International Journal for Parasitology 39 (2009) 1465–1473

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Expression of gonadotropin subunits in roach (*Rutilus rutilus*, Cyprinidae) infected with plerocercoids of the tapeworm *Ligula intestinalis* (Cestoda) ☆

Achim Trubiroha [a,*], Sven Wuertz [b], Sabrina N. Frank [a,c], Bernd Sures [c], Werner Kloas [a]

[a] Department of Aquaculture and Ecophysiology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, D-12587 Berlin, Germany
[b] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 289, 4050-123 Porto, Portugal
[c] Department of Applied Zoology/Hydrobiology, University of Duisburg/Essen, Universitätsstrasse 5, D-45141 Essen, Germany

ARTICLE INFO

Article history:
Received 28 February 2009
Received in revised form 27 April 2009
Accepted 7 May 2009

Keywords:
Host–parasite interaction
Roach
Rutilus rutilus
Ligula intestinalis
Plerocercoid
Reproduction
Gonadotropin
Host sterilization

ABSTRACT

Plerocercoids of the tapeworm *Ligula intestinalis* (Cestoda: Bothriocephalidea) have been reported to inhibit gametogenesis of their intermediate fish hosts. However, mechanistic studies are rare and the proximate cues leading to impaired reproduction still remain unknown. In the present study we investigated the effects of infection by *L. intestinalis* on reproductive parameters of roach (*Rutilus rutilus*, Cyprinidae), a common fish host of this parasite. Field studies on roach demonstrated that in both genders infection prevented gonad development. As revealed by quantitative PCR, infection was accompanied by essentially lower pituitary expression of follicle-stimulating hormone β-subunit (FSHβ) and luteinizing hormone β-subunit (LHβ) mRNA compared with uninfected roach, providing clear evidence for gonadotropin-insufficiency as the cause of arrested gametogenesis. Under controlled laboratory conditions infected roach showed lower mRNA levels of FSHβ but not of LHβ, despite histology revealing similar gonad stages as in uninfected conspecifics. These findings indicate the involvement of FSH rather than LH in mediating effects of infection early during gonad development in roach. Moreover, the impact of *L. intestinalis* on reproductive parameters of roach appeared to be independent of the parasite burden. Together, these data provide valuable information on the role of FSH and LH as mediators of parasite-induced sterilization in a vertebrate and implicate the selective inhibition of host reproduction by *L. intestinalis* as a natural source of endocrine disruption in fish.

1. Introduction

Parasites contribute remarkably to the biomass in ecosystems and play a considerable role in food webs (Lafferty et al., 2006; Kuris et al., 2008). Additionally, they may have a strong influence on ecosystem function by inducing a variety of behavioural and physiological changes in their hosts (Poulin, 1999). In particular, parasites that impair host reproduction consume high amounts of energy and can have significant impacts on host population dynamics (Kennedy et al., 2001; Kuris et al., 2008). Reduction of host reproductive capacity is frequently reported from diverse host–parasite systems and this phenomenon is usually based on two different mechanisms. Direct destruction of reproductive organs is, for example, a common characteristic in molluscs infected with trematode larvae such as sporocysts and radiae (Poulin, 2006). Apart from such obvious damage, in many cases physiological changes underlie impaired reproduction (Hurd, 2001). The allocation of host resources distinct from reproductive gains might be crucial for certain parasites in order to ensure a sufficient supply of nutrients and to reduce the harm exerted on the host (Ebert et al., 2004). However, in an evolutionary context it is often difficult to distinguish between a directed parasitic strategy, simple nutritional competition and specific compensatory responses of the host (Hurd, 2001). Investigations on the mechanisms underlying reduced host reproduction provide important insights into the physiology of host–parasite interactions and represent a substantial contribution to estimate the adaptive nature of this phenomenon. Indeed, studies in particular on molluscs and arthropods revealed that parasites are able to interact in very specific ways with the host endocrine system controlling reproduction (Beckage, 1993; Webb and Hurd, 1999; Jong-Brink et al., 2001).

In fish, larval stages (plerocercoids) of the tapeworm *Ligula intestinalis* are suggested to manipulate the host endocrine system leading to reproductive dysfunction (Arme, 1997). This cestode is characterised by a life cycle involving three hosts with copepods as the first and fish as the second intermediate host. Final hosts are piscivorous birds such as gulls or grey herons. The parasites

☆ Nucleotide sequence data reported in this paper are available in the GenBank℠ database under accession numbers **FJ769335** (*Rutilus rutilus* rpL8) and **EF486694** (*Rutilus rutilus* αGSU).

* Corresponding author. Tel.: +49 30 64181 614; fax: +49 30 64181 799.
E-mail address: trubiroha@igb-berlin.de (A. Trubiroha).

0020-7519/$36.00 © 2009 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2009.05.003

persist in the gut of birds for only a few days to reach sexual maturity and to reproduce (Dubinina, 1980). Plerocercoids are frequently found in the body cavity of cyprinid fish, where they survive for several years. As reported for different infected cyprinid species, irrespective of sex, season and age, gonads of infected fish remain immature and only early germ cell stages are present (Arme, 1968; Arme and Owen, 1968). Thus, it seems reasonable that the parasite *L. intestinalis* might contribute, as a natural source, to the recent ecotoxicological issue of endocrine disruption in fish (Kloas et al., 2009).

In vertebrates, reproduction is under endocrine control of the hypothalamus–pituitary–gonad[1] (HPG) axis and the pituitary gonadotropins, namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH), play a pivotal role in the regulation of cell differentiation, proliferation and steroidogenesis of gonad tissues (Richards, 1994). FSH and LH are heterodimeric glycoprotein hormones consisting of a non-covalently linked common glycoprotein-hormone α-subunit (αGSU) and a specific β-subunit (either FSHβ or LHβ) that exert their action via membrane-bound G-protein coupled receptors (FSH-receptor and LH-receptor) (Richards, 1994). In fish, as in mammals, the existence of two gonadotropins (FSH and LH) is now well accepted and both are thought to play a differential role in gonad development (Schulz et al., 2001; Swanson et al., 2003). Based on extensive studies in salmonids (Prat et al., 1996; Gomez et al., 1999), FSH is considered to stimulate early development of the ovarian follicle and spermatogenesis in the testes, while LH initiates final gamete maturation leading to ovulation in females and spermiation in males. However, especially in multiple spawning teleosts, the expression of gonadotropin subunits during gametogenesis shows different profiles. For example in goldfish (*Carassius auratus*), FSHβ and LHβ mRNA levels increase in parallel during the spawning period (Sohn et al., 1999). Thus, although well-characterised in salmonids, the role of both gonadotropins for gonad development may differ between fish species.

Irrespective of the precise differential functions of FSH and LH during gonad development, gonadotropins undoubtedly regulate reproduction. Interestingly, following hypophysectomy, gametogenesis in fish is blocked at the same maturational stages as reported for infections with *L. intestinalis* (Arme, 1968; Arme and Owen, 1968). This led to the hypothesis that *L. intestinalis* induces stagnation of gametogenesis by a deficiency of pituitary gonadotropins and, indeed, histological observations showed a reduction in the number and activity of putative gonadotropic cells in the pituitary of infected roach (*Rutilus rutilus*; Kerr, 1948; Arme, 1968). Moreover, in a recent study, Carter et al. (2005) demonstrated decreased contents of pituitary LH protein and mRNA in infected roach and, consequently, evidence suggested that *L. intestinalis* ceases host gonad development and reproduction via pituitary gonadotropins. However, despite the pivotal role of FSH for early gametogenesis in fish, expression of FSHβ in infected roach has not yet been investigated and the precise mechanisms involved in the stagnation of host gametogenesis and the endocrine status of infected fish are still poorly understood. Since gonadotropin synthesis and secretion change in the course of gametogenesis, differences between uninfected and infected individuals may reflect the difference between two gonadal stages, concealing initial parasite effects. Consequently, investigations during distinct reproductive periods and especially before the appearance of any differences in developmental stages of gametes, represent a promising approach to shed more light on the roles played by FSH and LH in mediating the impact of *L. intestinalis* on host gametogenesis.

In the present study, quantitative PCR (qPCR) assays for all three gonadotropin subunits (αGSU, FSHβ, LHβ) of roach and for a reference gene, ribosomal protein L8 (rpL8), were developed. Subsequently, to our knowledge for the first time, the effect of *L. intestinalis* on the expression of gonadotropin subunits in roach was analysed in the context of morphological, parasitological and reproductive parameters of infected individuals. To clarify which gonadotropin, FSH or LH, is the preferred target of *L. intestinalis* during early gametogenesis we compared the expression of gonadotropins between infected and uninfected roach of the same gonadal stage in a laboratory study.

2. Materials and methods

2.1. Animals and sampling

Adult roach (*Rutilus rutilus*) were caught by electrofishing from Lake Mueggelsee during October 2007. Lake Mueggelsee (52° 26′ N, 13° 39′ E) is a polymictic and eutrophic shallow lake (surface area of 7.3 km^2; mean depth of 5 m) to the southeast of Berlin (Germany) which is flushed by the River Spree (Driescher et al., 1993). Infected roach could be identified in many cases by their distended abdomen (Supplementary Fig. S1) and individuals were caught selectively. Roach selected showed comparable size parameters (i.e. length, see Table 1). Fish were separated into two groups. For field studies, fish were sampled on the day of catching ("field group"); for the "laboratory group", roach were held in aquaria for further investigation under controlled laboratory conditions. After acclimatisation in a 300 L tank, these fish were transferred to 40 L aquaria (four to six individuals) connected to a partial recirculation system and fed with commercial trout food (DAN-Ex 1750, Dana Feed) at approximately 2% of the body mass/day. Food was supplemented with living *Chaoborus* larvae three times per week. Rearing conditions were at 25 ± 1 °C water temperature and a light/dark ratio of 12/12 h. Sampling of the laboratory group was carried out five months after capture. All experiments were conducted in compliance with the institutional guidelines for the care and use of animals.

For sampling, roach were anaesthetised individually with ethyl 3-aminobenzoate methansulfonate (MS222, Sigma) and killed by decapitation. Pituitaries were removed, immediately frozen in liquid nitrogen, and stored at −80 °C until further processing. The age of fish in the field group was determined by scale annuli. Upon sampling, the following biometric and parasitological parameters were measured: fish total length (to the nearest mm); fish total mass and somatic mass (to the nearest 0.1 g); gonad mass of fish and parasite mass (to the nearest mg); number of parasites per fish. Morphological and parasitological indices were calculated as follows: condition factor (CF) = fish somatic mass × 100/(fish total length)^3; gonadosomatic index (GSI) = (fish gonad mass/fish somatic mass) × 100; parasitisation index (PI) = (parasite mass/fish somatic mass) × 100.

2.2. Gonad histology

Samples of gonads of all individuals were preserved in Bouin's fixative for 12 h, dehydrated in a graded series of ethanol and subsequently embedded in paraffin. Sections were cut at a thickness of 5 μm and stained with H & E. Phenotypic sex was confirmed by histology and staging of gonads was performed according to Nolan et al. (2001).

[1] *Abbreviations:* FSHβ, follicle-stimulating hormone β-subunit; LHβ, luteinizing hormone β-subunit; FSH, follicle-stimulating hormone, LH, luteinizing hormone; HPG, hypothalamus–pituitary–gonad; αGSU, glycoprotein-hormone α-subunit; CF, condition factor; PI, parasitisation index; GSI, gonadosomatic index; GLM, general linear model; GH, growth hormone; IGF, insulin-like growth factor; GnRH, gonadotropin-releasing hormone; RIN, RNA integrity numbers.