

Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 43 (1986)
Heft: 3

Artikel: Ascorbic acid induced immune-mediated decrease in mortality in "Ichthyophthirius multifiliis" infected rainbow-trout ("Salmo gairdneri") : short communication
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DOI: <https://doi.org/10.5169/seals-313639>

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Ascorbic acid induced immune-mediated decrease in mortality in *Ichthyophthirius multifiliis* infected rainbow-trout (*Salmo gairdneri*)

Short communication

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Ichthyophthirius multifiliis, a holotrichous ciliate, parasitizes both, the epidermis and the gill-tissue of fish. The parasite infects a broad spectrum of freshwater-fish and can cause considerable losses especially in fish farms. *I. multifiliis* has a direct life-cycle which consists of both, non-feeding free-living stages (adult trophonts, cysts, tomites) and stages feeding on the host (immature trophonts). Until now, all measures of control are limited to the free living stages of the parasite. The methods and chemicals used do not attack the parasitic stages on the host, since those are protected by fish tissue. In addition, the most effective drug, malachit-green-oxalate, is suspected to form residues in treated fish and to be cancerogenic.

By looking for new ways of treatment, trout were found to acquire immunity to *I. multifiliis* after having survived a macroscopically detectable infection at 12–18°C (Wahli, 1985; Wahli and Meier, 1985). The observation of an immune phenomenon led to the question whether an immune stimulation or even a vaccination could be induced as an alternative to a drug treatment. In support of this idea is the observation, that mortality of channel-catfish infected with the bacterium *Edwardsiella* sp. is decreased when the fish is fed vitamin C (Lovell, 1984). Li and Lovell (1985) suggested an influence of vitamin C on the immune response of fish. Therefore we have investigated the influence of ascorbic acid (AA) on artificially *I. multifiliis*-infected trout.

The experiments were carried out in six 200 l glass aquaria with a constant flow of freshwater (15°C). 50 trout (12–15 cm) were set in each aquarium. Fish of three tanks were fed a trout-diet containing 5 g AA/kg trout diet. Fish of three other tanks received a special trout-diet (free of AA; all diets prepared by Hoffmann-La Roche Ltd., Basel). 5 days after feeding the special diet, trout of

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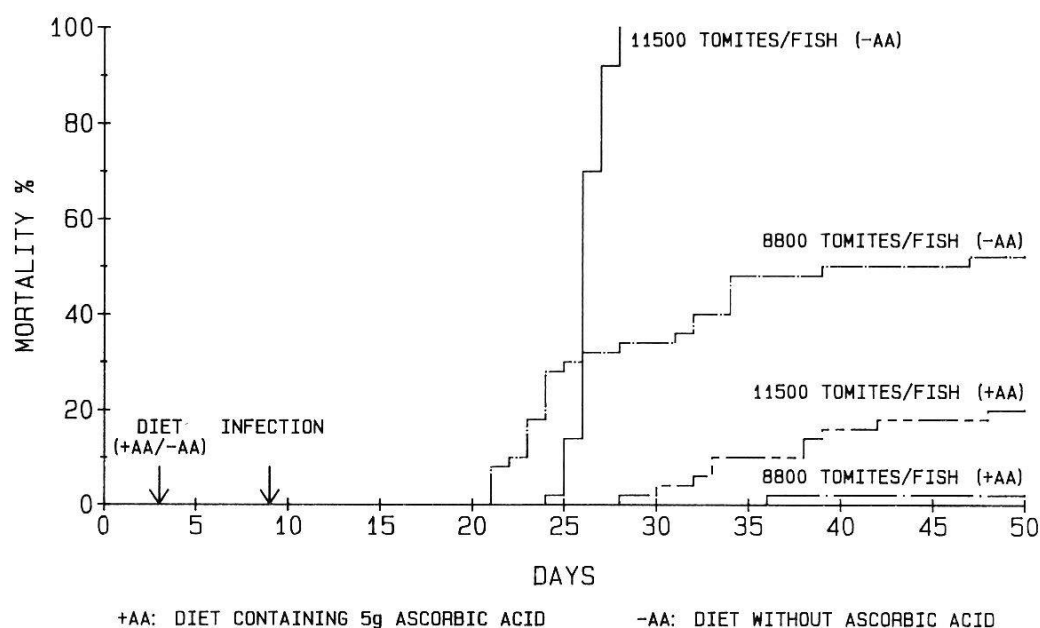


Fig. 1. Ascorbic acid and course of mortality of trout infected with *Ichthyophthirius multifiliis*.

two tanks each were infected with 8,800 and 11,500 tomites per fish, respectively. Fish of two tanks served as uninfected controls. 50 days post infectionem no *I. multifiliis*-parasites could be detected on fish which had survived the infection. The rate of mortality (in %) is shown in Fig. 1. An AA-containing diet reduced the mortality of *I. multifiliis*-infected trout as follows: in fish exposed to the lower dose of parasites (8,800 tomites per fish) the reduction was 50%, whereas in the higher infection group (11,500 tomites per fish) the reduction was 84%.

To prove a possible effect of AA on the immune response, serum samples from experimentally infected fish were collected and examined using the following three methods:

1. An in vitro test developed for *Paramaecium* sp. (Sonneborn, 1950) and modified for *I. multifiliis* (Hines and Spira, 1974; Wahli, 1985) was used to evaluate the tomite-immobilizing activity. All sera from fish which had survived a clinically obvious *I. multifiliis*-infection revealed an immobilizing activity up to a 1:2560 dilution in water. Previous experiments (Wahli, 1985) have demonstrated that fish with immobilizing titres $\geq 1:80$ were protected against reinfection. In sera from dying or from control fish the titres were found to be $\leq 1:40$.
2. A few sera with high immobilizing activity were examined for the presence of IgM. This immunoglobulin, of a tetramer configuration, represents the only antibody-class of teleost fish (Dorson, 1984). Using the Ouchterlony-method, identical precipitation lines were clearly visible between a monovalent rabbit-anti-trout-IgM antiserum and the various testsera of formerly *I. multifiliis*-infected trout, whereas sera from uninfected controls did not show any reaction.

3. A double-sandwich IFAT was carried out (antigen = acetone-fixed *I. multifiliis*-tomites) which showed that sera from recovered fish had positive titres $\geq 1:80$, thus revealing the presence of specific antibodies against *I. multifiliis*. By contrast sera from controls did not react positively.

These findings definitely indicate the presence of an immune response including specific anti-*I. multifiliis* antibodies of trout when surviving an *I. multifiliis*-infection. The results, together with the observation that fish fed an AA-containing diet showed a higher surviving capacity, suggest an influence of AA on the immune response of the host. However, the mechanisms remain unclear, and further experiments are required to explore possible effects of AA on the immune response.

Acknowledgments

This work is a result of research sponsored by Hoffmann-La Roche Ltd., Basel. The authors wish to thank Dr. P. E. Vestergård-Jørgensen for the kind supply of the rabbit-anti-trout-IgM antiserum and Miss B. Balsiger for her assistance in the laboratory.

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