PROTECTION OF PIGS WITH CYSTICERCOSIS FROM FURTHER INFECTIONS AFTER TREATMENT WITH OXFENDAZOLE

ARMANDO E. GONZALEZ, CESAR GAVIDIA, NESTOR FALCON, TERESA BERNAL, MANUELA VERASTEGUI, HECTOR H. GARCIA, ROBERT H. GILMAN, VICTOR C. W. TSANG, AND THE CYSTICERCOSIS WORKING GROUP IN PERU*

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

Abstract. Cysticercosis, the infection by the larvae of Taenia solium, is a major cause of acquired epilepsy in the world; it also causes significant economic loss because of contaminated pork. This disease is endemic in most developing countries and no control strategy has yet been proven efficient and sustainable. To further evaluate the full potential of single-dose oxfendazole treatment for pigs as a control measure, 20 pigs with cysticercosis were treated with oxfendazole and later matched with 41 naïve pigs and exposed to a natural challenge in a hyperendemic area. New infections were found by serologic testing in 15 of the 32 controls (47%), and by the presence of cysts at necropsy in 12 of them (37%). Only minute residual scars were detected in the carcasses of oxfendazole-treated pigs. Pigs with cysticercosis, once treated with oxfendazole, are protected from new infections for at least three months.

INTRODUCTION

Taenia solium cysticercosis is endemic in most developing countries. It increases the prevalence of epilepsy in humans, and causes significant economic loss because of damaged infested pork. The life cycle of T. solium includes the pig as the normal intermediate host and humans as the definitive host, who harbor the adult tapeworm. Although the rates of porcine infection with T. solium are variable, in disease-endemic areas more than 50% of the pigs may be seropositive for T. solium antigens. As the intermediate host and only reservoir source for new adult tapeworms, the pig is a key link in the transmission cycle that has been overlooked in control strategies. Several groups are working on the development of vaccines against porcine cysticercosis, but an effective one has not yet been developed.

Besides interrupting transmission, successful treatment of pigs with an inexpensive, effective drug may give peasant farmers access to commercial markets, with better prices for their animals. The latter could be a strong incentive for farmers to treat their pigs, ensuring community cooperation and promoting long-term compliance with control measures.

We have shown that oxfendazole (OFZ), an inexpensive benzimidazole, is almost 100% effective when used as a single-dose therapy for porcine cysticercosis. The present study was designed to determine if pigs with cysticercosis can acquire new infections after being treated with OFZ. This information is vital for evaluating the full potential of this drug in field control programs.

MATERIALS AND METHODS

Design. This is a prospective, controlled study that compares rates of new infections by cysticerci in previously infected pigs that had been treated with OFZ with naïve, uninfected control pigs, upon subsequent exposure.

Study settings. The study was performed in Casacancha, a small village in the central highlands of Peru. The village is located near Huancayo, 300 km east of Lima, at an altitude of 3,400 meters, and was selected on the basis of known cysticercosis endemicity and long-term successful collaboration with villagers during previous surveys.

Animals. Twenty pigs naturally infected with cysticerci (positive by tongue palpation) were bought in informal markets in Huancayo, a disease-endemic area, and 41 cysticerci-negative pigs were purchased eight weeks later from a cysticerci-free commercial farm in Huancayo City. Infection status was confirmed by serologic antibody test with the enzyme-linked immunoelectrotransfer blot (EITB).

Methods. Infected animals were purchased, transported to our specific pathogen-free (SPF) facility in Lima (San Marcos Veterinary School), and treated with a single oral dose of 30 mg/kg of OFZ given as a veterinary aqueous suspension. Treated pigs were kept here for eight weeks after treatment to ensure that all muscle cysts were killed by the drug. Uninfected animals of similar age and weight were purchased, and each treated pig was matched by age and sex with two uninfected animals to increase precision. All pigs were vaccinated against hog cholera, transported to the disease-endemic village, and boarded with village families (three pigs per family: one treated and two uninfected controls). At this time, blood samples were taken from all village pigs to establish baseline seroprevalence of cysticercosis.

Three months later, all study animals were re-purchased from villagers at market prices, transported back to the SPF facility in Lima, and kept for an additional three months, equivalent to the amount of time that cysts take to achieve full maturity. Pigs were then killed humanely in a commercial abattoir and a detailed dissection was performed to determine parasitic burden. Serologic status of all pigs was again determined by EITB after the challenge period and at the time of necropsy.
Serology. Blood samples were taken from the cava vein and processed by EITB as previously described. Briefly, this assay uses seven purified *T. solium* glycoprotein antigens (diagnostic bands GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13) in an immunoblot format to detect infection-specific antibodies in pigs. Reactions to at least one band are considered positive.

Determination of parasitic burden. Excised cysts were separated into apparently healthy or degenerated cysts. The total number of viable cysts and degenerating cysts was determined for each pig.

Control pigs were considered infected if viable or degenerating cysts were found in the carcasses at necropsy. Treated pigs were defined as re-infected if viable cysts were present in the carcasses. Although brains were examined, the number of viable cysts in the brains was not considered in the analysis because cerebral cysts may occasionally survive single-dose OFZ therapy. Thus, pigs with only viable cysts in their brains may not represent successful re-infection but treatment failure.

Macroscopically degenerated cysts are rarely, if ever, viable. Because of the small numbers of cysts found in this experiment, we decided to save the material for histopathologic examination instead of using it to test viability by evagination.

Analysis. The main outcome was the number of exposed pigs that became infected during the challenge exposure period. Infection was recognized in control pigs at two levels, acquisition of cysts (viable or degenerated) or seroconversion by EITB. The definition for new cases in the treated pigs was the presence of viable cysts in the muscles. Association between variables was investigated using the chi-square test or Fisher’s exact test as appropriate.

RESULTS

The seroprevalence of cysticercosis in native pigs in Casacancha was 75% (73 of 97 animals). Of the original 61 experimental pigs, 51 (84%) were recovered at the end of the study, as were 19 (95%) of 20 of the treatment group and 32 (78%) of 41 of the naive controls. One treated and nine control pigs were not recovered because of various reasons, mostly because villagers sold or slaughtered them for consumption.

New infections were detected by serology in 15 (47%) of 32 control pigs, and by the presence of cysts in 12 (38%) of 32. Among these 12 pigs, viable cysts were found in seven carcasses (viable only = 3 and viable and degenerated = 4), and only degenerated cysts were found in another five animals. The numbers of cysts in these newly infected animals ranged between five and 30 per pig. Conversely, no viable cysts were found in the carcasses of the 19 treated pigs (12 of 32 versus 0 of 19; \( P = 0.001\), by Fisher’s exact test).

Multiple, minute residual scars were found in 15 of the treated pigs, but most of these were thoroughly absorbed and almost totally replaced with muscular tissue (Table 1). In the remaining four treated pigs, no traces of infection were visible.

Viable cysts were found in the brains of two of the seven newly infected control pigs that had viable cysts in their carcasses, and in three of 19 pigs in the OFZ-treated group.

<table>
<thead>
<tr>
<th>Findings at necropsy*</th>
<th>OFZ-treated pigs (n = 19)</th>
<th>Naive control pigs (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cysts only</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Viable and degenerated cysts only</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Degenerated cysts only</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Residual scars only</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>No cysts</td>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

* No viable cysts were found in the carcasses of any of the 19 treated pigs (12 of 32 versus 0 of 19; \( P = 0.001\), by Fisher’s exact test).

All of these animals had viable cysts (controls) or residual scars (OFZ-treated group) in their muscles.

DISCUSSION

The control or eradication of *T. solium* cysticercosis has only been achieved in a few countries by significantly improving sanitary conditions and developing functional slaughterhouse control systems. In disease-endemic areas of developing countries, the life cycle of *T. solium* is sustained because pigs have access to infected feces, and cysticerci-infested pork is available for consumption. Therefore, control in developing countries has been limited by economic and sanitary conditions, and despite intervention trials with massive chemotherapy, immunotherapy, and health education, no intervention to date has proven effective and sustainable. Furthermore, the current inspection and condemnation policies of many countries encourages high rates of infection by targeting the slaughterhouse only, and failing to provide financial market incentives to reimburse the farmer.

The use of OFZ as a single-dose effective therapy for porcine cysticercosis provides a new tool for control in field conditions. This study demonstrates that infected pigs treated with OFZ are not re-infected for at least three months after therapy and thus the life cycle of the parasite is interrupted at this point. In field conditions, most pigs live approximately nine months. Cysts take about two months to develop, so it is reasonable to assume that pigs will be infective only after 3–4 months of age. The results of this study imply that, if treated at this time, cured pigs will not be re-infected until at least seven months of age, and it is very probable that this protection will extend for longer periods and thus cover the remaining lifetime of the pig.

A minor drawback in the use of OFZ is that some cysts may survive in the pig’s brain after treatment. This limitation is relatively inconsequential because brains are seldom, if ever, consumed in an undercooked state. Therefore, the life cycle would be interrupted even in those cases. Another problem is that infected meat (muscle) in treated pigs needs at least eight weeks for all the cysts to degenerate and up to 12 weeks to achieve a clear, acceptable appearance for human consumption.

Whether the low numbers of viable and degenerating cysts found in the control pigs are due to exposure to infection after weaning cannot be evaluated from the findings of this study. However, it is significant that approximately 50% of...
the control pigs showed evidence of exposure or infection during the study (47% seroconverted and 38% were parasite positive at necropsy), while none of the OFZ-treated pigs showed signs of re-infection. The presence of degenerated cysts in 15% of the control pigs may be caused by several factors. First, these pigs were exposed to parasites later in life, while most porcine cysticercal infection occurs early, when the animals are much younger. Second, cyst degeneration also occurs in naturally infected pigs, especially those with a low cyst burden (Gonzalez AE, unpublished data). Third, sentinel pigs (three-month old pigs from a non-endemic area) placed in an endemic area to monitor infection, or infection models in non-natural (non-porcine) hosts also have degenerate cysts, or unsuccessful or aborted infections. Possible explanations include differences in individual host responses and partial immunity from previous exposures.

The use of sentinel pigs as controls was developed to monitor infection in an endemic area over time. In the current study, experiments with sentinel pigs allowed us to evaluate immunity against cysticercosis. We showed that protection can be achieved by treating infected pigs. This could mean that OFZ is potentially an effective control agent because once treated, pigs are refractory to re-infection even if the source of infective eggs (adult tapeworm carrier) is still present. Although other concomitant measures are still needed since seronegative pigs still remain susceptible to infection, our findings demonstrate the potential for developing a viable vaccine for porcine cysticercosis. Meanwhile, the treatment of infected pigs with OFZ should be considered an important, cost-effective addition to the control of cysticercosis.

Financial support: This work was supported by grant number 1-U01 A135894-01 from the National Institutes of Health, and Emerging Infectious Diseases funding from the Centers for Disease Control and Prevention.

Authors’ addresses: Armando E. Gonzalez, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Av. Circuncvalacion s/n, Salamanca de Monterrico, Lima, Peru and A. B. PRISMA, Carlos Gonzalez 251, San Miguel, Lima, Peru. Cesar Gavidia, Nestor Falcon, and Teresa Bernal, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Av. Circuncvalacion s/n, Salamanca de Monterrico, Lima, Peru. Manuela Verastegui, Nestor Falcon, and Teresa Bernal, School of Veterinary Medicine, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin de Porras, Lima, Peru. Hector H. Garcia, Departments of Microbiology and Pathology, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin de Porras, Lima, Peru and Department of Transmissible Diseases, Instituto Nacional de Ciencias Neurologicas, Jr. Ancash 1271, Barrios Altos, Lima, Peru. Robert H. Gilman, A. B. PRISMA, Carlos Gonzalez 251, San Miguel, Lima, Peru. Departamentos de Microbiología and Pathology, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin de Porras, Lima, Peru and Department of Transmissible Diseases, Instituto Nacional de Ciencias Neurologicas, Jr. Ancash 1271, Barrios Altos, Lima, Peru, and Department of International Health, Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205. 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.

REFERENCES


