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Prevalence of *Clonorchis sinensis* infection in market-sold freshwater fishes in Jinzhou city, Northeastern China

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Clonorchis sinensis is an important human parasite in parts of the world, in particular in southeastern Asia, including China. In China's northeastern Liaoning province, the high prevalence of *C. sinensis* infection in humans has been reported. However, limited information is available for the prevalence of *C. sinensis* in its second intermediate host (freshwater fishes). Hence, the prevalence of *C. sinensis* infection in market-sold freshwater fishes was investigated in Jinzhou city, Liaoning Province, China between July and August 2011. A total of 607 fish representing 22 species were collected from Jinzhou city, Liaoning Province, and were examined for the presence of *C. sinensis* metacercariae by digestion technique. The average prevalence of *C. sinensis* infection in freshwater fishes was 36.1%. While the prevalences of *C. sinensis* metacercariae in some fish were very high, such as 100% in *Pseudorasbora parva* and *Mastacembelus aculeatus*, no *C. sinensis* metacercariae was found in *Macropodus ocellatus*, *Opsariichthys bidens*, *Monopterus albus*, *Misgurnus anguillicaudatus* and *Parabramis pekinensis*. The present investigation revealed a high prevalence of *C. sinensis* in its second intermediate host (freshwater fishes) in Jinzhou city, Liaoning province, northeastern China, which provides relevant "base-line" data for conducting control strategies and measures against clonorchiasis in this city.

Key words: Clonorchiasis, *Clonorchis sinensis*, metacercariae, intermediate host, freshwater fish, prevalence, Northeastern China.

INTRODUCTION

Clonorchis sinensis, the oriental liver fluke, is considered to be one of the major fish-borne zoonotic trematodes in some parts of Asia, including China, Korea and North Vietnam (Chen et al., 2011; Kim et al., 2009). Human

beings and other piscivorous mammals become infected with *C. sinensis* when they consume raw or undercooked freshwater fishes and shrimp infected by *C. sinensis* metacercaria (Zhou et al., 2008). The parasite causes clonorchiasis and is often associated with many human diseases such as biliary calculi, cholecystitis, liver cirrhosis, and even cholangiocarcinoma (Pak et al., 2009; Xu et al., 2010). Approximately 35 million people worldwide are estimated to be infected with *C. sinensis*, of

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whom about 15 million people are in China (Lun et al., 2005).

The first intermediate snail hosts for *C. sinensis* are mainly species of *Parafossarulus* and *Bithynia* (Zhang et al., 2007), and the prevalence is very high in some regions of China. Numerous species of freshwater fishes and some species of shrimp serve as the second intermediate hosts for *C. sinensis* (Zhou et al., 2008) while humans and piscivorous mammals serve as reservoir hosts for *C. sinensis* (Rim, 2005).

C. sinensis infection in freshwater fishes has been reported in some provinces in China, such as Guangdong, Guangxi, Hubei and Liaoning provinces (Chen et al., 2010; Shen et al., 2010; Luo et al., 2011; Liu et al., 2011) and its investigation in freshwater fishes has important implications for the prevention and control of *C. sinensis* in humans and animals. However, the majority of these papers were published in local Chinese journals which are not readily accessible to international readers.

In order to evaluate transmission status of *C. sinensis* and to reduce the risk of human infection, the objective of the present study was to estimate the prevalence of *C. sinensis* infection in market-sold freshwater fishes in Jinzhou city, Liaoning province, northeastern China. This would provide "base-line" data for the improved prevention and control of clonorchiasis in this region.

MATERIALS AND METHODS

The study site

Jinzhou city, Liaoning province is located in the northeastern part of mainland China. This city has a temperate climate, with an average annual temperature of 8 to 9°C. The average annual rainfall ranges from 540 to 640 mm. The city has an area of approximately 10 thousand square kilometers, and has a population of approximately 3 million (<http://www.jz.gov.cn/lnjz/>).

Collection and examination of freshwater fishes

Between July and August 2011, 22 species of freshwater fish ($n = 607$) sampled randomly from the market (Jinzhou city, Liaoning province) were examined for the presence of *C. sinensis*. Head, scales, bones, and viscera of the fish were removed to obtain net fish meat for analysis. After weighing, the fish meat was minced and placed in an enamel bowl. Then artificial gastric juice (4 g pepsin, 4.8 g sodium chloride, 40 ml hydrochloric acid, and 360 ml distilled water) with ten times volume of the fish meat was added. The fish meat was incubated at 37°C for more than 1 h with intermittent stirring. The fluid containing digested fish meat was filtered with a 80-well/inch copper sieve to remove the large fragments. The filtered fluid was placed into a 500 ml graduate cylinder with tap water being added. Standing for 15 to 20 min, the supernatant was replaced with fresh water. This process was repeated several times until the supernatant was clear. Finally, the supernatant was removed, and the sediment was examined under a microscope to examine the *C. sinensis* metacercaria (Zhang et al., 2007). Specimens were fixed in 75% ethanol after being cleared and species identification was carried out according to existing descriptions and keys (Kaewkes, 2003). The identity of three

representative recovered metacercaria samples was ascertained by the amplification and subsequent sequence analysis of the internal transcribed spacers (ITS) of nuclear ribosomal DNA following methods reported previously (Kang et al., 2008), and the mitochondrial cytochrome c oxidase subunits 1 (cox1) (Liu et al., 2012).

Statistical analysis

Differences in prevalence of *C. sinensis* in different freshwater fish were analyzed using Chi Square Test in SPSS for Windows (Release 17.0 standard version, SPSS Inc., Chicago, USA). The differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

A total of 607 freshwater fish were examined, and 219 (36.1%) of them were found to be infected by *C. sinensis* metacercaria (Table 1). The ITS and cox1 sequences of the metacercaria had 97-98% similarities with the corresponding sequences of *C. sinensis* (data not shown). This overall prevalence of *C. sinensis* in market-sold fish in Jinzhou city, Liaoning province was significantly higher than that found in some other provinces of China (Shen et al., 2010; Luo et al., 2011), this may be due to difference in different ecological conditions.

The present investigation revealed that the prevalences of *C. sinensis* metacercariae in some fish were very high, such as 100% in *Pseudorasbora parva* and *Mastacembelus aculeatus*, but no *C. sinensis* metacercariae was found in *Macropodus ocellatus*, *Opsariichthys bidens*, *Monopterus albus*, *Misgurnus anguillicaudatus* and *Parabramis pekinensis* (Table 1), and significantly different prevalences were observed for different fishes (Table 1). This may be due to different susceptibility of fish and the different ecological conditions.

The prevalence of *C. sinensis* in *P. parva* in the present study was consistent with that in Dalian city reported recently by Chen et al. (2008). Interestingly, the same fish species may have different prevalences in different rivers of the same area. For example, the prevalence of *Ctenopharyngodon idellus* (26.92%) in the Xiaoling river of Jinzhou area was higher than that (10.42%) in the daughter river (Nver river) of Jinzhou area (Liu et al., 2008).

Conclusions

The present investigation revealed the high prevalence of *C. sinensis* infection in freshwater fishes in Jinzhou city, Liaoning province, northeastern China, which poses significant public health. Therefore, integrated strategies and measures should be implemented to better control *C. sinensis* infection in freshwater fishes in this city, which will reduce the risk of infection in humans with *C. sinensis* in this area.

Table 1. Prevalence of *Clonorchis sinensis* infection in market-sold freshwater fishes in Jinzhou city, northeastern China.

Species of fish	No. (examined)	No. (positive)	Prevalence (%)
<i>Pseudorasbora parva</i>	46	46	100
<i>Mastacembelus aculeatus</i>	33	33	100
<i>Pelteobagrus fulvidraco</i>	25	20	80
<i>Hypseleotris swinhonis</i>	28	22	78.6
<i>Perccottus glehni</i>	25	18	72
<i>Saurogobio dabryi</i>	24	17	70.8
<i>Erythroculter ilishaeformis</i>	26	13	50
<i>Abbottina rivularis</i>	31	14	45.2
<i>Ctenopharyngodon idellus</i>	26	7	26.9
<i>Hypophthalmichthys nobilis</i>	17	4	23.5
<i>Parasilurus asotus</i>	20	4	20
<i>Cyprinus carpio</i>	21	4	19.0
<i>Rhodeus ocellatus</i>	27	5	18.5
<i>Leuciscus waleckii</i>	27	4	14.8
<i>Hypophthalmichthys molitrix</i>	15	2	13.3
<i>Ophicephalus argus</i>	24	3	12.5
<i>Phoxinus lagowskii dybowskii</i>	28	3	10.7
<i>Macoropodus chinensis</i>	38	0	0
<i>Opsariichthys bidens</i>	42	0	0
<i>Monopterus albus</i>	36	0	0
<i>Misgurnus anguillicaudatus</i>	25	0	0
<i>Parabramis pekinensis</i>	23	0	0
Total	607	219	36.1

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