BRIEF REPORT

# EFFICACY OF TWO LOCALLY PRODUCED OXFENDAZOLE FORMULATIONS FOR THE TREATMENT OF CYSTICERCOSIS IN NATURALLY INFECTED PIGS

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## ABSTRACT

The efficacy of two locally produced oxfendazole (OFZ) formulations against cysticercosis at 22,5% and 10%, versus a commercial formulation (Synanthic 9,06%) was evaluated in twenty-two naturally infected pigs that received a single oral dose of 30 mg/kg. Pigs were sacrificed at eight weeks post-treatment to evaluate the cysts found in their carcasses, and to determine the cysticidal efficacy, which was defined as the proportion of degenerated cysts over total cysts. Only degenerated cysts were found in muscle, heart, and tongue of pigs treated with OFZ in all groups, which shows an efficacy of 100%. Viable and degenerated cysts were found in brains, being the efficacy lower in all groups (65% [commercial OFZ], 47% [local OFZ 22.5%] and 31% [local OFZ 10%], p = 0.355). Locally produced OFZ formulations were similarly effective to the commercial formulation and may provide a practical alternative for the treatment of porcine cysticercosis.

**Keywords:** Cysticercosis; Dosage; Cysts; Porcine; *Taenia solium;* Peru (source: MeSH NLM).

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## INTRODUCTION

*Taenia solium* is a zoonotic and endemic cestode in developing countries. The life cycle of *T. solium* involves humans and pigs as definitive and intermediate hosts, respectively. In humans, the adult stage of the taenia develops in the small intestine when people ingest raw or undercooked pork meat infected with cysts. On the other hand, the larval stage (cysticercus) develops in pigs when they ingest human feces containing *T. solium* eggs [(1)]. Humans can also develop cysticercosis by accidentally ingesting these eggs [(1)]. Neurocysticercosis (NCC), the infection of the central nervous system by the larval form (cysticercus), is the leading cause of human-acquired epilepsy worldwide [(2)] and has a negative economic impact on patients due to hospitalization costs and productivity losses [(3)].

Combined interventions targeting both human and swine populations have the potential to interrupt the transmission cycle of *T. solium*. Oxfendazole (OFZ) chemotherapy in swine has


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shown to be highly effective against porcine cysticercosis [4]. OFZ (5 - [(phenyl-sulfinyl)-1H-benzimidazole-2-yl] carbamic acid methyl ester) is a broad-spectrum benzimidazole against nematodes and trematodes in various animal species at doses ranging from 2 to 10 mg/kg [5]. Controlled studies demonstrated the cysticidal efficacy of OFZ against porcine cysticercosis at a single oral dose of 30 mg/kg [6] and subsequent trials with OFZ, alone or combined with vaccination against porcine cysticercosis, have confirmed its efficacy [4,7,8].

The pharmacokinetics of OFZ in pigs receiving a single oral dose of 30 mg/kg of a commercial formulation (Synanthic 9.06%, Pfizer, Mexico) and a 22.5% local experimental formulation showed very high plasma concentrations 2 hours after treatment [9]. Both the maximum concentration and the area under the concentration-time curve were approximately 30% higher for the commercial formulation; however, it was not stated whether these differences can explain its cysticidal efficacy. OFZ is currently licensed for use against porcine cysticercosis in Africa [8]. In Peru, OFZ is marketed at concentrations of 10% or less. These low concentrated formulations require a large volume of medication per pig and have the risk of aspiration and death of the animal during treatment. More concentrated formulations of OFZ would be easier to administer in mass swine chemotherapy programs, as they decrease the risks of aspiration, but are not commercially available. If more concentrated local formulations of OFZ demonstrate similar efficacy, they could provide a practical alternative. This controlled experiment compared the efficacy of two locally produced OFZ formulations (22.5% and 10%) and a commercial formulation against porcine cysticercosis in naturally infected pigs.

### KEY MESSAGES

**Motivation for the study:** Oxfendazole (OFZ) is an effective antiparasitic against porcine cysticercosis and a valuable tool for its control, although the volume required per animal at the usual concentration of 9% makes it impractical for mass administration.

**Main findings:** Locally produced formulations of OFZ were equally effective as the commercial formulation.

**Implications:** Local formulations of OFZ may provide an alternative for the treatment of porcine cysticercosis in rural areas.

## THE STUDY

This experimental study was carried out at the facilities of the Faculty of Veterinary Medicine of the Universidad Nacional Mayor de San Marcos (FMV-UNMSM) in Lima, Peru. Twenty-two pigs, older than 12 months, infected with cysticercosis and diagnosed by tongue examination [10], were purchased in Huancayo, a cysticercosis endemic area of the Peruvian highlands [11]. The pigs were transported to the experimental facilities, vaccinated against hog cholera virus and ear-tagged with identification numbers two weeks before the start of the study. Pigs were randomly assigned (in strata by weight) to receive a single oral dose of 30 mg/kg of commercial OFZ (Synanthic 9.06%, Pfizer, Mexico® [n = 8]), or locally produced OFZ (at 22.5% [n = 8] and 10% [n = 6]).

Local formulations were prepared at the Department of Pharmacology, Universidad de San Marcos, using OFZ p.a (Spectrum Laboratory Products Inc., Gardena, CA, USA) with the following surfactants: sodium carboxymethylcellulose, citric acid, propylparaben, methylparaben and glycerin. Both methyl and propyl paraben were dissolved in deionized water at 85 °C by stirring; similarly, a solution containing sodium carboxymethyl cellulose and citric acid was prepared with deionized water at room temperature, then glycerin was added and dissolved by stirring. To this solution, OFZ (according to the formulations) was added in parts and mixed by vigorous stirring. Once the OFZ solution was completely homogenized, the solution with methyl and propyl paraben was added and mixed by stirring until total incorporation. This emulsion was passed through a roller mill to homogenize the mixture, which was completed to a final weight of 100 kg by the addition of deionized water. All manufacturing and storage procedures were carried out under protection from direct light. The products were used within a maximum period of one month after manufacture.

Oral administration of OFZ (according to the weight of the pigs) was carried out with a dosing syringe, under the supervision of a veterinarian. The formulations were administered while fasting; the pigs were monitored throughout the experiment for any side effects, such as anorexia and lethargy, previously described [12]. Pigs

were euthanized eight weeks after OFZ treatment using a combination of ketamine (10 mg/kg) plus xylazine (2 mg/kg) intramuscularly and an overdose of sodium pentobarbital (120 mg/kg) intravenously. A detailed necropsy was performed on each carcass, and all cysts were removed from skeletal muscle, heart, tongue and brain.

Cysts were classified as viable (vesicles with clear fluid and a clearly visible scolex) or degenerated (dense white vesicles without fluid or scolex) and were analyzed using the geometric mean (GM), according to sample type and treatments. The percentages of efficacy in each pig (number of degenerated cysts / number of total cysts × 100) and number of cysts (healthy and degenerated), according to treatments were compared by Kruskal Wallis analysis, using the statistical package RStudio v1.0.143. The Animal Ethics Committee of the FMV-UNMSM approved the study protocols, according to the guidelines established by the Office of Animal Welfare Laboratories (National Institutes of Health [NIH], USA).

# FINDINGS

No adverse reactions were observed during the entire experimental period. Two pigs, with identification numbers 9148 (OFZ commercial formulation) and 9264 (22.5% local formulation), respectively, did not show cysts or degenerated lesions at all, and were excluded from the analyses.

Only degenerated cysts were found in the skeletal muscle, heart and tongue samples (Table 1, Figure 1A and 1B); no viable cysts were found in these samples, demonstrating 100% cysticidal efficacy for all three OFZ formulations. Also, parasite loads with degenerated cysticerci were not different between groups ($p = 0.837$). Viable and degenerated brain cysts were found in pigs treated with all OFZ formulations (Table 1), with efficacy percentages being 65% for the commercial formulation, 47% and 31% for the local formulations at 22.5% and 10%, respectively ($p = 0.355$, Figure 1C). The parasite load in those pigs with viable brain cysticerci was lower in the group treated with the commercial formulation of OFZ (GM: 2) compared to those treated with the locally produced OFZ formulations at 22.5% (GM: 3.6) and 10% (GM: 5.7), respectively, although these differences were not statistically significant ($p = 0.185$).

# DISCUSSION

Our study demonstrated similar efficacy between the two local and the commercial OFZ formulations against muscle cysts, as has been consistently demonstrated in previous studies [6, 7]. Regardless of the formulation used, all muscle cysts were eliminated after a single dose of 30 mg/kg, and eight weeks later only degenerated cysts were observed in pig muscle [12]. Two pigs treated with the commercial and local formulation of 22.5% OFZ, respectively, had no cysts or lesions at necropsy. It is possible that these pigs were misdiagnosed with the tongue test; but it is also possible that the elimination of all traces of infection resulted from mild infections, since the effective time to kill cysts in the pig depends mainly on the intensity of infection [12, 13].

Compared to other benzimidazoles such as albendazole, OFZ is more easily absorbed from the gastrointestinal tract, which correlates with the high plasma concentration of the drug observed in pigs as early as two hours after treatment [9]. This rapid absorption of OFZ in pigs is also favored by their monogastric structure, in contrast to ruminants, where a significant amount of the drug is retained together with the digested feed in the rumen [14]. The aromatic substitution at the sulfur atom in the chemical structure of OFZ also provides a prolonged systemic exposure time of the drug to the parasite, which may explain its enhanced cysticidal efficacy [9]. Although drug manufacturing procedures may influence the pharmacokinetics of OFZ formulations, as previously described [9], a high plasma concentration after a dose of 30 mg/kg is sufficient to kill all cysticerci in the muscle.

Low levels of efficacy of OFZ against brain cysts have been previously reported [15]; however, the reasons are still not entirely clear. All three OFZ formulations tested in our study were highly effective against muscle cysts, but showed lower efficacy against brain infections (viable cysts were found in pig brains during necropsies). We observed that the group treated with commercial OFZ had fewer viable cysts in the brain than the groups treated with local OFZ. However, a true difference in efficacy against brain cysts cannot be excluded, as the non-statistical differences between treatments may reflect the lack of statistical power due to a small sample size. In any case, the low efficacy of OFZ against brain cysts is not a drawback for its applications in cysticercosis control programs [16].


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**Table 1.** Detailed distribution of viable and degenerated cysts in pigs, and percentages of efficacy according to tissue samples and treatment groups.

| Group | Pig number | Skeletal muscle | | Heart | | Tongue | | Brain | |
|---|---|---|---|---|---|---|---|---|---|
| | | Viable cysts | Degenerated cysts | Viable cysts | Degenerated cysts | Viable cysts | Degenerated cysts | Viable cysts | Degenerated cysts |
| 1 | 302 | 0 | 390 | 0 | 16 | 0 | 17 | 0 | 1 |
| | 312 | 0 | 8,236 | 0 | 114 | 0 | 268 | 1 | 8 |
| | 314 | 0 | 977 | 0 | 12 | 0 | 23 | 0 | 2 |
| | 271 | 0 | 132 | 0 | 0 | 0 | 7 | 0 | 4 |
| | 222 | 0 | 1,130 | 0 | 0 | 0 | 43 | 1 | 0 |
| | 301 | 0 | 38 | 0 | 0 | 0 | 9 | 8 | 0 |
| | 7200 | 0 | 9,281 | 0 | 75 | 0 | 70 | 0 | 0 |
| | GM [a] | - | 773.1 [a] | - | 35.8 [a] | - | 29.6 [a] | 2.0 [a] | 2.8 [a] |
| | Efficacy (%) [b] | - | 100.0 | - | 100.0 | - | 100.0 | - | 64.8 |
| 2 | 303 | 0 | 6,943 | 0 | 113 | 0 | 115 | 2 | 0 |
| | 308 | 0 | 2,943 | 0 | 67 | 0 | 63 | 7 | 11 |
| | 313 | 0 | 1,187 | 0 | 32 | 0 | 34 | 44 | 6 |
| | 349 | 0 | 173 | 0 | 19 | 0 | 11 | 0 | 0 |
| | 230 | 0 | 244 | 0 | 7 | 0 | 23 | 1 | 0 |
| | 9622 | 0 | 6,724 | 0 | 4 | 0 | 78 | 0 | 0 |
| | 9623 | 0 | 3,456 | 0 | 0 | 0 | 36 | 1 | 4 |
| | GM [a] | - | 1,572.7 [a] | - | 22.5 [a] | - | 40.4 [a] | 3.6 [a] | 6.4 [a] |
| | Efficacy (%) [b] | - | 100.0 | - | 100.0 | - | 100.0 | - | 47.3 |
| 3 | 304 | 0 | 1,128 | 0 | 17 | 0 | 26 | 0 | 1 |
| | 306 | 0 | 5,014 | 0 | 13 | 0 | 50 | 4 | 11 |
| | 309 | 0 | 516 | 0 | 9 | 0 | 110 | 43 | 2 |
| | 0032 | 0 | 4,088 | 0 | 38 | 0 | 67 | 2 | 0 |
| | 7185 | 0 | 5,376 | 0 | 203 | 0 | 167 | 6 | 26 |
| | 7186 | 0 | 380 | 0 | 0 | 0 | 10 | 3 | 1 |
| | GM [a] | - | 1,702.7 [a] | - | 27.4 [a] | - | 50.2 [a] | 5.7 [a] | 3.6 [a] |
| | Efficacy (%) [b] | - | 100.0 | - | 100.0 | - | 100.0 | - | 30.6 |

Efficacy: Proportion of degenerated cysticerci divided by total cysticerci (healthy + degenerated).
1: Commercial formulation of oxfendazole (Synanthic® 9.06%); 2: Local formulation of oxfendazole (22.5%); 3: Local formulation of oxfendazole (10%).
[a] Geometric mean (GM), [b] mean.

Our study has some limitations to consider. Due to the small number of viable brain cysts, we did not evaluate the effect of local formulations on cyst viability. In addition, we were unable to differentiate degenerated cysts due to treatment or natural evolution of parasitosis. Although we were unable to estimate the initial parasite load in pigs, analysis of the number of degenerated cysticerci at necropsy showed no significant differences between groups, suggesting that parasite loads were similar. Our results were obtained under controlled infection-free conditions, which may limit our ability to establish whether local formulations are as effective as commercial formulations under natural conditions where pigs are repeatedly infected. A previous study showed that pigs with cysticercosis treated with a commercial formulation of OFZ were protected from reinfection for at least three months [17].

The protection that results after OFZ treatment appears to be a consequence of the activation of the immune system against degenerating cysticercus antigens [17, 18] and is apparently independent of the formulation used. In addition, it is necessary to evaluate whether local formulations of OFZ at 30 mg/kg are also effective against nematodes in pigs, as has

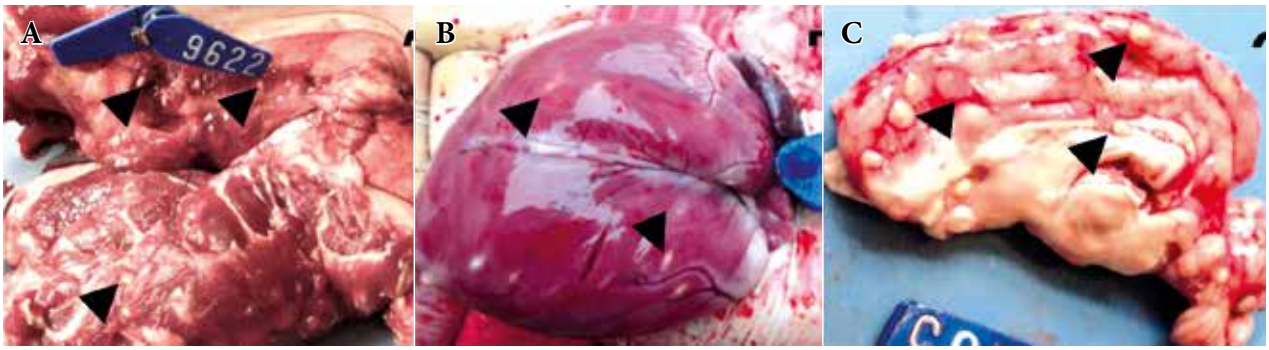


**Figure 1.** Macroscopic view of cysts in pig carcasses eight weeks after treatment with oxfendazole. A) Degenerating cysticerci in skeletal muscle (black arrows). B) Degenerating cysticerci in cardiac muscle (black arrows). C) Degenerated cysticerci in brain (black arrows).

been reported with a commercial formulation previously [19].

Compared with other antiparasitics that require multiple doses or have side effects [20], OFZ is safe and highly effective against porcine cysticercosis at a dose of 30 mg/kg. Local formulations of OFZ are easy to prepare and do not require sophisticated procedures beyond the use of surfactants to improve their stability. From a practical standpoint, highly concentrated local OFZ formulations that require less volume per dose may provide a practical alternative for dosing drugs to pigs in rural areas and reduce the risk of aspiration pneumonia during administration. Our results demonstrate that local formulations of OFZ are highly effective against porcine cysticercosis and further studies are required to corroborate their efficacy under field conditions.

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