Nutr Hosp. 2017; 34(3):675-679 ISSN 0212-1611 - CODEN NUHOEQ S.V.R. 318

Nutrición Hospitalaria

Trabajo Original

Otros

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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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.

Key words:

Metorchis orientalis. Endemic characteristics. Huainan area.

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 pollos, 9,8% (4/41) en gansos, 5,0% (1/20) en gatos y 5,3% (1/19) en perros. Sesenta y siete cercarias de *M. orientalis* fueron identificadas en 1.000 *Parafossarulus striatulus,* con una tasa de infección natural del 6,7%, y 19 en 300 *Pseudorasbora parva,* con una tasa de infección natural del 6,33%. La actividad de las cercarias 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, y se produjo aproximadamente en 89 días en caracoles, 28 días en peces y 20 días en patos.

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

Palabras clave:

Metorchis orientalis. Características endémicas. Área de Huainan.

Received: 11/10/2015
Accepted: 31/10/2015

Zhan X, Li C, Wu H, Sun E, Zhu Y. Investigation on the endemic characteristics of *Metorchis orientalis* in Huainan area, China. Nutr Hosp 2017;34:675-679

DOI: http://dx.doi.org/10.20960/nh.1333

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INTRODUCTION

Metorchis orientalis (M. orientalis) belongs to the family *Opisthorchiidae,* subfamily *Metorchiinae.* 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 stritulus*, 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 from the host snails, and rinsed as previous technique (2) for next use.

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. oritentalis* 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 80 ± 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 carmalum 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, *Bithynia tentaculata, Radix swinhoei* and *Galba pervia,* are growing in the lake. In addition, the local villagers (fishermen and farmers) have planted plots of economic trees, including poplars and Chinese scholar trees, and farm crops along the lakeshores, which are also overgrown with shrubs and weeds, and crude latrines built by the villagers.

The second sampling field, the Yaohe river, bordering on the Gaotan lake, has a similar ecological environment. And the third sampling site, the Jiaogang lake, which covers some 1,000 hectares of water, lies to the north of the Fengtai county. The local farmers live by pisciculture in purse seine. A large quantity of freshwater shellfish, such as *Parafossarulus striatulus*, field snail, *Parafossarulus* 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. orientallis* (100%) by detection of the eggs in stool, and adults of *M. orientalis* were seen in the hepatobiliary 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.

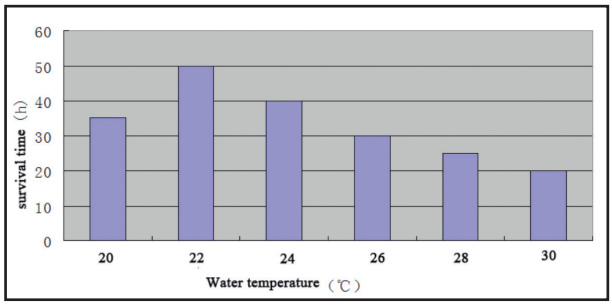

Figure 1.

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

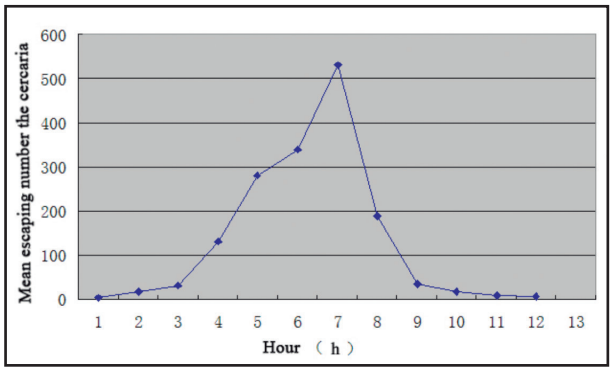

Figure 2.

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

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 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 anteriorly 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 testises 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 bunchy, lying on both sides of the body. The egg, 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.

DISCUSSION

Metorchis orientalis, belonging to the subfamily *Metorchiinae* and family *Opisthorchiidae,* lives in complex life cycles, and has *Parafossarulus striatulus* as its first-intermediate hosts. Its second-intermediate hosts involve freshwater fishes, such as *Pseudorasbora parva, Abbottina rivularis, Cichlasoma managuense* and *Pseudogobio rivularis*, 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 *Parafossarulus striatulus*, *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 *(Clonorchis singnsis, 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 *Biihynia* 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 funhsiana,* 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 striatulus* that were collected in the suburb of Xiamen and Zhangzhou in the Fujian province, China. Our work showed that only *Parafossarulus striatulus* were infected with *M. orientalis*, and other freshwater snails, such as *Ramshorn, Lymnaea* and *Bithynia fuchsianus,* are immune to this species, which was also confirmed by our artificial infection. This findings suggest that the *M. orientalis* is specific to its first-intermediate host, and has unique epidemic characteristics. The second-intermediate hosts for *M. orientalis* are involved in *Pseudorasbora parva, Pseudogobio rivularis, Aphyocypris shantungensis* and *Puntius semifabsciatus*. Infection of these fish with *M. orientalis* is possibly associated with their behaviors, since cercaria *M. orientalis* tend to swim on the water surface, which makes more frequent contact of the fishes. Hong et al. (1964) (27) reported that 60% of *Pseudorasbora parva* were infected with *M. orientalis* in the Yushan area in Shanghai, China. This indicated that the second-intermediate hosts in this area were highly infected with *M. orientalis.* Worse enough, once digesting the fish containing encysted cercariae of *M. orientali,* the poultry would be infected. The definitive hosts for *M. orientalis* may include domestic chicken and geese, except for domestic ducks. In addition, *Stirix unalensis, Milvus korchun lineatus, Colymbus ruficollis, C. cristatus, Bubulcusibis coromandus, Phasianus torguatus, Eurystomus 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 Sheyang 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 Luohe 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 or wintering grounds for various kinds of waterfowls. The local fishermen and villagers have been living on the river bank or in their fishing boat, as well as raising a larger number of poultry and livestock, for a long time. Still, the villagers planted large quantities of economic plants such as poplar and Chinese scholar trees, under which massive shrubs and weeds are growing. In the recent years, the poultry and livestock industries show a trend towards rapid development in the Huainan area. Once the poultry and livestock are infected with the flukes, the animals will be prone to develop the symptoms, including anemia and weight loss, which will directly refrain the growth of young poultry and livestock, even resulting in death. 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.

ACKNOWLEDGEMENTS

The authors are thankful for the generous assistance of teachers and some of the undergraduates from the Department of Pathogenic Biology, School of Medicine, of the Anhui University of Science & Technology.

REFERENCES

1. Chen B, Zhang H, Zhifang LL. Research progress of Metorchis orient disease. Strait J Prev Med, 2013;10(5):18-20.
2. Chen Y, Zhang W, Tian M. Investigation of snails transmitting parasitic diseases in Yunnan Province. J Pathog Biol 2009;4(3):211-4.
3. Kang J, Yang W. Investigation and prevention of duck Clonorchis sinensis in Huainan city, Anhui province. Chin J Veterin Parasitol 1996;4(3):58.
4. Cai R, Li Ch-P, Wang J, et al. Investigation of freshwater fish infected with metacercariae of Clonorchis sinensis in Huainan. Chin J Parasitol Parasitic Dis 2005,23(1):39.
5. Li Ch-P, Gao XZ. Atlas of medical parasitic. Beijing: People's Medical Publishing House; 2012. pp. 197-8.
6. Zhang JS, Gao XZ. Clinical examination of parasites. Beijing: People's Medical Publishing House; 2009.
7. Chen XT. Platyhelminthes Trematoda Digenea. Beijing: Science Press; 1985.
8. Li ChP, Wang J. Preliminary study on ecology of Metorchis Orientalis in Huaihe river basin in China. Chin J Parasit Dis Con 2003;16(2):108-9.
9. Hang B, Shen J. Clasific atlas of parasites for livestock and poultry in China. Beijing: China Agricultural Science and Technology Press;2006.
10. Wu BH. Zhejiang zoography about Trematoda. Hangzhou: Zhejiang Science and Technology Publishing House; 1991. pp. 362-4.
11. Sun X, Li ChP, Zhang JS. Applied medical parasitology. Beijing: People's Medical Publishing House; 2005.
12. Qian DX, Du YP, Mao YX. A list of duck parasite in Guizhou Province. Guizhou Anim Sci Veterin Med 1996;20(5):15-6.
13. Li YC. Preliminary investigation of poultry trematodes in Wuhu area. Poultry Trematoda fauna in Wuhu area. Chin J Zool 1965,3:132-5.
14. Xu Y-Ch. A case of Clonorchis sinensis disease after the ducks outbreak. Poultry 1982;(5):22.
15. Lu F. Anhui Province livestock and poultry parasites list. Chin J Veterin Parasitol 2004;12(1):17-24.
16. Li Ch. Trichobilharzia fluke in basin of Huaihe River. Chin J Zoonos 1996; 12(3)54:79-181.
17. Wang K, Li Ch-P, Guo J. Preliminary investigation in the Metorchis taiwanensis infection in basin of Huaihe River. Chin J Zoonos 2005;21(10):917-9.
18. Zhu Y, Sun E. Metorchis orientalis in Huainan area. J Wangnan Med Coll 2012;31(2):143-4.
19. Zhu Y, Sun E, Li Ch. Survey on natural nidus of Metorchis orientalis in Huaihe river basin. Chin J Parasitol Parasit Dis 2012;10(1):44-51.
20. Hsu HF, Chow CY. Studies on helminth of fowls on the second intermediate host of Metorchis orientalis and M. taiwanensis, liver flukes of duck. Chin Med J Suppl 1938;2;433-40.
21. Hsu HF, Chow CY. Studies on helminths of fowls. Some trematodes of fowls in Tsingkinpu, Kiangsu, China. Chin Med J Suppl 1938;2:441-2.
22. Morishta K. Some avian trematodes from Japan, especially from Formosa, with a reference list of all know Japanese species. Annot Zool Japan 1929;12(1):143-74.
23. Yamaguti S. Studies on the helminth fauna of Japan. Part I. Trematodes of birds, reptiles and mammals. Jap J Zool 1933;5:62.
24. Yamaguti S. Studies on the helminth fauna of Japan. Part III. Avian trematodes II. Jap J Zool 1934;5:545.
25. Tao CS. Notes on the study of life cycle of Melorchis orientalis and M. taiwanensis. Chin Rev Trop Med 1948;1(1):9-14.
26. Zhang Y, Tang Z, Tang Ch. Studies on the life histories of three species of heteropryid trematodes. Chin J Parasitol Parasit Dis 1985;3(1):12-6.
27. Lin J, Li Y, Zhou X. Gu J, ed. Food borne parasitic disease emoticon. Beijing: People's Medical Publishing House; 2009. pp. 15-8.