

NOTE

Effects of immunosuppressive agents on common carp infected with the haemoflagellate *Trypanoplasma borreli*

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ABSTRACT: The effects of immunosuppressive agents (irradiation and administration of hydrocortisole) on carp *Cyprinus carpio* L. experimentally infected with the haemoflagellate *Trypanoplasma borreli* Laveran & Mesnil, 1901 were monitored. Treatment with immunosuppressive agents led to significantly increased parasitemia and subsequently high mortalities among infected carp.

Trypanoplasma borreli, a haemoflagellate, occurs in the blood of European cyprinids (Lom 1979). It has been reported to cause death in naturally infected carp (Hofer 1904), and in experimentally infected goldfish (Lom 1979) and carp (Lom et al. 1986).

The importance of environmental stressors in disease outbreaks in fish has been pointed out by several authors (e.g. Snieszko 1974). Environmental stressors are accompanied by rapid changes in the concentration of several plasma chemicals especially corticosteroids (Mazeaud et al. 1977, Pickering & Duston 1983). A recent study on rainbow trout *Salmo gairdneri* with cortisol implants revealed an increased susceptibility of these fish to experimental infection with the haemoflagellate *Cryptobia* (syn. *Trypanoplasma*) *salmositica* (Woo et al. 1987). An increased susceptibility of goldfish *Carassius auratus* to infections with bacteria and parasites was also reported after X-ray irradiation (Shechmeister et al. 1962, Purdom & Woodhead 1973). In this communication, data on *Trypanoplasma borreli* infections of carp treated with hydrocortisole and X-rays are reported.

Materials and methods. One-year-old 25 g carp were obtained from laboratory raised stock, kept in 80 l tanks at 20 °C and fed daily on fish food pellets (F-45, Rheinkrone, Wesel, FRG). *Trypanoplasma borreli* was isolated from naturally-infected carp and maintained in the laboratory by fish-to-fish passage. A clone, established from this isolate, was also cultivated (by syringe-passages) in laboratory-raised carp using the method

described by Kruse et al. (1989). Carp were infected with *T. borreli* by intramuscular inoculation of 10 000 trypanoplasms from the clone and parasitemia was monitored by counting the number of *T. borreli* mm⁻³ blood in wet mount preparations. To compare the parasitemia of experimental groups Student's t-test was used.

Carp were tranquillized using iced water, and blood samples from infected and uninfected carp were taken at Days 3, 7, 11, 14, 21, and 28 post inoculation (p.i.) by scarifying gill vessels and collecting the blood in heparinized microhaematocrit tubes. Water-soluble hydrocortisole Prednisolon (Friesocortin, German Wellcome) and X-rays were used as immunosuppressive agents. A dosage of 42 mg kg⁻¹ body weight of hydrocortisole in phosphate-buffered saline (PBS) was inoculated i.p. (Levy 1963) followed by 20 mg kg⁻¹ body weight inoculations every 4 d for the next 24 d. A control group was inoculated with PBS in the same manner. Carp were exposed to X-rays of 500 R (129 × 10⁻³ C kg⁻¹) as described by Egami & Etoh 1962. During irradiation carp, kept in a plastic tank (12 × 18 cm) containing 5 cm of water, were placed 75 cm from the focus of the X-ray source (Radiation factors: 200 kV, 1.20 mA, 0.35 mm Cu filtration). Carp were inoculated with *Trypanoplasma borreli* 4 d after irradiation and the first hydrocortisole injection. The inoculation schedule is given in Table 1.

Results and discussion. All carp inoculated with *Trypanoplasma borreli* developed parasitemia. Carp treated with hydrocortisole showed significantly ($p < 5\%$) higher numbers of *T. borreli* in blood samples than control carp inoculated with PBS (Fig. 1) Similarly, carp exposed to X-rays exhibited a significantly ($p < 5\%$) higher parasitemia than untreated controls (Fig. 2). A comparison of mortalities suggested that death of treated carp occurred more often and in shorter periods than

Table 1. *Trypanoplasma borreli*. Inoculation schedule and mortalities in experimental groups. Hydrocortisol dosage: initial inoculation 42 mg Prednisolon kg^{-1} body weight then every 4d 20 mg Prednisolon kg^{-1} body weight. X-ray dosage: $129 \times 10^{-3} \text{ C kg}^{-1}$ body weight (=500 R)

Immunosuppressive agent	<i>T borreli</i> inoculation	No. carp	No. dead carp
Hydrocortisol	10^4	5	5 ^a
	0	5	0
Phosphate buffer	10^4	5	2 ^b
	0	5	0
X-ray	10^4	5	3 ^b
	0	5	0
Untreated	10^4	5	2 ^b
	0	5	0

^a 3 died in Wk 3 post inoculation 2 in Wk 4
^b All died in Wk 4 post inoculation

death of control carp (Table 1). Carp exposed to immunosuppressive agents and not infected with *T. borreli* did not develop any pathological signs. These results suggested that the administration of hydrocortisole and X-rays increased carp parasitemia, and mortality in *T. borreli* infections.

In goldfish, *Trypanoplasma borreli* infections caused inflammatory reactions (Dyková & Lom 1979). The effects of glucocorticoids, e.g. hydrocortisole, on the immune response is based mainly on the suppression of inflammatory processes. Our results confirmed the findings of Woo et al. (1987) with *Cryptobia* (syn. *Trypanoplasma*) *salmositica* infections of rainbow trout. Cortisol implants caused a decreased immune response to *C. salmositica*, which led to considerable mortality of implanted fish. Irradiation of fish affected both humoral

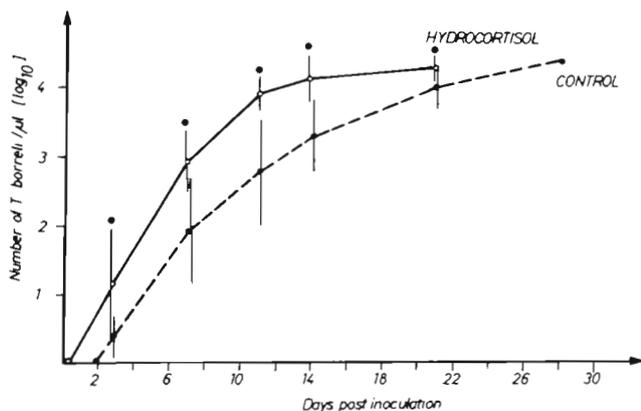


Fig. 1. *Trypanoplasma borreli*. Effect of hydrocortisole on development in experimentally-infected carp. Depicted are mean and standard deviation of *T. borreli* counts of treated and control (inoculated with phosphate-buffered saline) carp. * Counts in treated group significantly higher (Students' t-test, $p < 5\%$)

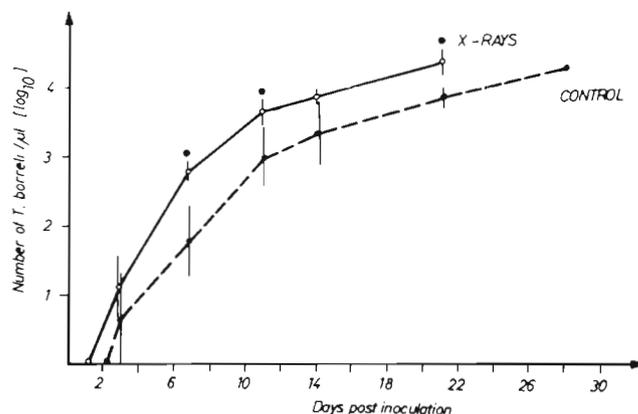


Fig. 2. *Trypanoplasma borreli*. Effect of X-rays on development in experimentally-infected carp. Depicted are mean and standard deviation of *T. borreli* counts of treated and control (untreated) carp. * Counts in treated group significantly higher (Students' t-test, $p < 5\%$)

and cellular immune response (Nakanishi 1986, Chilmonczyk & Oui 1988). Gamma-irradiated rainbow trout exhibited decreased leucocyte numbers and higher susceptibility to viral and bacterial pathogens (Chilmonczyk & Oui 1988).

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