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Alimentary Tract

# Efficacy of albendazole against *Anisakis simplex* larvae in vitro

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**Abstract**

**Background.** The growing popularity in western countries of eating uncooked seafood has resulted in an increased incidence of anisakidosis.
**Aim.** To study the in vitro activity of different concentrations of albendazole against *Anisakis simplex* larvae under different media pH.
**Methods.** *A. simplex* larvae were obtained from fresh hakes acquired from the fish market of Madrid. They were divided into groups and placed in culture dishes (15 larvae each) containing RPMI-1640, in the presence or absence of different concentrations of albendazole (300, 400 and 500 μg/mL).
**Results.** Albendazole dose-dependently reduced the survival of the larvae, its maximum activity being at 500 μg/mL when it killed almost all larvae at 48 h. Acidic medium pH significantly reduced the efficacy of albendazole.
**Conclusion.** Albendazole is effective in killing *A. simplex* larvae at different pH in vitro, suggesting that this molecule could be useful in treating clinical manifestations of human anisakidosis.
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*Keywords:* Albendazole; *Anisakis*; Anisakiasis; Anthelminthics

## 1. Introduction

*A. simplex* is a nematode that in its adult stage parasitises sea mammals. Its life cycle involves several intermediary hosts, including codfish, hake, sardine and anchovy [1]. Humans can be accidental hosts by eating raw or undercooked fish that contains the third-stage larvae. *A. simplex* larvae can penetrate the digestive tract and produce allergic manifestation and a variety of abdominal symptoms that can mimic other gastrointestinal disturbances [2].

Antibiotics, anticholinergics and/or corticosteroids have been occasionally used for the treatment of anisakiasis [3,4] without clear efficacy. Albendazole is a benzimidazole derivative that has been used widely to treat intestinal parasites since the late 1980s, and it seems to have activity against *Anisakis* [5].

The aim of the present study was to provide additional proof of in vitro activity of albendazole against *A. simplex* and to determine its efficacy at different concentrations and at different media pH.

## 2. Materials and methods

Third-stage viable larvae of *A. simplex* were obtained from the muscle and peritoneum of fresh hakes (*Merluccius merluccius*), purchased from the fish market of Madrid.

The worms had an average length of 20 mm, and were collected with the help of a needle with a blunt tip, placed in a Petri dish with 0.9% NaCl solution and washed in it several times. The majority of the larvae were encysted, but they quickly became excysted in the washing NaCl solution. They were observed individually under an inverted microscope and those which showed any kind of internal or external damage were discarded.

The larvae were identified according to morphological features, divided into groups and placed in culture dishes (15 larvae each) containing RPMI-1640 plus 20% (v/v) heat-inactivated foetal bovine serum, in an atmosphere of

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95% $O_2$/5% $CO_2$, at 37 °C. These culture conditions have shown to provide maximum development and survival of *A. simplex* [6]. The media were supplemented with penicillin (100 IU/mL), streptomycin (100 mg/mL) and amphotericin B (10 μg/mL), and different concentrations of albendazole (0, 300, 400 and 500 μg/mL). Either hydrochloric acid (HCl) or sodium hydroxide (NaOH) was added to some of the media to adjust their pH to 2 or 8, respectively.

Survival and mobility of the larvae were assessed at 6, 24, 48 and 72 h, and at day 7 using a stereomicroscope. Larvae status was scored as death, poor mobility or normal mobility by two different investigators in a blinded fashion.

Each experiment was performed in quadruplicate. Kaplan–Meier nonparametric techniques were used for the comparison of survival times between groups. Differences were assessed using a two-sided log-rank test.

## 3. Results

*A. simplex* larvae were not measurably affected by 7-day culture in RPMI-1640, even at alkaline pH.

Albendazole impaired both motility and survival of *Anisakis* in any of the medium pH tested, showing a dose-dependent activity (Figs. 1 and 2).

The maximum activity of albendazole was seen at 500 μg/mL, being able to kill almost all larvae at 48 h. Lower concentrations showed little efficacy, killing just about 20% *Anisakis* at 48 h (Figs. 1 and 2).

Efficacy of albendazole was also dependent on the medium pH, being less efficacious in acidic pH than in pH 7.4 or 8. In fact, almost 20% of *Anisakis* larvae remained alive at pH 2 after 48 h of 500 μg/mL albendazole (Fig. 2) although they exhibited poor motility.

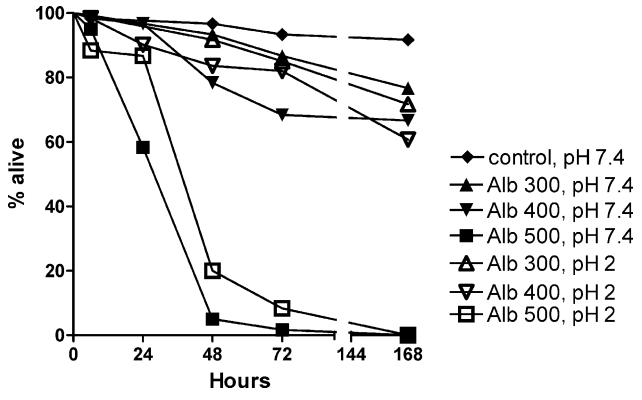


Fig. 1. Kaplan–Meier estimates of overall survival time for *A. simplex* larvae cultured in RPMI-1640, at either pH 2 (log-rank test, $\chi^2 = 137.7.8$; d.f., 3; $P < 0.0001$) or 7.4 (log-rank test, $\chi^2 = 155.8$; d.f., 3; $P < 0.0001$) in the presence or absence of 300, 400 and 500 μg/mL albendazole.

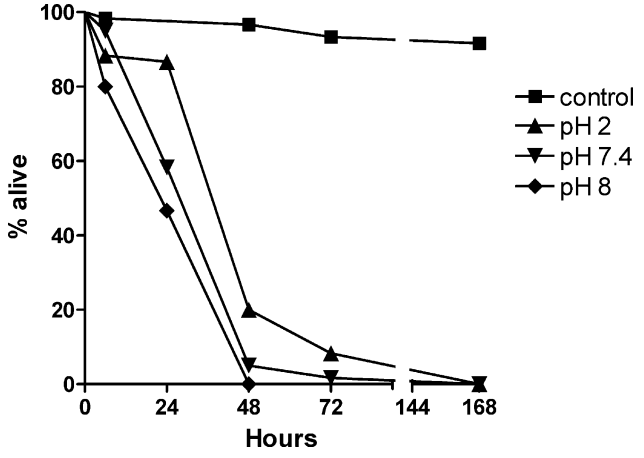


Fig. 2. Kaplan–Meier estimates of overall survival time for *A. simplex* larvae cultured in RPMI-1640 at pH 2, 7.4 and 8, in the presence or absence of 500 μg/mL albendazole (log-rank test, $\chi^2 = 98.3$; d.f., 3; $P < 0.0001$).

## 4. Discussion

Although the usual evolution of anisakiasis is towards spontaneous resolution, there are cases which develop serious complications such as perforation and peritonitis [7], or end up requiring surgery [2] because of ileus or malignancy suspicion.

The availability of an anthelminthic efficacious against *Anisakis* larvae could shorten the clinical course and prevent mechanical complications. In addition, the presence of several allergens [8] and proinflammatory molecules in larval excretory–secretory products is responsible for the systemic and local hypersensitivity manifestations to *A. simplex*. An antiparasitic agent might thus also be able of ameliorating clinical manifestations of hypersensitivity.

Albendazole has shown activity against *Anisakis* in vitro and in guinea pigs [5], and its use has been reported in treating isolated cases of human intestinal anisakiasis [9] with an apparent therapeutic benefit. In this study, we have found that albendazole is able to kill *A. simplex* larvae in a time- and dose-dependent fashion.

The acidity of the stomach appears to facilitate the growth of *Anisakis* larvae [6], and this may explain why acute gastric anisakiasis has rarely been reported in patients over 60 years of age [10] or following gastric surgery. In addition, the nematode hyaluronidases are postulated to have a role in mucosal invasion, and hyaluronidase from *A. simplex* larvae has a pH optimum of 4 [11].

*A. simplex* larvae suffer drastic changes in their environment pH during their transit through the digestive tract. In our study, the survival of the larvae in acidic pH in the presence of 500 μg/mL albendazole was superior than in neutral or alkaline pH. This finding is in agreement with low pH being most favourable to the parasite. This is noteworthy, especially in view of the pharmacokinetics of the albendazole molecule, whose solubility is markedly improved at low pH due to protonation [12].

Our in vitro results might be extrapolated to the therapy of human anisakiasis because the parasite usually remains in the digestive tract wall [10] where high concentrations of the drug are easily attained. Although 48 h were enough for eradication of most parasites, the fact that a significant proportion of *Anisakis* larvae remain alive at pH 2 after that period suggests that therapy should be continued for several days.

In conclusion, albendazole is effective in killing *A. simplex* larvae at different pH in vitro, suggesting that this molecule could be useful in treating clinical manifestations of human anisakidosis.

## Conflict of interest statement

None declared.

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