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# THE IMPACT OF ANIMAL AND PLANT PROTEASES ON NEMATODA 3rd STAGE *ANISAKIS SIMPLEX* LARVAE

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## Abstract

The influence of two animal and plant proteases was investigated *in vitro* on 3rd stage *Anisakis simplex* B larvae. The larvae were placed in 3 series of 5 sterile test-tubes containing 0.1% solutions of tyrosine, pepsin and papain. Physiological saline solution was used as control. It was found that *Anisakis simplex* B larvae are the most resistant to pepsin treatment.

## INTRODUCTION

One of the major mechanisms protecting intestinal parasites from being digested by the host's proteolytic enzymes is their production of a substance inhibiting these enzymes. During the present century it has been found that extracts of nematodes and tapeworms impair the host's proteolytic enzyme activity. Brand (1966), and Mendel and Blood (1910) noted the fact that an extract of *A. suum* impaired the activity of the pancreatic proteolytic enzymes. Rhodes et al. (1963) found inhibitors to be present not only in the skin-flesh bag of *A. suum* but in other body parts as well, e.g. intestines, ovaries, uterus, ovum thecas and body cavity fluid. According to Hawley and Peanasky (1992), and Kadłubowski and Ochęcka (1986), an isolated inhibitor from *A. suum* showed a high specificity towards particular

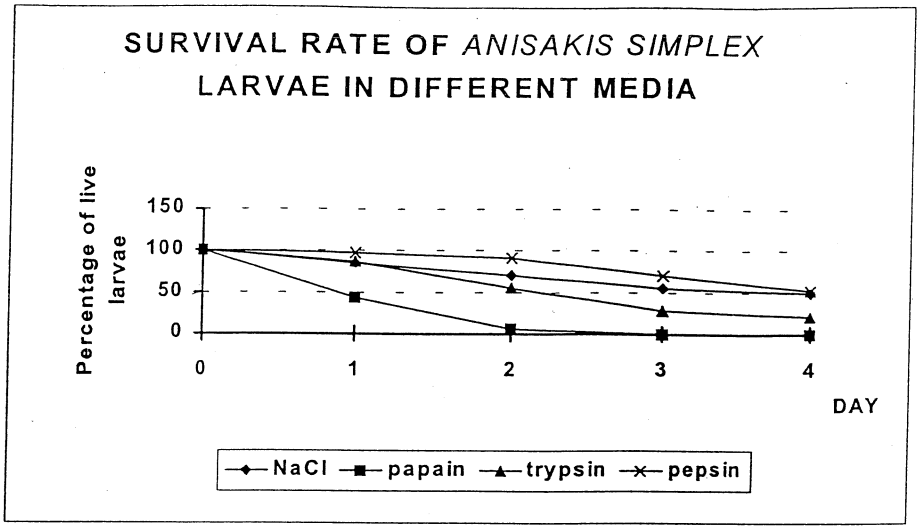


Fig. 1. Survival rate of *anisakis simplex* larvae in different media

proteolytic enzymes. This might be one of the elements limiting the parasite's specificity in relation to its host. Juhasz and Matskasi (1979) incubated *A. suum* in media with the addition of porcine trypsin and chymotrypsin. After 24 and 48 hours they found the enzyme activity had dropped as compared to the control samples not containing worms. Juhasz (1979) revealed that *A.suum* individuals secreted protease inhibitors into the incubation medium but not into the proteolytic enzyme. The trypsin and chymotrypsin inhibitor's activity almost doubled after 48 hours of incubation. Hawley and Peanasky (1992) investigated the resistance of *A. suum* in vitro to animal proteases (human and porcine trypsin) and plant proteases (papain and ficin). The incubated worms survived the 3 weeks of the experiment in an isotonic solution containing some porcine trypsin. In a medium containing human trypsin, however, they lived only up to 5 days, later undergoing digestion. In media containing added plant enzymes – papain or ficin – the worms began to die after only 1.5 h. Plant proteases, and trypsin from an unnatural host (human trypsin) digest both mature individuals and larvae of *A. suum* due to the lack of enzyme inhibitors in those worms. These authors thus maintain that it would be impossible for porcine worms to survive in humans. The larvae, which wander into the alimentary canal after being swallowed twice, do not produce inhibitors which would protect them against digestive enzymes in the intestines. Kadłubowski and Ochęcka (1986) incubated *A. suum* in a medium with proteases from *Candida albicans* and *Bacillus subtilis* and then showed that those proteases were not able to digest worms *in vitro*. The addition of 0,1% papain solution into the medium led to damage and mortality in the worms. Moreover Berger and Asenjo (1940) and Hogan

(1980) observed that plant proteases added to the medium led to damage and mortality in the worms by digestion. Martzen *et al.* (1985) incubated live *A. suum* in an isotonic medium containing trypsin and porcine chymotrypsin. They noted that the enzymes penetrated different body parts of the worm and were inactivated by the enzymes' internal inhibitors.

There are no papers available dealing with the influence of animal and plant proteases on 3rd stage *Anisakis simplex* larvae .These live in the alimentary system of their final hosts, which are marine mammals or an occasional host e.g. man. In the final host they moult for the last time and reach sexual maturity.

## MATERIAL AND METHODS

The aim of the present experiment was to investigate the influence of animal proteases such as bovine trypsin (akt. ab. 1000 BAEE, Polish Chemical Reagents, Gliwice), porcine pepsin (14 mA/mg Serva, New York) and plant proteasis-papain (1:350, Loba Chemie, Vienna) on 3rd stage *Anisakis simplex* larvae *in vitro*. 0.1% trypsin and papain solutions were prepared in Sörensen buffer at pH 7.6, as was a 0.1% pepsin solution in 0.1M citrate buffer at pH 3. The L3 larvae of *A. simplex* were obtained from herrings caught in the Baltic Sea. Before use, the larvae were kept in a sterile NaCl 0.85% solution containing antibiotics and fungicides. At the start of the experiment the larvae were rinsed in sterile 0.85% NaCl. 10 larvae were placed in three series of 5 sterile test-tubes each containing 2ml of the respective enzyme solutions. Physiological saline was used as control. After the addition of antibiotics and fungicides (penicillin, streptomycin, nystatin), the test tubes were incubated at 37°C, as Sommerville and Davey (1976) stated that this is the optimum temperature for *A. simplex* growth. These authors do not recommend that any nutrients such as glucose or proteins be added to the medium since L3 larvae do not feed at all before the next moult. Every day the live and intact larvae were calculated as a percentage and the number compared to the onsetting figure. Dead or damaged larvae were removed.

## RESULTS

After the first day the greatest number of live larvae were found in the pepsin solution (98%). The smallest number were in the papain solution (42%). In this medium all dead and some live larvae showed bodily damage. The number of live larvae in the trypsin and the control NaCl solutions was 86%.

On the second day most of the live larvae (90%) were found in the pepsin solution. No partial digestion of the bodies of the live or dead larvae had occurred. 56% of the larvae in the trypsin solution were alive. On a few live and dead larvae

no bodily damage was found. In the papain solution all had suffered bodily damage. All the remaining larvae had been digested to a significant extent. The control test contained over 70% of live larvae; among the dead larvae no bodily damage had occurred. On subseguent days the percentage of live larvae found in all solutions decreased. No bodily damage was found on larvae placed in pepsin and NaCl. The larvae in those solutions were the most mobile.

## DISCUSSION

It was found that the *A. simplex* larvae the were most resistant to porcine pepsin. In the literature available no papers have been found commenting pepsin inhibitors, which must be produced by these parasites and for which the present experimental results provide indirec evidence. They were less resistant to bovine trypsin. The phenomenon resembled the case of *Ascaris suum* (Hawley and Peanasky 1992) which were resistant to porcine trypsin (specific host) but not to human trypsin, in which they were able to survive for a short time only, after which they were digested. *A. simplex* remained alive for 48 hours in bovine trypsin, after which they underwent digestion. The trypsin inhibitor produced by them (Morris and Sakanari 1994) protects them for a short period of time. It is a Kunitz -type inhibitor and impairs bovine trypsin and human elastasis, and also proteasis secreted by *A. simplex* into the medium (Morris and Sakanari 1994). The inhibitor protects them against proteases in the final hosts e.g. marine mammals. The *A. simplex* larvae survive for about 3 months in an occasional host e.g. man. It has not been explained what happens to them after that; they might well be digested by human proteases.

Like *Ascaris suum* or *Ascaridia galli A. simplex* larvae are not resistant to plant enzymes. One hour after incubation onset partial digestion was observed on the larval bodies. This points to the fact that, like other Nematoda, they do not produce these inhibitors of these enzymes.

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