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Trabajo Original

Investigation on the zoonotic trematode species and their natural infection status in Huainan areas of China

Investigación sobre las especies de trematodos zoonóticos y su estado natural de infección en las zonas de Huainan en China

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Abstract

Background: To investigate the species of zoonotic trematodes and the endemic infection status in the domestic animals in Huainan areas, north Anhui province of China, we intent to provide evidences for prevention of the parasitic zoonoses.

Methods: The livestock and poultry (definitive hosts) were purchased from the farmers living in the water areas, including South Luohe, Yaohe, Jiaogang and Gaotang Lakes, and dissected the viscera of these collected hosts to obtain the parasitic samples. Then the specimens were microscopically identified, with reference to the descriptions in previous literatures for counting the zoonotic species found in these areas.

Results: A total of 41 species were detected in the domestic samples, in which 23 were zoonotic trematodes, and 18 were internal trematodes of animals. Of the 41 species, 38 were novel records in Huainan areas, and 12 were newly detected in Anhui province, including *Metorchis anatinus*, *Echinostoma hortense*, *E. cinetorchis*, *E. angustitestis*, *E. lindoensis*, *E. nordiana*, *E. ilocanum*, *Metagonimus yokogawai*, *Prosthogonimus gracilis*, *P. skrjabini*, *P. anatinus* and *Trichobilharzia sp.* which generally occurred in definitive hosts of chicken, ducks, geese, dogs, cattle, buffaloes, sheep, goats and pigs, respectively.

Conclusion: A large quantity of livestock and poultry are fed by the local farmers living along the river banks in Huainan area, suggesting that the population in that area are at higher risks of natural focus of zoonotic infections, since these animals are favorable definitive hosts to the zoonotic trematodes.

Key words:

Livestock. Poultry. Host. Zoonoses. Parasitosis. Huainan.

Resumen

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Introducción: para investigar las especies de trematodos zoonóticos y el estado de infección endémica en los animales domésticos en áreas de Huainan, al norte de la provincia de Anhui, China, tenemos la intención de proporcionar evidencias para la prevención de enfermedades zoonóticas.

Métodos: el ganado y las aves (hospedadores definitivos) fueron adquiridos a los campesinos que viven en las zonas con agua, incluyendo el sur de Luohe, Yaohe, Jiaogang y Gaotang Lagos, y se diseccionaron las vísceras de estos anfitriones recogidos para obtener las muestras parasitarias. Las muestras fueron identificadas microscópicamente, con referencia a las descripciones de la literatura revisada para contar las especies zoonóticas que se encuentran en estas áreas.

Resultados: se detectaron un total de 41 especies en las muestras nacionales, de las que 23 eran trematodos zoonóticos y 18 eran trematodos internos de los animales. Estas especies representaron 22 géneros en 12 familias de 4 órdenes. De las 41 especies, 38 fueron registros nuevos en las áreas de Huainan y 12 fueron detectadas recientemente en la provincia de Anhui, incluyendo *Metorchis anatinus, Echinostoma hortense, E. cinetorchis, E. angustitestis, E. lindoensis, E. nordiana, Euparyphium ilocanum, Metagonimus yokogawai, Prosthogonimus gracilis, P. skrjabini, P. anatinus y Trichobilharzia sp., generalmente producidos en huéspedes definitivos de pollo, patos, gansos, perros, vacas, búfalos, ovejas, cabras y cerdos, respectivamente.*

Conclusión: los agricultores locales que viven a lo largo de las orillas del río se alimentan de gran cantidad de ganado y aves de corral de la zona de Huainan, lo que sugiere que la población en esa área tiene mayor riesgo ante el foco natural de las infecciones zoonóticas, ya que estos animales son huéspedes definitivos favorables a los trematodos zoonóticos.

Palabras clave:

Ganadería. Aves de corral. Anfitrión. Zoonosis. Enfermedades parasitarias. Huainan.

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INTRODUCTION

Zoonotic trematodes, belonging to the Trematoda in Platyhelminthes, live in complex life cycles, and primarily consist of two alternate hosts that represent sexual and asexual reproductions (1-3). The asexual reproduction commonly occurs in the freshwater shellfishes, mollusks and fishes as well as poikilothermic vertebrates and arthropods, whereas the sexual reproduction occurs mainly in the homeothermic vertebrates such as birds, mammals and human body (2,3). And the parasitism may take place either in the internal organs of domestic livestock and poultry as well as wild animals and humans, resulting in the sustaining transmission between man and animals, even endemic prevalence or outbreak of the parasitic zoonoses (1). This spreading modality demonstrates, on the one hand, that overwhelming majority of the zoonotic flukes in humans are animal sources, and on the other, the epidemic nature of the zoonotic trematodes further complicate prevention of this infections. Importantly, epidemic characteristics of the zoonoses fully prove that vertebrates in nature are reservoir hosts.

Prevalence of the zoonosis is typically associated with endemicity, seasonal variation and natural focus, and these factors are important prerequisites for planning rational strategies to prevention and control of the zoonotic infections. Huainan city, located in the north central Anhui Province, lies to the south of the middle reaches of Huaihe River, with a climate of subtropical monsoon and distinct four seasons. The hilly landforms and plentiful biological resources in this area make optimal living environment for wide range of parasites. In order to supply evidences for the health providers as well as veterinarians and biologists in Huainan area in practicing zoonosis prevention and control, it is necessary to understand the status of the zoonotic trematode infections, for which we conducted an investigation on the species at the natural focus.

MATERIALS AND METHODS

SAMPLING OF THE HOSTS

Sampling of the reservoir hosts were decided in accordance with the epidemic nature of the parasitic flukes and in reference to previous literatures and monographs (1-3).

SAMPLE COLLECTION AND TREATMENT

All livestock and poultry were purchased from the farmer households in the South Luohe, Yaohe, Jiaogang Lake and Gaotang Lake in Huainan area. The reservoir hosts, including chicken (n = 47), ducks (n = 60), geese (n = 32), dogs (n = 19), cattle (n = 18), buffaloes (n = 32), sheep (n = 21), goats (n = 32) and pigs (n = 50), were sacrificed to obtain the parasite specimens via dissecting relevant organs. The specimens were maintained in a flask containing adequate volume of saline and fully washed by gentle oscillation, and then stored in 70% alcohol for following use.

PREPARATION OF THE SPECIMENS

Preparation of the specimens were performed as the standard procedures described by Li (4) handling in order of washing, staining, distaining, dehydration, compression, translucent-treatment and fixation till successful slide. All specimens were maintained in the Department of Pathogen Biology, School of Medicine, Anhui University of Science & Technology.

SPECIES IDENTIFICATION

The specimens were morphologically examined and measured for the size under a microscope or dissecting microscope, and photographed. Identification was performed by matching with the drawings and details described by Huang & Shen (3) and Li & Gao (1). Classification of the species followed the taxonomy by Li et al. (4) besides the characterized morphology of the flukes being taken into consideration.

STATISTICS OF THE INFECTIONS

Infection rate for the reservoir hosts was estimated by formula:

$$p = \frac{a}{n} \times 100\%$$

(where *p* represented the host infection rate with the trematodes, and *a*, individuals detected in a single host, and n, the total number of hosts examined.

RESULTS

HOST SPECIES

A total of 9 species of hosts were investigated in the South Luohe, Yaohe, Jiaogang Lake and Gaotang Lake in Huainan area, and identified as common definitive hosts for zoonoses, which included chicken, ducks, geese, dogs, cattle, buffaloes, sheep, goats and pigs. The infection rate was ranked maximally in the ducks (28.33), followed by chicken (21.28%), geese (21.88%), pigs (18.00%), dogs (15.79%), buffaloes (15.63%), sheep (14.29%), goats (12.50%) and cattle (11.11%). Besides, we also detected the same fluke species in different hosts, such as *Echinoparyphium recurvatum* being found in the guts of both chicken and ducks and geese as well. Likewise, the liver flukes were uniformly isolated from the biliary ducts of the cattle, bufflaoes, sheep and goats.

FLUKE SPECIES IDENTIFIED

Totally, 41 species of the flukes were detected in the domestic samples we collected in the areas mentioned above. Twenty-three species were verified as zoonotic trematodes, and only 18 inhabit-

ed in animals. These species represented 22 genera in 12 families of 4 orders. Thirty-eight species of the 41 were novel records in Huainan areas, and 12 were first detected in Anhui province, including *Metorchis anatinus*, *Echinostoma hortense*, *E. cinetorchis*,

E. angustitestis, E. lindoensis, E. nordiana, Euparyphium ilocanum, Metagonimus yokogawai, Prosthogonimus gracilis, P. skrjabini, P. anatinus and Trichobilharzia sp. The species were listed in compliance with order, family, genus and species in table I.

Table I. The species of zoonotic trematodes borne in 9 species of domestic animals

Order	Family	Genus	Species
Opisthorchiata	Opisthorchiidae	1. Clonorchis	(1) C. sinensis ■
		2. Metorchis	(2) M. orientalis ■ (3) M. taiwanensis ■ (4) M. anatinus ▲
		3. Amphimerus	(5) A. anatis ▲
		4. Opisthorchis	(6) O. anatinus ▲
Echinostomata	Echinostomatidae	5. Echinostoma	(7) E. revolutum ■ (8) E. miyagawai ■ (9) E. hortense ■ (10) E. paraulum ■ (11) E. cinetorchis ■ (12) E. angustitestis ■ (13) E. lindoensis ■ (14) E. robustum ▲ (15) E. stromi ▲ (16) E. nordiana ▲ (17) E. pekinensis ▲
		6. Euparyphium	(18) E. ilocanum ■
		7. Echinoparyphium	(19) E. recurvatum ■
		8. Echinochasmus	(20) E. liliputanus ■
		9. Hypoderaeum	(21) H. conoideum ■
	Fasciolidae	10. Fasciolopsis	(22) F. buski ■
	Paramphistomatidae -	11. Paramphistomum	(23) P. cervi ▲
		12. Fasciola	(24) F. hepatica ■
	Gastrothylacidae	13. Fischoederius	(25) F. elongatus ■
	Notocotylidae	14. Notocotylus	(26) N. intestinalis ▲ (27) N. attenuatus ▲ (28) N. naviformis ▲
Plagiorchiata	Heterophyidae	15. Metagonimus	(29) M. yokogawai ■
	Dicrocoeliidae	16. Eurytrema	(30) E. pancreaticum ■ (31) E. coelomaticum ■
	Prosthogonimidae	17. Prosthogonimus	(32) P. gracilis ▲ (33) P. skrjabini ▲ (34) P. anatinus ▲ (35) P. anatinus ▲ (36) P. cuneatus ▲
	Paragonimidae	18. Paragonimus	(37) P. westermani ■
Strigeata -	Schistosomatidae -	19. Schistosoma	(38) S. japonicum ■
		20. Trichobilharzia	(39) T. sp. ■
	Strigeide	21. Apatemon	(40) A. gracilis ▲ (41) A. minor ▲

 $[\]hbox{``$\blacksquare$''} indicates the zoonotic trematodes; \hbox{``\blacksquare''} represents the species merely inhabits in domestic animals.$

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DISCUSSION

Livestock and poultry are associated with many species of helminthes (trematode, tapeworm, nematode and thornheaded worm), especially species of trematoda. Shen & Huang (5) described as many as 232 species of trematodes, belonging to 95 genera in 24 families, in A List of Parasites for Livestock and Poultry in China. Although Lu (6) reported 111 species of flukes detected in the livestock and poultry in Anhui province of China, yet she merely described 3 species, including Clonorchis sinensis, Amphimerus anatis and Metorchis taiwanensis in Huainan area (6). However, we isolated 41 species of tremadoes in the domestic animals fed by the farmers in Huainan area, in which 38 are novel records in that area and 12 are newly found in Anhui province. Of the 41 species, 23, particularly *Clonorchis sinensis*, *Metorchis* orientalis and M. taiwanensis, are recognized as the pathogens causing zoonoses. Most of all, C. sinensis is quite prevalent in the population of China. Another 18 species of flukes, such as Paramphistomum, P. skrjabini and P. anatinus, are also conventional in China, but there are few reports on the humans infected with these species.

The waters in Gaotang Lake, Luohe River, Yaohe River and Jiaogang Lake in Huainan area are in direct outlets to the Huaihe River, with unique ecological environment. Along the river/lake-sides, there grow a variety of economic trees and shrubs, weeds and crops; and in the water grow numerous aquatic plants, including Potamogeton maackianus, Myriophyllum spicatum L. Chlorophyta, Bacillariophyceae and yellow water chestnut, as well as live a plenty of freshwater shellfishes, such as mussel, Radix auricularia, Bithynia manchourica, B. tentaculata and vivipara and fish stocks. Still, the waters and river sides provide favorable habitats to various wild fowls, and the local residents, mostly fishermen who have long years been living on the river bank or in their fishing boat, are feeding a number of livestock and poultry. Such ecological chains do give the freshwater snails as eligible intermediate hosts for the trematodes described above, and similarly, the lake marsh and overgrowing shrubs and weeds are beneficial natural environments to the growth and development of the those intermediate hosts. Importantly, the domestic animals feeding on the banks and wet lands, once accidentally ingested the intermediate hosts containing metacercariae of the flukes or foods contaminated by the metacercariae, are prone to becoming infected. Therefore, we considered that the special ecological environment at the rivers and lakes in Huainan area should be the optimal natural foci for the zoonotic trematodes. In addition, human infections with majority of the zoonotic flukes, as a result of having ingested the raw or undercooked aquatic snails, were repeatedly reported in China (1,6-22). In order to prevent the domestic animals from infection with the flukes, it is essential to make the local residents understand the risks by feeding the animals with raw fish or raw feeds as well as the importance to rear the livestock in pens, so as to putting the zoonoses under control.

Our investigation preliminarily demonstrated the current natural infection status for zoonotic trematodes and the endemic features in the livestock and poultry infected with parasitic flukes in Huainan area, and also revealed the local population in that area are at greater health risks and risks of economic losses in the aquaculture industry because of the potential sustaining transmission of the natural focus diseases. This shall call for much attention of the local administrations in disease prevention through strengthening the management of the livestock and health education of the local residents.

The taxonomy in our work for the zoonotic trematodes complies to the conventional systems based on the adult morphology. In order to simplify the classification level for handy access, the name and categories for parasitic flukes are indicated by phylum, class, order, family and genus. Besides, we also added the surname who first described the parasite and the year reported to the species by references to the International Code of Zoological Nomenlature (23), and by exhaustive access to the published literatures and monographs (1-6) containing zoonotic species described in Huainan area so as to ensure the recognized nomenclature and completeness for our zoonotic trematodes. However, detailed classification on the zoonotic trematodes remains to be settled due to current controversial opinions towards the taxonomy in this field.

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