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Research Article

Occurrence and Effects of *Diplostomum* sp. Infection in Eyes of *Acanthobrama marmid* in Keban Dam Lake, Elazığ, Turkey

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Abstract: Infection with the parasite *Diplostomum* sp. can lead to severe ocular pathology and may result in host mortalities in commercial fish farming. Here we show that such infection is rife in a major lake used for fishing in Turkey. In a study of *Acanthobrama marmid* fish in Keban Dam Lake, Elazığ, Turkey, we found the infection period was normally between May and September each year, and that the prevalence and abundance of eye lens parasites reached a maximum in September. Typical results of metacercariae occurrence in the fish eye included exophthalmia, local haemorrhage and lens cataract. There was a significant positive correlation between the body condition of fish and the parasite burden (Spearman rank correlation coefficient, R=0.625, n=100, p<0.05). SDS-PAGE of solubilised insoluble proteins showed that infected lenses had an increase in high molecular weight species, suggestive of increased levels of crosslinked proteins giving rise to cataracts.

Key Words: cataract, lens, exophthalmia, parasites, fish, SDS-PAGE

Keban Baraj Gölü (Elazığ, Türkiye)'nde Bulunan *Acanthobrama marmid* Gözlerinde *Diplostomum* sp. Enfeksiyonunun Bulunuşu ve Etkileri

Özet: Ticari balık çiftliklerinde *Diplostomum* sp. enfeksiyonu ağır oküler bozukluğa yol açabilir ve konakçının ölümü ile sonuçlanabilir. Biz burada, Türkiye'de balıkçılık yapılan büyük bir gölde hüküm süren böyle bir enfeksiyonu işaret ediyoruz. Keban Baraj Gölü (Elazığ, Türkiye)'nde bulunan *A. marmid* balıklarında yapılan çalışmada, enfeksiyon periyodunun normal olarak yılın mayıs ve eylül ayları arasında olduğu ve göz lensi parazitlerinin yüzde ve yoğunluğunun eylül ayında maximuma ulaştığı bulundu. Balık gözlerinde metacercariae bulunuşunun tipik sonuçları exoftalmus, lokal hemoraji ve lens kataraktını içerdiği tespit edildi. Parazit yoğunluğu ile balığın kondisyon durumu arasında anlamlı pozitif korelasyon vardı (p<0.05). Serbest hale getirilen membran proteinlerinin SDS-PAGE analizleri, enfekte lens proteinlerinde kontrole göre agregasyon sonucu oluşan yüksek molekül ağırlıklı bantların katarakt oluşumunu hızlandırdığı anlaşılmaktadır.

Anahtar Sözcükler: Katarakt, lens, exoftalmus, parazit, fish, SDS-PAGE

Introduction

Among digeneans, the larval stages, especially cercariae and metacercariae (e.g. of *Diplostomum spathaceum*), are the main agents of important diseases in fish. The metacercariae of *D. spathaceum* live in the eye lenses of many freshwater fish without undergoing encystation. They are pathogenic to the fish, causing opacity of the eye lens (worm star). This parasite may lead to particular problems in trout farming and in commercially growing cyprinid. Species of the genus *Diplostomum* are widely distributed and are common parasites in aquatic environments (1). They require three hosts in their life cycle. *Diplostomum* mature in the small intestine of piscivorous birds and pass through snails and fish during their life cycles. They inhabit the lens, retina and aqueous humour of fish eyes as well as the brain, spinal cord and nasal spaces (2,3). Infection with *Diplostomum* spp. leads to severe ocular pathology and can result in host mortalities in commercial fish farming. The disease caused by invasion of the eye by larval digeneans which become established and grow in the lens,

retina or humour is called diplostomiasis, dipostomatosis and parasitic cataract of eyefluke disease (4).

The metacercarial stage of *Diplostomum spathaceum*, which is primarily a parasite of the lenses of many freshwater fish species and is the organism associated with cataracts or blindness, has been the major focus of research (4). The objectives of our study were to report *Diplostomum* spp. infection and determine the intensity of infection in *Acanthobrama marmid* and the pathological effects of the infection on fish eyes.

Materials and Methods

A total of 100 fish were caught by gill nets in 1997 and 1998 in Keban Dam Lake, Elazığ, Turkey. They were photographed and immediately transported to the Fish Disease Laboratory in Firat University. The fish were measured (weight and fork length) and examined and photographed under a dissection microscope for *Diplostomum* sp. as described previously (5). The prevalence and intensity of parasites were determined as described (6).

Dissected lenses were placed into preweighed Eppendorf tubes and their weights recorded and then frozen at -70ºC. Lenses were homogenised in a volume of one-tenth lens weight of Tris- EDTA (Tris, 5 mM, EDTA, 1 mM, sodium azide, 0.05% and b-mercaptoethanol, 5 mM) at 4ºC with a glass Dounce homogeniser and then centrifuged at 30,000 g for 15 minutes at 4ºC. Water soluble and solubilised water-insoluble proteins were assayed by the modified method of Laemmli (7) using SDS-PAGE (SDS-polyacrylamide gel electrophoresis). In brief, the gel system consisted of 2.5 ml of 1.5 M Tris-HCl, pH 8.8, 0.1 ml of 10% SDS and 7.5 ml of acrylamide/water. Polymerisation was initiated by the addition of 50 ml of 100 mg/ml ammonium persulphate solution and 15 µl TEMED.

Relationships between body condition and parasite burden were examined by means of pearson product moment correlation coefficient using MINITAB Statistical Software Release 10.

Results and Discussion

One hundred *Acanthobrama marmid* (weight range: 10-150 g, mean= 79.38±33.92 g and length range: 9.5-24 cm, mean= 17.95±3.05) were used in this study.

Kennedy (8) showed that a species of eyefluke introduced into new specific environments can become established in a population of fish very rapidly. We found the overall prevalence of *Diplostomum* sp. (Figure 1) infection in the sample of *Acanthobrama marmid* to be 78% (78/100 fish). The frequency distribution of metacercariae in individual fish is presented in Figure 2. Although previous studies (9-11) have shown an overdispersion of infection, the degree of overdispersion was not high in this study (variance: mean ratio= 1.3). The accumulation of eyeflukes in large numbers in fish

Figure 1. Metacercarial stage of *Diplostomum* sp. in the eyes of Acanhobrama marmid. The bar represents 25 mm.

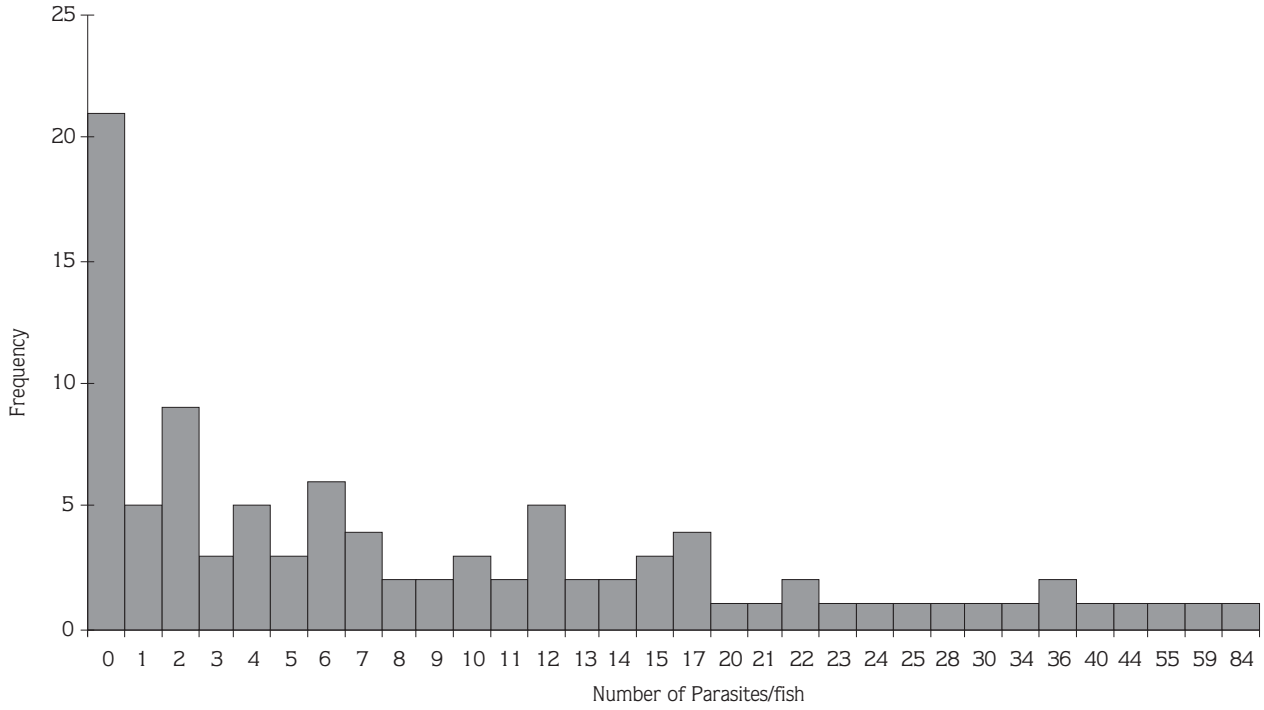

Figure 2. The frequency distribution of *Diplostomum* sp. in the eyes of *Acanthobrama marmid*.

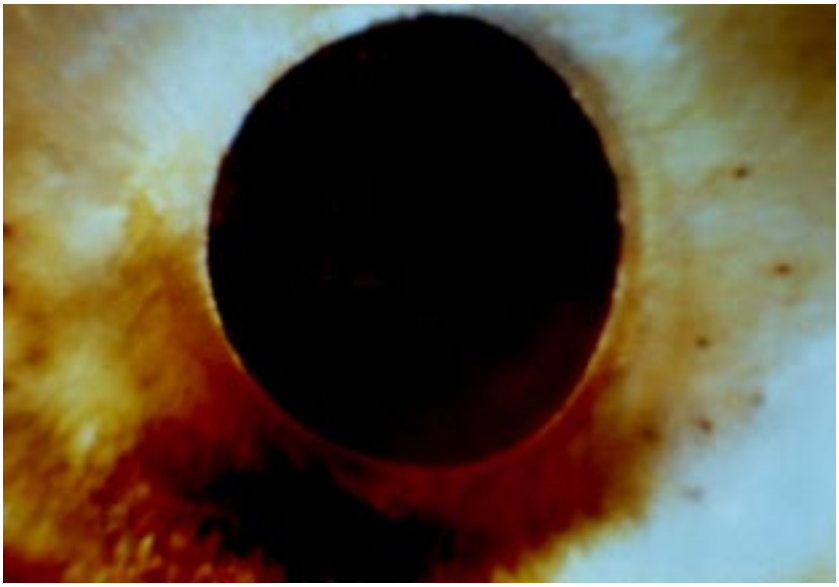

Figure 3. Microscopic feature of eye of *Acanthobrama marmid* infected with *Diplostomum* sp. Magnification x 4.

eyes without significant loss and limitation of fish or lens size on establishment of the parasite has been shown by Chappell (12), who recovered 231 *Diplostomum spathaceum* from the lenses of a six-year-old roach. Wootten (13) found over 550 metacercariae from mature rainbow trout. We found the infection period was normally between May and September each year; however, the prevalence and abundance of eye lens parasites reached a maximum in September. In our study, mean intensity (number of worms per infected fish) was 13.5, ranging from 1 to 84, and only one fish harboured the highest number. Most of fish examined had 2 or 6 metacercaria (Figure 2). A significant positive correlation was detected between the condition factor of fish and parasite burden (Spearman rank correlation coefficient, $R= 0.625$, $n=100$, $p<0.05$).

A high pathogenity of the metacercarial stage of diplostomatids in the eyes of both wild and farmed fish has been suggested (14). In our study, typical pathological signs of a metacercarial occurrence in the eye (Figure 3) included exophthalmia, local haemorrhage and lens cataract. Cloudy eyes (Figure 4) are an indication of cataract in fish eyes caused by heavy infection of metacercariae.

Figure 5 shows the SDS gels of insoluble proteins from noninfected (control) and infected fish lenses. There are increased levels of high molecular weight stained bands in samples of infected fish. This suggests an increase in high molecular crosslinked protein causing insolubility, which results in cataracts from many aetiologies (15-17).

Figure 4. The eyes of Acanhobrama marmid, uninfected (-) and infected (+) with *Diplostomum* sp.

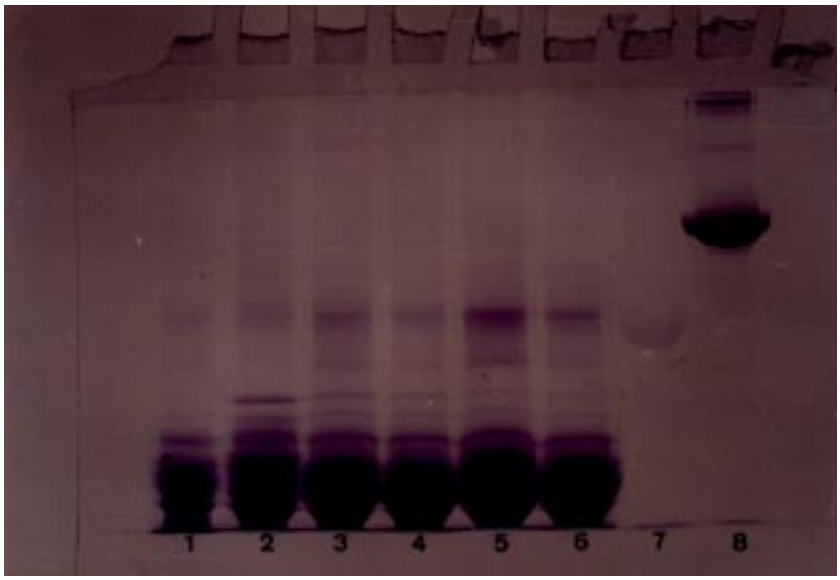

Figure 5. SDS-PAGE analysis of total water insoluble proteins (WIP) of fish lenses.

Lane 1: noninfected (control); lanes 2-6: infected lenses; lanes 7 and 8: molecular weight standards (34 and 45 kDa respectively).

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