Effects of the tapeworm *Ligula intestinalis* on biomarkers of endocrine disruption (ED) in roach (Cyprinidae, *Rutilus rutilus*)

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Anthropogenic compounds in the aquatic environment are an important cause for endocrine disruption (ED) in wildlife. Due to complex exposure scenarios and several biotic and abiotic factors modulating the endocrine system, under field conditions, correlations between suspected chemicals and observed ED-like effects are difficult to prove. An additional factor rarely considered in ecotoxicological studies is the ability of certain parasites to modulate the physiology of their hosts. In fish, plerocercoids (larval stages) of the tapeworm *Ligula intestinalis* are certainly the most frequently reported parasites causing reproductive dysfunction. This parasite is characterized by a complex life cycle involving three host species (Fig. 1). Infections by plerocercoids usually occur in cyprinid fish and result in an inhibition of host gonadal development. As a consequence, infection by *L. intestinalis* is a potential source of bias in ecotoxicological research. Therefore, reproductive parameters including gonad histology, plasma sex steroid levels, and expression of key endocrine genes of infected and uninfected roach (Cyprinidae, *Rutilus rutilus*) were investigated.

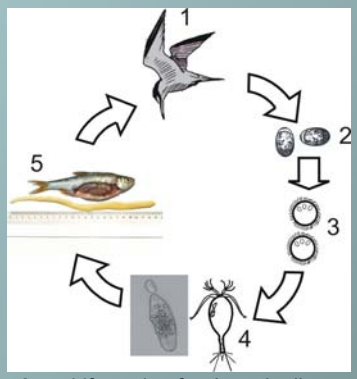

Fig.1. Life cycle of *L. intestinalis*.

1. bird (final host), 2 . tapeworm eggs, 3. coracidium, 4. copepod (first intermediate host) infected with procercoid, 5. fish (second intermediate host) infected with plerocercoid.

Material und Methods

Roach (3-6 old), infected and uninfected by *L. intestinalis* were captured from Lake Mueggelsee (Berlin, Germany) during autumn. Tissue and plasma samples were taken for analysis of reproductive parameters. Hormone levels were determined by ELISA. Using real time RT-PCR, mRNA-expression of the following genes was analyzed: Follicle-stimulating hormone β (FSHβ), luteinizing hormone β (LHβ), and glycoprotein-hormone α (αGSU) in pituitary; vitellogenin (VTG) in liver; estrogen receptors 1, 2a, 2b (Esr1, Esr2a, Esr2b), and androgen receptor (AR) in liver; gonad-type aromatase (Cyp19a) and brain-type aromatase (Cyp19b) in brain.

Results

Infection of roach by *L. intestinalis* resulted in an inhibition of gonadal growth and development. Only early germ cell stages could be found in gonads of both genders of infected roach (Fig 2).

Levels of 17β-estradiol (E2) and 11-ketotestosterone (KT) but not of testosterone (T) were significantly reduced in infected females. In males, infection resulted in lower plasma levels of E2, T, and 11-KT (Fig 3).

Analysis of gonadotropin subunit mRNAs in pituitary demonstrated substantially lower expression of FSHβ and LHβ in infected roach compared to uninfected conspecifics (Fig 3). This clearly indicates that gonadotropin insufficiency is the cause of depressed gonadal development.

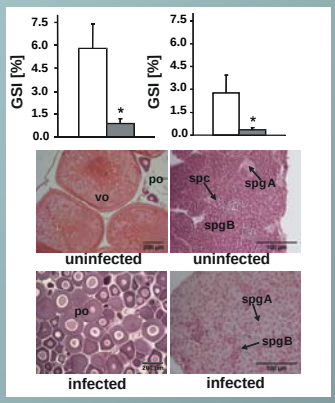

Fig 2: GSI and corresponding gonad histology of uninfected (white bars) and infected (grey bars) roach. Asteriks indicate significant differences between individuals of the same sex (N=12, p<0.05, t-test). po: oocyte in primary growth stage; spg A: spermatogonia A; spg B: spermatogonia B; spc: spermatocytes; vo: vitellogenic oocyte

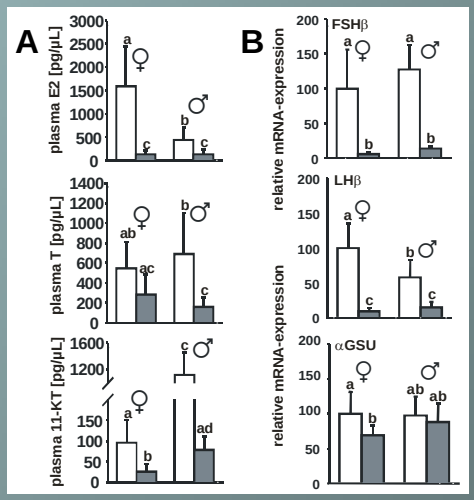

Fig 3: Plasma levels of E2, T, and 11-KT (A) and relative pituitary mRNA-expression of FSHβ, LHβ, and αGSU (B) in uninfected (white bars) and infected (grey bars) roach. Different letters indicate significant differences (N=12, p<0.05, Tukey).

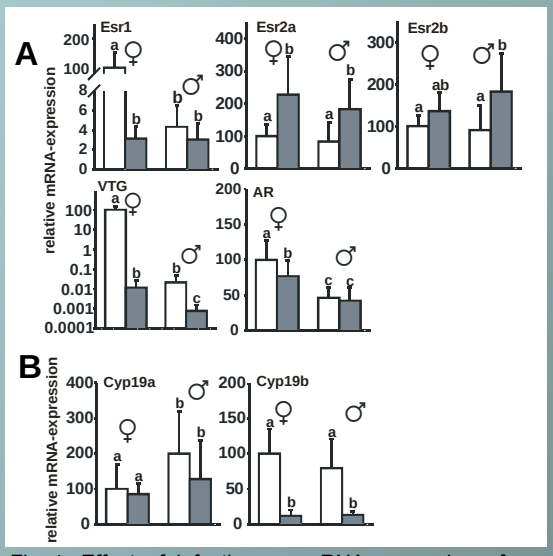

Fig 4: Effect of infection on mRNA-expression of sex steroid receptors (Esr1, Esr2a, Esr2b, AR) and VTG in liver (A) and aromatases (Cyp19a, b) in brain (B). white bars: uninfected; grey bars: infected; Different letters indicate significant differences (N=12, p<0.05, Tukey).

Consistent with lower plasma E2, mRNA-expression of the known estrogen depended genes VTG in liver and Cyp19b in brain was decreased in both genders of infected roach (Fig 4). Estrogen receptors were differently affected by infection. In females but not in males, Esr1-mRNA was severely suppressed in liver. In contrast, infected roach of both genders showed higher mRNA-levels of Esr2a in liver. For Esr2b a similar pattern could be observed but this was significant only in males. AR-mRNA was significantly affected only in liver of females.

Conclusion

Parasites like *L. intestinalis* can induce ED-like effects in fish and modulate classical biomarkers of ED.

Given the widespread distribution range of *L. intestinalis* it remains to determine to what extent parasites contribute to ED in wildlife

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