Sharma et al., 1983; Partanen et al., 1984; Potasman et al., 1986; Brooks et al., 1987; Decoster et al., 1988). With toxoplasmosis, there are also indications that IgG activity can severely block that of IgM. Furthermore, the early rise in IgM activity generally persists for more than 6 months after initial infection. One study (Sharma et al., 1983) showed that in 20% of cases there was demonstrable, specific IgM activity more than 12 months after the onset of lymphadenopathy.

The specific IgM activity for *T. solium* antigens in pigs appears to be much more transient. With the exception of Pig 2 (Fig. 2B), IgM activity became undetectable after 9 weeks. In Pig 2 IgM activity persisted until Week 11 before disappearing. Another unique feature of the *T. solium* system is that IgM activity appears to be directed almost entirely against GP97. One basic assumption of antibody blockade is that both IgG and IgM antibodies must share specificity. In Pigs 1 and 3 (Fig. 2A,B), GP97 was recognized by IgG and IgM simultaneously during the early stages of infection. The EITB strips in all of our experiments are quantitatively and qualitatively identical in terms of antigen load. They were exposed to the same amounts and concentrations of whole serum. Isotypic differentials were provided solely by the specific enzyme-conjugated probes (second antibodies). The fact that both IgG and IgM were detected simultaneously indicates that, at least by EITB, neither isotype was present in sufficient concentration or high enough affinity to overwhelm the other. This same logic applies to the specific antibody activities directed against GP50, GP42, and the lower molecular weight LL-GPs. However, with these LL-GPs, IgG rather than IgM dominates and appears to persist throughout the infection.

For cysticercosis in pigs, transient IgM anti-GP97 activity appears to be associated with early infection. Persisting IgG activity anti-GP50, anti-GP42, and anti-lower-molecular-weight LL-GPs is associated with late stages of infection. The transient antibody activity against GP97 invites speculation about its biological function in *T. solium* larval infections. Would anti-GP97 activity, if made to persist, be detrimental to the survival of this parasite? Does IgM anti-GP97 activity in human serum samples indicate early infections? These and other questions can only be answered with experiments based on much larger populations. The performance of the EITB with our test population suggests that this test may well prove useful for screening pigs in epidemiological settings.

REFERENCES

Brooks, R.G., McCabe, R.E. and Remington, J.S., 1987. Role of serology in the diagnosis of toxoplasmic lymphadenopathy. Rev. Infect. Dis., 9: 775–782.

Decoster, A., Darcy, F., Caron, A. and Capron, A., 1988. IgA antibodies against P30 as markers of congenital and acute toxoplasmosis. Lancet, II: 1104–1106.

Naot, Y., Guptill, D.R. and Remington, J.S., 1982. Duration of IgM antibodies to *Toxoplasma gondii* after acute acquired toxoplasmosis. J. Infect. Dis., 145: 770–776.

Nash, T.E. and Neva, R.A., 1984. Current concepts: Recent advances in the diagnosis and treatment of cerebral cysticercosis. N. Engl. J. Med., 311: 1492–1496.

Partanen, P., Turunen, H.J., Paasivuo, R.T.A. and Leinikki, P.O., 1984. Immunoblot analysis of *T. gondii* antigens by human immunoglobulins G, M, and A antibodies at different stages of infection. J. Clin. Microbiol., 20: 133–135.

Potasman, I., Araujo, G.D. and Remington, J.S., 1986. Analysis of *Toxoplasma gondii* antigens recognized by human sera obtained before and after acute infection. J. Infect. Dis., 154: 650–657.

Rhoads, M.L., Kamanga-Sollo, E.I.P., Rapic, D., Murrell, D.D., Schantz, P.M. and Beus, A., 1987. Detection of antibody in humans and pigs to *T. solium* metacestodes using an antigenic fraction from *T. hydatigena* metacestodes. Vet. Arch., 57: 142–150.

Richards, Jr., F.O., Schantz, P.M., Ruiz-Tiben, E. and Sorvillo, F.J., 1985. Cysticercosis in Los Angeles County. J. Am. Med. Assoc., 254: 3444–3448.

Sharma, S.D., Mullenax, J., Araujo, F.G., Erlich, H.A. and Remington, J.S., 1983. Western blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol., 131: 977–983.

Sotelo, J., Torres, B., Rubio-Connadieu, F., Escobedo, F. and Rodriguez-Carbajal, J., 1985. Praziquantel is the treatment of neurocysticercosis: Long-term follow-up. Neurology, 35: 752–755.

Tsang, V.C.W., Peralta, J.M. and Simons, A.R., 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymol., 92: 377–391.

Tsang, V.C.W., Hancock, K. and Maddison, S.E., 1984. Quantitative capacities of glutaraldehyde and sodium m-periodate coupled peroxidase-anti-human IgG conjugates in enzyme-linked immunoassays. J. Immunol. Methods, 70: 91–100.

Tsang, V.C.W, Hancock, K., Wilson, M., Palmer, D.F., Whaley, S.D., McDougal, J.S. and Kennedy, S., 1986. Enzyme-linked immunoelectrotransfer blot technique (Western blot) for human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) antibodies. Monograph: Immunology Series No. 15, Procedural Guide. USDHHS, PHS, CDC, Atlanta, GA, pp. 1–25.

Tsang, V.C.W., Brand, J.A. and Boyer, A.E., 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigen for diagnosing human cysticercosis (*Taenia solium*). J. Infect. Dis., 159: 50–59.

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Epidemiology of Taeniasis and Cysticercosis in a Peruvian Village

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To determine the prevalence of cysticercosis in a rural area where the disease is endemic, the authors studied the seroepidemiology of human and porcine cysticercosis in a Peruvian jungle community (Maceda, Peru) in 1988 using an enzyme-linked immunoelectrotransfer blot (EITB) assay. Of the 371 sampled inhabitants, 30 (8%) were seropositive, most of whom were asymptomatic. After niclosamide therapy, four *Taenia* species worms were identified in the seropositive group, compared with one in the control group ($p = 0.06$). Pigs were frequently infected: 44 of 133 (33%) were found positive for *Taenia* by tongue examination and 57 of 133 (43%) were positive by EITB. In 69% of the sampled households that had pigs, there was at least one seropositive pig. The number of pigs diagnosed positive by the tongue examination was significantly greater in households that had latrines than in those that did not. Cysticercosis is a common but usually asymptomatic infection that affects both humans and pigs in the high jungle areas of Peru. *Am J Epidemiol* 1992;135:875–82.

cysticercosis; environmental monitoring; immunoblotting; swine; *Taenia*; toilet facilities; transfer blot

Cysticercosis is a disease endemic in most developing countries where pigs are raised (1). In both Mexico and Peru, more than 10 percent of neurologic patients receiving acute care have serologic evidence of *Taenia solium* infection (2, 3). Humans acquire cysticercosis by ingesting *T. solium* eggs from human feces either directly or indirectly, through contaminated food or water. The eggs mature into larvae, which invade the intestinal wall, muscle, brain, and other tissues. Pigs also acquire cysticercosis in the same manner. Humans develop taeniasis after ingesting raw or poorly cooked pork or beef that contains the larval form of *T. solium* or *Taenia saginata*. Larvae invaginate

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Abbreviations: EITB, enzyme-linked immunoelectrotransfer blot.

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the small intestine of humans and develop into adult tapeworms. The tapeworm produces eggs after 3 months and may survive for years, thus spreading cysticercosis in both humans and pigs (4, pages 609–25).

Rational programs to control cysticercosis cannot be developed until the epidemiology of the disease is better understood. The true incidence of cysticercosis in both human and pig populations is unknown. The prevalence of porcine infection, based on meat inspections in Mexican slaughterhouses, has been estimated at 0.005–10 percent (5). However, many infected pigs are raised by small farmers, who often sell their pigs in informal markets where meat inspection is not performed. Since governmental and international agencies base their statistics for porcine cysticercosis on slaughterhouse statistics (6, 7), clandestine sale of meat results in a considerable underestimation of the true prevalence rates of porcine cysticercosis. For example, in Huancayo, a city of 500,000 inhabitants in the highlands of Peru, no pigs were registered as infected with cysticercosis (8), yet field surveys revealed that more than 30 percent of the pigs were infected with *T. solium* (9).

A reliable serologic test to determine the true prevalence of infection in both humans and pigs was lacking until the enzyme-linked immunoelectrotransfer blot assay (EITB) was developed for the detection of antibodies to *T. solium* in sera (10, 11, 12). We used this highly sensitive (98 percent) and specific (100 percent) assay (11) to study the epidemiology of human and porcine cysticercosis in a community in the high jungle of Peru where the disease is endemic. We also compared seropositive inhabitants with an age- and sex-matched group of seronegative subjects to determine whether seropositivity was associated with an increased risk of neurologic symptoms.

MATERIALS AND METHODS

Study site

Case reports from the hospital and slaughterhouse of the city of Tarapoto in the division of San Martin in the Peruvian jungle have shown that cysticercosis is endemic in this area (13). The nearby (20 km) rural village of Maceda (population, 421) was selected for this study because of its accessibility by an all-weather road and its convenient size. Maceda is typical of villages populated by descendents of the Chanca tribes, who until recently spoke only Quechua. Most of the Chanca villages are located along tributaries of the Huallaga river, such as the Mayo river where Maceda is located. Maceda, based on its size, agricultural economy, and riverside location, is a representative village of the high jungle area. Temperatures in the zone vary from 23 to 27°C, and the rainy season extends from November through May. Maceda contains simple adobe houses built on hillsides along the river. Pigs and other animals wander freely in the houses, which all have dirt floors. There are three natural geographic strata in the village: near the river basin, on the slope of the hills, and at the top of the hillside near the road.

Like other villages in the zone, Maceda does not have electricity or piped water. Water is obtained from the river. A number of the residences have borehole latrines in the backyard; other households use the fields for defecation. Pigs eat human feces deposited in the fields and at the sides of the latrines. Pigs are rarely corralled, and the majority are permitted to feed freely throughout the village. Most pigs are raised for sale in Tarapoto, but some are consumed by villagers on festival days.

Study design

The study was performed in two stages. First, we conducted an epidemiologic survey to determine prevalence rates and clustering in humans and pigs. A study was then conducted to compare the prevalence of neurologic disease in seropositive versus seronegative individuals. Studies were approved by the Committee on Human Volunteers and the Animal Experimentation Committee of the Universidad Peruana Cayetano Heredia and the Johns Hopkins University, and by the village council of Maceda. Informed consent was obtained from all participants.

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Epidemiologic survey

Village meetings were held to explain the purpose of the study. After constructing a map of the village, we visited each household, counted the number of humans and pigs, and noted the presence of latrines. Two teams performed the survey. The first team collected stool and serum samples from villagers. A second team of veterinarians captured pigs and examined their tongues for cysts, and obtained blood from their jugular veins for the EITB test. Pigs under 4 months of age were excluded from the study, in order to exclude the presence of maternal antibody and because of difficulty in [illegible]

Examination of stool specimens

Stool specimens were collected, and a sample was examined for the presence of parasites by microscopy, both directly and after formalin ether concentration and sedimentation (4, pages 1124–79). Stool examination detects less than 20 percent of infected individuals (14).

Soil samples

A core of soil was taken from five areas considered most likely to be contaminated with *T. solium* eggs. These included the sides of dirty latrines or boreholes as well as open household areas used for open-air defecation. Soil was transported to the laboratory and processed as follows: Soil samples were diluted and strained through gauze; after 20 minutes, the sediment in the filtrate was examined by microscope for the presence of eggs. When used to examine stool specimens, this method is tenfold more sensitive than direct microscopic examination (15).

Water studies

Five samples of river water obtained at different points were pumped through a 0.10-μm nylon filter. The quantity of water sampled ranged from 50 to 100 gallons, depending on the amount of sediment present in the water. Filters were placed in a small amount of 10 percent formalin and backwashed with pressurized nitrogen, and the sediment was examined for the presence of *Taenia* species eggs. This method has been used primarily for the recovery of oocysts of cryptosporidium (16).

EITB analysis

Antibodies to *T. solium* species in blood samples from humans and pigs were examined using the method developed by Tsang et al. (11, 12). Briefly, the assay uses purified glycoprotein antigens (LL-GP) in an immunoblot format to detect infection-specific antibodies. Peroxidase-labeled goat anti-[illegible] antibody (KPL, Gaithersburg, MD) [illegible] at a molar ratio of 1:2 (immuno-[illegible] (peroxidase) diluted at 1/100. Bound antibodies were visualized using a hydrogen [illegible] 3,3-diaminobenzidine hydrochloride substrate system. The sensitivity of this assay varied from 94 to 98 percent, and the specificity was 100 percent (11, 12).

Diagnostic criteria

There are seven glycoprotein bands commonly recognized by antibodies from serum of human cases of cysticercosis (9). These bands are GP50, GP42–39, GP24, GP21, GP18, GP14, and GP13. Quality control of the EITB assay was performed in collaboration with the laboratory at the Centers for Disease Control, Atlanta, Georgia.

Cross-sectional comparison of the prevalence of neurologic disease

The second study was initiated 2 months after the initial survey. Using the census data, each individual whose EITB test was positive ($n = 30$) was matched by age and sex with a seronegative individual. A neurologist who was unaware of the participants' serologic status examined each pair and took detailed clinical histories, including the history of seizures, headaches, and subcutaneous nodules. In addition, all individuals were given niclosamide (2 g, by mouth) followed by a magnesium sulfate purge. Stools were collected and examined for the presence of *Taenia* species eggs. Villagers found to be infected with other helminths were treated whenever possible.

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Data analysis

Either the chi-square or the McNemar test was used to test categorical variables. Clustering was measured using Smith and Pike's test (17). This test was performed to determine whether there was an increased rate of seropositivity among individuals living in the same house, in households located within 15 meters of each other, and in different topographic locations.

RESULTS

EITB results in humans

At the time of our survey, Maceda had 421 inhabitants living in 71 separate households (mean ± standard deviation (SD), 5.75 ± 2). Serum was obtained from 371 villagers (88 percent) (table 1) and stool specimens were obtained from 305 (72 percent). Children aged less than 1 year were significantly ($p < 0.05$) underrepresented in our sampling. Many parents would not allow us to take blood from their small children. Thirty inhabitants (8 percent)—18 females and 12 males—were seropositive. None of the 62 children younger than 6 years of age were seropositive. Children 6–15 years of age had significantly lower rates of seropositivity in comparison with older individuals (table 1).

In two houses, more than one inhabitant was seropositive. The rate of human seropositivity was not increased by the presence of another infected household member, proximity to a household with an infected individual, or the topographic location of the house. The presence of a latrine and the size of the family also were not significantly associated with a positive EITB result. Families owning infected pigs did not have a higher risk of seropositivity than families who either did not own pigs or owned noninfected pigs.

Only one of the stool samples (0.3 percent) from the 305 individuals who were examined before being treated with niclosamide contained *Taenia* species eggs. Serum from this inhabitant was EITB positive.

Results from tongue and EITB tests in pigs

Nearly 90 percent (64/73) of the Maceda households owned pigs (mean number of pigs per household, 3.7 ± 3.7 (SD); range, 1–11 per household). More than 80 percent of the pigs aged 4 months or more (133/151) were sampled; 43 percent (57/133) were seropositive for *Taenia* by the EITB, and 33 percent (44/133) were found positive by the tongue examination (table 2). We examined pigs at 52 of the 64 households (81 percent) that had pigs. Of these 52 households, 36 (69 percent) had at least one seropositive pig, and 28 (54 percent) had a pig positive by the tongue examination. Of the houses with latrines, 76 percent had at least one pig positive by the tongue examination,

TABLE 1. Age-stratified rates of seropositivity for antibodies to *Taenia solium* detected by the enzyme-linked immunoelectrotransfer blot (EITB): Maceda, Peru, 1988

Age (years)	Total no. of subjects	Sampled subjects		Seropositive subjects	
		No.	%	No.	%
<1	18	10	55	0	0
1–5	72	62	86	0	0
6–10*	67	64	96	4	6
11–15*	58	56	97	3	5
16–30	79	66	84	7	11
31–40	45	39	87	5	13
41–50	29	26	90	2	8
>50	53	48	91	9	19
Total	421	371	88	30	8

* There were significantly fewer seropositive results among children aged 6–15 years than among older villagers (7/120 vs. 23/179; $p < 0.05$ by the chi-square test).

TABLE 2. Comparison of results of enzyme-linked immunoelectrotransfer blot (EITB) tongue examination for *Taenia solium* infection in pigs*: Maceda, Peru, 1988

Results of tests for *T. solium* (EITB/tongue examination)†	No. of pigs	%
+/+	37	28
−/+	7	5
+/−	20	15
−/−	69	52
Total	133	100

* McNemar's test shows a significant ($p < 0.01$) difference between the results of the EITB and the tongue examination.
† +, positive for *T. solium*; −, negative for *T. solium*.

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TABLE 3. Association between household latrine status and detection of *Taenia solium* in pigs by two methods*: Maceda, Peru, 1988

Household latrine status	No. of households	No. of households with pigs	Households with pigs positive for *T. solium*			
			By EITB		By tongue examination	
			No.	%	No.	%
With latrine	31	25	20	80	19†	76
Without latrine	42	27	16	59	9	33
Total	73	52	36	69	28	54

* Enzyme-linked immunoelectrotransfer blot (EITB) and tongue examination, both performed by veterinarians.
† Significantly associated with latrines, $p < 0.05$ by the chi-square test.

as compared with 33 percent of the houses without latrines. This difference was statistically significant ($p < 0.05$) (table 3). However, there was no difference in the total number of pigs found positive for *Taenia* by each method in households with latrines and those without latrines: Of the 76 pigs tested from households with latrines, 27 were found positive by tongue examination and 29 were found positive by the EITB; of the 56 pigs tested from households without latrines, 16 were found positive by tongue examination and 27 were found positive by the EITB. (Note: The household status of one of the 133 pigs examined could not be identified.)

Environmental samples

No *Taenia* eggs were found in either the five water or the five soil samples.

Cross-sectional comparison of the prevalence of neurologic disease

We compared signs and symptoms of neurologic disease in 28 of the seropositive persons and 29 of the seronegative controls. Two of the seropositive subjects and one of the controls were not available for the neurologic examination. No difference was found between the two groups (table 4). Headaches were common in both groups, and except for one seronegative patient with seizures, neurologic symptoms were limited to signs of peripheral neuropathy (table 4).

As part of the study of the prevalence of neurologic disease, 21 stool samples from the 30 subjects seropositive for *Taenia* by the EITB and from 22 of the 29 seronegative controls were examined for *Taenia* species eggs after niclosamide treatment. *Taenia* species eggs were observed in stools from 4 of 21 infected persons (19 percent) and from 1 of the 22 controls (5 percent) ($p = 0.06$). We were unable to distinguish the eggs of *T. solium* from those of *T. saginata*. *T. saginata* infection occurs in Peru, but the finding for cysticercosis by the EITB in individuals infected with this cestode is negative (12). The eggs observed in the stool of the single control person are probably from a beef tape worm.

TABLE 4. Comparison of neurologic symptoms in villagers seropositive for antibodies to *Taenia solium* by the enzyme-linked immunoelectrotransfer blot and seronegative villagers matched for age and sex: Maceda, Peru, 1988

Symptoms	Seropositive villagers ($n = 28$)		Seronegative villagers ($n = 29$)		p value
	No.	%	No.	%	
Headache	16	57	12	41	>0.05
Seizures	0	0*	1	3	>0.05
Hyporeflexia or areflexia	2	7	0	0	>0.05
Hypesthesia	1	4	1	3	>0.05
Paresthesia	2	7	1	3	>0.05

* One patient in the seropositive group had a history of episodes of loss of consciousness.

DISCUSSION

The EITB test utilizes glycoprotein antigens purified by lectin affinity chromatography. This test is highly sensitive (98 per-

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cent) and specific (100 percent), with a positive predictive value greater than 90 percent (11, 12). It does not cross-react with other parasites, including other cestodes such as *T. saginata*, *Hymenolepis nana* and *Echinococcus granulosus* (11, 12).

This study utilized the EITB test to detect antibodies to *T. solium* in persons and pigs in communities where *T. solium* is endemic. Infection was common; 8 percent of the inhabitants and 43 percent of the pigs were seropositive. Cysts were detected on the tongues of 33 percent of the pigs, confirming the high prevalence rates found serologically.

The seroprevalence rates for infection with *Taenia* species in Maceda are among the highest in the world. They are surpassed only by those in New Guinea, where seroprevalence in villages endemic for this disease is about 20 percent (18). In Mexico, serosurveys using the enzyme-linked immunosorbent assay (ELISA), a less sensitive method (12), have found that 5 percent of the population had positive results (19).

In our studies, clustering of infected humans or pigs was not evident, but in similar studies in Mexico, infected individuals tended to be clustered by household (19). The considerable difference in patterns of village transmission and presentation are probably due to differences in cultural behavior. The discrepancy between low rates of tapeworm infection and high rates of cyst infections in humans has also been noted in India (20), New Guinea (18), and Mexico (19).

The high prevalence of pigs that were seropositive and/or positive by tongue examination strongly suggests that these animals often eat human feces containing *T. solium* eggs. Infection can be severe; some pigs have as many as 2,500 cysts per kilogram of meat (21). Large numbers of eggs must be ingested for pigs to acquire this degree of infection, since at least one egg must be ingested to get one cyst (4, pages 609–25). In areas where pigs are so heavily infected, environmental contamination must be intense, yet environmental surveys rarely demonstrate *Taenia* species eggs (19).

In pigs, agreement between the results of the EITB and of tongue examination has previously been shown to be good (9). The sensitivity of tongue examination is generally lower than that of the EITB. However, in the present study, seven pigs found positive for *Taenia* infection by the tongue examination were negative by the EITB. In pigs, the discrepancy between the findings of the tongue examination and the EITB may be the result of a lack of antibody production in lightly infected animals. Another possible explanation is that the pigs in this study were allowed to range and graze freely about the surrounding thickets, where thorns abound in the vegetation. Some of the palpated "cysts" may actually have been scar tissue from old injuries acquired during feeding.

In this study in a tropical hillside village, the only identifiable risk factor associated with *T. solium* infection in humans was age; in pigs, the presence of a latrine was a risk factor. Antibodies in pigs appear to develop within several months of infection (9, 22). In our study, seropositivity in humans did not begin until after school age. Reasons for the delay in seropositivity may include social factors and dose- and exposure-related variables. Perhaps infection occurs primarily outside the household, or young children are less susceptible to *T. solium* infection.

Our finding that latrines were associated with increased positivity on the tongue examination in pigs suggests that a poorly designed latrine may be more of a hazard than a help in controlling cysticercosis. The lack of an association between latrines and seropositivity in both humans and pigs, however, indicates generalized exposure to *T. solium*.

None of the seropositive villagers had symptoms that were clearly due to cysticercosis or that required therapy or hospitalization. No difference in the rate of headache was noted between infected persons and controls. Signs and symptoms of peripheral neuropathy (hypesthesia, hyporeflexia and/or paresthesia) were present in five subjects. Tropical peripheral neuropathy is usually secondary to toxins such as organophosphorus compounds (23) rather than to space

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occupying lesions such as cysticercosis (3).

The high rate of asymptomatic infection is consistent with previous reports of prevalence rates of 0.2 percent found in autopsies of presumably asymptomatic individuals killed by trauma (24). The percentage of asymptomatic seropositive individuals who will eventually develop symptomatic disease is unknown. Some British soldiers stationed in India first developed symptoms 30 years after returning home (25).

Stool examination was not an efficient method for diagnosing *T. solium* infection. Only one of six individuals with taeniasis was detected when a routine single stool examination was performed. This poor result is consistent with that of another study, in which routine microscopy examination of stool detected less than 20 percent of infected individuals (14). While the EITB test is a useful marker for exposure to *T. solium* antigen, it cannot distinguish between the tapeworm and cyst stages of *T. solium*.

Serologic studies in humans and pigs are useful for 1) determining areas where the disease is endemic, 2) defining and targeting high-risk families by *T. solium* antigen contact, and 3) monitoring the success of control programs by determining the incidence of new cysticercosis infections. Needed however are assays for the detection of carriers of tapeworm and detailed local studies concerning the consumption and sale of pork in zones where the disease is endemic. In addition, studies examining the effect of specific interventions on infections in pigs and humans are required. Such studies will assist in the construction of a rational control program for the prevention of *T. solium* infection.

We have shown the EITB to be a useful tool for defining the epidemiology of cysticercosis in both humans and pigs. In areas where *T. solium* infection is endemic, it is present in 5–10 percent of individuals, most of whom are usually asymptomatic. *T. solium* infection was also seen in 10 percent of Peruvian neurologic patients (3). What is not known is the proportion of infected individuals who will eventually develop symptoms. Even when this proportion is lower than 1 percent, the number of cases of neurologic disease may be high because of the high prevalence of *T. solium* infection in the population. Cysticercosis is a significant public health problem that will require intervention programs that are culturally acceptable and within the economic reach of third world countries.

REFERENCES

1. Mahajan RC. Geographical distribution of human cysticercosis. In: Flisser A, Willms K, Laclette JP, et al., eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, 1982:39–46.
2. Flisser A. Neurocysticercosis in Mexico. Parasitol Today 1988;4:131–7.
3. Garcia HH, Martinez M, Gilman RH, et al. Diagnosis of cysticercosis in endemic zones. Lancet 1991;338:549–51.
4. Belding DL. Textbook of parasitology. 3rd ed. New York: Appleton Century Crofts, 1965:609–25.
5. Aluja A. Frequency of porcine cysticercosis in Mexico. In: Flisser A, Willms K, Laclette JP, et al., eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, 1982: 55–62.
6. Benson AJ. El control de las enfermedades Transmisibles. (Spanish). Publicacion cientifica. Washington, DC: Pan American Health Organization, 1983:442.
7. Matyas Z, Pawlowski Z, Soulsby EJL, et al. Guidelines for surveillance, prevention and control of cysticercosis/taeniasis. Geneva, Switzerland: World Health Organization, 1983:71–2. (WHO/VPH 83.49).
8. Ministerio de Agricultura. Unidad de Servicio Pecuario. (Spanish). Lima, Peru: Ministerio de Agricultura, 1986.
9. Gonzalez AE, Cama V, Gilman RH, et al. Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. Am J Trop Med Hyg 1990;43:194–9.
10. Tsang VCW, Peralta JM, Simons AR. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymol 1983;92:377–91.
11. Tsang VCW, Brand J, Boyer AE. Enzyme-linked immunoelectrotransferency blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J Infect Dis 1989;159:50–9.
12. Diaz F, Garcia H, Gilman RH, et al. Immunodiagnosis of human cysticercosis (*Taenia solium*): a comparison of ELISA and EITB assays. Am J Trop Med Hyg. (In press).
13. Llontop R, Nolte C, Urbina R. Cisticercosis en cerdos beneficiados en el camal frigorifico No 37

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de Tarapoto (Spanish). Tarapoto, Peru: Ministerio de Agricultura y Alimentacion RA XI, Laboratorio de Sanidad Animal y Zoonosis, 1980.
14. Pawlowski ZS, Schultz MG. Taeniasis and cysticercosis (*Taenia saginata*). Adv Parasitol 1972;10:270–343.
15. Lumbreras H, Cantella R, Burga R. Acerca de un procedimiento de sedimentacion rapida para investigar huevos de *Fasciola hepatica* en heces. Su evaluacion y uso en el campo. (Spanish). Revista Medica Peruana 1962;31:167–74.
16. Musial CE, Arrowood MJ, Sterling CR, et al. Detection of *Cryptosporidium* in water by using polypropylene cartridge filters. Appl Environ Microbiol 1987;53:687–92.
17. Smith PG, Pike MC. Generalizations of two tests for detection of household aggregation of disease. Biometrics 1976;32:817–28.
18. Muller R, Lillywhite J, Bending J, et al. Human cysticercosis and intestinal parasitism amongst the Ekari people of Irian Jaya. J Trop Med Hyg 1987;90:291–6.
19. Sarti-Gutierrez EJ. *Taenia solium* taeniasis and cysticercosis in a Mexican village. Trop Med Parasitol 1988;39:194–8.
20. Tandon PN. Cerebral cysticercosis. Neurosurg Rev 1983;6:119–27.
21. Chavera A, Gonzales AE, Cama V, et al. Cisticercosis porcina: Aspectos clinicos y patologicos en la Sierra Central del Peru. (Spanish). Anales XII Congreso Panamericano de Ciencias Veterinarias, May 1988:27.
22. Tsang VC, Brand JA, Zhou W, et al. Efficacy of the immunoblot assay for cysticercosis in pigs and modulated expression of distinct IgM/IgG activities to *Taenia solium* antigens in experimental infections. Vet Immunol Immunopathol 1991;29:69–78.
23. Senanayake N, Roman GC. Toxic neuropathies in the tropics. J Trop Geogr Neurol 1991;1:3–15.
24. Escalante S. Neurocisticercosis. (Spanish). Unpublished doctoral dissertation. Universidad Peruana Cayetano Heredia, Lima, Peru, 1973:16.
25. Dixon HBF, Hargreaves WH. Cysticercosis (*Taenia solium*): A further ten years' clinical study, covering 284 cases. Q J Med 1944;13:107–21.

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IMMUNODIAGNOSIS OF HUMAN CYSTICERCOSIS (*TAENIA SOLIUM*): A FIELD COMPARISON OF AN ANTIBODY-ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), AN ANTIGEN-ELISA, AND AN ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (EITB) ASSAY IN PERU

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Abstract. We compared results of an enzyme-linked immunosorbent assay (ELISA) and an enzyme-linked immmunoelectrotransfer blot (EITB) assay for the diagnosis of cysticercosis in sera and cerebrospinal fluid (CSF). Sera from 34 patients with confirmed cysticercosis were tested by both ELISA and EITB assays. Cerebrospinal fluid from some of these patients was also tested by ELISA for the presence of antibody (AB-ELISA) (n = 21) and antigen (AG-ELISA) (n = 15). Specificity in sera was examined by testing 51 serum samples from Bangladesh, where cysticercosis is not endemic. Cross-reactivity was evaluated in sera from patients with *Echinococcus granulosus* (hydatid) and *Hymenolepis nana* infections. Sensitivity in detecting cysticercosis in sera was 94% by EITB and 65% by AB-ELISA ($P < 0.01$). Sensitivities in the CSF tested by EITB, AB-ELISA, and AG-ELISA were 86%, 62%, and 67%, respectively. The specificity of the EITB was 100%, while that of AB-ELISA was 63% ($P < 0.01$). Cross-reactions occurred in the AB-ELISA with 11% and 20% of sera from hydatid and *H. nana* patients, respectively. Our results demonstrate that the EITB is the best assay available for the diagnosis of cysticercosis in both sera and CSF.

Cysticercosis, the disease produced by the larval stage of *Taenia solium*, is present worldwide. High rates of infection are found in India, Indonesia, Africa, Mexico, Peru, and other South American countries.[1–3] In both Peru and Mexico, cysticercosis accounts for over 10% of neurologic hospital admissions.[3]

Cysticercosis is frequently misdiagnosed because of its highly variable presentation. Computed tomography scan and magnetic resonance imaging can be helpful in diagnosing neurocysticercosis, but their use in developing countries is limited because of their high cost and limited availability.[4]

Three new serologic techniques have been reported to be useful in the diagnosis of cysticercosis: an enzyme-linked immunosorbent assay (ELISA) using a crude antigen for the detection of antibodies to *T. solium* (AB-ELISA) in serum or cerebrospinal fluid (CSF),[5] an antigen-detection ELISA limited to the CSF (AG-ELISA),[6] and an enzyme-linked immunoelectrotransfer blot (EITB) assay used to detect antibodies in the serum or CSF.[7]

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We conducted a prospective study to provide a realistic field trial for these three assays and to compare their diagnostic sensitivity and specificity. Sera and CSF samples were obtained from both tissue-confirmed cases of neurocysticercosis and from individuals who were infected with *T. solium* or *T. saginata* tapeworms. We also determined the rates of cross-reactivity with sera from patients with *Echinococcus granulosus* and *Hymenolepis nana* infection.

MATERIALS AND METHODS

Human sera and CSF

Serum and CSF specimens were obtained from tissue-confirmed cases of neurocysticercosis. All specimens were coded and tested in a blinded fashion. Duplicate specimens from a portion of the patients were sent to the Parasitic Diseases Branch (PDB) of the Centers for Disease Control (CDC) for quality control testing.

Clinical classification

The following definitions were used for clinical classification of the study participants: 1 = confirmed cysticercosis, patients in whom cysticercosis was confirmed by means of a biopsy, surgery, or presence of ocular cysticercus (n = 34); 2 = negative controls, persons from countries where cysticercosis is not normally transmitted (n = 11 from the United States and 51 from Bangladesh); and 3 = taeniasis patients, persons who eliminated a tapeworm or proglottides identified as *T. saginata* (n = 4), *T. solium* (n = 14), or *Diphyllobothrium* species (n = 2).

Antibody detection by ELISA (AB-ELISA)

Whole *T. solium* cysticerci were excised from the skeletal muscle of naturally infected pigs. Ten grams of the larvae were washed in 30 ml of phosphate-buffered saline (PBS) (0.137 M NaCl, 1.47 mM KH_2PO_4, 10.9 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4) containing 0.25 M phenylmethylsulfonyl fluoride as a protease inhibitor. The larvae were homogenized in this solution at 4°C in a glass tissue homogenizer. The homogenate was sonicated three times (3 min/pulse at 20 kHz) on ice with 60-sec cooling intervals. The sonicate was centrifuged at 25,000 *g* in a SA-600 rotor (Dupont, Wilmington, DE) for 30 min at 4°C, and the supernatant was used directly as the crude antigen preparation.

The antigen obtained was diluted in carbonate buffer (pH 9.6) to a concentration of 1 μg/ml. The wells of microtiter plates (Immulon 1; Dynatech, Alexandria, VA) were coated with antigen by overnight incubation at 4°C. The plates were blocked with 5% nonfat milk in PBS with 1% Tween-20, and incubated at 37°C for 1 hr. Serum was diluted 1:2,500 with PBS containing 0.05% Tween 20 (PBS-Tween). After the samples were washed five times with PBS-Tween, serum was added to all wells, and the plates were incubated at room temperature for 2 hr. The plates were then washed five times, 100 μl of a 1:2,000 dilution of peroxidase-conjugated goat anti-human antibody (Kirkergaard and Perry, Gaithersburg, MD) was added to each well, and the plates were incubated for 1 hr at room temperature. The plates were then washed five times, and 100 μl of *o*-phenylenediamine peroxidase substrate (Sigma, St. Louis, MO) was added to each well. After a 15-min incubation, 100 μl of 0.1 M sulfuric acid was added to each well to stop the reaction. Optical density (OD) values at 492 nm were determined with a Multiscan (Flow Laboratories, McLean, VA) ELISA reader. Cutoff values were determined by calculating the mean and the standard deviation of the OD of four determinations from the negative control serum pool. A serum sample was considered positive when its OD was greater than the control mean plus two standard deviations. The same control pool was used in all determinations.[8]

Antigen detection by ELISA (AG-ELISA)

Cerebrospinal fluid was diluted 1:160 in bicarbonate buffer, and 100 μl was placed in duplicate wells of microtiter plates (Immulon 1). The plates were incubated overnight at 4°C and washed five times with PBS-Tween. Rabbit antisera to cysticercosis (100 μl) at a 1:5,000 dilution in PBS-Tween was incubated in the wells for 1 hr at 37°C. The plates were then washed five times with PBS-Tween and 100 μl of peroxidase-conjugated goat anti-rabbit IgG (1:1,000 dilution) was added. The plates were incubated for 1 hr at 37°C, washed five times with PBS-Tween, and 100-μl volumes of freshly prepared substrate (0.4 mg of *o*-phenylenediamine/ml plus 0.06% H_2O_2 in citrate-phosphate buffer, pH 5) were added. The reaction was allowed to proceed for

TABLE 1

*Sensitivity of the enzyme-linked immunoelectrotransfer blot (EITB) and the enzyme-linked immunosorbent assay (ELISA) with serum and cerebrospinal fluid (CSF) of Peruvian patients with confirmed cysticercosis**

Patient description (clinical groups)	EITB No. positive/no. tested (%)		AB-ELISA No. positive/no. tested (%)		AG-ELISA† No. positive/no. tested (%)
	Serum	CSF	Serum	CSF	CSF
Subcutaneous cysts	12/14 (88)	7/8 (89)	12/15 (80)	6/8 (78)	2/5 (40)
Ocular cysts	3/3 (100)	1/1 (100)	2/3 (67)	0/1 (0)	0/1 (0)
Subcutaneous and ocular cysts	2/2 (100)	1/1 (100)	2/2 (100)	1/1 (100)	0/0 NA
Cysts at other locations	15/15 (100)	9/11 (82)	6/14 (43)	6/11 (55)	8/9 (89)
All confirmed cases	32/34 (94)‡	18/21 (86)§	22/34 (65)	13/21 (62)	10/15 (67)

* AB-ELISA = antibody ELISA; AG-ELISA = antigen ELISA; NA = not available.
† Only CSF can be used in the AG-ELISA.
‡ $P < 0.05$ versus AB-ELISA for serum, by chi-square test.
§ $P > 0.05$ versus AG-ELISA for CSF, by chi-square test.

5 min at room temperature, and was stopped by the addition of 25 μl of 1 M sulfuric acid. The OD was measured at 492 nm in a photometer. A sample was considered positive when its OD was greater than the mean of the negative control plus two standard deviations.

Antibody detection by immunoblot (EITB)

Glycoprotein antigens were prepared as previously described.[7] Cysts were dissected and washed as described above, drained, and blotted dry. They were suspended in 20 ml (two times the weight of the cysts) of 8 M urea in Tris HCl buffer (0.05 M Tris HCl, 0.1 M NaCl, pH 8.0), homogenized, and sonicated as described above. Fifty percent freon was added, and the material was centrifuged for 30 min at 15,000 rpm (SA-600 Rotor) at 4°C. The supernatant was collected, centrifuged at 250,000 *g* for 2 hr, desalted with Sephadex G-25, and passed through a lentil-lectin Sepharose 4B column at room temperature. Lentil-lectin–bound glycoproteins were eluted from the column with 0.2 M α-methyl-mannoside in Tris HCl buffer. After the protein concentration was determined, the glycoprotein antigens were stored in glycerol.

Lentil-lectin–purified glycoprotein antigens were used in an immunoblot format to detect infection-specific antibodies in serum and CSF, as previously described.[7] Peroxidase-labeled goat anti-human antibody was used at a 1:1,000 dilution. Bound antibodies were visualized using the H_2O_2/DAB substrate system.[7] Seven glycoprotein bands are commonly recognized by antibodies from serum of cysticercosis patients.[7] These glycoprotein bands have molecular masses of 50, 42–39, 24, 21, 18, 14, and 13 kD, respectively. Quality control analysis of the EITB assay was performed in collaboration with the PDB laboratory at CDC.

TABLE 2

Specificity of the enzyme-linked immunoelectrotransfer blot (EITB) and the antibody enzyme-linked immunosorbent assay (AB-ELISA) for antibody to Taenia solium *in serum samples of negative controls from Bangladesh and the United States*

Serum source (negative controls)	EITB No. negative/no. tested (%)	AB-ELISA* No. negative/no. tested (%)
Bangladesh	51/51 (100)	32/51 (63)
United States	11/11 (100)	10/11 (91)

* The AB-ELISA is significantly more specific ($P < 0.05$ by chi-square test) with serum samples from the United States than with those from Bangladesh.

Data analysis

The sensitivity and specificity of the ELISA and EITB with sera and CSF were calculated. Only confirmed cysticercosis cases and negative controls were used in the calculation of specificity and sensitivity. The chi-square test was used for

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TABLE 3

Sensitivity and specificity of the enzyme-linked immunoelectrotransfer blot (EITB) and the enzyme-linked immunosorbent assay (ELISA) for antibody to Taenia solium *in serum and cerebrospinal fluid (CSF) of patients**

Parasitologically confirmed disease†	EITB No. positive/no. tested (%)		AB-ELISA No. positive/no. tested (%)		AG-ELISA‡ No. positive/no. tested (%)
	Serum	CSF	Serum	CSF	CSF
T. solium with neurologic symptoms	4/4 (100)	3/3 (100)	4/4 (100)	2/3 (67)	1/3 (33)
T. solium without neurologic symptoms	10/10 (100)	1/1 (100)	4/6 (67)	0/1 (0)	0/1 (0)
Total *T. solium*	14/14 (100)	4/4 (100)	8/10 (80)	2/4 (50)	1/4 (25)
T. saginata	0/4 (0)	0/1 (0)	2/4 (50)	0/1 (0)	0/1 (0)
Echinococcus granulosus	0/18 (0)	—	2/18 (11)	—	—
Hymenolepes nana	1/59 (2)	—	12/59 (20)	—	—
Diphyllobothrium sp.	0/2 (0)	—	0/2 (0)	—	—

* AB-ELISA = antibody ELISA; AG-ELISA = antigen ELISA.
† All patients either passed worms or had tissue-confirmed disease.
‡ Only CSF can be used in the AG-ELISA.

comparison of data sets. *P* values less than 0.05 were considered significant.[9]

RESULTS

Sensitivity and specificity

The EITB was the most sensitive test with either sera or CSF specimens (Table 1). The sensitivity with CSF was similar for both the AB-ELISA and AG-ELISA tests. Surgery patients with hydrocephalus tended to have more false-negative results in the AB-ELISA assay than did patients with other clinical presentations (Table 1).

The EITB assay was also highly specific (Table 2). In contrast, the AB-ELISA gave relatively nonspecific results, especially with Bangladeshi serum samples. With the serum samples from the United States, the AB-ELISA showed a higher specificity (Table 2), but its specificity was still not comparable to that of the EITB. The specificity of the AG-ELISA, which requires CSF, was not studied because CSF samples from heterologous infections were not available.

Cross-reactivity

Eighteen Peruvian patients with tissue-confirmed *E. granulosus* (hydatid) infections and 59 Peruvian patients with *H. nana* infections were tested (Table 3). Two (11%) hydatid and 12 (20%) *H. nana*-positive serum samples reacted in the AB-ELISA. In contrast, when these sera were tested by EITB, none of the patients with hydatids and only 1 with a *H. nana* infection had a positive result, which consisted of three bands specific for cysticercosis (GP50, GP42-39, and GP24). Because these 18 patients reside in an area where cysticercosis is endemic, the one with *H. nana* may also be infected with cysticercosis.

Patients with intestinal taeniasis

All serum samples from the four patients with neurologic symptoms who had passed proglottid segments identified as *T. solium* were positive by both AB-ELISA and EITB (Table 3). Ten persons with *T. solium* worms were neurologically asymptomatic. Serum samples from these patients were all positive by EITB, but only six (60%) were positive by AB-ELISA. Serum samples from four patients who had passed *T. saginata* proglottids were negative by the EITB test, but two reacted positively on the AB-ELISA test. Neither of the serum samples from the two patients who had passed *Diphyllobothrium* sp. was positive by EITB or AB-ELISA (Table 3).

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Reproducibility

Twenty-one serum and CSF samples were sent to CDC for analysis by EITB. All positive samples were also positive at CDC (100% concordance). Concordance of the bands in confirmed patients was 91%. All sera were retested by AB-ELISA by an independent observer. No significant difference was observed in the two sets of results.

Test costs

We estimated the cost per 100 tests to be $53 for the EITB and $15 for the two ELISAs. We excluded the cost of labor, shipping, depreciation, and equipment. The EITB test uses over ten times more conjugate than does the ELISA. This difference represents the major cost difference between the two tests. Other significant costs in the EITB test are the nitrocellulose paper and the purified antigen. The power source and chambers for the EITB cost about the same as the cheapest ELISA reader on the market.

DISCUSSION

All reagents for each assay were individually assayed for both sensitivity and specificity. Our results demonstrate that the EITB is the best available test for the diagnosis of cysticercosis infection. The test has high sensitivity and specificity for antibody to *T. solium* in both serum or CSF specimens. Since the EITB detects antibody with equal efficacy in both serum and CSF, it can be used with nonhospitalized patients, and is highly applicable for epidemiologic studies.

Neither the AB-ELISA, using a crude *T. solium* antigen, nor the AG-ELISA was nearly as sensitive as the EITB test. In addition, the AB-ELISA was not very specific. Previous studies on porcine cysticercosis in Peru also demonstrated that the EITB is a more sensitive and specific test than the ELISA.[10] Several previous studies have reported a high sensitivity for the AG-ELISA. However, these studies may be biased because they selected as their positive controls cysticercosis patients who had had antibody detected by another test.[6, 11]

Cross-reactive components in antigen preparations are the most important obstacles to overcome in producing a reliable test. The current ELISA uses a crude or slightly purified antigen that shows cross-reactivity. This phenomenon occurs even with the lentil-lectin–purified antigens used for the EITB assay; however, in this test, irrelevant bands are easily distinguished from those of the specific antigens by the differences in their molecular mass. In the ELISA, specific reactions cannot be differentiated from cross-reactions.

Sera from patients infected with *H. nana*, a common helminthic infection, frequently cross-react in the AB-ELISA.[12] In areas where this parasite is endemic, high rates of false-positive results will occur. In contrast, the EITB test for cysticercosis does not show cross-reactivity with *H. nana* or any other helminthic infections in patients from areas without endemic cysticercosis. The single patient with an *H. nana* infection whose serum reacted in the EITB was a resident of Peru, an area where cysticercosis is endemic. Although the patient has no direct evidence of this disease, we cannot rule out an occult coinfection by *T. solium* in this individual.

The selection of populations to study test specificity is a critical issue in tropical diseases. Our selection of the Bangladeshi negative controls, who are also from a tropical developing country and are similar to our Peruvian patients in terms of socioeconomic background but do not have any risk for cysticercosis, is an attempt to achieve the ideal negative control. Since the development of the EITB for cysticercosis, over 12,000 assays have been performed, and not a single false-positive case has been documented. The 100% specificity of this assay can be tested properly only with carefully selected negative controls from the developing world.

Our laboratory in Peru can perform 300–500 EITB tests per week. If conjugates are prepared in-house, the cost of the EITB is similar to that of ELISA. The EITB assay is the best current immunodiagnostic test for cysticercosis and/or taeniasis. The next task is to develop inexpensive immunologic tests that can be performed in the field or in minimally equipped laboratories, while retaining the high sensitivity and specificity of the EITB.

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REFERENCES

1. Mahajan RC, 1982. Geographical distribution of human cysticercosis. Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. *Cysticercosis: Present State of Knowledge and Perspectives.* New York: Academic Press, 39–46.
2. Schenone H, Ramirez R, Rojas A, 1973. Aspectos epidemiologicos de la neurocisticercosis en America Latina. *Bol Chil Parasitol 28:* 61–72.
3. Schenone H, Villarroel F, Rojas A, Ramirez R, 1982. Epidemiology of human cysticercosis in Latin America. Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. *Cysticercosis: Present State of Knowledge and Perspectives.* New York: Academic Press, 25–38.
4. Nash TE, Neva FA, 1984. Recent advances in the diagnosis and treatment of cerebral cysticercosis. *N Engl J Med 311:* 1492–1496.
5. Diwan A, Coker-Vann M, Brown P, Subianto DB, Yolken R, Desowitz R, Escobar A, Gibbs Jr C, Gajdusek C, 1982. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to cisticerci of Taenia solium. *Am J Trop Med Hyg 31:* 364–369.
6. Estrada J, Kuhn R, 1985. Immunochemical detection of antigen of larval Taenia solium and anti-larval antibodies in the cerebrospinal fluid of patients with neurocysticercosis. *J Neurol Sci 71:* 39–48.
7. Tsang VCW, Brand J, Boyer E, 1989. Enzyme-linked immunoelectrotransferency blot assay and glycoprotein antigens for diagnosing human cysticercosis (Taenia solium). *J Infect Dis 159:* 50–59.
8. Larralde C, Laclette JP, Owen CS, Madrazo I, Sandoval M, Bojalil R, Sciutto E, Contreras L, Arzate J, Diaz ML, Govezensky T, Montoya RM, Goodsaid F, 1986. Reliable serology of Taenia solium cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am J Trop Med Hyg 35:* 965–973.
9. Armitage P, Berry G, 1987. *Statistical Methods in Medical Research.* London: Blackwell Scientific Publications, 472.
10. Gonzales AE, Cama V, Gilman RH, Tsang VCW, Pilcher JB, Chavera A, Castro M, Montenego T, Verastegui M, Miranda E, Bazala H, 1990. Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. *Am J Trop Med Hyg 43:* 194–199.
11. Tellez-Giron E, Ramos MC, Dufour L, Alvarez P, Montante M, 1987. Detection of Cysticercus cellulosae in cerebrospinal fluid by dot enzymne-linked immunosorbent assay (DOT-ELISA) and standard ELISA. *Am J Trop Med Hyg 37:* 169–173.
12. Castillo R, Grados P, Carcamo C, Guevara A, Verastegui M, Gilman RH, 1991. Effect of treatment on serum antibody to Hymenolepis nana detected by enzyme-linked immunosorbent assay. *J Clin Microbiol 29:* 413–414.

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Diagnosis of cysticercosis in endemic regions

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Taenia solium cysticercosis is a frequent cause of neurological disease in developing countries. Specific diagnosis of cysticercosis is difficult. We obtained serum and/or CSF samples from 204 consecutive patients admitted to a neurological ward in Lima, Peru, and looked for antibodies specific for *T solium* with the enzyme-linked immunoelectrotransfer blot (EITB) assay. 21 (12%) of 173 serum samples from these patients were EITB-positive. In contrast, only 2 (1·5%) of 135 patients attending a public endoscopy clinic and 1 (1%) of 88 patients attending a private endoscopy clinic were seropositive. 1 (1%) of 98 pregnant women living in a Lima shanty town was EITB-positive. 15 (58%) of 26 neurology patients diagnosed clinically as having cysticercosis were seronegative. Routine screening by EITB of all patients with neurological symptoms from areas of endemic cysticercosis would avoid misdiagnosis of this common and treatable disease.

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Introduction

The life cycle of the cestode *Taenia solium* involves an intermediate host, normally the pig, for the cystic form of the parasite and a definitive host, normally man, for the adult form or tapeworm. However, persons infected by *T solium* eggs can also serve as hosts for the cystic form, which frequently attacks the central nervous system. Cysts may also be found in the subcutaneous tissue, muscle, and eye,[1,2] and, more rarely, in other parts of the body.[3,4] *T solium* cysticercosis is common in countries where free-ranging pigs are raised.[5-7]

Many factors affect the clinical manifestations of the disease, including the number, size, location, and form of the parasites, and the immune response of the host. The interaction of these factors produces a spectrum of neurologic symptoms.[1-6,8-12]

Correct diagnosis of cysticercosis is important since most patients respond to treatment with albendazole or praziquantel. However, diagnosis is difficult. The disease is usually diagnosed on the basis of clinical symptoms, including the occasional presence of subcutaneous nodules, and confirmed by computed tomography (CT) or nuclear magnetic resonance imaging (MRI); but CT and MRI are rarely available in endemic areas. Until recently serological tests, including the enzyme-linked immunosorbent assay (ELISA), were not sensitive and specific enough to confirm the diagnosis, especially in areas where other cestode infections are endemic.[12-14]

Development of the enzyme-linked immunoelectrotransfer blot (EITB) assay,[15] has provided a precise method of diagnosing *T solium* infection in neurological patients. Here we report use of this new immunological test to detect antibodies to *T solium* in patients admitted to an acute-care neurological ward in Lima, Peru.

Patients and methods

Patients

All new patients (n = 231) admitted to the male neurological ward at the Instituto Nacional de Ciencias Neurológicas (INCN) in Lima, Peru, between September, 1988, and June, 1989, were entered into the study. The INCN is the neurological reference centre for Peru, serving mainly lower and middle class sections of the population. Serum samples for EITB assay were taken from all patients at admission, and when cerebrospinal fluid (CSF) was

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TABLE I—COMPARISON OF SEROPOSITIVE AND SERONEGATIVE PATIENTS WITH NEUROLOGICAL SYMPTOMS

—	Sero-positive (n = 21)	Sero-negative (n = 183)	Significance
Mean (SD) age (yr)	44 (17·9)	42 (20·8)	NS (Student's *t* test)
Mean (SD) hospital stay (day)	45 (43·4)	29 (25·6)	NS (Mann-Whitney test)
No (%) with headache	11 (52)	83 (45)	NS (χ^2 test)
No (%) with seizures	10 (48)	54 (30)	NS (χ^2 test)
No (%) with motor deficit	6 (29)	82 (45)	NS (χ^2 test)
No (%) with cranial nerve compromise	4 (19)	34 (19)	NS (Fisher's test)
No (%) with clinical diagnosis of cysticercosis	11 (52)	15 (8)	..
No (%) without clinical diagnosis of cysticercosis	10 (48)	168 (92)	..

NS = not significant.

obtained for clinical reasons a sample was set aside for assay. Informed consent was obtained from all patients. The study was approved by the ethical review boards of the Universidad Peruana Cayetano Heredia, Lima, and the Johns Hopkins University, Baltimore, USA.

Clinical histories and results of physical examinations were reviewed, and, when available, the diagnosis obtained after CT scan was recorded. CT scans are done infrequently in Peru because of their cost; MRI is not available in Peru. After the initial clinical diagnosis had been recorded, EITB results were made available to doctors managing the patients.

Patients may be free of symptoms of cysticercosis but seropositive. To determine the proportion of seropositive patients without neurological disease, we used EITB to assay serum samples from two groups of patients attending endoscopy clinics for treatment of gastrointestinal symptoms. One group were 135 poor or lower-middle-class patients who visited a public endoscopy clinic (34 men, 101 women; mean [SD] age 42 [17·9] years, range 18–94), and the other group were 88 middle or upper class patients attending a private endoscopy clinic (48 men, 40 women; mean age 48 [16·1] years, range 16–82). To determine the rate of symptom-free cysticercosis in individuals born or living in Lima, we surveyed the sera of 98 pregnant women (mean age 28 [5·81], range 17–43) from a shanty town in Lima.

EITB assay

The EITB assay for antibodies specific for *T solium* was done as described previously.[14] Briefly, seven lentil-lectin purified *T solium* glycoprotein antigens are used in an immunoblot to detect specific antibodies in serum or CSF. Antibody reactions against these glycoproteins are visualised with H_2O_2/diaminobenzidine substrate, and reactions to one or more glycoprotein are considered a positive result. The assay was repeated when results were ambiguous. Tests were done at the Laboratory of Parasitology of the Universidad Peruana Cayetano Heredia, and duplicate specimens were sent to the Centers for Disease Control, Atlanta, USA, for quality control.

Definitions

Clinical diagnosis—The clinical diagnosis of cysticercosis was not standardised but rather reflected the consensus opinion of the attending neurologists. In general, patients admitted to the neurological ward who had lived in areas of endemic cysticercosis and had late-onset seizures or raised intracranial pressure were diagnosed as having cysticercosis.

CT scan diagnosis—CT scans are indicated in all patients with chronic or focal neurological disease. However, in Peru many patients cannot afford a CT examination; thus, most scans were done based on medical and economic criteria. The following radiological criteria, any one of which was compatible with a diagnosis of neurocysticercosis, were used: one or more cystic images, two or more calcifications, and/or one or more nodules visualised after injection of contrast medium. The last criterion is compatible with a diagnosis of either tuberculosis or cysticercosis.

TABLE II—RESULTS OF EITB IN PATIENTS WHO HAD A CEREBRAL CT SCAN

CT finding	EITB-positive	EITB-negative
Normal (n = 14)	1	13
Cysticercosis (n = 14)	11	3
Vascular disease (n = 14)	3	11
Neoplasia (n = 7)	0	7
Other infectious diseases (n = 5)	0	5
Hydrocephalus (n = 2)	1	1
Total (n = 56)	16	40

Results

Of the 231 patients admitted to the neurological ward, 204 (88%) provided serum (n = 173, 75%) and/or spinal fluid (n = 166, 72%) for testing. 21 (12%) serum samples and 18 (11%) CSF samples were EITB-positive. Matched CSF and serum samples were obtained from 135 patients—19 (14%) of these serum samples and 17 (13%) CSF samples were EITB-positive; thus, 2 CSF samples were negative when the matching serum sample was positive. No serum sample was negative when the matching CSF sample was positive. There were no differences in age or clinical characteristics between EITB-positive and EITB-negative patients (table I). Although the length of hospital stay was longer for seropositive patients than for seronegative patients, this difference was not significant. The mean [SD] age of the 27 patients who did not provide samples was similar to that of the 204 individuals who were sampled (44 [19] years, range 19–79 *vs* 42 [18], range 14–88). However, the unsampled patients had a significantly shorter mean [SD] length of hospital stay (8 [8·8] days *vs* 31 [28·4]; $p < 0·01$, Mann-Whitney test).

Of the 204 patients who provided samples for testing, 26 had a clinical diagnosis of cysticercosis and 11 (42%) of these 26 patients were seropositive. 10 (6%) of the 178 patients not given a clinical diagnosis of cysticercosis were seropositive (table I). Of the 10 seropositive patients not initially diagnosed as having cysticercosis, 1 was a *T solium* adult worm carrier; 1 had previous surgery for cerebral cysticercosis (not recorded on the admission history), 3 had cerebral CT scans showing multiple cystic images, 1 had a CT image of an expanding temporal process, and another had severe hydrocephalus with non-specific pachymeningitis at necropsy. The CT scans were done before results of EITB assay were known. The 3 remaining patients were admitted for diverse clinical signs: 1 for seizures, another for trauma but with a history of occipital lobe seizures, and the third for a stroke. These 3 patients did not have CT scans or parasitological stool examinations.

Clinical findings in the 56 patients admitted to the neurological ward who had a cerebral CT scan are shown in table II. The EITB-positive patient with a normal CT scan had an ocular cysticercus and an adult tapeworm. 6 of the 15 seronegative patients clinically diagnosed as having cysticercosis had a cerebral CT scan; 3 scans were diagnosed as showing cysticercosis (calcifications as the only CT scan finding), 1 showed a neoplasia, and 2 scans were normal.

2 (1·5%) of the 135 patients attending a public endoscopy clinic were EITB-positive. 1 of the patients was symptom-free and no history was available for the other. 1 (1%) of 88 patients seen at the private endoscopy clinic was EITB-positive. This individual was without neurological

symptoms and had a normal CT scan. Only 1 (1%) of the 98 pregnant women was EITB-positive and she was also without neurological symptoms. All seropositive subjects in the comparison groups were born in rural pig-raising areas.

Discussion

In developing countries, investigation of patients with neurological symptoms is limited by economic factors. Routine analyses are reduced to those strictly necessary and special radiological studies, such as CT scans, are often available only to privileged members of the population. In Peru, Mexico, and Brazil, cysticercosis is found during 6–7% of necropsies done at neurological centres.[14-18] Patients suspected of having cysticercosis occupy 10–15% of the beds in neurological wards (M. Martinez, personal communication). Besides being common, hospital admissions for cysticercosis can be longer than for most other neurological diseases.[16] The combination of high prevalence and prolonged hospital admissions gives rise to high health-care costs.

Cysticercosis is frequently misdiagnosed clinically. The EITB technique is a sensitive and specific assay for the diagnosis of cysticercosis that is as sensitive in serum as it is in CSF.[15] We detected a high prevalence of cysticercosis (11%) by EITB in patients admitted to a neurological ward. Cysticercosis was not the initial diagnosis in most of these patients. CT scan, although highly specific, was relatively insensitive compared with EITB for diagnosing cysticercosis. In addition, most patients diagnosed clinically as having cysticercosis did not have serological evidence of this infection. None of the 6 seronegative patients who had a clinical diagnosis of cysticercosis and a CT scan had any radiological evidence of active cysticercosis lesions. 3 of these 6 patients had multiple calcifications; thus, we cannot exclude the possibility that these patients converted from seropositive to seronegative after their lesions calcified.

In our laboratory, the EITB assay has 95% sensitivity and 100% specificity.[20] The EITB assay will give positive results with patients who are infected with either the tapeworm or the cystic form. In a region of endemic cysticercosis, many seropositive individuals may be symptom-free if they are infected with occult cysts or intestinal tapeworms. To control for silent infection, we examined patients from different social classes who came to hospital for endoscopy. Prevalence (positive EITB) was seven times higher in neurological patients than in those coming for endoscopy, suggesting strongly that seropositivity and neurological symptoms are linked. Additionally, only 1 of 14 neurology patients with normal CT scans was EITB-positive. It appears that most EITB-positive patients admitted to the neurological ward had symptomatic neurocysticercosis.

Patients who did not provide serum or CSF samples had a shorter hospital stay than those who did. This unsampled group may have had a lower proportion of EITB-positive cases. However, even if all 27 unsampled patients were EITB-negative, and we had included them in the analysis, the EITB-positive rate would still be 9·5%.

None of our patients with neurological symptoms had a positive serological test for syphilis or human immunodeficiency virus. Although less than 0·5% of neurological patients admitted to the INCN have positive syphilis serology according to hospital records, syphilis screening is done routinely. Cysticercosis is 10–20 times more frequent than syphilis in Peru, but routine screening is not done. We recommend that in endemic areas all patients with neurological symptoms be screened routinely for cysticercosis by EITB to avoid misdiagnosis of this treatable disease.

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REFERENCES

1. Loo L, Braude A. Cerebral cysticercosis in San Diego: a report of 23 cases and a revision of the literature. *Medicine* 1982; 61: 341–59.
2. Lozano D. Ophthalmic cysticercosis. In: Palacios E, Rodriguez-Carbajal J, Taveras J, eds. Cysticercosis of the central nervous system. Springfield, USA: Charles C. Thomas, 1983: 84–100.
3. Andrews R, Mason W. Cysticercosis presenting as acute scrotal pain and swelling. *Pediatr Infect Dis J* 1987; 6: 942–43.
4. McCormick GF, Zee CS, Heiden J. Cysticercosis cerebri. Review of 127 cases. *Arch Neurol* 1982; 39: 534–39.
5. Nash TE, Neva FA. Recent advances in the diagnosis and treatment of cerebral cysticercosis. *N Engl J Med* 1984; 311: 1492–96.
6. Mahajan RC. Geographical distribution of human cysticercosis. In: Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, 1982: 39–46.
7. Flisser A. Neurocysticercosis in Mexico. *Parasitol Today* 1988; 4: 131–37.
8. Trelles JO, Trelles L. Cysticercosis of the nervous system. In: Wynken PJ, Bruyn GW, eds. Handbook of clinical neurology. Amsterdam: North Holland Publishing, 1978; 35: 291–320.
9. Rangel R, Torres B, Del Brutto O, Sotelo J. Cysticercosis encephalitis: a severe form in young females. *Am J Trop Med Hyg* 1987; 36: 387–92.
10. Neva J. La cisticercosis del sistema nervioso central. *Salud Publica Mex* 1983; 25: 297–300.
11. Salazar A, Sotelo J, Martinez H, Escobedo F. Differential diagnosis between ventriculitis and fourth ventricle cyst in neurocysticercosis. *J Neurosurg* 1985; 63: [illegible]
12. Chopra JS, Kaur U, Malhotra RC. Cysticercosis and epilepsy; a clinical and serological study. *Trans R Soc Trop Med Hyg* 1981; 75: 4–7.
13. Flisser A, Woodhouse E, Larralde C. Epidemiology of human cysticercosis in Mexico. In: Palacios E, Rodriguez-Carbajal J, Taveras J, eds. Cysticercosis of the central nervous system. Springfield, USA: Charles C. Thomas, 1983: 7–17.
14. Mohammad I, Heiner D, Miller B, Goldberg M, Kagan I. Enzyme-linked immunosorbent assay for the diagnosis of cerebral cysticercosis. *J Clin Microbiol* 1984; 20: 775–79.
15. Tsang VCW, Brand J, Boyer B. Enzyme-linked immunoelectrotransblot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J Infect Dis* 1989; 159: 50–59.
16. Schenone H, Villarroel F, Rojas A, Ramirez R. Epidemiology of human cysticercosis in Latin America. In: Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, 1982: 25–38.
17. Ascojo A. Setenta y dos casos de cisticercosis en el Instituto de Neurocirugia. *Rev Neuropsiquiatr* 1950; 13: 337–53.
18. Takayanagui O, Jardim E. Aspectos clinicos da neurocisticercose. Analise de 500 casos. *Arq Neuropsiquiatr* 1983; 41: 50–63.
19. Cysticercosis Working Group in Peru and the CDC. Serological diagnosis of cysticercosis—comparison of the ELISA assay for antibody (EAB) and antigen detection (EAG) compared to the enzyme immuno-transfer blot (EITB). 38th Annual Meeting of the American Society of Tropical Medicine and Hygiene; December, 1989; Hawaii (abstract 388).

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