The study of the plerocercoid of diphyllobothriidae (cestoda, pseudophyllidea) in two cyprinid hosts, *Abramis brama* and *Alburnoides bipunctatus* from north and northwest of Iran

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Abstract:

BACKGROUND: The plerocercoid stage of Pseudophyllidean cestoda infected a wide range of fresh water fish, particularly the members of the Cyprinidae family. The parasite species are the most common pathogens that have severe effects on fish. **OBJECTIVES:** The aim of the present study is to determine the occurrence and distribution of the plerocercoid of Diphyllobothriidae in two freshwater fish from north and northwest of Iran. Finally, we discuss the role and dynamics of these species of fish in the transmission of infection. **METHODS:** This study was carried out from September 2011 to September 2012on a total of 883 *A. bipunctatus* and 418 *A. brama* from north and northwest of Iran. The samples were analyzed to find the plerocercoid infection. **RESULTS:** From a total number of 883 *A. bipunctatus* and 418 *A. brama* fish samples, 558 fish (63.19%) of the former and 67 fish (16.02%) of the latter were infected. The rate of infection was significantly lower in winter ($p<0.01$). Also, the weight of infected fish was significantly lower than non-infected ones ($p<0.01$). Moreover, the infection in northwest of Iran was significantly higher than north of Iran ($p<0.01$). **CONCLUSIONS:** The family of Diphyllobothriidae is an important cestode and the prevention programs to break the cycle of infection are essential. More suitable solutions to tackle the problem, further epidemiological studies on other fresh water sources of Iran are needed.

Introduction

The family of Diphyllobothriidae is a significant family of parasites of freshwater fish that belongs to the pseudophyllidea order. These tapeworms have a complex life cycle with three hosts. In the free living phase, coracidium develops in the egg, hatches and then is swallowed by a copepod and finally develops in to a procercoid. The second host is usually a cyprinidean fish. The procercoid develops to a plerocercoid in the abdominal cavity (Innal et al., 2007).The cycle is completed when the final host, like piscivorous birds, mammalians, or amphibians prey the fish and plerocercoid matures in the intestine of the final host. Then, theirs eggs are shed in water along with the birds' feces to renew the cycle (Hoole, 1994). During the plerocercoid development process, the plerocercoid damages the gonads of the host. The physical damage puts pressure on the viscera and also infuses chemicals that affect the brain, hypophysis and gonad axis. It also inhibitsthe LH hormone infusion that finally leads to the immature gonads and immaturity (Choudhury and Dick, 1995; Jalali et al., 2008). This family has significant genera like *Ligula intestinalis*, *Digramma interrupta*, *Spirometra mansoni*, and *Schistocephalus solidus*. The plerocercoid of *L. intestinalis* and *D. interrupta* has a wide global distribution (Loot et al., 2002; Brown et al., 2002; Barson and Marshall, 2003; Koyun, 2006; Ergonul, et al, 2005; Innal andKeskin, 2006; Pazooki et al., 2007). The presence of the plerocercoid of *L. intestinalis* and *D. interrupta* were reported in several studies.

However, there is limited information about the infection and epidemiological aspects of it in *A. bipunctatus* and *A.brama*.

The aim of the present study is to determine the occurrence and distribution of the plerocercoid of Diphyllobothriidae in two freshwater fish from north and northwest of Iran. Finally, we discuss the role and dynamics of these species of fish in the transmission of infection.

Materials and Methods

Area of study: This study was carried out in Bandar Anzali (37.28'N, 49.27'E) in the Guilan province, in Ramesar (36.54'N, 50.39'E) in the Mazandaran province at north of Iran. The same was undertaken in Maragheh (37.23'N, 46.14'E) in the East Azerbaijan province and in the Aras dam (39.05'N, 45.24'E) in the West Azerbaijan province in the northwest of Iran (Figure 1).

The Aras dam is located in the downstream of Poldasht in the West Azerbaijan province, along the border of Iran and Azerbaijan.

Sampling: This study was carried out from September 2011 to September 2012 in north and northwest of Iran. A total of 1301 fish including 883 *A. bipunctatus* and 418 *A. brama* were collected. The samples were captured through angling with a gillnet of various mesh sizes (10,12,14,17,21,27,32 and 40 mm) in order to catch different fish sizes. *A. bipunctatus* samples were obtained from the fresh water source of the East Azerbaijan (Maragheh) and Mazandaran province (Ramesar). In Maragheh, due to the frozen water, sampling could not be undertaken in winter. Also, samples of *A. brama* were captured from the water source of the West Azerbaijan (Aras Dam) and Guilan province (Bandar-e-Anzali). The samples were put in ice boxes and immediately transferred to the laboratory. The sample size for this study was determined using $n= (z^2p(1-p))/d^2$. Where n is the estimated sample size, z is the standard value of confidence level at 95% which is 1.96, p is the estimated prevalence of Diphyllobothriidae plerocercoid in fish which is considered to be 15% and d is the margin of error set at 5% (0.05=>n=288) (Daniel, 1999).

Morphometric and morphological identification: The species of all fish samples were identified based on the taxonomical keys. The weight of each sample was measured using a digital scale and the total weight and length of each fish was recorded. The abdominal cavity was autopsied with a scalpel and the body cavity was examined for the presence of plerocercoid. If any plerocercoid was found, its weight and length of would be recorded, and the same time the rate of infection and the morphometric and morphological characteristics of the plerocercoid were also determined. The parasites were fixed in 70% alcohol so that their characteristics could be determined. The samples terminal segments stained with aceto-carmineand were mounted with the Canada balsam. The morphological characterization of the plerocercoid was undertaken by observing them under the light microscope. The species identification of each parasite was made through the taxonomic keys (Chubb et al., 1987).

Statistical Analysis: Obtained data were analyzed by t-test and Chi square in SPSS (software version 16; SPSS, Inc., Chicago, Illinois, USA). Statistical significance was set at $p<0.001$.

Results

From a total number of 883 *A. bipunctatus* and 418 *A. brama* collected samples, 558 (63.19%) of the former and 67 fish (16%) of the latter were infected. The plerocercoid of *L. intestinalis* and *D.interrupta* were detected in this study. The plerocercoid of *L. intestinalis* was isolated only from *A. bipunctatus* and the plerocercoid of *D.interrupta* was found only in *A.brama*. The infection rate of the fish species in different seasons was shown in (Figure 2,3). The prevalence of infection was significantly lower in winter ($p<0.01$). The infection in northwest of Iran was significantly higher than north of the country ($p<0.01$). In the Maragheh dam site, the sampling was undertaken in two locations: at the dam reservoir and the rive contributing to it. In the reservoir, 65 out of

Figure 1. Four different geographical region of sampling.

71 samples were infected whereas none of 153 fish captured from the river was infected. In addition, the infection rate in fish collected from the reservoir was significantly higher than those from the river ($p<0.01$) (Figure 4). The weight ratio of parasite to host in *A.brama* was significantly lower than *A.bipunctatus* ($p<0.01$). Also, the weight of infected fish was meaningfully lower than non-infected ones ($p<0.01$).

There existed 1-7 plerocercoid per infected fish. The mean length and weight of each plerocercoid of *L. intestinalis* were 22±7.0 Cm and 1.62±0.3 gr, respectively. These figures for each plerocercoid of *D. interrupta* were 45±19.0 Cm and 22.55±5.0 gr, respectively. The largest plerocercoid was a *D. interrupta* that had a length of 112.5 Cm. The mean weight of infected and non-infected *A. brama* was 197±63.0 gr and 220±70.1 gr, respectively. These figures for infected and non-infected *A. bipunctatus* were 3.1±0.6 gr and 4.78±0.75 gr, respectively.

Discussion

In a study undertaken in the Sattarkhandam in Ahar (i.e. the East Azerbaijan province, Iran), the rate of infection to *L.intestinalis* in *A. bipunctatus* and Alburnus filippi fish were reported to be 80% and 88%, respectively (Mortazavi-Tabrizi et al., 2004). In a similar survey in the northwest of Iran, *L.intestinalis* and *D. interrupta* were reported in some species of freshwater fish (Pazooki et al., 2007). The prevalence of *L.intestinalis* in *A. brama* in the Aras dam was 45% with a significantly higher infection rate in autumn compared to winter (Nezafat-Rahimabadi et al., 2008). These results show that *A. bipunctatus* and *A. brama* are important hosts for the plerocercoid stage of *L.intestinalis* and *D. interrupta*, respectively.

The *L.intestinalis* is a common fish parasite, especially in Cyprinidae in southwest of France (Loot et al., 2002). In France, the highest prevalence was seen at the end of summer and throughout autumn (Brown et al., 2002). In Zimbabwe, the highest seasonal infection rate was reported in summer. The results regarding the weight of infected and non-infected fish showed that the weight of infected fish was significantly lower than non-infected fish (Barson and Marshall,2003). Brown revealed that the weight of infected fish to *L.intestinalis* was significantly lower than non-infected ones (Brown et al, 2002). In a survey in Turkey with a 16% prevalence of *L. intestinalis*, the most infection rate was reported in August and September, and no infection was seen in winter (Koyun, 2006). According to another seasonal survey in Turkey, it was shown that autumn with 73% infection had a significant difference with other seasons (Turgut et al., 2011). The findings of the current study are completely in agreement with the results of the mentioned studies. Britton found only one parasite per each fish (Britton et al 2009), whereas Cowx observed up to 8 plerocercoids per each fish (Cowx et al., 2008). In this study, up to 7 parasites were observed in each fish (Figure 5).

Studies undertaken on the distribution and prevalence of *L.intestinalis* and *D. interrupta* in Iran show that most of the infections have been from west and northwest of the country; in contrast few reports show the source of infection from the north region (Parsa-Khanghah et al., 2011; Parsa, 2010; Haji Rostamloo, 2008; Pazooki et al., 2007; Jalali and Barzegar, 2006; Mortazavi-Tabrizi et al., 2004). It should be noted that, according to the obtained data, there are few infection reports from the fish samples of the Caspian Sea; however, some of them are originated from the Aras dam (West Azerbaijan) and transported for sale to north of Iran (Youssefi et al., 2002; Nezafat-Rahimabadi et al., 2008).

In the present study, none of collected cyprinidae fish from north of Iran (*A. brama* and *A. bipunctatus*) were infected. In contrast, the rate of infection in northwest of Iran was remarkably high. It is thought that one of probable reasons for this is that the

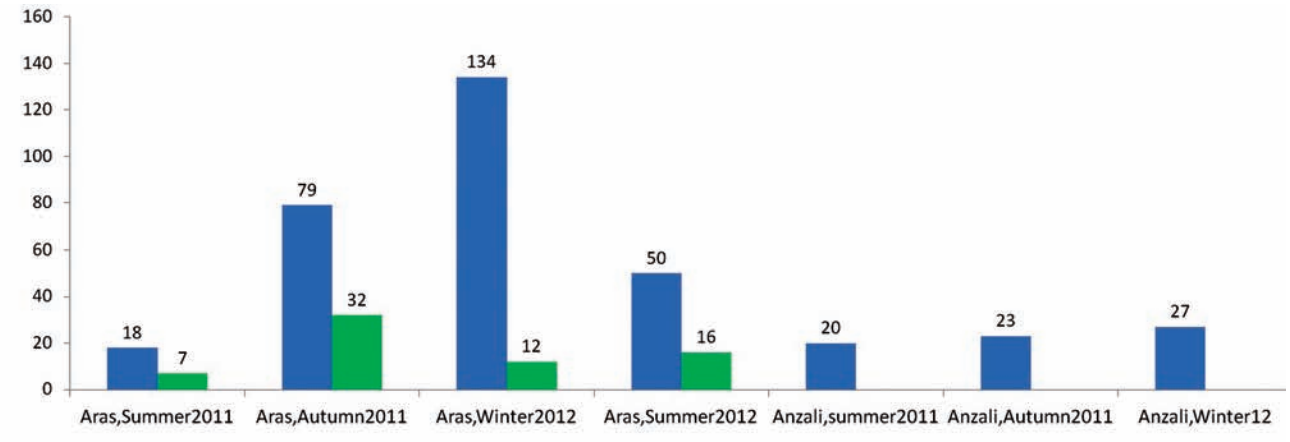

Figure 2. Prevalence of *Digramma interrupta* plerocercoid in *Abramis brama* in different seasons. Negetive Positive

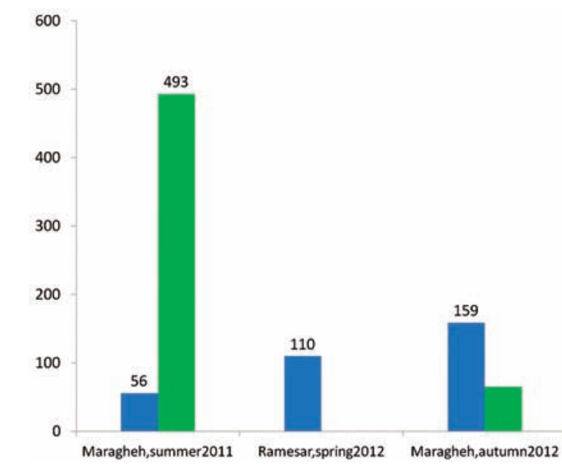

Figure 3. Prevalence of *Ligula intestinalis* plerocercoid in *Alburnoides bipunctatus* in different seasons.
Negetive Positive

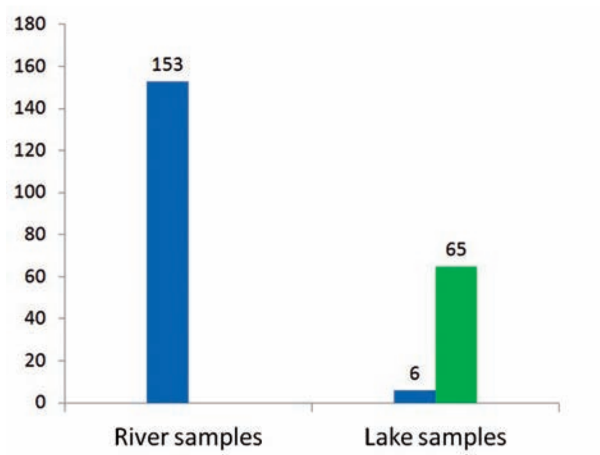

Figure 4. *Ligula intestinalis* prevalence in *Alburnoides biponctatus* in two region of the Maragheh dam.
Negetive Positive

Figure 5. An infected *Alburnoides bipunctatus* with plerocercoids of *Ligula intestinalis*.

infection of fish has affected other species of fish and the habitat. Bouzid et al., (2008) revealed that geographically isolated lineages and host specificity could be direct results of the adaption to local host fauna. Also, it is notable that *L. intestinalis* in Kenya is highly host specific (Britton et al., 2009). Another reason for this may be associated to the fact that the northwest region is in the vicinity of Turkey where this infection is highly endemic(Turgut., et al. 2011; Ozbek and Ozturk, 2010; Korkmaz and Zencir, 2009; Aydogdu., et al. 2008; Tekinozan., et al. 2008; Innal., et al., 2007; Ergonul., et al, 2005;Innal andKeskin, 2006; Koyun, 2006;Yavuzcan., et al., 2003). It is important to note that the migration of fish-eating birds (as the final host) causes the extension of infection too (Kennedy, 1998; Wyatt and Kennedy, 1989). The Aras river originates from the Turkey mountains and extends along the border of northwest of Iran, and the Aras dam is the most important dam on the way of this river. Therefore, infected fish could be carrier of parasite and also re-infection in these fish could be occurred (Eslami, 2006). That is another probable reason for the high infection rate in northwest of Iran. On the other hand, it seems that a low infection rate in the fish samples of north of Iran has two major reasons. First, in the south coast of the Caspian sea, migratory birds that choose lagoons for overwintering, come from Siberia where a low infection rate of the Diphyllobothriidae plerocercoid exists. That is because of low temperature and the freezing of water sources especially in winter. Second, the marine water is not much suitable for this parasite (Eslami, 2006; Scholz et al., 2009).The unique event seen in this study is related to the Maragheh region where the samples were obtained from two different sites including the reservoir of the Maragheh dam and its upstream river. The infection rate in the specimen from the reservoir was significantly higher than those isolated from the river. The parasite prevalence was significantly correlated with the habited occupancy. There are two mechanisms that may explain this. The first one is that in the reservoir, infection caused by the parasites resulted in behavioral modification that increased an opportunity for bird predation; and the second one is that fish were more exposed to the parasite infection in certain habitats (britton et al., 2009). Also, it may be due to the fact that the first intermediated hosts (cyclops) often live in the slow-flowing parts of the water (backside of the reservoir) (Elgmork, 2004). These reasons not only seem to be logical for a higher infection rate in those areas, but also could be an alternative cause of the negative results in the samples collected from a local river in Ramsar.

Previous published reports in Iran and other parts of the world indicate that *L.intestinalis* is one of the most important and widespread infection of freshwater fish, while the other genera of Diphyllobothriidae family such as *D. interrupta* has a lower prevalence that is in accordance with our findings. In conclusion, the results of this study at northwest and north of Iran showed that infection with plerocercoid was highly correlated with the habitat and also was host specific. However, *A. bipunctatus* and *A. brama* must be considered as important hosts in the ecological components in the transmission of *L. intestinalis* and *D. interrupta,* respectively.

According to the results of this study *A. bipunctatus* is an important host of *L. intestinalis* in the northwest of Iran. *L.intestinalis* and *D. interrupta* can have serious effects on fish as an important source of human nutrition. Also, considering the fact that the Diphyllobothriidae family is zoonose, the prevention programs for breaking the cycle of infection is essential. To have more appropriate solutions to tackle this problem, more comprehensive studies in other fresh water sources of Iran are needed.

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مجله طب دامی ایران، ۱۳۹۲، دوره ۷، شماره ۲، ۱۰۹-۱۰۳

مطالعه پلروسرکوئید انگل‌های خانواده دیفیلوبوتریده (راسته پزودوفیلیده آ) در دو ماهی سیم (*Abramis brama*) و خیاطه (*Alburnoides bipunctatus*) در حوضه‌های آبی شمال و شمال غربی ایران

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چکیده

زمینه مطالعه: مرحله پلروسرکوئید سستودهای راسته پزودوفیلیده آ طیف وسیعی از ماهیان آب شیرین به ویژه اعضای خانواده کپورماهیان را آلوده می کنند. گونه های انگلی از شایع ترین بیماریهای ماهیان بوده و اثرات شدیدی بر روی آنها به جای می گذارند. **هدف:** مطالعه مرحله پلروسرکوئید انگل های خانواده دیفیلوبوتریده در دو ماهی سیم (*Abramis brama*) و خیاطه (*Alburnoides bipunctatus*) در حوضه‌های آبی شمال و شمال غربی ایران و نقش این ماهیان در چرخه انتقال آلودگی می باشد. **روش کار:** این مطالعه از شهریور ۱۳۹۰ لغایت مهر ۱۳۹۱ صورت گرفت. در مجموع ۸۸۳ قطعه ماهی خیاطه و ۴۱۸ قطعه ماهی سیم از حوزه های آبی شمال و شمال غرب ایران جمع آوری شد. سپس نمونه‌ها از نظر وجود پلروسرکوئید بررسی شدند. **نتایج:** از مجموع ۸۸۳ قطعه ماهی خیاطه و ۴۱۸ قطعه ماهی سیم، به ترتیب ۵۵۸ (۶۳/۱۹٪) و ۶۷ نمونه (۱۶/۰۲٪) آلوده بودند. شیوع آلودگی در فصل زمستان به طور معنی داری کمتر از سایر فصول سال بود ($p<0/01$). همچنین وزن ماهیان آلوده نسبت به وزن ماهیان غیرآلوده، به طرز معنی داری کمتر بود ($p<0/01$). شیوع آلودگی در حوزه های آبی شمال غرب کشور به طور معنی داری بالاتر از حوزه آبی شمالی بود ($p<0/01$). **نتیجه‌گیری نهایی:** بر اساس نتایج به دست آمده از مطالعه حاضر، با توجه به اهمیت اقتصادی و بهداشتی این انگل ها، انجام برنامه های پیشگیرانه برای شکستن چرخه آلودگی از اهمیت بسیاری برخوردار است. در این راستا انجام مطالعات جامع همه‌گیری شناسی در حوزه های آبی ایران ضروری است.

واژه های کلیدی: خانواده دیفیلوبوتریده، ماهی سیم، ماهی خیاطه، حوزه‌های آبی شمال و شمال غرب

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