Investigation on the endemic characteristics of *Metorchis orientalis* in Huainan area, China

*Investigación sobre las características endémicas de Metorchis orientalis en Huainan, China*

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**Key words:**

*Metorchis orientalis*. Endemic characteristics. Huainan area.

**Abstract**

**Objective:** To investigate the endemic characteristics of *Metorchis orientalis* (*M. orientalis*) in the Huainan area, Anhui province, China.

**Methods:** The first-intermediate host, second-intermediate host and reservoir hosts were collected, and the endemic characteristics of *M. orientalis* were examined through field investigation and artificial infection.

**Results:** Investigation was completed in 89 domestic ducks, 156 domestic chicken, 41 domestic geese, 20 domestic cats and 19 dogs. The infection rate of *M. orientalis* was 18.0% (16/89) in ducks, 12.2% (19/156) in chicken, 9.8% (4/41) in geese, 5.0% (1/20) in cats and 5.3% (1/19) in dogs. Sixty-seven cercariae of *M. orientalis* were identified in 1,000 *Parafossarulus striatulus*, with a natural infection rate of 6.7%, and 19 cercariae occurred in 300 *Pseudorasbora parva*, with a natural infection rate of 6.33%. The activity of the cercariae of *M. orientalis* was associated with light intensity and temperature. The full life cycle of *M. orientalis* ranged from 120 to 140 days; it occurred approximately in 89 days in snails, 28 days in fish and 20 days in ducks.

**Conclusion:** *M. orientalis* is prevalent in the Huainan area, and it may complete its life cycle in *Parafossarulus striatulus*, *Pseudorasbora parva* and natively raised ducks.

**Resumen**

**Objetivo:** Investigar las características endémicas del *Metorchis orientalis* (*M. orientalis*) en el área de Huainan, en la provincia de Anhui, China.

**Métodos:** fueron recogidos el primer huésped intermediario, el segundo huésped intermediario y el reservorio, y se examinaron las características endémicas del *M. orientalis* a través de investigación de campo e infección artificial.

**Resultados:** la investigación se llevó a cabo en 89 patos domésticos, 156 gallinas domésticas, 41 gansos domésticos, 20 perros y 19 gatos domésticos. La tasa de infección del *M. orientalis* fue del 18.0% (16/89) en patos, 12.2% (19/156) en gallinas, 9.8% (4/41) en gansos, 5.0% (1/20) en perros y 5.3% (1/19) en gatos. Sesenta y siete cercarieas de *M. orientalis* fueron identificadas en 1,000 *Parafossarulus striatulus*, con una tasa de infección natural del 6.7%, y 19 cercarieas ocurrieron en 300 *Pseudorasbora parva*, con una tasa de infección natural del 6.33%. La actividad de las cercarieas de *M. orientalis* se asoció con la intensidad de la luz y la temperatura. El ciclo de vida completo del *M. orientalis* osciló entre 120 y 140 días; se produjo aproximadamente en 89 días en caracoles, 28 días en peces y 20 días en patos.

**Conclusión:** *M. orientalis* es prevalente en el área de Huainan, y puede completar su ciclo de vida en *Parafossarulus striatulus*, *Pseudorasbora parva* y patos autóctonos.
INTRODUCTION

Metorchis orientalis (M. orientalis) belongs to the family Opisthorchidae, subfamily Metorchininae. It was originally described in Japan, Russia and China, where the prevalence of M. orientalis was reported from Heilongjiang, Jilin, Beijing, Tianjin, Shanghai, Jiangsu, Zhejiang, Fujian, Jiangxi, Guangdong, Guangxi, Sha’anxi, Sichuan, Taiwan and Anhui province in China (1). M. orientalis occurs in wider hosts, and primarily parasitizes in the hosts’ hepatic duct and gallbladder, consequently resulting in enlarged gallbladder, thickened cystic wall, desquamation of epithelium, vascular congestion and hemorrhage in internal organs of the infected hosts. Although this species involves wider hazards, its life cycle is less reported in China. The current study was undertaken to investigate the life cycle of M. orientalis and its epidemiological characteristics in the Huainan area of the Anhui province, China.

MATERIALS AND METHODS

SURVEY ON THE LOCAL ECOLOGICAL SYSTEM FOR M. ORIENTALIS

Investigation on the local ecosystems for M. orientalis was performed through interview with the local residents, field survey, sample collection, video and document recording, as well as observation on the surroundings of plants and animal feeding and practice of fishery, agriculture and livestock raising.

INVESTIGATION OF THE HOSTS

Samples of Parafossarulus striatulus, the first-intermediate host of M. orientalis, were collected from the waters (Luohe river, Yaohe river, Jiaogang lake and Gaotang lake) in the Huainan area with scoop net. Then, the individual snail was placed in the disposable dish and maintained in an eco-box at 18 °C ± 2 °C to observe escaping of the cercaria under the dissecting microscope once every 120 min. Cercariae were microscopically isolated for isolation of the adult worms from their common bile ducts and hepatic ducts. Cercariae were also isolated from the Parafossarulus striatulus.

Pseudorasbora parva, the second-intermediate hosts, were captured from the above lakes and rivers with net. The flesh parts of the fish were cut into fine pieces, washed and precipitated before microscopical isolation of the encysted cercariae. Then, the artificial pancreatic juice was applied to digest the bony parts and scales, which were subjected to repeated rinsing and precipitation for isolation. Finally, encysted cercariae of fluke were separated under microscope. The cercariae obtained from the previous samples were cleansed and maintained for their next use as described previously (3,4).

Selection of the definitive host or reservoir hosts was performed in terms of the epidemic characteristics of M. orientalis with the previously described method (5-7). Sampling was carried out in 89 chicken, 156 ducks, 41 geese, 20 cats and 19 dogs that were raised by fishermen living along the areas of the Luohe and Yaohe rivers and Jiaogang and Gaotang lakes of the Huainan city. All animals were sacrificed, and the corresponding internal organs were taken for isolation of the parasites. The specimens were rinsed in saline, and maintained in 70% alcohol solution for next use.

ARTIFICIAL INFECTION EXPERIMENT

The eggs were obtained from the gallbladder of the ducks; the cercariae, from Parafossarulus striatulus; and the metacercaria, from the flesh of positive Pseudorasbora parva. The negative experimental animals, including young Parafossarulus striatulus, Pseudorasbora parva, and ducklings, were artificially infected following the procedures described in related documents (8). Eggs of M. orientalis were used for artificial infection with the first-intermediate host at a density of 60 ± 20 in water temperature of 18 °C ± 2 °C. The whole process, developing upon intake of the eggs by Parafossarulus striatulus into miracidiae, sporocysts, rediae and cercariae, took approximately 89 days, and by the 90th day, cercariae were obtained. Artificial infection of the second-intermediate hosts was performed in the cercariae that emerged from its snail hosts by a density of 100 ± 20 in the Pseudorasbora parva. Both the cercariae and the fish were maintained in a beaker containing 200 ml of water, and stored in an eco-box at 18 °C ± 2 °C by exposure to the daylight lamp. It took approximately 28 days for the cercariae to develop mature in the fish. Artificial infection with the definitive hosts was carried out in the domestic ducks by feeding them with the cercariae isolated from M. orientalis at a density of 60 for each animal. The feces of the infected ducks were collected after 20 days, and the younger ducks were microscopically dissected for isolation of the adult worms from their common bile ducts and hepatic ducts. Cercariae were also isolated from the Parafossarulus striatulus.

ACTIVITY PROFILE OF CERCARIAE AND THE INFLUENCE OF LIGHT ON CERCARIAE ESCAPING

Examination of the cercariae activity profile was performed in positively infected Parafossarulus striatulus by dividing them into two groups (n = 5 for each group). Parafossarulus striatulus in group A were maintained in a weighing bottle containing 50 ml of water; then, the bottle was stored in an eco-box at 1-32 °C with the daylight lamp on. The activity of cercariae was observed every two hours for determination of the duration of cercariae survival after emerging. Those in group B were also kept in a weighing bottle with 50 ml of water, which was placed outdoors and subjected to observation every two hours for determination of the effects of diurnal variation on cercaria escaping. The dish containing positive Parafossarulus striatulus was kept in an eco-box at 18 °C ± 2 °C for four hours with the daylight lamp on and off alternately in order to observe the quantity of the cercaria escaping and the effect of light on its escaping.
SPECIMEN PREPARATION

The specimens obtained in different stages, including cercariae, encysted cercariae and adults, were stained with carmalm solution, initially discolored in 1% hydrochloric acid solution, and rinsed in clean water. Then, the discoloration continued in the alcohol in gradient manner till 70%. The M. orientalis was compressed thinly and smoothly, and fixed in Bowen’s fluid and dehydrated by 95% alcohol (where necessary, re-staining is required). Then, the specimens were subjected to dehydration in 100% alcohol. After transparency with oil of wintergreen, the specimens were moved onto the glass slide that was mounted with Canadian gum and dried in a drying oven for the next use.

SPECIES IDENTIFICATION

Morphological identification of the species was performed under the conventional microscope or dissecting microscope in compliance with the previous description (9-11). The data were maintained pertaining to the taxonomy, definitive host, parasitic sites and distribution of this species.

RESULTS

ECOLOGICAL ENVIRONMENT

The Gaotang lake, one of our sampling sites, is located between the Huainan city, Fengyang, Dingyuan and Changfeng county, and close to the Shangyao town of Huainan city. The whole natural water body covers approximately 100 hectares, and across the lake a dam was built, along which the local fishermen are living and practicing fisheries. Likewise, a large number of poultry and livestock are being raised, including ducks, geese, dogs, cats, pigs, cattle and sheep. Occasionally, some children are seen to graze the cattle on the lakeshore, where herds of poultry and livestock are also breeding freely at the dam or by the lake. A large quantity of hares, field mice and wild cats are living in the shrubs and weeds, and crowds of wild ducks and water fowls are seen flying over the lake or playing or seeking for foods in the water. A variety of aquatic plants or weeds, such as yellow water chestnut, and various freshwater shellfish, such as mussel, Parafossarulus striatulus, field snail, Pseudorasbora and Bithynia tentaculata, breed in the water weeds, and herds of pigs and domestic ducks feed themselves on the lakeshores. This place has a similar ecosystem to the Gaotang lake. The ecosystem of the Luohe river, our fourth sampling site, is in general identical to that of the above three areas.

INFECTION OF THE INTERMEDIATE HOSTS

Cercariae of M. orientalis occurred in 67 of the 1,000 Parafossarulus striatulus (the first-intermediate host) detected in the Huainan area (Luohe river, Yaohe river, Jiaogang lake and Gaotanghu lake), and encysted cercariae of M. orientalis occurred in 19 of the 300 Pseudorasbora parva (the second-intermediate host). The natural infection rate was 6.7% (67/1,000) and 6.33% (19/300), respectively. Infection of the definitive hosts with M. orientalis was 18.0% (16/89) in domestic ducks, 12.2% (19/156) in chicken, 9.8% (4/41) in geese, 5.0% (1/20) in cats, and 5.3% (1/19) in dogs, in which the domestic ducks were most affected. The density of M. orientalis was 135 ± 2.38 in ducks, 110 ± 4.33 in chicken, 86 ± 2.06 in geese, 165 ± 4.27 in cats and 149 ± 1.78 in dogs on average.

ARTIFICIAL INFECTION OF THE HOSTS

In the artificial infection experiment with the first-intermediate, cercariae were detected in six of the 100 Parafossarulus striatulus with an infection rate of 6.0%, from which six cercariae were isolated. After artificial infection of the second-intermediate in 30 days, we detected the encysted cercariae in 19 of the 60 Pseudorasbora parva. The infection rate was 31.7%, and 38 encysted cercariae on average were isolated from each host. Sixty domestic ducks were totally infected with M. orientalis (100%) by detection of the eggs in stool, and adults of M. orientalis were seen in the hepatobilary systems in the domestic ducks, with an average of eleven for each ducks.

CERCARIA ACTIVITY REGULARITY AND THE EFFECT OF LIGHT ON CERCARIA ESCAPING

By observation of the behavior of M. orientalis cercaria, we found that cercariae escaping from the water outlet of the Parafossarulus striatulus. The survival time for cercariae in group A was negatively correlated with water temperature (Fig. 1), and the number of emerged cercariae in group B was higher in daytime (Fig. 2). One hundred and thirty-two cercariae emerged from the Parafossarulus striatulus with the light on, whereas no single cercaria occurred with the light off in the same ambient temperature, suggesting that light may greatly affect the activity of the cercariae.
about 0.029 mm × 0.015 mm in diameter, with claybank color, egg
Clonorchis sinensis, is oval and sized somewhat resembling that of
gonopore, which is located at the anterior ventral sucker. The vitellaria
tubular uterus containing eggs twists through the ovary towards the
ovary and is slightly curved, measuring 0.357 mm × 0.333 mm. The
 seminal receptacle arises at the back of the
testis and has oval shape, measuring 0.196
mm × 0.261 mm. The esophagus is short, and the two intestines run through both
part. The ventral sucker anteriorly occurs at the 1/4 parts of its body,
and abdomen flattened and densely distributed in small cuticular
spines. The body measures 2.894-5.943 mm in length, 0.712-1.733
mm in width, with length to width ratio of 2.99~4.49 on average. The
oral and ventral suckers look almost round and measure 0.204 mm × 0.211 mm and 0.189 mm × 0.209 mm, respectively. The oral
sucker has well developed sarcoplasma, and arises from the anterior
part. The ventral sucker look almost round and measure 0.204 mm × 0.211 mm and 0.189 mm × 0.209 mm, respectively. The oral
sucker has well developed sarcoplasma, and arises from the anterior
part. The ventral sucker occurs at the 1/4 parts of its body, and the pharynx appears spherical, measuring 0.082 mm × 0.049
mm. The esophagus is short, and the two intestines run through both
sides of the body. Two testes present generally in petaloid fashion,
and arrange in tandem at the posterior 1/4 of the body. The ovary
is located in front of the testis and has oval shape, measuring 0.196
mm × 0.261 mm. The seminal receptacle arises at the back of the
ovary and is slightly curved, measuring 0.357 mm × 0.333 mm. The
tubular uterus containing eggs twists through the ovary towards the
gonopore, which is located at the anterior ventral sucker. The vitellaria
is granular and bunched, lying on both sides of the body. The egg,
which somewhat resembling that of Clonorchis sinensis, is oval and sized
about 0.029 mm × 0.015 mm in diameter, with claybank color, egg
cover, small spines at the rear end and miracidium in it.

MORPHOLOGICAL CHARACTERISTICS FOR M. ORIENTALIS

The adult worms of M. orientalis are oval, shoe-like, with the back
and abdomen flattened and densely distributed in small cuticular

Figure 1.
Survival time (LD50) of cercaria of M. orientalis and water temperature.

Figure 2.
Tendency for the quantity of M. orientalis cercariae emerging within 24 hours.

DISCUSSION

Metorchis orientalis, belonging to the subfamily Metorchiinae
and family Opisthiorchidae, lives in complex life cycles, and has
Parafossarulus striatus as its first-intermediate hosts. Its sec-
ond-intermediate hosts involve freshwater fishes, such as Pseu-
dorasbora parva, Abbottina riviulare, Cichlasoma managuense
and Pseudogobio riviulare, as well as the definitive hosts, poultry,
certain birds, dogs, cats and other mammals or occasional human
beings. In the present study, we successfully isolated the cercaria,
encysted cercariae and adults of M. orientalis from the Parafossa-
ruulus striatus, Pseudorasbora parva and poultry collected in the
Huainan area of the Anhui province, in China. The findings comply
with the life cycle of this species. Huang et al. (9) described 232
species of flukes (95 genera under 24 families) in the Chinese
poultry and livestock, and found that adults and larvae can live
in livestock. Lu (15) reported 665 species that can parasitize in
the poultry and livestock in the Anhui province through extensive
literature review, in which 111 species were trematodes. Although
only three species are associated with the Huainan area (Clonor-
chis signins, Amphimerus anatis and Metorchis taiwanensis), yet
our results further confirmed that M. orientalis are prevalent in
this area. Tao (1948) (25) used to hypothesize that Bithynia
should be the first-intermediate host for M. orientalis, yet his artificial
infection with this host was unsuccessful. In order to confirm the
hypothesis, we failed to detect any cercariae of M. orientalis in the
Bithynia fuchsianar, and also failed to perform the artificial infection
in this species. Zhang (1985) (26) reported natural infection of the
cercariae of M. orientalis with the Parafossarulus striatus that
were collected in the suburb of Xiamen and Zhangzhou in the
Fujian province, China. Our work showed that only Parafossarulus
striatus were infected with M. orientalis, and other freshwater
snails, such as Ramshorn, Lymnaea and Bithynia fuchsianar, are
immune to this species, which was also confirmed by our artificial
infection. This findings suggest that the second-intermediate hosts in this area were highly
infected. The definitive hosts for M. orientalis may include domes-
ritic chicken and geese, except for domestic ducks. In addition,
Strix unalensis, Milvus korchun lineatus, Colymbus ruficollis, C.
cristatus, Bubulcusibis coromandus, Phasianus torquatus, Eurys-
tomus orientalis and Anas platyrhynchos, as well as wild ducks
and waterfowls, have also been reported as the definitive hosts
for M. orientalis, which suggests that this species is not specific
to selection for its definitive host (20-24).
The fact that domestic ducks are prone to infection with *M. orientalis* cannot be neglected, and high infection rate in certain areas can lead to heavy economic loss. Although low-grade infection with *M. orientalis* will not always cause death to the host, yet the parasitism occurs mostly in vital organs of the digestive system. This may result in lesions in the gallbladder and bile duct, inflammation, degenerated bile, congestion of the bile duct, hepatic pathological changes or serious damage to the liver function. Once the ducks are infected with such flukes, they tend to be emaciated due to poor digestion of the feeds, thus leading to reduced egg production. Terribly, if the endemic area is poorly managed, outbreak of the infection could be possible, and may eventually cause death of large amounts of animals (12,13). It had been reported that 198 of the 660 (30%) ducklings died from intake of the viscera of fish infected with *M. orientalis* in the Shexiang county of the Jiangsu province, China.

In our study, the poultry (chicken, geese and ducks) were purchased from the villagers and dissected by ourselves, and the internal organs of the livestock were ordered from the local butchers through field collection of the bile ducts, intestinal canals, oviducts and pancreatic ducts. These samples were brought back to our laboratory for isolation of the flukes with dissecting needle, spatula and hairbrush. This sampling method made hard to ensure the complete set of the internal organs in individual animals for measurement of the infection density. The sampling sites, including Luoke river, Yaohe river, Gaotang lake and Jiaogang lake, belong to the water system of the Huaihe river, and have their unique natural environment. A large quantity of phytoplankton, such as diatoms, green algae, yellow silk grass, *Myriophyllum spicatum* and yellow *Trapa bicornis*, and a variety of freshwater snails (such as *Parafossarulus striatulus*, field snails, *Bithynia tentaculata* and *Radix auricularia*) are growing or living in the river or lake (16,17). Apart from that, those water areas are critical habitats for various kinds of waterfowl, birds, reptiles and mammals. Jap J Zool 1933;5:62.

In our investigation, we found that the infected species and infection rate in chicken, ducks and geese were relatively higher, especially in the free-ranged ducks along the river bank. This may be associated with the fact that free-ranged ducks have easy access to the freshwater snails containing encysted cercariae (18,19). In compliance with the epidemiological characteristics of the *M. orientalis*, specificity to the first-intermediate host, blocking the transmission route, can rely on the eradication or reduction of the density of first-intermediate hosts by applying molluscicide. This can be appropriately done in the spring and summer seasons, when the *Parafossarulus striatulus* begin to multiply and are active (27), and the control effect can be effectively assessed. In addition, providing health education to the local fishermen and villagers could be important to reduce the infection of the flukes.

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