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Exploring the Potential Transmission Risk of Schistosomiasis Japonica in the Lower Reaches of the Yangtze River, China

Xuedong Wang[1] --- Chunli Cao[2] --- Jing Gao[3] --- Eniola Michael ABE[4] --- Qungang Wang[5] --- Ling Jiang[6] --- Feng Huang[7] --- Shizhu Li[8]

[1,5,6,7]Zhangjiagang Center for Disease Control and Prevention, Jiangsu Province (Zhangjiagang City, People's Republic of China)
[2,3,8]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention; Key Laboratory of Parasite and Vector Biology, MOH; WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis, Shanghai, People's Republic of China
[4]Department of Zoology, Federal University Lafia, Lafia, Nasarawa State, Nigeria

Abstract

Vector snails are important in the life cycle of schisosomiasis, the need to understand the ecologic factors that could enhance snails' survival and trigger schistosomiasis transmission necessitated this study. Therefore, the potential risk of schistosomiasis transmission was explored in Zhangjiagang region, a non-endemic area in lower reaches of Yangtze River, eastern of China. The key indictors, including snail survival rate, spawn rate, hatching rate and gland development, were investigated through the designed experiments, routine snail and infectious source surveillance. The results showed that there was no significant difference in surviving rate, spawn rate, hatching rate and gland development between groups of simulated environments in laboratory, similar finding in field experiments, which suggested that snails stand a high possibility to survive in these non-endemic areas once they spread into these areas from other places. And no snails and infectious source were found either in the previous routine monitoring in the past decades and the snail surveillance we conducted from 2007 to 2013. Therefore, there is little risk in the study areas in the lower reaches of the Yangtze River. However, the sporadic and imported cases are still seen in a few areas adjacent to the endemic or transmission interrupted areas as the important infectious source, thus become a risk of schistosomisis transmission or re-emergence in these areas where the snail exists. Hence, maintaining routine monitoring and surveillance can be one of the effective and efficient ways to prevent the re-emergence of Schistosomiasis.

Keywords: Potential risk, Schistosomiasis japonica, Oncomelania hupensis, Surveillance, Ecologic factors, Lower reaches of the Yangtze River.

1. Introduction

Schistosomiasis japonica, caused by the parasite *Schistosoma japonicum* and a snail-borne zoonosis, continues to be one of the serious public health problems in China [1]. With highly endemic status, it was responsible for high mortality rate along the Yangtze River basin and left millions of people living with disabilities from the early 1950s [2]. For the great health and economic burden, the government has made enormous endeavors for schistosomiasis control over the past 60 years [3], especially after the massive implementation of integrated control strategy mainly targeting infectious sources during the past 10 years [4]. Currently, both the incidence and prevalence of schistosomiasis has reduced greatly compared with historical data [5].

According to the annual report of 2012, Schistosomiasis were endemic in 12 provinces of China, in which five of the provinces reached the criteria of transmission interruption since the end of last century (no infected human and livestock for consecutive five years), three of them reached the criteria of transmission control (both human and livestock prevalence are less than 1%) and the remaining four provinces were infection control (both human and livestock prevalence are less than 5%) [6]. All of the last four provinces, including Hunan, Hubei, Jiangxi and Anhui provinces, are all located in the marshland and lakes region along the Lower regions of the Yangtze River that harbors the largest areas of Oncomelania snail habitats.

Oncomelania hupensis is the only intermediate host of *S. japonica*, and plays an important role in Schisotomiasis transmission in China [7]. Therefore, the endemicity of S. japonicum is governed by the distribution of its intermediate host snail *O. hupensis*, and socio- economic and behavioral determinants [3, 8], and snail control and surveillance used to serve as the most significant strategy in China decades ago and it remains important in routine activity carrying out every spring and autumn [9, 10]. Because the climate and ecological factors such as rainfall, temperature, floods, humidity, vegetation, and water level influences *O. hupensis*`s subsistence [11], the distribution of snail will be decided by the these ecological factors, especially by land surface temperature, rainfall and their derived factors [8]. A WHO report showed an increase of 2ºC in China was likely to result in a potential increase of 50-100% of the geographic distribution of *O. hupensis* [12]. Furthermore, the effects of construction of Large-scale water conservancy project such as Three Gorges Project and the South-to-North Water Transfer Project on changing ecological factors has proven to support snail distribution and Schistosomiasis transmission [13, 14]. Therefore, it is very important to monitor the snail survival in the areas sensitive ecological factors changing or the areas adjacent to the endemic areas [15, 16].

Jiangsu province is located in the lower parts of the Yangtze River basin and it was known to be one of most endemic area for schistosomiasis with the highest mortality and morbidity. With decades great control effort for the disease control, it has reached the criteria of transmission control at the end of 2010. However, the possible risk of schistosomiasis spreading northward or re-emergence still exists widely due to the ecological and climate factor changing. Therefore, this study explored the potential impact of risk of schistosomiasis transmission by conducting a longitudinal surveillance on the host snail and infectious source survey and snail survival experiments at four selected counties which adjacent to endemic areas in Jiangsu province of China.

2. Materials and Methods

2.1. Study Site

A total of five study sites were selected from the 4 counties in Jiangsu provinces for this study. The sites of Shuangshan and Qiganhe in Zhangjiagang City, Wangyuhekou in Changshu City and Liuhekou in Taicang county in historically non-endemic areas of schistosomiasis served as experimental group which along with the Yangtze River adjacent to the endemic areas. The site of Jiangxinzhou in Changzhou city in historically endemic areas of schistosomiasis served as the control group of the snail breeding experiment in Lab. The geographical co-ordinates (latitude/longitude) of each study site were recorded using Global Positioning System (GPS, Garmin Map76). The study sites list and distribution are showing as table 1 and figure 1, respectively.

Table-1. Study sites of experiment on snail biology

Group	County	Study sites
Experimental group (non-endemic areas)	Zhangjiagang	Shuangshan
		Qiganhe
	Changshu	Wangyuhekou
	Taicang	Liuhekou
Control group (endemic area)	Changzhou	Jiangxinzhou

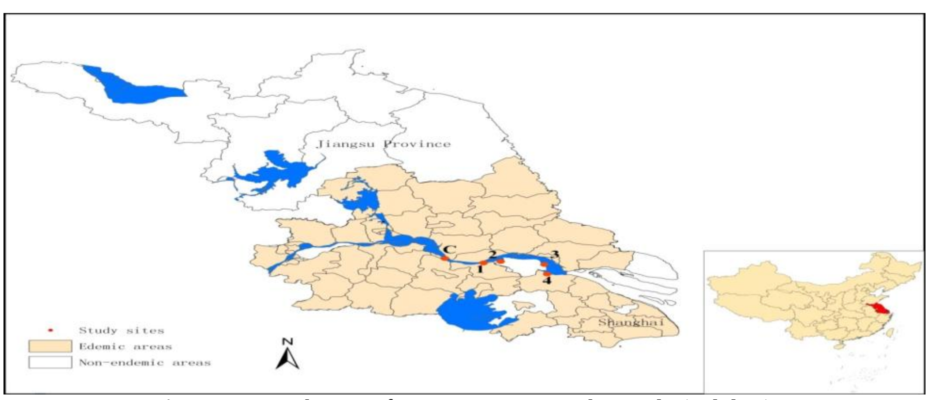

Figure-1. Distribution of investigate sites in this study (red dots).
(1. Shuangshan and 2.Qiganhe in Zhangjiagang, 3.Wangyuhekou in Changshu, 4.Liuhekou in Taicang; and c. Jiangxinzhou in Changzhou served as control site)

2.2. Breeding Experiments of Snail

To test the snail breeding ability, *O. hupensis* was collected from Nanjing Marshland along the Yangtze River to conduct the snail breeding experiments in laboratory and the selected field environment.

In laboratory, 50 live snails (25 male and 25 female) were raised in a plastic tray, 8 trays per group. A total of five groups were set up by paving with the soil collected the above five field environments and soaked wet with river water. In each group, two of them were used to observe the survival rate and the remaining six trays were used to observe reproduction. Survival rate, spawning rate, hatching rate and gland development status were calculated as the main indicators to reflect the breeding ability of the snail.

On the other hand, for the field experiment, the snails were raised in nylon net cages that were put into the environment of the channel connecting Yangtze River. The nylon cage, 60cm×60cm×60cm in size, was made of steel gauze and covered by nylon net, underlain with mud and grass in the bottom of the cage. The lower part about 1/3 of breed cage was put under the river and the left part was put on the bank. A total of 12 nylon cages were used in the field trial, among them six cages were used for breeding second-generation snails from laboratory, and another

6 cages for breeding snails from field. The field trial started in October 2008, and the survival rate, gonad development status and reproduction of new generation of O. hupensis snails were observed every two months, for one year.

2.3. Snail Surveillance

Five sentinel sites were randomly selected from each non-endemic county that contributed to 15 sites in the end. Annual snail survey was conducted by systematical sampling with a square frame of 0.11 m2. The frame was set every 20 meters in potential snail habitats, at selected sentinel sites each spring. All the snails, if find, inside the frame were collected, counted, crushed and examined by microscopy to detect sporocysts and cercariae. Data collection was carried out from 2007 to 2012 in the three selected study counties.

2.4. Infectious Source Surveillance

Both residents and migrants between the ages of 6 and 60 years old who have lived in the sentinel sites for more than one month were recruited in the study between 2007 and 2010. Recruited population was screened for schistosoma japonica infection using Indirect Hem-agglutination Assay (IHA) [17], and stool samples of individuals who tested positive by IHA were collected for further Kato–Katz thick smear test [18].

2.5. Statistical Analysis

Descriptive analysis was used to analyze general characteristics, Chi-square and Ranksum test were performed using the statistical software package SPSS (Version 11, SPSS Inc. Chicago, IL, USA) to test for difference between experimental and control group.

3. Results

3.1. Breeding Experiments of Snail

3.1.1. Snail Breeding and Reproduction in Laboratory

After 6 months' of raising snails in the laboratory, the survival rates of snails raised on the soil collected from the experimental group (Liuhekou, Wangyuhekou, Qiganhe and Shuangshan) were 90.0%, 94.0%, 90.0%, and 92.0%, respectively, 91.5% in average. While the corresponding survival rate dropped to 50.0%, 52.0%, 48.0% and 46.0% after 12 months in laboratory, 49% in average. Soil collected from Jiangxinzhou, which represented the endemic areas, served as the control group, while the survival rate was found to be 92.0% and 50.0% after six and 12 months respectively. No statistically significant difference was observed in the survival rate between experimental and control group in 6 months ($\chi 2 = 0.013$, p=0.909) and 12 months ($\chi 2 = 0.016$, p=0.899). (Table 2)

Regarding the laying eggs per female snail, it reached the peak during March and April, and then reduced after April. No egg was found from June to September, while few eggs were found during October and November. Both the total number of eggs and average number of eggs per female snail in the experimental group are higher than that of control group, except for Qiganhe. The average number of eggs per female snail spawned of four experimental groups is 71.06±13.17. No statistic significant was found between experimental and control group (t=1.601, p=0.104).Moreover, the average hatching rate of snail egg was 84.5% in the experimental group, while the highest was found in Wangyuhekou (90.0%) and the lowest was found in Qiganhe (76.0%). There was no statistical difference between experimental and control group ($\chi 2 = 1.182$, p=0.277). (Table 2)

Table-2. Snail breeding and reproduction in laboratory

Groups	Study sites	Survival rate (%)*		Average no. of eggs per female snail**	Egg hatching rate（%）*
		6 months	12 months		
Experimental group	Liuhekou	90	50	61.56	85.0
	Wangyuhekou	94	52	88.44	90.0
	Qiganhe	90	48	38.31	76.0
	Shuangshan	92	46	95.91	87.0
Control group	Jiangxinzhou	92	50	49.96	80.0

*Pearson's Chi-square test
**t-test

3.1.2. Gland Development

The gland development was observed by dissecting gland in adult snails from each group monthly. It was found that all glands started to develop from September. For female snails, eggs was first observed in ovary from October and reached the peak in March, with its color gradually turning into yellow. The average size of ovary was 2.446 mm in length and 0.618 mm in width, while for male snails, the testis turned yellow and the spermatophore turned as the chubbiest, with the average size of testis was 2.590 mm in length and 0.446mm in width. However, both the glands of male and female snails began their atrophy from late May and reached complete atrophy at the end of June with branches no longer clear and color turning light. Nevertheless, both ovary and testis begin their second round of development in September, revealing an obvious pattern of seasonal change.

3.2. Snail Biology in the Field

3.2.1. Survival Rate in the Field

As shown in table 3, the survival rate dropped continuously with time for both groups except for snails collected from Nanjing Marshland at the time point of six months. The average survival rate of young snails from Nanjing

Marshland (92.22%) and their second generation raised in the laboratory (92.5%) was similar, and no significant difference was observed between them ($\chi 2$ =2.550, p=0.110).

The difference between two groups at each point of time was tested by Fisher's exact test, but no significant difference was found between them at all six months' time points.

Table-3. Snail survival rate after raised in the field channel

Time	Snails from Nanjing Marshland			Second generation from laboratory		
	No. snail observed	No. survived	Survival rate (%)	No. snail observed	No. survived	Survival rate (%)
1st(2 monthes)	60	59	98.33	60	58	96.67
2nd(4monthes)	60	55	91.67	60	58	96.67
3rd(6monthes)	60	57	95.00	60	56	93.33
4th(8monthes)	60	55	91.67	60	54	90.00
5th(10monthes)	60	53	88.33	60	54	90.00
6th(12monthes)	60	53	88.33	60	53	88.33
Total	360	332	92.22	360	333	92.5

3.2.2. Gland Development

The development of gland was also observed in snails collected from both Nanjing Marchland and the second generation from laboratory. Results showed that gland development started in January and reached the mature peak in March while the ovary of female snails (2.43 mm in length and 0.61 mm in width) was observed yellow and chubby with eggs. For male snails, the testis was orange in colour and the spermatophore became chubby with the average size of 2.58 mm in length and 0.44mm in width. Both the testis and ovary started atrophy in May, but all revealed a second growth in October.

3.2.3. Snail Reproduction Rate

Snails from both groups started reproduction from the 8th month and lasted till the 12th month (table 4). By the 12th month, the average reproduction rates of snails from Nanjing Marshland and laboratory were 25.83 and 26.0 per female snail, respectively. No significant difference was found in average reproduction rate between the two groups (t-test, t=0.046, p>0.05).

Table-4. Snail reproduction rate e situation

Observation turn	Snails from Nanjing marshland			Snails from laboratory		
	No. of snails raised	No. of snails observed	Reproduction rate per snail	No. of snail raised	No. of snails observed	Reproduction rate per snail
1st(2months)	60	60	0	60	60	0
2nd(4months)	60	60	0	60	60	0
3rd(6months)	60	60	0	60	60	0
4th(8months)	60	800	12.33	60	911	14.18
5th(10months)	60	1334	21.23	60	1326	21.10
6th(12months)	60	1610	25.83*	60	1620	26.00*

* t-test, t=0.046,P>0.05

3.3. Snail Surveillance

There is an increasing trend in the size of snail surveillance in Zhangjiagang, Changshu and Taicang as shown in table 5. Zhangjiagang has the highest diversity in the snail habitats compared to other two counties. However, snails were not found in all the sentinel sites from the counties already mentioned from 2007 to 2013. A total of 1512 batches of floating substance with gross weight of 18 144 kg were detected, and no *O. hupensis* snails were found in the floating materials along the Yangtze River.

Table-5. Annual snail surveillance in non-endemic areas, 2007-2013(m2)

Year	Zhangjiagang					Changshu		Taicang		
	Marshland	River channel	Ridge	Canal	Total	Marshland	Total	Marshland	River channel	Total
2007	13500	29500	3600	9900	56500	1698214	1698214	127500	64380	191880
2008	20600	29700	3630	9980	63910	1946625	1946625	84500	65840	150340
2009	20500	29650	3680	9920	63750	1989420	1989420	35000	136290	171290
2010	21200	29800	3650	9950	64600	1253090	1253090	51000	35250	86250
2011	21600	29700	3670	9930	64900	735500	735500	53000	33800	86800
2012	21300	29900	3660	9960	64820	787900	787900	72500	34900	107400
2013	23500	29800	3700	9970	66970	353500	353500	79000	21700	100700

3.4. Infectious Source Surveillance

Human surveillance to determine prevalence of *S. japonica* was done from 2007 to 2013, no IHA positive cases were found in all sentinel sites. Zhangjiagang have the largest population under surveillance compared to Changshu and Taicang each year. Meanwhile, migrant population accounted for the most participants for human surveillance in each site.

Table-6. Annual surveillance of human infection of Schistosomiasis in non-endemic areas, 2007-2013

Year	Zhangjiagang			Changshu			Taicang		
	Residents	Migrant	Total	Residents	Migrant	Total	Residents	Migrant	Total
2007	1	5999	6000	0	1091	1091	1281	925	2206
2008	335	5665	6000	0	1000	1000	1139	1621	2760
2009	1453	5012	6465	0	591	591	428	1644	2072
2010	3389	3164	6553	0	584	584	300	988	1288
2011	5746	1068	6814	0	651	651	300	893	1193
2012	4123	2377	6500	0	652	652	700	1136	1836
2013	4764	239	5003	0	606	606	1129	89	1218

4. Discussion

The pattern of schistosomiasis distribution relies heavily on the surroundings, due to its nature of biological attributions. Climate change and water system are the main drivers of the changing pattern of the host snail distribution and schistosomiasis transmission [19]. In China, according to the climate change prediction and water transfer projects, risks are identified that snails may move northward to non-endemic areas that may trigger the transmission of the disease to the north part of China [15, 20]. Therefore, surrounding with endemic areas and along with the Yangtze River in Jiangsu province, Zhangjiagang region is facing the potential risk of the host snail invasion that could increase the potential risk of schistosomiasis transmission.

In this study, the snail breeding ability was tested in the simulation and field environments in Zhangjiagang region. The key indicators, including survival rate, spawn rate, hatching rate and gland development, were investigated through the designed experiments. There was no significant difference in surviving rate, spawn rate and hatching rate between groups of simulated non-endemic and endemic environments in laboratory, which suggested that snails stand a high possibility to survive in these non-endemic areas once they spread into these areas from other places. Similar finding attenuated in experiments we conducted in the field study. No snails were found either in the previous routine monitoring in the past decades and the snail surveillance we conducted from 2007 to 2013.

The non-endemic areas in zhangjiagang region of the estuary of Yangtze river is a long and narrow band, including Zhangjiagang, Changshu and Taicang county, with only 40km away from the upstream endemic areas and 7km from the most closest one [21]. The close linkage between the two types of areas decided their affinity of environmental factors, which provided a relatively similar habitat for *O. hupensis* snail. However, the interesting results of snail monitoring did not show any clues about the snail invasion or existing in these areas. Therefore, besides the evident effects of ecological characteristics such as water, soil, temperature, etc., the survival and reproduction of O. hupnesis may also be influenced by the velocity, direction and rate of flow, tides, etc [22, 23]. Geographically, Located in the upstream of Zhangjiagang region, the Ebizui estuary is the last natural node along Yangtze River. The sudden narrowness of this area increases the speed of flow rate, which becomes a natural barrier for the movement of snails coming from upstream. Previous study found both the flow velocity and tide level were significantly higher than the downstream at this point of river course, which also increased the fallen rate of snails [21]. Hence, with the similar ecological factors and positive biological experiment results, studies have shown that no snail is found in these areas.

The key point of whether environmental factors changing will affect the risk of schistosomiasis transmission relies not only on the possibility of snail immigration, but also on the importing of infectious source [24]. Previous works in Africa have reported the association between building of large dams and transmission of Schistosomiasis mansoni and Schistosomiasis haematobium [25-28]. Same as the snail monitoring, a negative result was found in the resident population surveillance during study period. It shown that it is not a rounded circulation for schistosomiasis transmission in above areas because of lack of the snail host and infectious source.

With the continuous reduction in the prevalence rate of Schistosomiasis in human, livestock and snails [29], China is expected to reach the goal of national transmission control by the year 2015 [30]. Although it's plain to see, there is little risk in the study areas in the lower reaches of the Yangtze River, the sporadic and imported cases are still seen in a few areas adjacent to the endemic or transmission interrupted areas as the important infectious source, thus become a potential risk of schistosomisis transmission or re-emergence in these areas where the snail exists [31]. Hence, continuous surveillance for both human and snails are necessary in those areas due to the changing of socio- or eco-factors. Thus, maintaining routine monitoring and surveillance can be one of the effective and efficient ways to prevent the re-emergence of Schistosomiasis [32].

5. Conclusions

In spite of the fact that no *O. hupensis* snails and no infectious source were found in the aforementioned sentinel sites along the lower reaches of the Yangtze River, the snail showed the ability to reproduce and breed in the simulated environment under laboratory conditions and field environment. It is necessary to maintaining routine monitoring and surveillance for schistosomiasis local transmission prevention in the lower reaches of the Yangtze River.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

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Author Contributions

Conceived and designed the experiments: Xuedong Wang, Jing Gao, Eniola Michael ABE, Chunli Cao and Shizhu Li. Performed the experiments: Xuedong Wang, Qungang Wang, Ling Jiang and Feng Huang. Analyzed the data: Xuedong Wang, Jing Gao, Chunli Cao and Shizhu Li. Contributed reagents/materials/analysis tools: Xuedong Wang, Jing Gao, Chunli Cao and Shizhu Li. Wrote the paper: Xuedong Wang, Jing Gao, Chunli Cao and Shizhu Li. All authors read and approved the final version of the manuscript.

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