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Evaluation of activity of triclabendazole against *Taenia solium* metacestode in naturally infected pigs

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Abstract

Objective: To assess the efficacy of triclabendazole (TCBZ) in porcine cysticercosis.

Methods: Eighteen naturally infected cysticercosis pigs were divided into 3 groups of 6 individuals each. The first group was treated orally with TCBZ at a single dose of 30 mg/kg of body weight, the second group was treated orally with oxfendazole at a single dose of 30 mg/kg of body weight and the third group received a placebo (control group). All animals were kept under the same management conditions. The pigs were euthanized 17 wk post-treatment and the number of surviving cysts in muscles was assessed and compared between groups.

Results: All pigs treated with oxfendazole had only degenerated cysts in their carcasses. In contrast, TCBZ had very little effect against the parasitic cysts. Cysts from pigs in the TCBZ group looked apparently normal after treatment. However, histological evaluation showed a mild to moderate degree of inflammation.

Conclusions: TCBZ is not an efficacious drug against *Taenia solium* cysticercosis in swine using a single dose.

Keywords

Taenia solium; Cysticercosis; Triclabendazole; Oxfendazole; Treatment

1. Introduction

The disease complex, taeniasis/cysticercosis caused by the pork tapeworm *Taenia solium* (*T. solium*), is a very important zoonotic disease in developing countries [1,2]. The adult *T.*

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Conflict of interest statement

We declare that we have no conflict of interest.

solium tapeworm localizes in the small intestine of humans, the definitive host. The eggs of *T. solium* are eliminated into gravid proglottids with the faeces, and then they are ingested by pigs, the intermediate host. The *T. solium* metacestode then develops in different organs of the pig, principally muscles [3,4]. However, humans can act as accidental hosts when they ingest eggs in contaminated food [3,5].

During the last decade, a variety of strategies have been performed for the control of *T. solium* cysticercosis including animal inspection, massive chemotherapy for *T. solium* in humans and pigs from endemic areas, health education, and pig vaccination [2,6-9]. For this purpose, many studies have been performed to evaluate the efficacy of different drugs against porcine cysticercosis [10]. Of these drugs, albendazole and oxfendazole (OFZ), members of the benzimidazole drugs, are efficacious for the treatment of the porcine cysticercosis [11]. Albendazole has been used in humans since 1979, and currently it is considered the drug of preference in humans for its availability, low cost and efficacy [5,11]. Albendazole does not seem to be a good option for field interventions due to the requirement for multiple doses [10]. OFZ, used in a single oral dose of 30 mg/kg, is the drug of choice for the treatment of porcine cysticercosis [12]. The widely used triclabendazole (TCBZ), another member of the benzimidazole family, is efficacious against adults and larval stages of a variety of helminthic parasites [13,14]. The aim of this study was to compare the efficacy of a single oral dose of TCBZ or OFZ against *T. solium* cysticercosis in naturally infected pigs.

2. Materials and methods

2.1. Study design and settings

The study was conducted at the animal facilities of the Laboratory of Veterinary Epidemiology and Economics, School of Veterinary Medicine, National University of San Marcos in Lima, Peru. Pigs were acquired in a cysticercosis-endemic area and transported to the animal facilities in Lima. Pigs were randomized in three similar groups (TCBZ, OFZ, and placebo) using a random numbers table. All pigs were coded and labelled with ear tags.

2.2. Animals

Eighteen adult pigs were acquired in Huancayo, a cysticercosis-endemic area in the Peruvian highlands [15]. All animals were positive to cysticercosis by tongue examination, and confirmed by antibody detection on serum enzyme-linked immunoelectro transfer blot [16,17]. Only animals with more than one cyst in the tongue and more than four antigen bands in enzyme-linked immunoelectro transfer blot were included in the study. All pigs were vaccinated against hog cholera immediately after purchase.

2.3. Treatment protocol

The pigs were randomly placed in three different groups of six pigs each. Pigs in group 1 received a single oral dose of 30 mg/kg body weight of TCBZ (Trisan 12%, Montana S.A., Peru). Pigs in group 2 were treated orally with OFZ (Synanthic® 9.06%, Fort Dodge, Mexico), using a single dose of 30 mg/kg body weight. Control pigs (group 3) received sugar water as a placebo. Each treatment group was housed in a single pen.

2.4. Necropsy

All animals were euthanized 17 wk after treatment, and necropsied at the School of Veterinary Medicine facilities in Lima, Peru. Pigs were anesthetized and sacrificed by intramuscular injection of a combination of ketamine (20 mg/kg) and xylazine (2 mg/kg) followed by intravenous injection of pentobarbital sodium overdose (60 mg/kg). Necropsy was performed immediately after euthanasia, and muscles from arms and legs were dissected, and all cysts were counted and classified. Cysts were classified as viable if a defined cystic structure with clear liquid content was still present, and degenerated if this had been replaced by semi-solid contents or an inflammatory scar (calcified nodule).

2.5. Histological analysis

Five randomized cysts from the muscles of each pig were fixed in 10% formalin, embedded in paraffin, then sectioned at 5 μ m and stained with haematoxylin and eosine (H&E), as well as Von Kossa stain to assess the presence of calcium.

2.6. Data analysis

Parasitic load was expressed in median with their respective ranges. A non-parametric one-way analysis of variance (Kruskal–Wallis) test was used to estimate the difference in the number of cysts between treatment groups. Data was analysed using STATA 10 statistical software (v10.0; StataCorp LP, College Station, TX). Differences were considered statistically significant at P -value <0.05 .

2.7. Ethical approval

The study was approved by the Animal Ethics Committee of the School of Veterinary Medicine, National University of San Marcos, Lima, Peru.

3. Results

During treatment and follow up, pigs from the treated groups involved in this experiment were apparently normal without apparent adverse events. All pigs used in this study were positive to cysticercosis at tongue examination as well as positive to 4 or more antibody bands enzyme-linked immunoelectro transfer blot. After necropsy, the overall parasite load including viable or degenerated cysts for each group was 1658 (131–2575) (median, interquartile range) cysts for control group, 1414 (511–4052) cysts for TCBZ treated group, and 259 (38–1953) cysts for OFZ treated group (Table 1). All remaining cysts in the OFZ pigs were evidently degenerated. Furthermore, the numbers of cysts were statistically significantly smaller in OFZ group compared with the TCBZ and control groups ($P < 0.01$).

TCBZ had very little effect against the parasitic cysts. Cysts from animals in the TCBZ group looked apparently normal after treatment. However, histological evaluation showed a mild to moderate degree of inflammation composed of eosinophils, macrophages, lymphocytes, plasmocytes, and fibroblasts with connective tissue in the surrounding parenchyma (Figure 1) in all samples from this group. On the other hand, OFZ killed 100% of cysts in treated pigs, and all animals in this group showed only degenerated cysts. The inflammatory reaction around cysts from the OFZ group was severe, and also demonstrated

areas of dystrophic calcification (Figure 2). No inflammatory reaction was observed in pericystic tissue samples from pigs in the control group.

4. Discussion

T. solium cysticercosis remains a public health issue in many developing countries, and therefore, the evaluation of new alternative drugs for its control is urgently required. A very large variety of antiparasitic drugs have been tested against cysticercus so far [10]. Although almost all these anthelmintic drugs are active against adult stages of cestodes, nematodes and trematodes, only a few drugs that have a positive effect against its larval stage [11,18].

TCBZ, a benzimidazole drug, has a potent effect against adult and larval stages of some tissue helminths such as *Fasciola* and *Paragonimus* [14]. Likewise, studies *in vitro* have been shown effective activity in some nematodes and cestodes [13,19]. On these bases, we evaluated the efficacy of TCBZ against porcine cysticercosis. TCBZ in a single dose of 30 mg/kg was not effective against cysticercosis in this group of naturally infected pigs. On necropsy, there were no apparent differences in cyst burden or cyst appearance between pigs treated with TCBZ and pigs in the control group.

Microscopic evaluation of cysts from treated pigs with TCBZ evidenced mild to moderate inflammatory reaction with presence of different types of leukocytes. This indicates that cysts are not found completely healthy, as observed at necropsy. This mild inflammatory reaction, macroscopically imperceptible, suggests that TCBZ has a slight effect against cysticerci raising the question of whether higher or multiple doses could be efficacious against cystic cestode larvae, as shown by Richter *et al.* [19]. In that study, TCBZ had a positive activity against *Echinococcus multilocularis* metacestodes *in vitro*, using a 20-d treatment regime. Increased doses or multiple doses would still make TCBZ poorly practical for use in porcine cysticercosis in field conditions.

Several antihelminthic drugs (*i.e.* albendazole, flubendazole, fenbendazole, nitazoxanide, and praziquantel) have been tested for the treatment of porcine cysticercosis. Albendazole was evaluated using a single (50 mg/kg) and multiple doses (30 mg/kg) in experimentally infected pigs, and it was effective using multiple doses (3 consecutive days). However, some pigs treated to single and multiple doses showed secondary effects as anorexia, lethargy, prostration and death [11]. Nitazoxanide was tested in naturally infected pigs using multiple doses (150 mg/kg) by 7 consecutive days, and it was not effective against cysts. Some pigs treated with nitazoxanide developed prostatic hypertrophy and bladder distention [11]. In this experiment, single doses of 30 mg/kg of either OFZ or TCBZ did not produce any adverse effects in pigs. They were apparently normal and did not show any signs of toxicity during and after treatment.

Flubendazole and fenbendazole have show efficacy against porcine cysticercosis when used in multiple doses, 40 mg/kg for 10 d and 5 mg/kg for 7 d, respectively, without secondary effects. Furthermore, praziquantel has been shown to have efficacy against cysticerci using daily doses of 50 mg/kg for 15 consecutive days [11,20]. However, animal treatments using multiple doses are impractical in field conditions and not suitable for mass chemotherapy

campaigns in endemic zones. In summary our findings do not support the use of TCBZ for porcine cysticercosis, and confirm the efficacy of a single dose of OFZ for muscle cysticercosis cysts.

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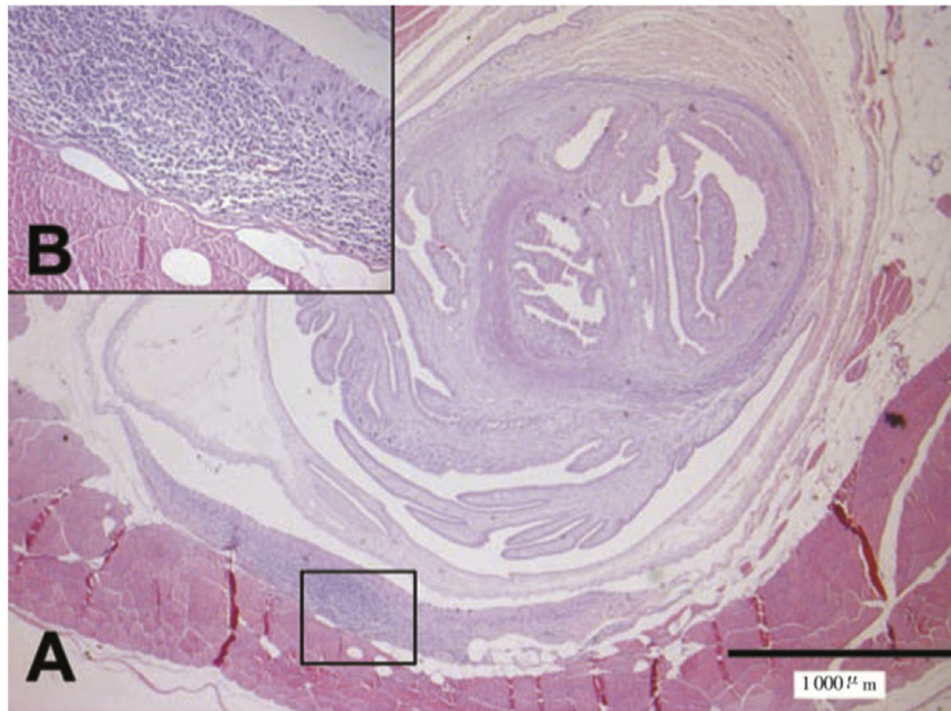


Figure 1. Histological section of cysts from a pig treated with TCBZ (H&E stain). It showed a significant inflammatory reaction. Multifocal lymphocytic myositis (A). Presence of lymphocytes, eosinophils, macrophages, plasmacytes, and fibroblasts (B).

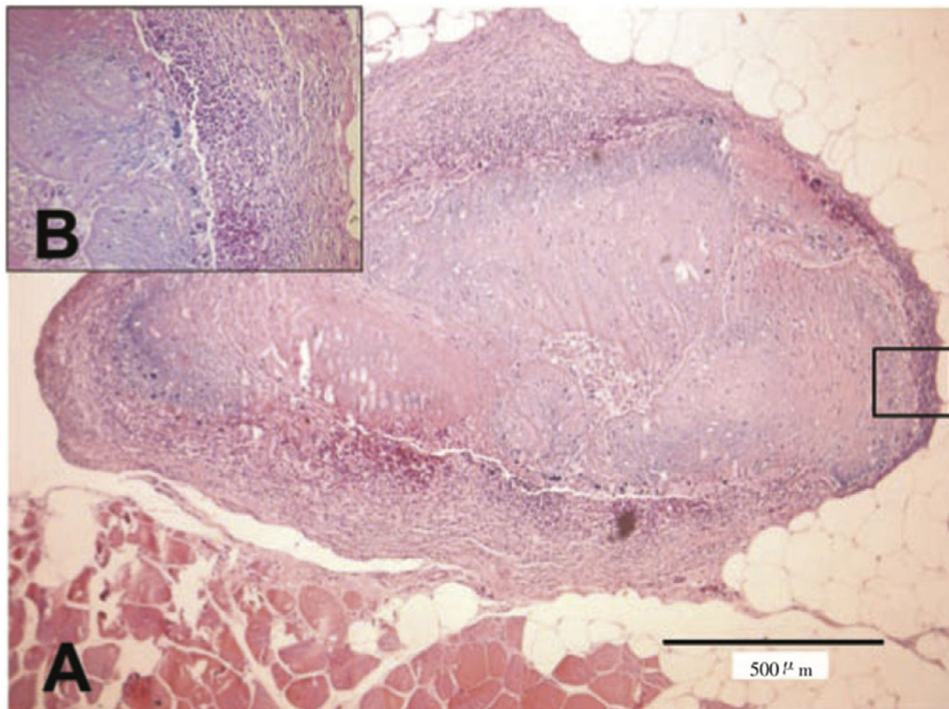


Figure 2. Histological section of cysts from a pig treated with OFZ (H&E stain). It showed moderate diffuse lymphocytic myositis and dystrophic calcification (A). Presence of lymphocytes, fibroblasts, connective tissue, tissue mineralization with basophilic granules (B).

Table 1

Median, range and feature of larval stage of *T. solium* after treatment of pigs.

Indexes	Group		
	TCBZ	OFZ	Control
Animals	6	6	6
Male	2	2	2
Weight (kg) ^a	77 (67–86)	95 (66–117)	81 (49–119)
Number of viable cysts	1414 (511–4052) ^b	0 ^b	1658 (131–2575) ^b
Number of degenerated cysts	0	259 (38–1953)	0

^aMean and range

^bSignificant difference between control and treatment (Kruskal–Wallis test, $P < 0.01$).