Infection with metacercaria of *Clinostomum complanatum* (Trematoda: *Clinostomidae*) in freshwater fishes from Southern Caspian Sea Basin

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SUMMARY

*Clinostomum complanatum* is a digenetic food borne trematode that is known to heavily infect fish-eating birds in northern Iran, adversely affecting their health. We performed the present study to investigate the infection status of freshwater fish with digenetic trematode metacercariae (*C. complanatum*) in the water systems of Shiroud, Tajan and Gorganroud Rivers of the Southern Caspian Sea Basin, Iran. A total of 849 fish belonging to 9 species were examined, namely *Alburnus alburnus*, *A. chalcoides*, *Alburnoides bipunctatus*, *Barbus plebejus*, *Capeota gracilis*, *Carassius auratus*, *Squalius cephalus*, *Neogobius fluviatilis* and *Cobitis cf taenia*. Of these, only fish in the Shiroud River were found to be infected with the parasite, with the species *Alburnoides bipunctatus*, *Capeota gracilis*, *Cobitis cf taenia* and *Squalius cephalus* being affected. To the best of the authors’ knowledge, this is the first time that *Alburnoides bipunctatus* and *Cobitis cf taenia* have been recorded as intermediate hosts of this parasite. Metacercariae were found mostly under the skin of the fish; however, one metacercariae was found in the brain of *A. bipunctatus*, with a curled tail suggesting that the parasite is able to migrate to the vital organs of fish and consequently affect their functions. To confirm the identity of the metacercariae, they were fed to two chickens to obtain adults which were identified as *Clinostomum complanatum*. The Shiroud River runs through highly populated areas and is the centre of major fishery activities, therefore the high prevalence of a parasite zoonotic such as *Clinostomum complanatum* is of high significance, and it is considered to be an important parasitic species infecting fish. They can cause low weight gain, low unmarketability and high mortality, and some may also have zoonotic importance.

**Keywords:** *Clinostomum complanatum*, Fish, Prevalence, Caspian Sea, Zoonose

Introduction

It is well known that freshwater fish are not only a major source of protein for humans but also common intermediate hosts for different parasitic species. The metacercariae of digenetic trematodes are considered to be one of the most prevalent parasitic species infecting fish. They can cause low weight gain, low unmarketability and high mortality, and some may also have zoonotic importance.

*Clinostomum complanatum* is a hermaphroditic parasite with an indirect life cycle. The adult worms typically live in the oral cavity and oesophagus of piscivorous birds which become infected after consuming infected fish or amphibians [7]. Metacercariae of *C. complanatum* are large, often yellow and encyst in a variety of sites in the body such as the oral cavity, gills, intestines, tail, muscles and eye sockets of fish or amphibians, causing the well-known “yellow grub disease”. In humans, when infection occurs after the consumption of raw fish, the fluke accidentally attaches to the surface of the mucus membrane of the throat, causing a clinical syndrome called *halzoun*. Many cases have been described in several countries, including Japan, Korea, Thailand and India [2, 11, 15, 20] and it is considered to be an important parasitic zoonosis for public health in these countries [9]. Although no human cases have yet been reported in Iran, this could be due health practitioner’s lack of knowledge about the occurrence of this parasite in local fish and/or substandard...
documentation and recording of human health data in the country.

There is a high prevalence of this zoonotic parasite in the relatively large populations of birds that exist in northern Iran, an area that also has a dense human population among whom seafood is popular [16]. Therefore, we were prompted to carry out the present study with the aim of finding the intermediate fish hosts of *C. complanatum*. Many rivers in the region are home to a broad variety of freshwater and anadromous fish species. Several commercially valuable fish species, such as *Rutilus frisii* (also known as Caspian kutum) and sturgeon migrate seasonally to breed and spawn. Many of these fish are edible and local diets may include the consumption of raw or undercooked small fish, either whole or in parts.

**Materials and methods**

**STUDY AREA**

Fish were collected from three rivers within the southern coast of the Caspian Sea Basin (Fig. 1) during the summer months. The Caspian Sea is the largest enclosed inland body of water on Earth by area. Iran has three highly populated provinces located on its southern coast, the populations of which have grown steadily during the last decades. It is estimated that at least 10 million people live in the southern Caspian Sea Basin. The region is bordered by Alborz Heights in the south, which also is responsible for its semitropical climate. Several rivers flow into the basin that are inhabited by numerous fish species from six families [14], which makes it one of the most important and primary production areas in Iran. These rivers are known to be the main spawning ground for several of Iran’s commercially valuable fish. Due to high rainfall, the rivers flow rapidly throughout the year. There are several private and government fish farms along the rivers or in close proximity to them. Table 1 shows details of the fish collected and their localities. The nomenclature of the fish investigated in the present study is based on Keivany et al. [10].

**FISHERY AND PARASITOLOGICAL EXAMINATION**

Fish were transferred to a laboratory in ice and were examined for infection with parasites according to standard protocols [5]. In the laboratory, all fish were first visually examined for any unusual features on their surface. Fish then were incised, transferred into a petri dish, tray or similar container and examined for anything unusual. They were cut open using sharp scissors from the anus to the area between the pelvic fins. The body wall was removed on the left side

<table>
<thead>
<tr>
<th>Family</th>
<th>Common name</th>
<th>Scientific name</th>
<th>Shiroud River (1)</th>
<th>Tajan River (2)</th>
<th>Gorganroud River (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobitidae</td>
<td>Spined loach</td>
<td><em>Cobitis cf taenia</em></td>
<td>101</td>
<td>35</td>
<td>-</td>
<td>136</td>
</tr>
<tr>
<td>Cyprinidae</td>
<td>Bleak</td>
<td><em>Alburnus alburnus</em></td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Danube bleak</td>
<td><em>A. chalcoides</em></td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Schneider</td>
<td><em>Alburnoides bipunctatus</em></td>
<td>101</td>
<td>30</td>
<td>9</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Barbel</td>
<td><em>Barbus plebejus</em></td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Goldfish</td>
<td><em>Capoeta gracilis</em></td>
<td>103</td>
<td>100</td>
<td>72</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>Chub</td>
<td><em>Squalius cephalus</em></td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Monkey goby</td>
<td><em>Neogobius flaviotilis</em></td>
<td>77</td>
<td>16</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td><em>Total</em></td>
<td></td>
<td>480</td>
<td>256</td>
<td>113</td>
<td>849</td>
</tr>
</tbody>
</table>

**Table 1:** List, number and location of sampling for fish examined in the present study. Numbers after rivers’ name corresponds to the numbers in Figure 1.
by making an incision above the pectoral fin and cutting along the lateral line to the anus. Before examining any organ for parasite infection, the outer layers of all internal organs, including the gut, gonads, swim bladder, liver, kidneys, pyloric caeca and heart were thoroughly visually examined for the presence of parasites.

Metacercariae were recovered from cysts using a sharp needle and fixed between two slide glass slides with 70% ethanol. The infected organs, number of cysts per fish and other important data such as the fish’s species and locality were recorded. Later, the metacercaria were stained with carmine. After de-staining in acid alcohol, they were dehydrated in alcohol series, cleared in xylol and mounted in Canada balsam. Drawings were made to scale with the aid of a camera lucida and measurements were made directly with an eyepiece micrometer, including body size, and oral suckers and acetabulum length and width. According to their morphological characteristics, parasites were identified to genus level [1]. Parasite prevalence and intensity were calculated according to Shamsi and Suthar [17].

EXPERIMENTAL INFECTION OF BIRDS

For specific identification, some live metacercariae were obtained from fish and fed to two 20-day old chickens. Although those chickens had never been fed with seafood of any sort, their faeces were examined for infection with parasite eggs prior to experimental infection to ensure no previous infection existed. After 5 and 7 days, chickens were euthanized by an overdose of clove oil and examined for infection with parasites. Parasites were collected in accordance with Shamsi et al. [16] and then processed in the same manner as metacercaria, i.e., preserved in ethanol and stained with carmine.

Results

Both the Tajan and Gorganrud Rivers are the major rivers in the southern Caspian Sea area. Some 256 fish belonging to nine species (Table 1) were examined from the Tajan River and 113 fish from belonging to five species (Table 1) were examined from Gorganroud River. None of these fish were found to be infected with metacercaria of *C. complanatum*.

In Shiroud River, 480 fish belonging to nine species were examined of which four species, including *Alburnoides bipunctatus*, *Capoeta gracilis*, *Cobitis cf taenia* and *Squalius cephalus*, were found to be infected with metacercaria of *C. complanatum* (Table 2). The highest rate of infection (24.3%; *n* = 103) was observed in *Capoeta gracilis*, followed by *Squalius cephalus*, *Alburnoides bipunctatus* and *Cobitis cf taenia*. The present investigation also found that fishes from Shiroud River were more heavily infected with *C. complanatum* than those from the other study sites.

The encapsulated metacercariae were found mostly under the skin, on the gills and within muscle tissues. The maximum number of metacercaria per fish (84) was found in a *Capoeta gracilis*. In one *A. bipunctatus*, with an abnormally curved spinal cord, a metacercaria was found in its brain.

All metacercariae found in the present study (Fig. 2) had similar morphology with an average length of 4.75 cm (*n* = 10) and width of 2.34 cm. Oral collars were visible. Ventral suckers (mean = 0.81 × 0.84 cm) were larger than oral suckers (mean = 0.31 × 0.43 cm). Intestinal caeca were lateral to ventral suckers and genital complexes. Testes (mean = 0.29 × 0.73 and 0.41 × 0.99 cm, for anterior and posterior testes, respectively) and ovaries (mean = 0.11 × 0.14 cm) were present.

![Figure 2: showing metacercariae of *C. complanatum* found in the present study. Scale bar = 500 μm.](image)

<table>
<thead>
<tr>
<th>Fish</th>
<th>No of examined fish</th>
<th>No of infected fish (frequency %)</th>
<th>Min./max. no of parasites per fish</th>
<th>Intensity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alburnoides bipunctatus</em></td>
<td>101</td>
<td>8 (7.9)</td>
<td>1-4</td>
<td>1.9</td>
</tr>
<tr>
<td><em>Capoeta gracilis</em></td>
<td>103</td>
<td>25 (24.3)</td>
<td>1-84</td>
<td>9.8</td>
</tr>
<tr>
<td><em>Cobitis cf taenia</em></td>
<td>101</td>
<td>5 (4.9)</td>
<td>1-2</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Squalius cephalus</em></td>
<td>6</td>
<td>1 (16.6)</td>
<td>2-2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

¹Intensity = number of parasites/number of infected fish

Table II: Infection status with *C. complanatum* metacercariae in fresh-water fishes collected from Shiroud River, Iran.

Revue Méd. Vét., 2018, 169, 7-9, 147-151
Adult worms were recovered from the mouths and pharynxes of the experimentally infected chickens. Microscopy and examination of the adults revealed their specific identity as *C. complanatum* according to Grabda-Kazubska [7] and Shamsi et al. [16].

**Discussion**

Despite examining fish from a broad geographical distance in the region (over 300 km; Fig. 1), only fish collected from Shiroud River were found to be infected with *C. complanatum* (Table 2). Although some fish, such as *Capoeta gracilis* and *Alburnoides bipunctatus*, were collected and examined from all three rivers (Table 1), only those collected from Shiroud River showed infection with the parasite, suggesting that only the Shiroud River has suitable environmental and/or biological factors for the completion of the parasite’s life cycle. A previous study [16], also conducted in the southern Caspian Sea Basin, showed that piscivorous birds can be heavily infected with the adult form of this parasite. Therefore, the results of the present study suggest that parasite eggs are distributed across the whole region through infected birds but can only reach fish, their second intermediate hosts, in the Shiroud River region. The climate of the study area is highly varied, being dry near Gorganroud River and subtropical near Shiroud River (Fig. 1). For example, the annual rainfall in the Gorganroud River area, located in the eastern side of the southern Caspian Sea Basin, ranges between 18 to 88 mm, while that of the Shiroud River region, located on the western side of the Southern Caspian Sea Basin, is 29 to 210 mm. This dramatic difference may affect the survival of parasite eggs and miracidium which require an aquatic environment to survive and snails as the first intermediate host. Given that both the infected fish in the present study and birds in the previous study [16], including *Ardea purpurea*, *Egretta alba*, *E. garzetta* and *Nycticorax nycticorax* have wide geographical distributions in the region and can also be found elsewhere, it is possible that the limited geographical distribution determined for this parasite in northern Iran is due to a limited distribution of suitable first intermediate hosts, i.e., snails. Currently, the first intermediate host(s) for *C. complanatum* in the region is (are) not known. It is only known that cercariae of *Clinostomidae* have been reported in *Lymnaea gedrosiana* [19]. Therefore, one important area for future study would be in the determination and specific identification of the natural first intermediate host(s) for the parasite in the region.

In other countries, metacercaria of *Clinostomum* have been reported in a wide variety of freshwater fish (the second intermediate host), as well as toads, frogs, salamanders and tritons. In Iran, *C. complanatum* has previously been reported in five species of fish from other regions, including *Aphanius aphanis* [8], *A. dispar* [6], *Chalcalburnuc chalcoides* [4], *Capoeta gracilis* and *Pseudorasbora parva* [13]. These fish are from various zoogeographical regions and from a variety of fish families suggesting that, as pointed out by several other studies [12], the metacercariae of *C. complanatum* have broad host specificity, making the findings of the present study significant due to the potential impacts on farmed fish and public health.

From a fish health impact point of view, the present study’s finding of *C. complanatum* metacercariae in the brain of *Alburnoides bipunctatus* is significant, as it shows the ability of the parasite to migrate to the vital organs of fish and, consequently, adversely affect their function. It would have been interesting to investigate the histopathology of the brain in the infected fish; however, due to the condition of the fish, which had been euthanized some time previously, the brain tissue was not suitable for histological study. In gross examination, the fish was found to have a curved spinal cord. We did not find any other parasites that are known to cause a similar effect, such as myxosporeans, in this fish. It is known that infection of fish by *Clinostomum* can result in behavioural changes, disease and death resulting in economic loss for fishing industries. Previous histopathological studies showed that metacercariae can cause infiltration of the immune cells at the site of cyst attachment, degenerative changes in the tissues around the cyst [18] and even mortality due to higher predation rate [3].

More importantly, *C. complanatum* is known to be zoonotic [15, 20]. The metacercaria is the infective stage. Humans become infected by consuming infected fish. Therefore, the high prevalence and abundance of parasite metacercaria found in edible fish from the Shiroud River are of high significance. The landscape of the lower reaches of this river is dominated and influenced by the city of Tonekabon, which has a population of over 200,000. The Shiroud River basin is located in the central sector consisting of 33,000 people living in 135 residential areas and villages [14]. More than 30 of these villages are located on the banks of the Shiroud River and, thus, there are direct interactions between people and the river. Interestingly, in the local area, it is believed that consumption of small freshwater fish is a natural remedy for jaundice, a disease that is relatively common among locals, particularly children. Small-sized fish are also quite commonly consumed whole, where internal organs such as the brain may remain undercooked. This greatly increases the risk of infection with this zoonotic parasite among the local population. The symptoms caused by infection with *C. complanatum* after consuming raw or semi-cooked infected fish varies between humans, making accurate diagnosis difficult, particularly as local medical doctors are not usually aware of this parasite. The most common condition is laryngopharyngitis where metacercariae firmly attach to the mucous membrane of the pharynx [11]. Injury to other organs such as the oesophagus due to attachment of metacercariae, anaemia and eye infections are among other conditions assigned to infection with the metacercariae of *C. complanatum* in humans [20]. Since local people are not aware of these parasites and the consequences of consuming infected fish, it is suggested that fisheries authorities in the region take the initiative to inform local fish farmers about preventing farmed fish becoming infected with this parasite.
by controlling snail and bird populations in local aquaculture systems. Medical authorities also should take the initiative to educate medical practitioners and the public about the risk C. complanatum may pose to public health.

Acknowledgement

We are grateful to Dr Celia Barril, from School of Agricultural and Wine Sciences & National Wine and Grape Industry Centre in Charles Sturt University for the French summary of this manuscript. This study had partial financial support from the Iranian Fisheries Research Organisation. Animal ethic guidelines developed by the Iranian Fisheries Research Institute were followed in this study.

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