

PORCINE ANTIBODY RESPONSES TO *TAENIA SOLIUM* ANTIGENS RGP50 AND STS18VAR1

SUKWAN HANDALI, ARMANDO E. GONZALEZ, KATHY HANCOCK, HECTOR H. GARCIA, JACQUELIN M. ROBERTS, ROBERT H. GILMAN, AND VICTOR C. W. TSANG

Immunology Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru; Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru; Cysticercosis Unit, Instituto de Ciencias Neurológicas, Lima, Peru; Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland

Abstract. Cysticercosis, a disease caused by the larval form of *Taenia solium*, is diagnosed by detection of specific antibodies or by imaging techniques. Our preferred immunologic assay for cysticercosis is the enzyme-linked immunoelectrodiffusion transfer blot, or immunoblot, using the lentil lectin bound antigens from larval cysts. Antibody reactivity with any one of seven glycoproteins is diagnostic for cysticercosis. To develop a simple antibody detection assay for field use, we have synthesized an 8-kD diagnostic antigen, sTs18var1 (a secreted protein with a mature size of 67 amino acids), and expressed a 50-kD membrane protein antigen, rGp50. We used these two diagnostic proteins in a quantitative Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA) to measure the antibody responses in Peruvian pigs with cysticercosis. Three study designs were used. First, we followed the kinetics of antibody responses against these two diagnostic proteins in pigs with cysticercosis that were treated with oxfendazole. Second, we measured antibody response in naive experimentally infected pigs. Third, we followed the maternal antibodies against rGp50 and sTs18var1 in piglets born from sows with cysticercosis. These studies showed that antibody responses against the two diagnostic proteins in the FAST-ELISA are quantitatively correlated with infection by viable cysts, with anti-sTs18var1 activity being most responsive to the status of infection.

INTRODUCTION

Taenia solium (pork tapeworm) has a two-host life cycle, with humans as the only definitive host carrying the adult tapeworm in the intestine and pigs as the intermediate host harboring the larval cyst in muscles or brain. Humans also occasionally become the intermediate host by harboring the metacestode/cysticerci of *T. solium* after ingesting the eggs of the tapeworm, a condition known as cysticercosis. *Taenia solium* cysticercosis is endemic in many developing countries.^{1,2} It increases the prevalence of epilepsy in humans and causes significant economic loss because of infested pork.

Since the pig is the intermediate host and the only reservoir source for this parasite, porcine cysticercosis is critical for the development of strategies to control cysticercosis and will benefit the rural poor by preventing pork condemnation.^{3–5} Prevalence of porcine cysticercosis is a reliable indicator of active transmission. In Peru, we use sentinel pigs to monitor transmission and measure efficacy of control programs. We prefer to use native sentinel pigs rather than importing animals from the city, even though seronegative animals are rarer, but native pigs are less expensive and more resistant to local pathogens than city pigs.³ Unfortunately, the use of native sentinel pigs is hampered by the longevity of transferred maternal antibodies from colostrum that can last up to eight months.^{4,6} Development of a quantitative, rapid, serologic assay such as the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA),^{7,8} to differentiate active from inactive infections will be better suited for field use. To develop a highly sensitive and specific FAST-ELISA, we have cloned, sequenced, and produced two of the seven lentil lectin-bound glycoprotein diagnostic antigens used in the enzyme-linked immunoelectrodiffusion transfer blot (EITB).^{9,10} The first one is a recombinant glycoprotein known as Gp50 (rGp50), a membrane-bound protein that we have expressed in insect cells,¹¹ and the second one is an 8-kD protein (with a mature size of 67 amino acids) known as *T. solium* 18 var 1

(sTs18var1) that we have chemically synthesized.¹² These two antigens represents two of three dominant antigens of the original diagnostic glycoproteins used the EITB.

In this study, we report the use of the FAST-ELISA with rGp50 and sTs18var1 to quantify the progress of infection in porcine cysticercosis and to differentiate between active and inactive infections. Understanding the progress of infection in porcine cysticercosis will allow us to learn more about the progress of infection of human cysticercosis, where the only available indicator for therapeutic successes is neuroimaging techniques that are not available in many parts of the world where the disease is endemic.¹³

METHODS AND MATERIALS

Sera samples. We used samples from three studies. The first was an oxfendazole (OFZ) treatment study designed to prove that infected pigs that were treated and cured with OFZ become immune to future infections.⁵ Briefly, 19 infected pigs (based on positive EITB and tongue inspection and purchased from an endemic village) were treated with single oral dose of 30 mg/kg of OFZ, a cysticercidal drug. The pigs were kept 20 weeks after treatment to ensure that all muscle cysts were killed. Each treated pig was then matched by age and sex with two uninfected pigs and send back to the endemic village. Three months later, all animals were repurchased from villagers, transported back to our specific pathogen-free facility in Lima, and kept for an additional three months, a sufficient time for cysts to achieve full maturity. Pigs were then killed in an abattoir and a detailed dissection of the carcasses was performed to determine parasite burden. Serologic status of all pigs was determined by the FAST-ELISA.

In the second study, 16 naive pigs were fed oncospheres or eggs (in proglottids). Sera were collected weekly for 12 weeks and the pigs were then killed as described in the first study. Four naive pigs were used as uninfected controls. Serologic status of all pigs was determined by the FAST-ELISA.

In the third study, 34 piglets from 4 sows with cysticercosis were followed for up to 145 days. Serologic status of all piglets and sows were determined by the FAST-ELISA.

Serology. Sera collected were analyzed by the FAST-ELISA as previously described.^{7,8} To ensure comparability of day-to-day measurement, we prepared a standard serum pool from the sera of five Peruvian pigs with confirmed cysticercosis and with high antibody activities against rGp50 and sTs18var1. We defined 1 μL of the standard serum pool as having 100 units of activity. A reference curve constructed from the standard serum pool was included with each assay. The diluent used was a negative United States pig serum pool obtained from Equitech (Kerville, TX).

Sticks (transferable solid phase screening, catalog # 445497; Nunc, Roskilde, Denmark) were sensitized with 1 $\mu\text{g}/\text{mL}$ of rGp50 and 2 $\mu\text{g}/\text{mL}$ of sTs18var1, respectively, in 96-well plates (Catalog # 269620; Nunc) for two hours at room temperature on a shaker (Titer Plate Shaker; Laboratory-Line Instruments, Melrose Park, IL) at speed 5. The diluent used was 0.01 M phosphate-buffered saline (PBS), pH 7.2. After sensitization, the sticks were washed with 0.01 M PBS, pH 7.2, containing 0.3% polyoxyethylene sorbitan monolaurate 20 (Tween 20; Sigma Chemical Company, St. Louis, MO) using a standard plastic garden sprayer. Sera to be assayed for antibodies against rGp50 and sTs18var1 were dispensed into the wells, and 4.5 μL of serum were diluted to a total volume of 150 μL in 0.01 M PBS, pH 7.2/0.3% Tween 20/5% nonfat dry milk. Sera with activities above the maximum standard curve were diluted further using the United States normal pig serum until the activities were within the boundaries of the standard curve. Sticks were exposed to the sera for five minutes at room temperature with mixing, washed again as in the previous step, and then exposed to a home-made antibody/enzyme conjugate (150 μL of affinity-purified goat anti-pig IgG [heavy and light chain activity] labeled with horseradish peroxidase) and diluted 1:1,000 in PBS/Tween 20. After exposure to the conjugate for five minutes at room temperature with mixing, and washed as before, the sticks were immersed in 150 μL of SureBlue 3,3',5,5'-tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) for five minutes at room temperature with mixing. The sticks were then removed and discarded. The absorbance of each well was measured at 650 nm using a ThermoMax microtitration plate (Molecular Devices, Sunnyvale, CA). The activity unit of each serum sample was derived from the standard curve and was expressed in units/microliter. All samples were run in triplicate.

Data analysis. The FAST-ELISA for rGp50 and sTs18var1 performance index (Youden J index) was determined.¹⁴ In principle, the J index is sensitivity (number of positive samples detected in the infected group divided by number of known positive samples) plus specificity (number of negative samples detected in the uninfected group divided by number of known negative samples) minus 1. The highest J index obtainable is 1.0 and occurs only when there is 100% sensitivity and 100% specificity.^{7,14} Optimizing the J index will, therefore, allow us to determine what assay cut-off point to use to differentiate between positive and negative samples. The mean number of weeks at which time antibody responses decreased below and increased above the detection level was determined by calculating Kaplan-Meier product limit estimates of the survival function. Pearson correlation coeffi-

cients were used to assess the association between infection and antibody response. All statistical analysis were performed using SAS version 8.02 (SAS Institute, Cary, NC).¹⁵

RESULTS

Determination of the J indices and the test cut-off points. The results from the assay of the pretreatment sera from the 19 OFZ experiment pigs (pigs positive for cysticercosis) and 27 control pigs gave a J index of 0.86 with a cut-off point of 0.40 unit/ μL with rGp50 and a J index of 1 with a cut-off point at 0.70 unit/ μL with sTs18var1, respectively. Therefore, sera with activity units ≥ 0.40 for rGp50 and ≥ 0.70 for sTs18var1 were considered positive.

Oxfendazole study. For analyzing the antibody responses after treatment with OFZ, only data from infected pigs will be reported. A total of 19 infected and treated pigs were observed in the study; 4 pigs (21%, 4 of 19) were found to have viable cysts at the end of the study and the rest of the pigs (79%, 15 of 19) had only degenerated or no remnants of cysts found. In those pigs with viable cysts, all viable cysts were found only in the brain. In all infected and treated pigs, the antibody responses against rGp50 and sTs18var1 showed similar patterns: increased antibody responses against rGp50 and sTs18var1 immediately after treatment and then mostly a decrease to below detection levels at a mean \pm SE of 40.9 ± 1.6 weeks for rGp50 and 20.7 ± 2.5 weeks for sTs18var1 (Figures 1 and 2). At end of the study, in pigs with only degenerated cysts, 40% (6 of 15) and 13% (2 of 15) of the antibody responses against rGp50 and sTs18var1, respectively, were above the cut-off point. For pigs with viable cysts, 50% (2 of 4) of the antibody responses against rGp50 and sTs18var1 were above the cut-off point by end of the study. The two pigs that tested negative were animals with only minimal numbers of cysts in the brain. In all instances, the ratio of rGp50/sTs18var1 was > 1 .

Experimental infection. In this study, 20 pigs were inoculated by several methods to produce porcine cysticercosis. In four pigs that received no infection (control pigs), all were free from cysticercosis and antibody responses against rGp50 and sTs18var1 were below detection limit. In 16 pigs that received infective eggs, two pigs had only degenerated cysts and they showed some increase of antibody responses to rGp50 and sTs18var1, but by end of the study, the antibody responses decreased below the cut-off point, except in one pig against sTs18var1.

There were 14 pigs with living cysts by end of the study (range = 1–590 alive cysts and 6–601 total cysts). The antibody responses against rGp50 and sTs18var1 followed the same patterns, and could be detected as early as three weeks, with mean \pm SE times for detection of 7.6 ± 0.8 weeks for rGp50 and 6.1 ± 0.6 weeks for sTs18var1 (Figures 3 and 4). By the end of the study, 70% of the pigs with viable cysts had a positive antibody response against rGp50 and 93% had a positive antibody response against sTs18var1. In this experiment, the ratio of rGp50/sTs18var1 was < 1 . The correlations between antibody responses at the time of necropsy and number of viable cysts were 0.62 and 0.86 for rGp50 and sTs18var1, respectively.

Sow-piglet study. In this study, 34 piglets from 4 sows with cysticercosis were followed for 145 days. The relationship be-

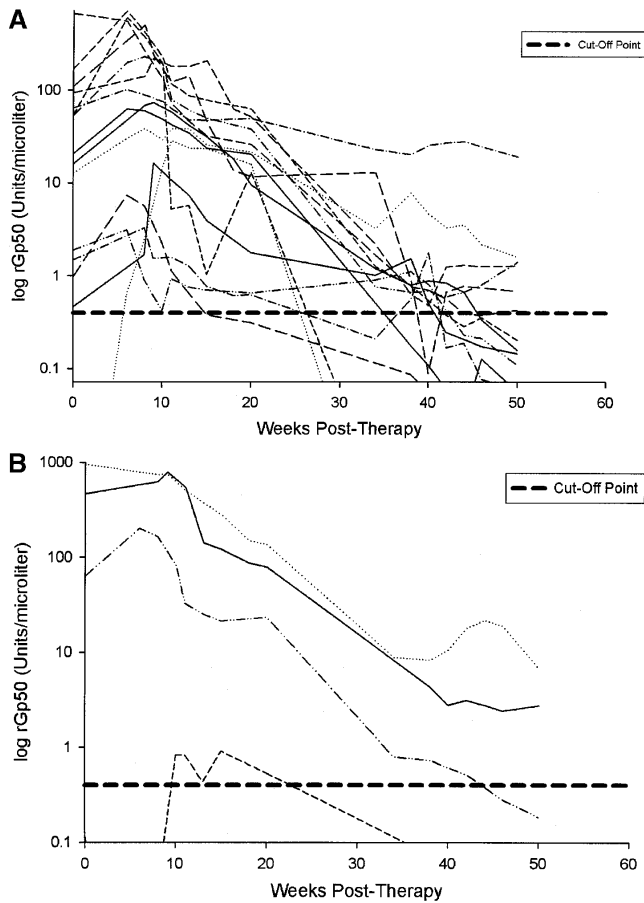


FIGURE 1. Antibody responses against rGp50 in oxfendazole-treated pigs detected by the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA). **A**, Pigs with degenerated cysts. **B**, Pigs with viable cysts. Samples were tested in triplicate and the average absorbance at 650 nm was converted into units of activity based on a standard curve. The horizontal dashed line represents the cut-off value of the FAST-ELISA.

tween antibody responses against rGp50 and sTs18var1 in sows and their piglets at time of delivery is shown in Table 1. Sows with higher antibody responses against rGp50 and sTs18var1 had piglets with higher levels of maternal antibodies. Maternal antibodies against rGp50 and sTs18var1 in piglets decreased below detection levels at a mean \pm SE of 74.4 ± 4.8 days and 31.1 ± 4.4 days, respectively (Figure 5).

DISCUSSION

The results of this study suggested that we could use antibody data from the FAST-ELISA for rGp50 and sTs18var1 to quantitatively follow the progress of infection, treatment of cysticercosis in pigs, and the fate of maternal antibodies in piglets. Antibody responses against rGp50 and sTs18var1 did not show significant differences. The only clear difference was that in pigs with established infections, the ratio of rGp50/sTs18var1 is >1 , but it was reversed in the early infections, such as the experimentally infected pigs. Antibody responses against rGp50 could be detected at seven weeks post-infection instead of at six weeks with sTs18var1 and waned later at 41 weeks instead of 21 weeks. Also, in piglets, mater-

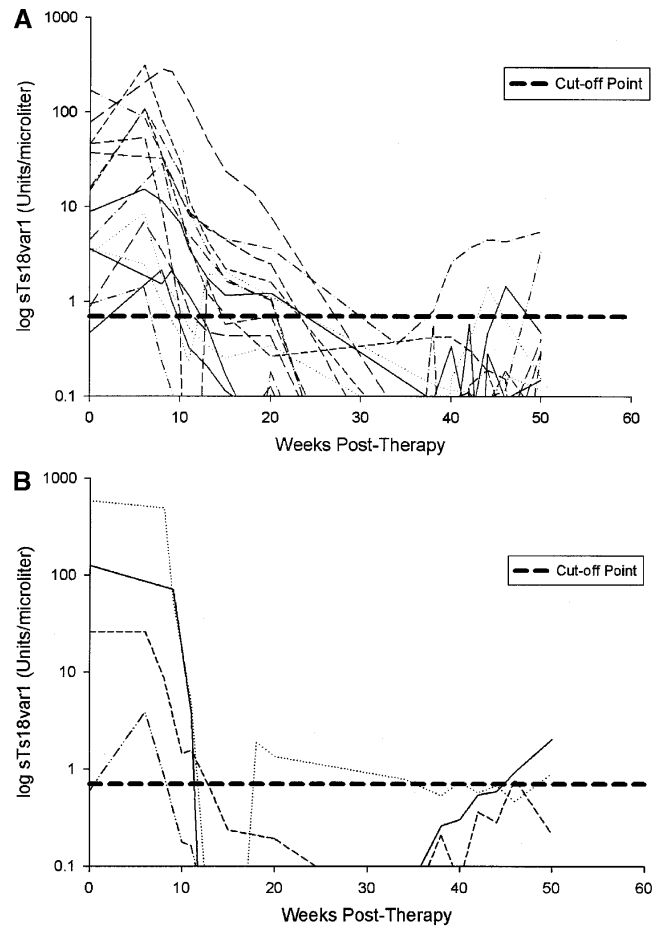


FIGURE 2. Antibody responses against sTs18var1 in oxfendazole-treated pigs detected by the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA). **A**, Pigs with degenerated cysts. **B**, Pigs with viable cysts (all in the brain). Samples were tested in triplicate, and the average absorbance at 650 nm was converted into units of activity based on a standard curve. The horizontal dashed line represents the cut-off value of the FAST-ELISA.

nal antibodies against rGp50 waned at nine weeks compared with four weeks against sTs18var1.

Data from OFZ-treated pigs with viable cysts showed that positive antibody responses against rGp50 and sTs18var1 could be detected in only 50% of the pigs. The viable cysts were found in only in the brain. We speculated that viable cysts in the brain did not induce high antibody responses due to the fact that the brain is considered a privileged site for immune functions.¹⁶

To answer the question of any difference in antibody responses against rGp50 and sTs18var1 between pigs with viable and non-viable cysts, data from the experimentally infected pig study showed that infection or cysts viability correlated well with antibody responses against sTs18var1 ($r = 0.86$), but not against rGp50 ($r = 0.62$). In the sow-piglet study, we found that although the piglets were not infected, they do have maternal antibodies against rGp50 and sTs18var1. The antibody against sTs18var1 however, waned after 31 days; this observation is important because it enables one to differentiate pigs with real infections from those maternal antibodies, an effort that is quite difficult with the EITB.⁴

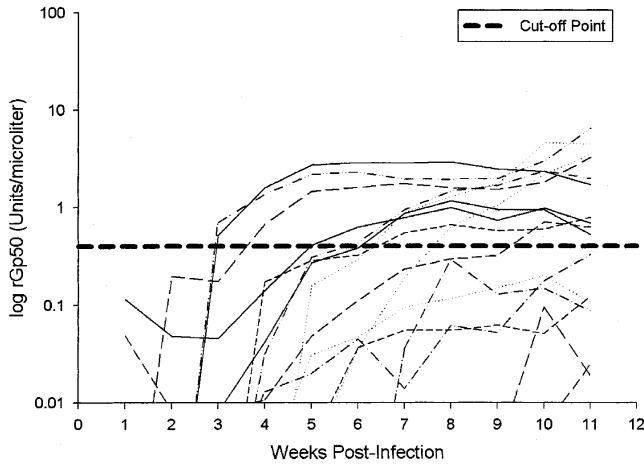


FIGURE 3. Antibody responses against rGp50 in experimentally infected pigs detected by the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA). Samples were tested in triplicate, and the average absorbance at 650 nm was converted into units of activity based on a standard curve. The horizontal dashed line represents the cut-off value of the FAST-ELISA.

There are several implications of this study. First, it is possible to use a simple, fast, quantitative FAST-ELISA to follow the progress of infection in porcine cysticercosis, correlate antibody responses with viability of cysts, and monitor the progress of maternal antibodies in piglets. We showed that the FAST-ELISA for sTs18var1 could give us more information than that for rGp50. The reasons are that antibody against sTs18var1 disappears early in treated pigs and piglets, is found in 93% of the infected pigs, and has a higher correlation with cyst viability ($r = 0.86$) and a J index of 1. Second, for the purpose of cysticercosis control programs, the EITB is important for estimating the burden of exposure (total exposure to cysticercosis),¹⁷ but the FAST-ELISA for sTs18var1 will give a better estimate of the true prevalence of cysticercosis (ability to detect infected pigs with live cysts and thus

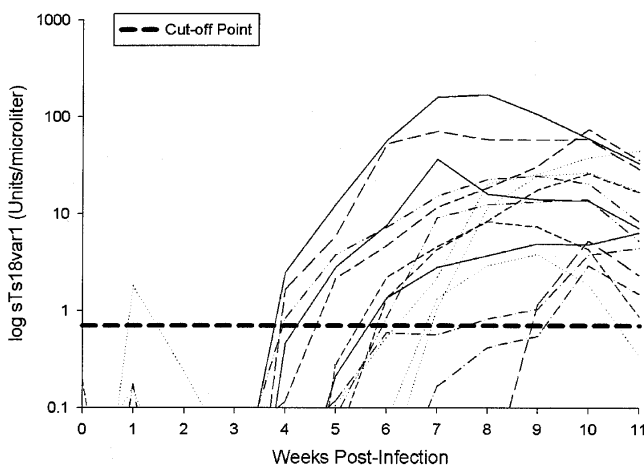


FIGURE 4. Antibody responses against sTs18var1 in experimentally infected pigs detected by the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA). Samples were tested in triplicate, and the average absorbance at 650 nm was converted into units of activity based on a standard curve. The horizontal dashed line represents the cut-off value of the FAST-ELISA.

TABLE 1
Relationship between antibody responses in sows and maternal antibodies in piglets against rGp50 and sTs18var1 at time of delivery*

| Subject† | Antibody responses against rGp50 (units/ μ L)* | Antibody responses against sTs18var1 (units/ μ L) |
|-----------------------|--|---|
| Sow 1 | 58.7 | 17.3 |
| Piglets (4) | 58.3 (15–101.9) | 13.8 (5.7–24.3) |
| Sow 2 | 0.4 | 3.9 |
| Piglets (8) | 0.07 (0.01–0.14) | 1.5 (0.8–2.1) |
| Sow 3 first delivery | 40.5 | 7.2 |
| Piglets (8) | 28.2 (10–48) | 9.7 (3.8–15.2) |
| Sow 3 second delivery | 47.1 | 8.6 |
| Piglets (4) | 71.3 (55.4–117) | 20.4 (12–30.5) |
| Sow 4 | 1.6 | 0 |
| Piglets (8) | 14.2 (9.4–18.7) | 0.04 (0–0.38) |

* Values are the mean (range) activity units of antibody responses of all piglets from the same sow.
† Values in parentheses are the number of piglets.

potential sources of infection) and also confirm treatment efficacy in the frame of intervention control programs. Combination of the EITB and FAST-ELISA for sTs18var1 will give a better picture of the cysticercosis problem.

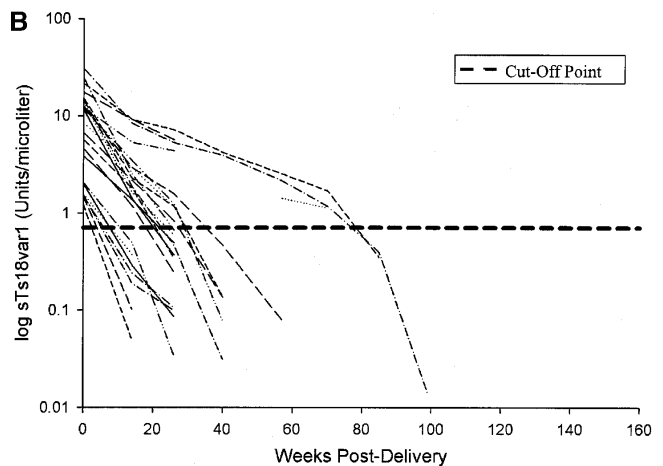
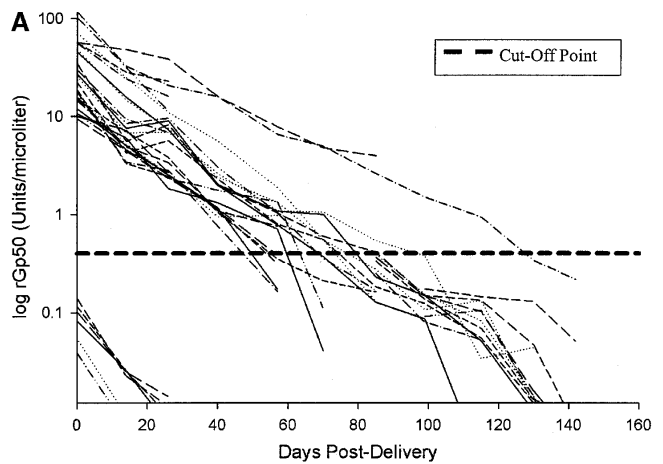


FIGURE 5. Antibody responses in piglets against **A**, rGp50 and **B**, sTs18var1. Pig sera of piglets from the sow-piglets study were tested by the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA). Samples were tested in triplicate, and the average absorbance at 650 nm was converted into units of activity based on a standard curve. The horizontal dashed line represents the cut-off value of the FAST-ELISA.

The only disadvantage of this FAST-ELISA system is that it failed to detect an antibody response in 50% of the pigs with viable cysts only in the brain. This situation will be crucial when it comes to diagnose human neurocysticercosis, but this problem exists also with the EITB.¹⁸

In conclusion, the FAST-ELISA for rGp50 and sTs18var1 could follow the progress of infection in porcine cysticercosis and the antibody responses against rGp50 and sTs18var1 correlated well with cysts viability, although the overall performance of the FAST-ELISA for sTs18var1 was better than for rGp50.

Received August 21, 2003. Accepted for publication April 1, 2004.

Financial support: This research was supported by International Collaborations in Infectious Disease Research—National Institutes of Health (NIH) grant U01 AI-35894, NIH National Institute of Allergy and Infectious Diseases Tropical Medicine Research Center grant 1 P01 AI-51976-01, Wellcome grant 063109, and Bill and Melinda Gates foundation grant 23981.

Authors' addresses: Sukwan Handali, Kathy Hancock, and Victor C. W. Tsang, Immunology Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Mailstop F-13, Atlanta, GA 30341-3724, E-mails: ahi0@cdc.gov, kyh7@cdc.gov, and vct1@cdc.gov. Armando E. Gonzalez, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Cuadra 29 Avenida Circunvalacion s/n, San Borja, Lima, Peru, E-mail: emico@terra.com.pe. Hector H. Garcia, Department of Microbiology, Universidad Peruana Cayetano Heredia, Avenida Honorio Delgado 430, San Martin de Porras, Lima, Peru and Cysticercosis Unit, Instituto de Ciencias Neurológicas, Lima, Peru, E-mail: hgarcia@terra.com.pe. Jacquelin M. Roberts, Data Management Activity, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Mailstop F-22, Atlanta, GA 30341-3724, E-mail: jmr1@cdc.gov. Robert H. Gilman, Department of International Health, Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205, E-mail: gilmanbob@yahoo.com.

REFERENCES

- Garcia HH, Gilman R, Martinez M, Tsang VC, Pilcher JB, Herrera G, Diaz F, Alvarado M, Miranda E, 1993. Cysticercosis as a major cause of epilepsy in Peru. The Cysticercosis Working Group in Peru (CWG). *Lancet* 341: 197–200.
- Garcia HH, Del Brutto OH, 2000. *Taenia solium* cysticercosis. *Infect Dis Clin North Am* 14: 97–119.
- Gonzalez AE, Gilman R, Garcia HH, McDonald J, Kacena K, Tsang VC, Pilcher JB, Suarez F, Gavidia C, Miranda E, 1994. Use of sentinel pigs to monitor environmental *Taenia solium* contamination. The Cysticercosis Working Group in Peru (CWG). *Am J Trop Med Hyg* 51: 847–850.
- Gonzalez AE, Verastegui M, Noh JC, Gavidia C, Falcon N, Bernal T, Garcia HH, Tsang VC, Gilman RH, Wilkins PP, 1999. Persistence of passively transferred antibodies in porcine *Taenia solium* cysticercosis. Cysticercosis Working Group in Peru. *Vet Parasitol* 86: 113–118.
- Gonzalez AE, Gavidia C, Falcon N, Bernal T, Verastegui M, Garcia HH, Gilman RH, Tsang VC, Cysticercosis Working Group in Peru, 2001. Protection of pigs with cysticercosis from further infections after treatment with oxfendazole. *Am J Trop Med Hyg* 65: 15–18.
- 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.
- Hancock K, Tsang VCW, 1986. Development and optimization of the FAST-ELISA for detecting antibodies to *Schistosoma mansoni*. *J Immunol Methods* 92: 167–176.
- Richards FO, Tsang VCW, Brand JA, Hancock K, 1989. Modification of the FAST-ELISA for field diagnosis of *Schistosomiasis mansoni* with serum of blood samples. *Trop Med Parasitol* 40: 332–334.
- Greene RM, Wilkins PP, Tsang VC, 1999. Diagnostic glycoproteins of *Taenia solium* cysts share homologous 14- and 18-kDa subunits. *Mol Biochem Parasitol* 99: 257–261.
- Greene RM, Hancock K, Wilkins PP, Tsang VC, 2000. *Taenia solium*: molecular cloning and serologic evaluation of 14- and 18-kDa related, diagnostic antigens. *J Parasitol* 86: 1001–1007.
- Hancock K, Patabhi S, Greene RM, Yushak ML, Williams F, Khan A, Priest JW, Levine MZ, Tsang VC, 2004. Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. *Mol Biochem Parasitol* 133: 115–124.
- Hancock K, Khan A, Williams FB, Yushak ML, Patabhi S, Noh J, Tsang V, 2003. Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. *J Clin Microbiol* 41: 2577–2586.
- Garcia HH, Gilman RH, Catacora M, Verastegui M, Gonzalez AE, Tsang VC, 1997. Serologic evolution of neurocysticercosis patients after antiparasitic therapy. *J Infect Dis* 175: 486–489.
- Youden WJ, 1950. Index for rating diagnostic tests. *Cancer* 3: 32–35.
- Elandt-Johnson RC, 1999. *Survival Models and Data Analysis*. New York: John Wiley & Sons, Inc.
- Xiao BG, Link H, 1998. Immune regulation within the central nervous system. *J Neurol Sci* 157: 1–12.
- Garcia HH, Gonzalez AE, Gilman RH, Palacios LG, Jimenez I, Rodriguez S, Verastegui M, Wilkins P, Tsang VC; Cysticercosis Working Group in Peru, 2001. Short report: transient antibody response in *Taenia solium* infection in field conditions—a major contributor to high seroprevalence. *Am J Trop Med Hyg* 65: 31–32.
- Garcia HH, Herrera G, Gilman RH, Tsang VC, Pilcher JB, Diaz JF, Candy EJ, Miranda E, Naranjo J, 1994. Discrepancies between cerebral computed tomography and Western blot in the diagnosis of neurocysticercosis. The Cysticercosis Working Group in Peru (Clinical Studies Coordination Board). *Am J Trop Med Hyg* 50: 152–157.