TIME-RESPONSE CURVE OF OXFENDAZOLE IN THE TREATMENT OF SWINE CYSTICERCOSIS

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Abstract. Human Taenia solium cysticercosis is a major cause of epilepsy in developing countries, and porcine infection causes widespread economic losses because of infected pork. Recently, the use of oxfendazole (OFZ) for porcine cysticercosis provided, for the first time, an effective, single-dose treatment. We performed a controlled study to determine the time required between treatment with a single dose of OFZ and the death of cysticerci to define its applicability as preslaughter treatment or as a field control measure. Twenty naturally infected pigs were included in this study. Sixteen received a single dose (30 mg/kg) of OFZ, and were killed in groups of four at one, two, four, and 12 weeks after treatment. Four untreated controls were killed at week 12. No adverse reactions to OFZ were noted. A clear decrease in viability and number of cysts was evident after the first week after therapy, but even at week 4 some viable cysticerci were found in all samples. Twelve weeks after treatment, all meat appeared clear and only minuscule scars remained, except in one animal that had viable brain cysts. This study confirms the efficacy of a single dose of 30 mg/kg of OFZ for porcine cysticercosis but demonstrates that preslaughter treatment of pigs with OFZ will not be useful in controlling cysticercosis. The inclusion of porcine treatment with OFZ in mass cysticercosis control programs is, however, highly promising because it is a simple, effective, inexpensive, and potentially sustainable method for decreasing the porcine reservoir of cysticercosis in disease-endemic countries.

Taenia solium is a common parasite in pig-raising areas of developing countries.1 The life cycle of this cestode includes pigs as the normal intermediate host, harboring the larval vesicles or cysticerci, and humans as the definitive host, harboring the adult form of the tapeworm. Humans can also serve as the intermediate host and develop the cystic form when they ingest T. solium eggs in fecal-contaminated materials.2 Porcine cysticercosis produces widespread economic losses because of damage by cysticerci in the meat,3 and human cysticercosis is an important contributor to neurologic abnormalities in disease-endemic areas.1,4

The rates of porcine infection in Peru are variable, but in highly disease-endemic regions, between one-third and more than half of the pigs reared in villages may be infected5 (Gonzalez AE, unpublished data). Most of the efforts related to the control of swine cysticercosis are based on veterinary inspection in the abattoirs. Once detected, T. solium-infected pigs are confiscated,6 thus prompting residents to avoid the use of official slaughterhouses. Consequently, the current system of control, or lack thereof, encourages clandestine marketing of pigs and permits the commercialization of infected pork, which is camouflaged either by red dye or by mixing it with uninfected meat.7

During the last decade, several approaches to the control of T. solium cysticercosis, including mass chemotherapy for T. solium in inhabitants of disease endemic zones, health education, and mass porcine vaccination8-11 have been tested in field conditions, with promising but incomplete results. Porcine chemotherapy was considered impractical because of partial efficacy, high cost, and the need for multiple doses.12-15 However, after the recent introduction of oxfendazole (OFZ) as the first effective, single-dose treatment for porcine cysticercosis,16 pig treatment (alone or in combination with other measures), must be considered as a potential intervention measure.

Survival of cysticerci in host tissues involves active immune evasion mechanisms.17,18 Previous studies using praziquantel12 and flubendazole13 have shown that cyst death is not immediate after treatment. It is hypothesized that although anthelmintic drugs affect parasite metabolism and damage the cyst, the death of the parasite occurs later as a result of direct attack on the damaged cyst by the immune system of the host.19 In the context of control programs, the time between therapy and cyst death is extremely important. If death is delayed beyond a few days, then OFZ treatment of infected pigs would not be a useful strategy for slaughterhouse control. Conversely, if the process is prolonged, both the time before slaughtering when the treatment must be administered and the potential for reinfection must be addressed. This controlled study determined the time period between pig treatment with a single dose of OFZ and the death of T. solium cysticerci.

MATERIALS AND METHODS

Animals. Twenty pigs reared by small-scale village farmers and sold for slaughter were obtained from Huancayo, a city in the Peruvian Sierra, and brought to our infection-free veterinary facilities in Lima for the duration of the study. All pigs had palpable nodules in the tongue, inferring heavy infection with cysticerci.19 In Lima, all pigs were weighed, vaccinated against hog cholera, and acclimated in the veterinary facility for a three-week period. The pigs were fed freely and no other medications were given. Four infected animals that received no treatment were defined as nontreated infected controls. The other 16 animals received a single dose of 30 mg/kg of OFZ and were randomly assigned into...
groups of four to be killed at one, two, four, and 12 weeks after treatment. This schedule was based on our previous study that showed that 12 weeks after OFZ treatment all cysts were dead and only visible as miniscule scars. The four untreated controls were killed at the end of the experiment (week 12).

Necropsy. Pigs were anaesthetized and humanely killed. In addition to standard necropsy procedures, the heart, tongue, brain, psoas, and anconeal muscles were removed. The left psoas and anconeal muscles and left half of the brain, tongue and heart were weighed and carefully dissected to evaluate parasitic burden. The number of cyst for each tissue sample was recorded and used to calculate the number of cysts and/or scars per 100 grams. Efficacy of therapy was measured by cyst evagination.

Evagination procedure. All cysticerci in each sample (up to 100 cysticerci) were removed from surrounding tissues and washed in sterile phosphate-buffered saline (0.02 M Na₂HPO₄/NaH₂PO₄, 0.15 M NaCl, pH 7.2). Washed cysts were then incubated in bovine bile salts at 37°C for 2 hr. The criteria used to define a positive evagination was the presence of a moving scolex outside the bladder wall. The number of cyst used and the proportion of cysts that evaginated was recorded for each tissue from every pig.

Serology. Serum samples were taken from the cava vein from every pig at baseline and at the time of necropsy. Enzyme-linked immunoelectrotransfer blot (EITB) assays were performed as previously described. Briefly, this assay uses seven purified *T. solium* glycoprotein antigens (diagnostic bands GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13, with the number indicating the respective molecular weight in kD) in an immunoblot format to detect infection-specific antibodies. Reactions to at least one band are considered positive. The EITB assay was interpreted with reference to the above described seven glycoprotein bands, commonly recognized by antibodies from serum in human and swine cases.

Data analysis. Outcome, independent, and potentially confounding variables were entered into a database and analyzed with the Statistical Package for the Social Sciences (SPSS+ 4.0) software (SPSS Inc., Chicago, IL). Differences among groups were identified by using a nonparametric one-way analysis of variance (Kruskall-Wallis). The function of time on cyst viability was calculated using the least squares estimators for the regression coefficients. The time to zero viability was estimated with the resulting regression equation.

RESULTS

Side effects. After OFZ therapy, no visible adverse reactions were noted. The pigs all fed normally and demonstrated no signs of illness.

Efficacy of therapy. A clear decrease in viability and number of cysts was noted after the first week after therapy (Tables 1 and 2), but even at week 4 some live cysticerci were found in all tissues. Twelve weeks after OFZ treatment, the meat examined was clear and only miniscule scars were observed (Figure 1), except in one animal that had viable cysts in the brain.

The predicted time to total viability decay depended on the organ. The time for zero viability in muscle and heart was 3.94 and 3.47 weeks, respectively. Interestingly, the time for the tongue, another voluntary muscle, was 4.7 weeks. Due to higher viability at week 4 than at week 2, and to the presence of live cysts in one sample, it is not possible to evaluate the time for total decay in brain cysts evagination, although three of four animals did not have viable brain cysts at week 12. Significant negative correlations between percent evagination and time were found for muscle (correlation index = −0.65, *P* < 0.05; Figure 2), heart (correlation index = −0.80, *P* < 0.05), and tongue (correlation index = −0.69, *P* < 0.05).

Serology. All sera samples were positive on the EITB assay, including those taken 12 weeks after therapy in the treatment control group.
The treatment of *T. solium*-infected pigs with OFZ as part of a control program for cysticercosis has the advantage of being relatively inexpensive, sustainable, and culturally acceptable. Oxfendazole is superior to other agents for this purpose because it is nearly 100% effective and safe when given as a single dose. This study demonstrates, however, that cyst death is not immediate but rather requires a period of at least one month after treatment before cyst viability is reduced to levels that will effectively control the *T. solium* life cycle.

Cysts do not die immediately after antiparasitic therapy of infected pigs. Apparently, anthelminthic drugs induce changes in the cysts (e.g., benzimidazole agents bind to parasite tubulin, inhibiting its polymerization into microtubuli, and alter glucose uptake), exposing hidden antigens that prompt an immune response that finally kills the larvae. Histopathologically, cysts treated with praziquantel are attacked first by eosinophils and then by lymphocytes, suggesting that cyst death is associated with an immunopathologic type of lesion. In sheep, the half-life of OFZ is 8–20 hr, and the drug or its metabolites are not detectable in plasma after one week when given at a dose of 10 mg/kg. Being monogastric, these time periods must be even shorter in swine, yet cysts remain viable for periods beyond four weeks after treatment. This delay in cyst death suggests that an immunologic reaction may be responsible for delayed cyst death.

Our pigs actually had less viable cysts at two compared...
with four weeks after therapy. We have no explanation for this except that it may be due to the variability that occurs with the small number of animals used per group. In a previous study, we have demonstrated that doses less than 30 mg/kg were not 100% effective in killing all cysts.\textsuperscript{20} In this study, we demonstrated again that all cysts except for some in one brain sample were killed by 12 weeks when OFZ was given as a single 30 mg/kg dose. This is the first study in which viable cysts have been found after treatment with a single dose of 30 mg/kg of OFZ. Survival of cysts in brain tissue may be explained by lower concentrations of the drug or reduced immune efficacies in the central nervous system because of the blood-brain barrier.\textsuperscript{27} Pig brain is not commonly eaten raw; thus, it is highly improbable that cysts that survive only in the brain will be ingested and perpetuate the cycle.

This study demonstrates that preslaughter treatment of pigs with OFZ will not be a useful strategy to control cysticercosis. Treatment with OFZ should be used eight or more weeks before a pig is brought to slaughter because killed cysts are still visible, thus making it difficult to market the meat.\textsuperscript{20} The use of OFZ as a control measure in the village is, however, highly promising. Most control programs to date are limited to the treatment of humans alone, leaving the huge pig reservoir of cysticercosis untouched and available to infect and complete the cycle in humans. Adding a pig treatment arm to control programs is that it uses economic pressure to drive the control program. Unlike human disease, porcine cysticercosis is felt by villagers as an important problem: \textit{T. solium}-infected pigs if sold in the formal market may be confiscated and in the informal market will get much lower prices than uninfected animals. This economic pressure may serve as an incentive for locally administered porcine treatment programs, providing both community support and sustainability long term. In summary, single dose OFZ treatment of pigs should be included in mass cysticercosis control programs as a simple but effective method of decreasing the porcine reservoir of cysticercosis in disease-endemic countries.

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