Soil-transmitted Helminthiasis, Intestinal Protozoa and Clonorchiosis Infections in Southeast China: A Cross-sectional Study

Yan Feng
Zhejiang Provincial Center for Disease Control and Prevention
Kegen Yu
Zhejiang Provincial Center for Disease Control and Prevention
Hualiang Chen
Zhejiang Provincial Center for Disease Control and Prevention
Xuan Zhang
Zhejiang Provincial Center for Disease Control and Prevention
Qiaoyi Lu
Zhejiang Provincial Center for Disease Control and Prevention
Xiaoxiao Wang
Zhejiang Provincial Center for Disease Control and Prevention
Xueying Zhang
Icahn School of Medicine at Mount Sinai
Linong Yao
Zhejiang Provincial Center for Disease Control and Prevention
Wei Ruan (wruan@cdc.zj.cn)
Zhejiang Provincial Center for Disease Control and Prevention https://orcid.org/0000-0003-3717-3055

Research article

Keywords: parasitic intestinal diseases, soil-transmitted helminth, protozoa infections, clonorchis sinensis, prevalence

Posted Date: November 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-99550/v1

Abstract

Background: The large scale epidemiology of parasitic diseases in Zhejiang Province has not been investigated since the second national survey.

Methods: We investigated the distribution of three types of parasitic diseases (i.g. soil-transmitted helminthiasis (STH), intestinal protozoa, and clonorchiosis) in Zhejiang province from late 2014 to 2015. Kato-Katz technique was used for STH and *Clonorchis*. *Sinensis* detection, transparent adhesive paper anal swab was used for pinworm detection, and iodine smear was for protozoa detection. Hookworm positive samples need to be cultured for identification of *Ancylostmoma duodenale* or *Necator americanus*.

Results: 23,552 participants were included in this study (19,935 from rural area and 3,617 from urban area). The overall prevalence of any intestinal helminth infection was 1.800%. Seven species of helminth were found: *A.duodenale*, *N.americanus*, *Trichuris trichiura, Ascaris lumbricoides*, *C. sinensis*, *Fasciolopsis buski* and pinworm. The prevalence of STH infection was 1.711% (1.941% in rural area, 0.442% in urban area). Hookworm was the most prevalent infection (1.584%). Only 2.79% children were found for pinworm infection in rural area. 0.396% of the rural participants were infected protozoa, and *Endolimax nana* was the most prevalent (0.231%). *C. Sinensis* infected only one man. 89.135% of participants had good personal hygiene habits. The awareness of *C. Sinensis* was 24.467% and 45.963% in rural and in urban area, respectively.

Conclusions: Comparing to the second national survey, the prevalence of STH and protozoa infections declined greatly and *C. Sinensis* infection was still rare in Zhejiang Province. However, hookworm, especially *N.americanus* was still a parasitic threat to population health. More health education about fertilization and farming habits should be provided in rural areas. The awareness of hookworm and *C. Sinensis* should be reinforced in both rural and urban areas.

Background

Parasite disease is a major component of Neglected Tropical Diseases (NTDs) that affect the health for more than one million population globally [1]. It especially affects low-income populations in developing regions of Africa, Asia, and the Americas [1]. Helminthiasis, protozoa, and clonorchiosis are three types of parasite diseases continuously found in China but their prevalence were likely to be underestimated because their symptoms are not obvious at early stage of infection.

Soil-transmitted helminth (STH) is widely distributed in tropical and subtropical areas. Moreover, the incidence of STH infection is highly overlapped with areas being lack of sanitation facilities. Thereby STH is prevalent among population in poverty [2]. In the year of 2010, approximately 5.3 billion people in total and 1.0 billion school-aged children lived in areas considered as habitats of at least one STH species, and the 69% of them were living in Asia [3]. In the year of 2003, the prevalence of all species of STH (there are four main species of STH: roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*) [4]) was highest in China, followed by Southeast Asia and India [5]. The data of STH species are limited. The data of South Asia and South East Asia show that in the year of 2018, roundworm was the most common cause of STH (18% of all cases), followed by whipworm (14%) and hookworm (12%) [6]. China conducted two national surveys in 1992 and 2001–2004 respectively. The overall prevalence of roundworm, whipworm and hookworm was 47.0%, 18.8%, and 17.2% in 1992 [7], 12.72%, 4.63% and 6.12% in 2003[8]. However, the prevalence of STH is lack of investigation in recent years. One possibility is that because the economy and sanitation improved unequally in different areas of China, the distribution of STH should shift from it in decades ago.

Similar to STH, lack of access to clean water, sanitation, and hygiene can facilitate the transmission of intestinal protozoa(e.g. *cryptosporidium, Giardia intestinalis (G.intestinalis), Entamoeba histolytica (E. histolytica)*)[9, 10]. Therefore, intestinal protozoa infections are predominantly occurring in developing countries.

Cryptosporidium, the cause of cryptosporidiosis, is one of the most common causes of diarrhea and gastroenteritis in humans and brings a great health burden to children and those who have impaired or weakened immune system [11–13]. The incidence rates of cryptosporidiosis range from 1.4% to 10.4% in different countries, and the rate was higher in low-income countries (5–10%) than in developed area (1%) [14]. *G.intestinalis* can cause giardiasis, which is another common cause of chronic diarrhea. The cases of giardiasis are mainly in developing countries and children. In developing countries, the childhood giardiasis prevalence is 20–30%, which is only 2–3% in high-income countries [13]. The amebiasis caused by *E. histolytica* affects 50 million people and causes 100,000 deaths people annually in worldwide [15]. In China, the infection rates of *G.intestinalis* was 2.52% and of *E. histolytica* was 0.95% according to the national survey conducted in 1992 [7], but the prevalence declined significantly in the national survey conducted during 2001–2004. For example, in Zhejiang province, the prevalence of *G.intestinalis* decreased from 3.85–1.30% and the prevalence of *E. histolytica* decreased from 1.5–0.14% between the two surveys [16].

Human liver fluke is another NTD threating people's health in East Asia. *Clonorchis sinensis* (*C. sinensis*) is the most common type of human liver fluke. The infection of *Clonorchis sinensis (C. sinensis)* often develop server consequence. The infection may result in the severe complication of cholangiocarcinoma which was classified as a group 1 biological carcinogen by the International Agency of Cancer Research in 2009 [17]. In addition, due to its high prevalence, *C. sinensis* caused a great health burden in East Asian countries such as China. Approximately 15 million people were infected with *C. sinensis* globally in 2004 [18], with 85% of infections were occurred in China [19]. In China, the infection rate of *C. sinensis* was 0.365% [20] in the year of 1992 and the rate was increased to 0.58% [8] in early 2000s. There were two epidemic zones of *C. sinensis* in China: the first zone was the Heilongjiang and Liaoning provinces in northern China, and the second zone was the Guangdong and Guangxi Provinces in southern China. Zhejiang province is located in the southeast area of China. The prevalence of *C. sinensis* is generally low in Zhejiang province. In the second national survey, the prevalence of *C. sinensis* infection for Zhejiang prevalence was only 0.01% [16], which was much lower than the national average 0.58% [8]. However, the transmission of parasites and the composition of population changed rapidly in the recent decades. It is necessary to conduct another survey to know if the distribution of *C. sinensis* in Zhejiang province has been changed consequentially [21].

To our knowledge, there is no large-scale study investigated the epidemiology of the three species of parasites introduced above (STH, intestinal protozoa, and clonorchiosis) since the two national surveys completed in 1992 and 2003 respectively. Moreover, the overall incidence of parasitic diseases decreased greatly due to the improvement of public health condition and monitoring system in China. However, specific areas might have increased risk for particular types of parasitic diseases because of the change of climate and demographics. The aim of this study was to describe the distribution of the three types of parasites in Zhejiang province. This study can provide reference for the health burden and prevention of intestinal parasitic infections in Zhejiang province.

Methods

Study Sites

An extensive field sample collection and survey were conducted in 34 counties of all 89 counties in Zhejiang province from late 2014 to 2015 (Figure. 1). Zhejiang province located in the southeast area of China, and it features a monsoon season, temperate climate, and mountainous landscape. Each study county had one study site, which was assigned to rural site or (and) urban site. All of study sites were investigated for STH, and *C. Sinensis*and rural sites were investigated for intestinal protozoa as well. The survey conducted in rural counties was primarily focusing on STH and intestinal protozoa, while the survey conducted in urban counties was primarily focusing on *C. Sinensis*. The urban study sites were selected by the national Center for Disease Control and Prevention based on previous study results of 27 provinces in 2004, and the smallest selected unit was the neighborhood. The rural study sites were selected based on a multi-stage sampling method: counties in Zhejiang province were divided into three or four levels based on the county-level per capita net income (GDP) (data from 2011 Zhejiang provincial statistic yearbook). In each GDP level, one town was randomly selected, and then, one village was randomly selected from the town. In each neighborhood of urban and each village of rural, two-hundred and fifty subjects were sampled by randomly selecting families. Once the family was selected, all of people in this family will be asked to participant. Additional file 1 and 1aditional file 2 Table showed the study sites in rural area and urban area, respectively.

Study Population and Sample Collection

Permanent residents in selected rural sites were screened for STH, *C. Sinensis* and intestinal protozoa infection. Children of 3-6 years old were additionally examined for pinworm infection. Permanent residents in urban sites were screened for both STH and *C. Sinensis* infection. About 30g stool sample were taken from each participant and an additional anal swab was taken from each child participant.

Ethics Approval and Consent to Participate

This study was part of the Epidemiology Survey of Important Parasitic Diseases in China, which has been approached by the Ethical Review Committee of National Institute of Parasitic Disease, Chinese Center of Disease Control and Prevention. The form of inform consent was not emphasized, written or verbal were both acceptable. Because some old participates, especially in rural areas were illiterate, in order to unify the inform consent form in Zhejiang Province, verbal informed consent was obtained from all participants before the sample collection and the face-to-face survey. Study staff, for example, the head of village, explained the content in presence of villagers, if they agreed to participate the study, they would sent the sample by themselves or on behalf of their children.

Sample Examination

For each stool sample, two thick-smear slides were processed using the modified Kato-Katz technique under light microscope at 10×and 40× objectives for eggs detection. Meanwhile, one slide was processed using iodine solution under light microscope at 40× objectives for cysts of protozoa. The mean number of eggs on two Kato-Katz slides was calculated if any helminth egg was detected. The result was reported as positive or negative for cysts of protozoa. Pinworm eggs were examined under light microscope at 10× using the transparent adhesive paper anal swab method and reported as positive or negative in detection.

Sample Examination

After collection, stool samples were cultured to detect hookworms using the following steps. About 0.5g of each stool sample was spread on a piece of spindly filter paper. This disposal was placed in a cone-shaped tube with cold boiled water at the bottom of the tube. Then, the sample was incubated for 4 days at 30°C. During the incubation period, water was added if necessary to prevent the tube drying up. Finally, after spontaneous sedimentation, the water at the bottom was collected and examined under an anatomical lens for *A. duodenale* or *N. americanus* larvae. The species name and the number of larvae were recorded. At most 50 stool samples conducted culture in a study county. The intensity of infection was measured by eggs per gram (EPG), EPG=mean eggs of two slides of one sample*24. Intensity of hookworm: 0<EPG<400, intensity=light; 400≤EPG≤3000, intensity= moderate; EPG>3000, intensity= heavy; intensity of *A.lumbricoides*: 0<EPG<5000, intensity=light; 5000≤EPG≤50000, intensity= moderate; EPG>50000, intensity= heavy; intensity of *T. trichiura*: 0<EPG<1000, intensity=light; 1000≤EPG≤10000, intensity= moderate; EPG>10000, intensity= heavy.

Quality control

Two trained laboratory staff read two Kato-Katz slides of each stool sample, and an parasitologist would check the slides when the results reported by the two trained staff were inconsistent. An experienced parasitologist would re-observed iodine slide if the cysts of protozoa was detected.

Questionnaire survey

The questionnaires (additional file 3 and additional file 4) were developed for this study and the surveys were conducted in 17 counties that randomly selected from the 34 study counties. 60 subjects among those under stool examination in each study unit were surveyed about the alimentary habits, but the sanitary behaviors were only surveyed in rural area.

Statistical analysis

Filled questionnaire and laboratory examination results were entered parallelly by two research staffs using Epi-Info 3.5.4 (CDC, GA, USA), and the data entered by them were compared to avoid discrepancies, logical errors and missing values. Statistical analysis was conducted using SPSS 24 (SPSS Inc., IL, USA) software. The study county distribution and hookworm infection distribution were mapped using open-source QGIS 2.18.27 software. Pearson's chi-squared test (c2) was used to compare the crude associations between binary and independent variable. For all the tests, the significance level was set to 5% (p<0.05).

Results

Characteristics of study sites

Seventy-eight villages in rural area and 14 urban neighborhoods were selected in this study. The landscapes of selected villages (rural study sites) were mountainous (35.9897%), hilly (32.051%), and plain (25.641%); and the main landscape of urban study sites was plain (42.86%). More than a half of (58.33%) study villages do not have modern toilet. Most villages (90.476%) can access to tap water. No villages have the habit of eating raw meat, but there was one village in Wenzhou city have the habit of eating raw freshwater fish and shrimp. Local drinking and eating habits are displayed in Table 1.

Table 1
Local drinking and food habits in rural area

Habits	The NO. (%) of villages	
	N	%
Major drinking water source		
Tap water	76	90.476
Well water	3	3.571
Pond water	2	2.381
Spring water	3	3.571
Raw meat diet		
Yes	0	0
No	84	100%
Raw freshwater fish & shrimp diet		
Yes	1	1.190
No	83	98.810
Raw vegetable diet		
Yes	50	59.524
No	34	40.476
Air-dried meat and fish		
Yes	28	33.333
No	56	66.667

Characteristics of participants

The characteristics of the study population were showed in Table 2. A total number of 23,552 participants were included in this study, among them, 19,935 were from rural sites and 3,617 were from urban sites. The number of female participants was than the number of male participants in both rural and urban sites. The average age of rural and urban participants was 53 years old and 49 years old, respectively. Ethnic Han accounted for 98.50% of the study population, followed by Ethnic She (1.44%), as a minority clustered in Zhejiang Province. Participants' job composition was different in rural and urban areas. Farming was the predominant type of occupation in rural areas. In urban, farming and being retired were the two most common occupations. The proportions of participants with illiteracy, completed primary school and middle school were similar in rural and urban areas, but the proportion of participants with degree of high school, college and above were significantly higher in urban than rural area.

Table 2
Characteristics of participants in rural area and urban area

	Rural area		Urban area		Total	
	N	(%)	N	(%)	N	(%)
Age						
1–17	2008	10.073	424	11.722	2432	10.326
18–44	3967	19.900	836	23.113	4803	20.393
45–60	7012	35.174	1062	29.361	8074	34.282
> 60	6948	34.853	1295	35.803	8243	34.999
Sex						
Female	10487	52.606	1968	54.410	12455	52.883
Male	9448	47.394	1649	45.590	11097	47.117
Ethnicity						
Ethnic Han	19619	98.415	3580	98.977	23199	98.501
Ethnic She	303	1.520	36	0.995	339	1.439
Others	13	0.065	1	0.028	14	0.059
Job						
Farmer	13922	69.837	869	24.025	14791	62.801
Worker	1346	6.752	420	11.612	1766	7.498
Housewife	1522	7.635	339	9.372	1861	7.902
Student	1250	6.270	320	8.847	1570	6.666
Preschoolers	856	4.294	147	4.064	1003	4.259
Officer	342	1.716	294	8.128	636	2.700
Retired	204	1.023	825	22.809	1029	4.369
Others	493	2.473	403	11.143	896	3.804
Education level						
Illiteracy	4228	21.209	482	13.326	4710	19.998
Primary school	8555	42.914	1204	33.287	9759	41.436
Middle school	5218	26.175	1099	30.384	6317	26.822
High school	1357	6.807	470	12.994	1827	7.757
College and above	577	2.894	362	10.008	939	3.987
Total	19935	100	3617	100	23552	100

Prevalence of helminth infection

The overall prevalence of any intestinal helminth was 1.800% in Zhejiang Province. Seven species of helminth were found: *A.duodenale*, *N.americanus*, *T. trichiura*, *A.lumbricoides*, *C. sinensis*, *Fasciolopsis buski* and pinworm. The prevalence of any STH infection was 1.711% higher in rural area (1.941%) than it in urban area (0.442%) ($p < 0.05$) (Table 3). Hookworm was the most prevalent parasite (1.584%), followed by *T. trichiura* (0.085%) and *A.lumbricoides* (0.055%). The prevalence of hookworm was much higher in rural area (1.791%) than it in urban area (0.442%). *T. trichiura* and *A.lumbricoides* were only found in rural area. Among participants with parasite infection, most of them has only one type of infection. Three participants (0.909%) had mix infection of *T. trichiura* infection and *A. lumbricoides.* No one had more than two types of infection. Figure 2 displayed the distribution of hookworm infection among all study counties. Hookworm infection had higher prevalence in counties located in the central and western Zhejiang. For example, Yongkang was a county in central of Zhejiang with the highest hookworm prevalence (10.246%), followed by Kaihua (6.796%) and Jiande County (6.570%). Hookworm infection was not detected in ten counties (29.412%). Most infections of hookworm (70.241%), *T.trichiura* (100%), and *A.lumbricoides* (92.308%) were of light intensity (Table 4). Male (60.590%), old (> 60) participants and farmers had higher prevalence of hookworm infection (Table 5). Three hundred and thirty of 357 hookworm positive stool samples in rural areas were cultured and larvae were found in 79.9% samples. *N. americanus* was the predominant (75.934%) species in Zhejiang Province (Table 6). The average number of hookworm eggs on the slide of larvae negative samples was 6.186. Although the maximal number of hookworm eggs was high (60), most of them (62.791%.) had less than 3 eggs on a slide.

Table 3
Prevalence of helminth infection

Parasite	The NO. and prevalence of infection							
	Rural area		Urban area		χ^2/ χ_c^2	*p*	Total	
	N	%	N	%			N	%
Any intestinal helminth	406	2.037	18	0.498			424	1.800
STH	387	1.941	16	0.442	40.901	<0.001	403	1.711
Hookworm	357	1.791	16	0.442	35.716	<0.001	373	1.584
T. trichiura	20	0.100	0	0	6.672*	0.010	20	0.085
A.lumbricoides	13	0.065	0	0	4.336*	0.037	13	0.055
Fertilized egg	8	0.040	0	0	2.668*	0.102	8	0.034
Unfertilized egg	6	0.025	0	0	/	0.600#	6	0.025
T.trichiura & *A.lumbricoides*	3	0.015	0	0	/	1.000#	3	0.013
C. sinensis	1	0.005	0	0	/	1.000#	1	0.004
Fasciolopsis buski	0	0	2	0.055	/	0.024#	2	0.008
Pinworm(anal swab)	18	2.786	NA	NA	NA	NA	18	2.786

*If theoretical frequency (T) in the table was between 1 and 5, correction for continuity (Yate's correction) would be used with χ_c^2.

#If T in the table was less than 1, exact probability method would be used with only Fisher's exact *P*.

NA = not available

Table 4
Infection intensity of STH

Intensity	**The NO. of Hookworm**		**Total**	**The NO. of *A.lumbricoides***		**Total**	**The NO. of *T. trichiura***		**Total**
	Rural area	**Urban area**		**Rural area**	**Urban area**		**Rural area**	**Urban area**	
Light	251	11	262	12	0	12	20	0	20
Moderate	88	4	92	1	0	1	0	0	0
Heavy	18	1	19	0	0	0	0	0	0
Total	357	16	373	13	0	13	20	0	20

Table 5
Age and job distribution of hookworm positive participants

Characteristics	Sex (n)		Total n(%)	NO. of subjects	Prevalence(%)
	Male	Female			
Age					
1–17	5	2	7	2432	0.288
18–44	4	10	14	4803	0.291
45–60	45	34	79	8074	0.978
> 60	172	101	273	8243	3.312
Job					
Farmer	197	124	321	14791	2.170
Worker	18	4	22	1766	1.246
Housewife	0	16	16	1861	0.860
Student	3	2	5	1570	0.318
Preschoolers	2	0	2	1003	0.199
Retired	5	0	5	1029	0.486
Others	1	1	2	1532	0.131
Total	226(60.590)	147(39.410)	373(100)	23552	1.584

Table 6
Coproculture result of hookworm positive stool sample in rural area

Coproculture result	The NO. (%)of fetal sample
Larvae positive	244 (73.939%)
N. americanus	183 (55.455%)
A. duodenale	58 (17.576%)
A.duodenale and *N.americanus*	3 (0.909%)
Larvae negative	86 (26.061%)
Total*	330 (100%)
* Not each positive stool was cultured in each study county.	

Six hundred and forty-six children were tested for pinworm only in rural area using transparent adhesive paper anal swab. Only 2.79% children were found to have pinworm infection. The number of boy infected was close to that of girl infected. Among children between three to six years old, six year-old children have the highest infection rate (3.846%, 8/208), and five year-old children have the lowest (0.617%, 1/162) infection rate.

Prevalence of protozoa infection

Nine species of protozoa were detected in rural area. There were 0.396% of rural participants were infected with protozoa, and *Endolimax nana* was the most prevalent (0.231%) species, followed by *Entamoeba histolytica* (0.045%) and *Entamoeba hartmani* (0.040%) (Table 7). *Entamoeba coli, Entamoeba polecki*, *Giardia intestinalis*, *Trichomonas hominis*, *Iodamoeba butschli*, *Blastocystis hominis* were also detected but the prevalence were low (Table 7). Three participants from different villages have the multiple infection of hookworm and protozoa.

Table 7
Prevalence of protozoa infection

Protozoa	**The NO. and prevalence**	
	N	**%**
Endolimax nana	46	0.231
Entamoeba histolytica	9	0.045
Entamoeba hartmani	8	0.040
Entamoeba coli	4	0.020
Trichomonas hominis	4	0.020
Entamoeba polecki	3	0.015
Giardia intestinalis	3	0.015
Iodamoeba butschli	1	0.005
Blastocystis hominis	1	0.005
Total	79	0.396

Prevalence of *C. Sinensis*

C. Sinensis infected only one man living in rural area. No one in urban area was infected with *C. Sinensis*. Clonorchiosis had a very low infection rate in Zhejiang province.

Sanitary behaviors and alimentary habits

There were 2,485 participants completed the questionnaire survey of the behaviors related to STH infection in rural study areas. The majority of them (89.135%) had good personal hygiene such as wash hands before eating or after using the toilet. However, there were still 12.515% people in rural area had the habits of drinking unboiled water. Moreover, 21.127% participants still used human stools to fertilize crops and 22.777% participants did not wear shoes when farming. These two kinds of behaviors could increase the risk of STH infection.

There were 2,485 in rural area and 483 participants in urban area completed the survey of the knowledge, risky behaviors, and alimentary habits related to *C. Sinensis* infection. The knowledge of C. *Sinensis*' transmission route and its damage to health showed significant difference between rural and urban participants (Table 8). The proportion of participants using separated cutting board for raw and cooked food was not significantly different between rural and urban. Small proportion of participants (14.004% in rural area 9.317% in urban area) have the habits of eating raw freshwater fish and shrimp. The vast majority people would like to take medicine if they were told to be infected with *C. Sinensis* (Table 8).

Table 8
Knowledge, infection risky behaviors and alimentary habits about *C. Sinensis*

Investigation	**Rural area**	**Urban area**	χ^2	***p***
	N (%)	**N (%)**		
Know of *C. Sinensis*	608 (24.467)	222 (45.963)	92.761	< 0.001
Know of *C. Sinensis* transmission route	357 (14.366)	123 (25.466)	36.751	< 0.001
Know of *C. Sinensis* infection damage to the health	432 (17.384)	127 (26.294)	21.000	< 0.001
Separate raw and cooked cutting board	832 (33.481)	175 (36.232)	1.365	0.243
Eating raw freshwater fish and shrimp	348 (14.004)	45 (9.317)	7.734	0.005
Would like to try raw freshwater fish and shrimp even knowing the infection risk	182(7.324)	59 (12.215)	12.969	< 0.001
Willing to buy anthelmintic if knowing infected by *C. Sinensis*	2332 (93.843)	457 (94.617)	0.427	0.513

Discussion

As a part of the third national parasitic disease survey, more than one third of counties in Zhejiang province were covered in this study. Zhejiang province is one of the smallest provinces in China with 74.63% lands are mountains and hills [22]. Generally, the study counties have access to clean water, fairly hygiene condition and rare special eating habits in Zhejiang province.

According to the sex and age distribution of participants, we found that women were more willing to participate the survey in either rural or urban area. The average age of rural participants was higher than that of urban area. It is due to that most old people in rural area stayed in hometown while most younger people moved to urban for work. The urban area in this study included many previous suburban areas because of the accelerated process of urbanization.

Therefore, the most common job among urban participants was still farming. In addition, to some extent, it could explain the reason that the rate of having low-level education rate was similar among rural and urban participants. However, people in urban area have better access to higher education, which may be the reason that urban participants have higher rates of high school or higher degrees than rural ones.

The overall prevalence of intestinal parasite infections in Zhejiang province decreased dramatically from 22.84% [16] in the second national survey (2003) to 2.123% in this study. Nationally,the prevalence of intestinal parasite infections was 3.93% in rural area in 2015 [23]. Compared to neighboring countries, the overall prevalence of STH infection was much higher: 59.9% in Malaysia [24], 75.6% in India [25], 31% in Philippines [26]. The prevalence of all types of STH infection was significantly higher in rural area than that in urban area. The causes of STH infection has changed greatly in Zhejiang since the second national survey [16]. In Zhejiang, there were 17 species of parasites detected in the second national survey [16] and 16 species of parasites (7 species of helminth and 9 protozoa) detected in this study. Although the number of species found in the two surveys were close, the prevalence showed great differences. The prevalence decreased dramatically from 6.84–0.055% for *A.lumbricoides*, from 3.64–0.085% for *T. trichiura*, from 8.18–1.584% for hookworm, from 24.42–2.79% for pinworm, from 0.14–0.045% for *Entamoeba histolytica*, from 1.00–0.015% for *Giardia intestinalis* [16]. The top cause of STH infection switched from multiple helminth species to hookworm, and the prevalence of hookworm was higher than that of those helminth species. *A.lumbricoides* and *T. trichiura* were no longer the leading cause of helminth infection in Zhejiang Province, but the hookworm did. In children, the infection of pinworm was well controlled. The substantial reduction of prevalence may attribute to the rapid economic development [27], increased access to tap water and sanitary toilet, as well as improvement of personal hygiene habit such as wash hands. According to the provincial statistical yearbook, the per capita income increased 452.4% for urban residents and increased 486.7% for rural residents. The rate of having access to tap water in rural area was 80% in nationwide at the year of 2017 [28]. In Zhejiang rural tap water supply rate reached 99% in 2017 [29]. A meta-analysis study showed that use of piped water was associated with reduced likelihood of *A.lumbricoides* infection and *T.trichiura* infection but little evidence for similar association for hookworm infection [30]. Although tap water was supplied to more and more villages, the habits of drinking unboiled water from well or pond may still introduce some protozoa infection. In addition to tap water, the rate of access to sanitary toilet showed a rapid growth from 20.9% in 1996 to 78.43% in 2015 in nationwide [31]. In Zhejiang, coverage rate of sanitary toilet had already reached 94.60% households in 2011 [32]. However, in some rural area, villagers, especially seniors, were not willing to use modern sanitary toilet and used to apply fresh stool as fertilization. Furthermore, the habit of barefooted farming would increase their hookworm infection risk. In rural area, man was the main labor force in the field. These habits and customs may explain the higher prevalence of hookworm in male, the old and farmers. Hookworm took the place of other STH and became the main parasitic threaten to Zhejiang people. The prevalence of hookworm increase inversely with latitude. In South Asia and South East Asia, hookworm (12%) is the third most prevalent type of STH; The country-level prevalence of hookworm was highest in Laos, Vietnam and Cambodia [6]. *A.duodenale* and *N.americanus* were both endemic in Zhejiang Province, but *N.americanus* was the predominant species. In neighboring Jiangxi Province, which located southwest of Zhejiang, *N.americanus* had a higher proportion of hookworm (80.41%) [33]. In Thailand, *N.americanus* was the main hookworm identified in northeast, while only *N.americanus* was detected in the south [34]. In the first national survey, there was already a trend for a higher prevalence of *N.americanus* in the south, while *A.duodenale* is predominant in the north [7].

Questionnaire survey about *C. Sinensis* revealed that although urban participants had more knowledge regarding the Clonorchiosis or *C. Sinensis* than rural participants, the overall awareness rate in urban participants was still less than a half. More than a half of participants would like to try raw freshwater fish and shrimp without knowing that those were the transmission route of parasites. Interestingly, 9.317% urban participants eaten raw freshwater fish and shrimp, and this rate increased to 12.215% among participants knowing the risk of *C. Sinensis* infection. However, the situation was opposite in rural participants. Maybe the knowledge and health education were not the main factors influencing people's behavior, curiousness in novelty, the confidence to cure the disease in urban area might enhance the courage of trying risky food.

Conclusion

In summary, the results of this study indicated that the prevalence of STH and protozoa infections had sharply declined and *C. Sinensis* infection was still rare in Zhejiang. However, hookworm, especially *N.americanus* was still a parasitic threat to population health. More health education about fertilization and farming habits should be provided, and the awareness of hookworm and *C. Sinensis* should be reinforced, and regular medication should be supervised instead of self-medication, in order to decrease hookworm infection further.

Abbreviations

STH: soil-transmitted helminthiasis; NTDs: Neglected Tropical Diseases; GDP: per capita net income.

Declarations

Ethics approval and consent to participate

The collection of samples used in this study and the questionnaire survey were conducted with the permission of participants or their parents. The research protocol was reviewed and approved by the Ethical Review Committee of National Institute of Parasitic Disease, Chinese Center of Disease Control and Prevention. Verbal informed consent was obtained from all participants before the sample collection and the face-to-face survey.

Competing Interest

The authors declare no conflicts of interest.

Consent for Publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets analyzed during the current study are not publicly available due to data sharing need the approval of affiliation but are available from the corresponding author on reasonable request.

Funding

This research received funding from Chinese Central Government's transferring payment.

Authors' contributions

YF, KgY, HlC, XZ, QyL, XxW performed the research. YF performed the data analysis and wrote the manuscript. WR and LnY performed the on-site organization and quality control. XyZ modified the grammar and words of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets analysed during the current study are not publicly available due to data sharing need the approval of affiliation but are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to thank Yingdan Chen, Changhai Zhou, Huihui Zhu, Menbao Qian, Tingjun Zhu for providing parasite identification training to this survey and all the CDCs of studied counties in Zhejiang province for providing laboratory and facility.

References

1. Hotez PJ, Alvarado M, Basáñez M-G, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM *et al*: **The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases**. *PLOS Neglected Tropical Diseases* 2014, **8**(7):e2865.
2. **Intestinal worms Epidemiology-geographical distribution** [https://www.who.int/intestinal_worms/epidemiology/en/]
3. Pullan RL, Brooker SJ: **The global limits and population at risk of soil-transmitted helminth infections in 2010**. *Parasit Vectors* 2012, **5**:81.
4. **Intestinal worms-What are intestinal worms (soil transmitted helminthiasis) ?** [https://www.who.int/intestinal_worms/disease/en/]
5. de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L: **Soil-transmitted helminth infections: updating the global picture**. *Trends Parasitol* 2003, **19**(12):547-551.
6. Silver ZA, Kaliappan SP, Samuel P, Venugopal S, Kang G, Sarkar R, Ajjampur SSR: **Geographical distribution of soil transmitted helminths and the effects of community type in South Asia and South East Asia - A systematic review**. *PLoS Negl Trop Dis* 2018, **12**(1):e0006153.
7. Xu LQ, Yu SH, Jiang ZX, Yang JL, Lai LQ, Zhang XJ, Zheng CQ: **Soil-transmitted helminthiases: nationwide survey in China**. *Bull World Health Organ* 1995, **73**(4):507-513.
8. Coordinating Office of the National Survey on the Important Human Parasitic D: **[A national survey on current status of the important parasitic diseases in human population]**. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2005, **23**(5 Suppl):332-340.
9. Painter JE, Gargano JW, Collier SA, Yoder JS, Centers for Disease C, Prevention: **Giardiasis surveillance – United States, 2011-2012**. *MMWR Suppl* 2015, **64**(3):15-25.
10. Speich B, Marti H, Ame SM, Ali SM, Bogoch, II, Utzinger J, Albonico M, Keiser J: **Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide**. *Parasit Vectors* 2013, **6**:3.
11. Checkley W, White AC, Jr., Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L *et al*: **A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium**. *Lancet Infect Dis* 2015, **15**(1):85-94.
12. Sangare I, Bamba S, Cisse M, Zida A, Bamogo R, Sirima C, Yameogo BK, Sanou R, Drabo F, Dabire RK *et al*: **Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso, Burkina Faso**. *Infect Dis Poverty* 2015, **4**:32.
13. Speich B, Croll D, Furst T, Utzinger J, Keiser J: **Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis**. *Lancet Infect Dis* 2016, **16**(1):87-99.
14. Wang JL, Li TT, Huang SY, Cong W, Zhu XQ: **Major parasitic diseases of poverty in mainland China: perspectives for better control**. *Infect Dis Poverty* 2016, **5**(1):67.
15. SL S: **Amoebiasis**. *Lancet* 2003, **361**(9362):1025-1034.
16. Xing-Guo TU, Yao LN, Huang XM, Chen HL, Ke-Gen YU, Jiang MG, Zhu WM, Chen YM, Liu BD, Lei CQ: **[Second Sampled Survey on the Distribution of Human Parasites in Zhejiang Province]**. *Chinese Journal of Parasitology and Parasitic Diseases* 2001, **19**(3):145.
17. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L *et al*: **A review of human carcinogens--Part B: biological agents**. *Lancet Oncol* 2009, **10**(4):321-322.
18. Lun ZR, Gasser RB, Lai DH, Li AX, Zhu XQ, Yu XB, Fang YY: **Clonorchiasis: a key foodborne zoonosis in China**. *Lancet Infect Dis* 2005, **5**(1):31-41.
19. Qian MB, Chen YD, Liang S, Yang GJ, Zhou XN: **The global epidemiology of clonorchiasis and its relation with cholangiocarcinoma**. *Infect Dis Poverty* 2012, **1**(1):4.

20. Yu S, Xu L, Jiang Z, Xu S, Han J, Zhu Y, Jiang C, Lin J, Xu F: **[Report on the First Nationwide Survey of the Distribution of Human Parasites in China]**. *Chinese Journal of Parasitology and Parasitic Diseases* 1994, **12**(4):241-247.
21. Toledo R, Esteban JG, Fried B: **Current status of food-borne trematode infections**. *Eur J Clin Microbiol Infect Dis* 2012, **31**(8):1705-1718.
22. **General Introduction of Zhejiang Province** [http://www.zj.gov.cn/col/col1568516/index.html]
23. Prevention NiopdCCFDCA: **[Report on the National Survey of Important Human parasitic Diseases in China (2015)]**. In., 1 edn. Beijing: People's Medical Publishing House; 2018.
24. R RNN, S SAA, KH KHCC, RM RMAA, SC SCLL, TK TKTT, MM MMSS, AF AFAA, M MRR, YA YALLL: **Patterns and Risk Factors of Soil-Transmitted Helminthiasis Among Orang Asli Subgroups in Peninsular Malaysia**. *Am J Trop Med Hyg* 2015(2):361-370.
25. S SGG, S SBB, S SKK, P PSS, K KBB, K KKK, P PKK, P PCC, R RSS, D DRR *et al*: **High prevalence of soil-transmitted helminth infections among primary school children, Uttar Pradesh, India, 2015**. *Infect Dis Poverty* 2017(1):139.
26. VG VGVPP, ER ERCdCdC: **Toxocara (Nematoda: Ascaridida) and other soil-transmitted helminth eggs contaminating soils in selected urban and rural areas in the Philippines**. *Scientific World Journal* 2014:2014:386232.
27. **Statistical Yearbook Data of 2018** [http://zjjcmspublic.oss-cn-hangzhou.aliyuncs.com/jcms_files/jcms1/web3077/site/flash/tjj/Reports1/2018%E7%BB%9F%E8%AE%A1%E5%B9%B4%E9%89%B4%E5%85%89%E7%9
28. Ministry of Water Resources PsRoC: **2017 Statistic Bulletin on China Water Activities**. In.; 2017: 9.
29. **[8.03 million quality water wiil be provided to rural residents]** [http://www.zjwater.gov.cn/art/2018/12/20/art_1590826_29872347.html]
30. Strunz EC, Addiss DG, Stocks ME, Ogden S, Utzinger J, Freeman MC: **Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis**. *PLoS Med* 2014, **11**(3):e1001620.
31. Yan-fen ZM-jYWF: **[Distribution disparity of sanitary latrines construction in rural China during 2006-2015]**. *Journal of Environment and Health* 2018, **35**(1):61-64.
32. CAI Jian-min CZ-j, LOU Xiao-ming, WANG Xiao-feng, CHEN Wei-zhong, REN Li-hua: **[A survey on the situation of rural environmental health conditions in Zhejiang province]**. *Modern Preventive Medicine* 2014, **41**(8):1380-1381+1390.
33. X XS-YS-Y, G GZ-HZ-H, C CZZ, H HC-QC-Q, L LW-MW-M, J JW-SW-S, Z ZX-JX-J: **[Current status of human hookworm infection in Jiangxi Province in 2014]**. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* 2018(4):446-448.
34. I IPP, PM PMII, T TTT, O OSS, P PJJ, P PLL, W WAA, N NMM, W WMM: **Molecular detection of Ancylostoma duodenale, Ancylostoma ceylanicum, and Necator americanus in humans in northeastern and southern Thailand**. *Korean J Parasitol* 2013, **51**(6):747-749.

Figures

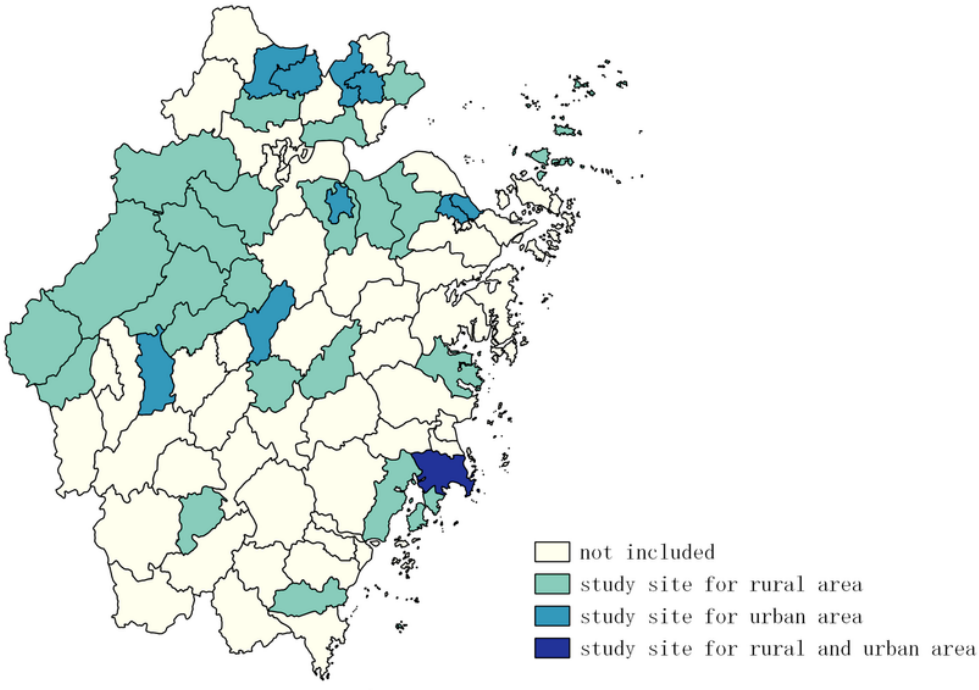

Figure 1

Distribution of the study counties in Zhejiang province. The base map of Figure 1 is obtained from the open source of National Geomatics Center of China(http://www.ngcc.cn/ngcc/html/1/index.html) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

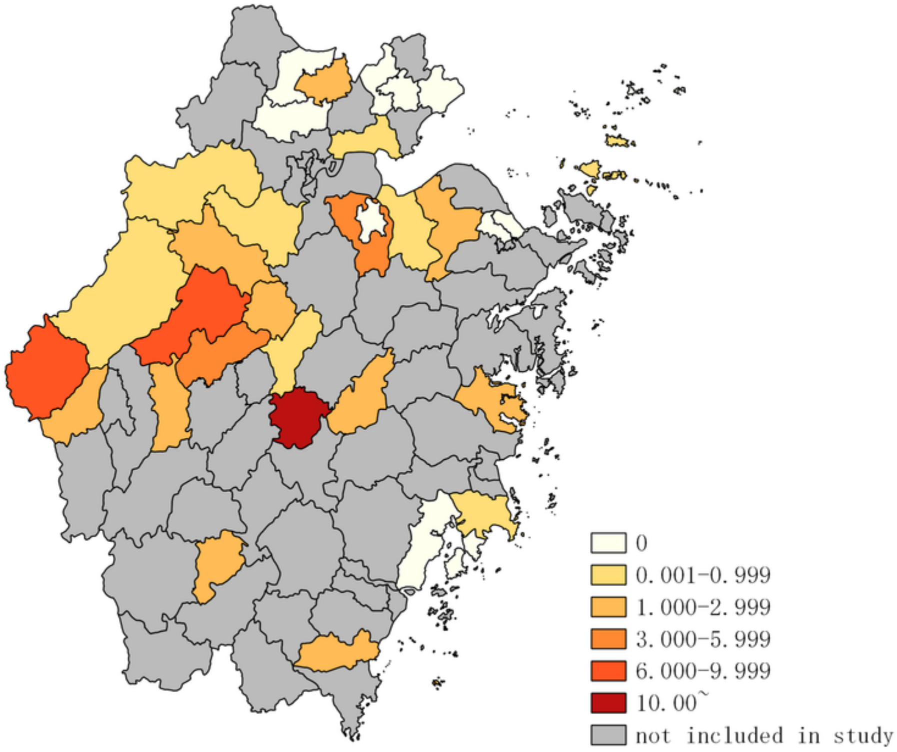

Figure 2

Hookworm infection distribution in Zhejiang province. The base map of Figure 2 is obtained from the open source of National Geomatics Center of China(http://www.ngcc.cn/ngcc/html/1/index.html) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx
- Additionalfile2.xlsx
- Additionalfile3.docx
- Additionalfile4.docx
- Additionalfile5STROBEchecklistcrosssectional.doc