Acta Tropica xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples

Qun-Bo Tong[a], Rui Chen[a], Yi Zhang[b,c], Guo-Jing Yang[e], Takashi Kumagai[d], Rieko Furushima-Shimogawara[d], Di Lou[a], Kun Yang[e], Li-Yong Wen[a], Shao-Hong Lu[a,*], Nobuo Ohta[d], Xiao-Nong Zhou[b,c,**]

[a] *Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences, Hangzhou, PR China*
[b] *National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, PR China*
[c] *Key Laboratory for Parasite and Vector Biology, MOH, PR China*
[d] *Section of Environmental Parasitology, Department of International Health Development, Division of Public Health, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*
[e] *Jiangsu Institute of Schistosomiasis, Wuxi, PR China*

ARTICLE INFO

Article history:
Received 8 March 2013
Received in revised form 16 January 2014
Accepted 21 January 2014
Available online xxx

Keywords:
Oncomelania hupensis
Schistosoma japonicum
Surveillance and response
Loop-mediated isothermal amplification (LAMP)
Risk map

ABSTRACT

Although schistosomiasis remains a serious health problem worldwide, significant achievements in schistosomiasis control has been made in the People's Republic of China. The disease has been eliminated in five out of 12 endemic provinces, and the prevalence in remaining endemic areas is very low and is heading toward elimination. A rapid and sensitive method for monitoring the distribution of infected *Oncomelania hupensis* is urgently required. We applied a loop-mediated isothermal amplification (LAMP) assay targeting 28S rDNA for the rapid and effective detection of *Schistosoma japonicum* DNA in infected and prepatent infected *O. hupensis* snails. The detection limit of the LAMP method was 100 fg of *S. japonicum* genomic DNA. To promote the application of the approach in the field, the LAMP assay was used to detect infection in pooled samples of field-collected snails. In the pooled sample detection, snails were collected from 28 endemic areas, and 50 snails from each area were pooled based on the maximum pool size estimation, crushed together and DNA was extracted from each pooled sample as template for the LAMP assay. Based on the formula for detection from pooled samples, the proportion of positive pooled samples and the positive proportion of *O. hupensis* detected by LAMP of Xima village reached 66.67% and 1.33%, while those of Heini, Hongjia, Yangjiang and Huangshan villages were 33.33% and 0.67%, and those of Tuanzhou and Suliao villages were 16.67% and 0.33%, respectively. The remaining 21 monitoring field sites gave negative results. A risk map for the transmission of schistosomiasis was constructed using ArcMap, based on the positive proportion of *O. hupensis* infected with *S. japonicum*, as detected by the LAMP assay, which will form a guide for surveillance and response strategies in high risk areas.

Introduction

Schistosomiasis is a neglected tropical disease and remains a serious health problem, affecting more than 200 million people worldwide (Steinmann et al., 2006). The World Health Organization (WHO) identified schistosomiasis as the second most important human parasitic disease in the world, after malaria (Croft et al., 2003). The causative organism, *Schistosoma japonica*, is a zoonotic parasite prevalent in Asia, including the People's Republic of China (PR China), Indonesia and Philippines. In PR China, *S. japonicum*, and its intermediate snail host, *Oncomelania hupensis*, are distributed along the middle and lower reaches of the Yangtze River, especially around southern tributaries, lakes and marshlands, and in some hilly and mountainous regions of Southwestern China (Liang et al., 2006; Li et al., 1999). Control efforts over the last 60 years have effectively controlled most infections in endemic areas; for instance, the disease has been eliminated in five out of 12 endemic provinces, including Fujian, Guangdong, Guangxi, Shanghai and Zhejiang provinces. The remaining endemic areas, located in lake regions of Hunan, Hubei, Jiangxi, Anhui, Jiangsu provinces and in mountainous regions of Sichuan, Yunnan provinces, have shown very low prevalences during last 5 years (Collins et al., 2012). For example, three provinces, Sichuan, Yunnan and Jiangsu, achieved

* Corresponding author.
** Corresponding author at: National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, PR China. Tel.: +86 2164738058.
E-mail addresses: llsshh2003@163.com (S.-H. Lu), ipdzhouxn@sh163.net (X.-N. Zhou).

0001-706X/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2014.01.006

the national criteria of transmission control by the end of 2010, which indicated that prevalence of schistosomiasis in all three provinces was less than 1% (Li et al., 2009; Hao et al., 2009, 2010; Lei et al., 2011). By the end of 2010, 32854 *S. japonica* infections had been reported, representing a reduction of 10.92% compared with those in 2009. Among these, only 43 acute cases were reported, representing a decrease of 44.16% over the number of acute cases in 2009 (Lei et al., 2011). However, schistosomiasis control is still vital, especially in regions with lakes and marshlands, where a large area of schistosomiasis transmission risk remains present. To achieve further reduction of the risk of human infection in this background of low prevalence, real-time surveillance and response systems are required to monitor and predict the risk areas of schistosomiasis transmission (Bergquist and Whittaker, 2012; Zhou et al., 2013). These surveillance and response systems require an innovative tool to rapidly locate infected snails so that they can be controlled using molluscicides in these areas, using a multidisciplinary approach, to reduce the risk of human infection through water contact (Zhou, 2012). The innovative tool should be affordable (require less expensive, simple equipment), sensitive, specific, user-friendly, rapid and deliverable to be used in the diseases control program in the field (Mabey et al., 2004; So and Ruiz-Esparza, 2012).

Molecular techniques, such as conventional polymerase chain reaction (PCR), are capable of detecting schistosomal DNA in samples obtained from snails, feces and vaginal lavage (Standley et al., 2010; Oliveira et al., 2010; Kumagai et al., 2010; Gomes et al., 2010; Kjetland et al., 2009). Real-time PCR and nested-PCR have also been established to detect *S. japonicum* infections (Lier et al., 2008; Tong et al., 2009). Although PCR-based assays provide excellent sensitivity and specificity, their dependence on expensive apparatus, professional skill and long reaction times restrict their widespread application for clinical diagnosis. Therefore, cost-effective, simple, and rapid detection methods need to be developed for the diagnosis of schistosomiasis.

Loop-mediated isothermal amplification (LAMP) is a rapid, simple and sensitive technique developed by Notomi et al. (2000), which does not require expensive equipment. This method enables the amplification of a few copies of DNA to 10^9 copies in less than 1 h under isothermal conditions. The amplification products can be observed by the naked eye. The LAMP reaction requires only a single enzyme, *Bst* DNA polymerase, which synthesizes a new strand of DNA while simultaneously displacing the former complementary strand, thereby enabling DNA amplification at a single temperature. Four primers are required for the LAMP reaction, namely, FIP (Forward Inner Primer), BIP (Backward Inner Primer), F3 (Forward outer primer 3), and B3 (Backward outer primer 3).

LAMP assays have been developed to detect parasites, including *Plasmodium* spp. (Han et al., 2007; Poon et al., 2006), *Trypanosoma* spp. (Thekisoe et al., 2007; Njiru et al., 2010), *Leishmania* spp. (Adams et al., 2010; Takagi et al., 2009), *Taenia* spp. (Nkouawa et al., 2009, 2010), *Dirofilaria immitis* (Aonuma et al., 2009), *Toxoplasma gondii* (Zhang et al., 2009a,b; Kong et al., 2012), and *Angiostrongylus cantonensis* (Chen et al., 2011). For *Schistosoma* spp., LAMP assays have been used to detect *S. mansoni*, *S. haematobium* and *S. japonicum* DNA by targeting Sm1-7, DraI and 28S rDNA, respectively. These LAMP assays are sensitive enough to detect infection from the first day after exposure to miracidia, which permits monitoring of prepatent snails (Abbasi et al., 2010). These LAMP assays could be adapted for application in field laboratories (Hamburger et al., 2013). LAMP assays targeting the SjR2 and 28S rDNA to detect *S. japonicum* DNA have been reported by Xu et al. (2010) and Kumagai et al. (2010). In addition to the above-mentioned applications, LAMP assays have also for detection from pooled samples, for example, to detect *Wuchereria bancrofti* and influenza viruses. LAMP detected *W. bancrofti* DNA in a mimic pooled sample of 60 *Culex* spp. mosquitoes containing 100 μL human blood and one microfilaria (Mf) (Takagi et al., 2011). Using pooled RNA samples extracted from influenza viruses corresponding to all 15 HA subtypes (in addition to other avian pathogenic viruses), the RT-LAMP system only amplified H7 AIV RNA (Bao et al., 2012).

Technological developments in geographical information systems (GIS) have provided scientists with the opportunity to develop risk maps of parasitic diseases, including malaria, schistosomiasis, trypanosomiasis and leishmaniasis, resulting in better designed and refined surveillance-impact strategies and early warning systems (De la Rocque et al., 2001; Bavia et al., 2005; Noor et al., 2012. Scholte et al., 2012; Yang et al., 2012; Omumbo et al., 2013; Tsegaw et al., 2013).

Based on a previously published LAMP assay (Kumagai et al., 2010) that targeted 28S rDNA, the present study aimed to apply LAMP assay to pooled samples in the field to provide a rapid and effective method to detect *S. japonicum* DNA in field-collected *O. hupensis*. This new assay will aid the establishment of a surveillance and response system toward schistosomiasis elimination in PR China. The risk map presented here will be useful for the spatial targeting of schistosomiasis control interventions and future efforts focusing on schistosomiasis elimination.

Materials and methods

Parasites

Clonorchis sinensis, *Paragonimus westermani*, *Schisotosoma mansoni* and *Angiostrongylus cantonensis* were prepared in the Zhejiang Academy of Medical Sciences, PR China. Three female New Zealand rabbits, weighing approximately 3 kg, were infected with 1000 *S. japonicum* cercariae for 45 days, anesthetized with Pelltobarbitalum Natricum. Adult worms were collected from the rabbits by perfusing their portal veins with physiological saline. The Institutional Animal Care and Use Committee (IACUC) approved the use of the animals.

Snails and pooled samples

From April to August 2010, *O. hupensis* snails were collected from 28 field sites in five endemic provinces, including Anhui, Hubei, Hunan, Sichuan, and Yunnan, in PR China. Two groups, e.g. individual and pooled samples, were prepared before DNA detection. For individual detection, the collected snails were crushed individually with tweezers classified as infected ones if cercariaes of the parasite were identified under a light microscope. For pooled sample detection, the maximum pool size (MPS) was calculated based on the following pooled sample formula (Gu, 1998):

$$1 - (1 - r)^n - nr(1 - r)^{n-1} < \alpha.$$

where r is the estimated infection rate of the snails, n is the MPS and α is the significant level of detection. Our previous data showed that r is approximately 0.26% (Dang et al., 2006), and α is 0.01; thus, n is about 50. Therefore, 50 snails were pooled and crushed together as one group for each site.

DNA extraction

Genomic DNA of *S. japonicum* from adult worms was extracted using a QIAmp DNA mini kit (QIAGEN, Dusseldorf, Germany), according to the manufacturer's instructions, and the concentration of DNA was measured by NanoDrop spectrometry (Thermo Fisher Scientific, MA, USA). DNA derived from *P. westermani*, *C. sinensis*, *S. mansoni* and *A. cantonensis* was used to confirm the specificity of the LAMP assay. To detect schistosomal DNA from snails, the

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006

Table 1
Specific primer sets used in this study.

Primers	Sequence (5′-3′)
Nested-PCR	F1: CATAGAAGCGATGTAGTC
	S1: TGGACTGAAACTGGTCAG
	F2: GAAATATAAAGGAACCGGTGG
	S2: ATCCTAAGCGAATGCCTCG
LAMP	F3: GCTTTGTCCTTCGGGCATTA
	B3: GGTTTCGTAACGCCCAATGA
	FIP: ACGCAACTGCCAACGTGACATACTGGTCGGCTTGTTACTAGC
	BIP: TGGTAGACGATCCACCTGACCCCTCGCGCACATGTTAAACTC

DNA extraction method used incorporated heated NaOH (Hirayama et al., 2004). Briefly, each crushed snail was placed into 200 μL of 50 mM NaOH and heated to 95 °C for 30 min. In mass detection, DNA was extracted from 50 crushed snails in 10 mL of 50 mM NaOH in a 50 mL tube and incubated at 95 °C for 60 min. After centrifugation, 50 μL of supernatant was recovered and mixed to an equal volume of 1 M Tris–HCl (pH 8.0). This solution was used directly as the template (0.5 μL) for nested-PCR and the LAMP assay.

LAMP assay

DNA from *S. japonicum* was amplified using both LAMP (Kumagai et al., 2010) and nested-PCR methods (Tong et al., 2009). The 28S rDNA gene (GenBank Accession No. Z46504) and the Sj-alpha-1 gene (GenBank Accession No. AF213692) sequences of *S. japonicum* were targeted for LAMP and nested-PCR (Table 1), respectively. The LAMP assay was carried out according to a previously reported procedure using a Loopamp DNA amplification kit (EikenSci, Tokyo, Japan). The LAMP reagents were transported at 4 °C and preserved at −20 °C. Briefly, the reaction was performed in 20 μL of reaction mixture containing 0.5 μL of extracted DNA solution, 5 pmol each of the F3 and B3 primers, 40 pmol each the FIP and BIP primers, 10 μL of 2× Reaction Mix, 0.8 μL Fluorescent Detection Reagent (FD) (EikenSci, Tokyo, Japan) and 0.8 μL of *Bst* DNA polymerase. The LAMP reaction mixture was incubated at a range of temperatures (60, 63 and 65 °C) and over a range of times (40, 50, 60 and 90 min) to optimize the reaction conditions for positive reactions. We used the nested-PCR assay as a comparative method to assess the performance of the LAMP assay. The nested-PCR assay was performed according to the published protocol (Tong et al., 2009). FD (a detection dyethatuses calcein as the fluorescence agent) was added to the reaction mix. Therefore, the positive results of LAMP amplification could be observed visually by a color change from orange to green. In addition, both the LAMP and PCR products were electrophoresed on 1% agarose gels, stained with GelRed™ (Biotium, Inc., CA, USA) and visualized under UV light. Together with the samples, a DL 2,000™ DNA Marker (Takara, Dalian, PR China) was used in the electrophoresis to determine the molecular weights.

Statistics and risk map construction

Three hundred snails collected from each field site were divided into six groups with 50 snails in each group, based on the MPS calculation. After conducting the LAMP test for each group of pooled samples, the proportion of LAMP positive pools (R_p) and the positive proportion (R_i) of each site were estimated based on the following formula:

$$R_p = \frac{\text{No. of LAMP positive pooled samples}}{\text{Total no. of pooled samples}}$$

and

$$R_i = \frac{\text{No. of LAMP positive pooled samples}}{\text{Total no. of snails detected}}$$

For example, if the result of one group out of 6 was positive, it indicated that at least one of 50 snails of this group was positive, so the positive proportion (R_i) of this monitoring site was 0.33% (1/300).

The risk map was constructed based on the proportion of *O. hupensis* infected with *S. japonicum*, as detected by the LAMP assay. First, we used Google Earth (http://www.google.com/earth/) to determine the coordinates of each monitoring site, and then ArcMap (ESRI® ArcGIS® ArcMap™, USA) was used to produce data for each field site, which was saved to the geodatabase with site name, infection rate and coordinates. Overlaying the monitoring sites data on the administrative division data constructed the risk map. Using the categories symbology function of ArcMAP, the positive proportion of each monitoring site could be displayed on a histogram on the risk map, in which the height of the bar represented grades of 0, >0.33%, >0.67% and >1.33%, respectively, according to the positive proportions of pooled samples detected by the LAMP assay. Since the positive proportions of different regions reflect a spatial correlation structure, the spatial correlation structure of the data was specified by fitting a variogram model, and then using kriging as a best linear unbiased prediction method to construct a smooth map of snail infections in all schistosomiasis endemic areas.

Results

LAMP assay

The LAMP reaction mixture was incubated at a range of temperatures (60, 63 and 65 °C) for different times (40, 50, 60 and 90 min). The most suitable conditions for the reaction were determined as 65 °C for 60 min (data not shown). To determine the detection limit of the LAMP assay, 10-fold serial dilutions spanning 100 pg to 10 fg of *S. japonicum* DNA were amplified by LAMP. The detection limit of the *S. japonicum* DNA template using the LAMP assay was 100 fg, which was consistent with a previously reported result (Kumagai et al., 2010). The LAMP assay was tested for specificity using heterogeneous DNA samples as controls. DNA was amplified only from *S. japonicum*, no amplification occurred from DNA samples of *P. westermani*, *C. sinensis*, *S. mansoni* or *A. cantonensis* (Fig. 1).

For individual detection, snails collected from endemic areas were crushed individually and checked under a microscope. The DNA of each snail was then extracted for the LAMP assay. As shown in Fig. 2, all samples that were positive microscopically were also positive by the LAMP assay.

For detection from pooled samples using the LAMP assay, *S. japonicum* positive snails were found in seven sites accounting for 25% of the 28 survey sites, while nested-PCR only detected positive snails in four sites (14.3%), indicating that the LAMP assay had greater sensitivity than nested-PCR (Table 2).

Proportion of LAMP positive pools and the positive proportion of O. hupensis

The proportion of LAMP positive pools and the positive proportion of *O. hupensis* of 28 field sites are listed in Table 3. According to the data, Xima village in Hubei Province was at a relatively high risk of *S. japonicum* infection. Heini and Hongjia in Hunan Province, Yangjiang in Hubei Province, and Huangshan in Anhui Province were at a moderate risk of infection, and Tuanzhou and Suliao in Hunan Province were at a lower risk of infection. The remaining 21

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006

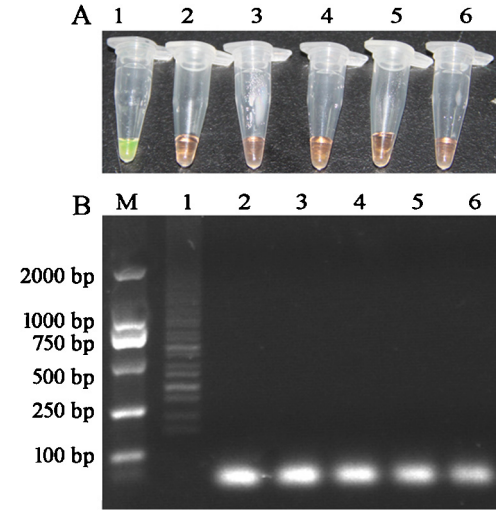

Fig. 1. Specificity test for *Schistosoma japonicum* using LAMP (A. Analysis using fluorescent detection reagent (FD) staining (tubes from left to right represent DNA amplified from 1: *S. japonicum*, 2: *Paragonimus westermani*, 3: *Clonorchis sinensis*, 4: *S. mansoni*, 5: *Angiostrongylus cantonensis*, 6: negative DNA control). B. Analysis by agarose gel electrophoresis (M: DNA marker DL2000 1: *S. japonicum*, 2: *P. westermani*, 3: *C. sinensis*, 4: *S. mansoni*, 5: *A. cantonensis*, 6: negative DNA control)).

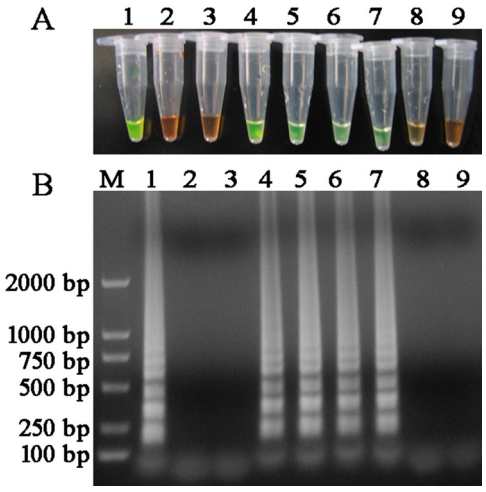

Fig. 2. Results of individual detection of field-collected snails. (A. Visualization by fluorescent detection reagent (FD) staining (1: *Schistosoma japonicum*, 2: negative DNA control, 3: uninfected snail control, 4–7: microscope (+) snails, 8–9: microscope (−) snails). B. Analysis by agarose gel electrophoresis (M: DNA marker DL2000, 1: *S. japonicum*, 2: negative DNA control, 3: uninfected snail control, 4–7: microscope (+) snails, 8–9: microscope (−) snails)).

monitoring field sites in the five provinces gave negative results, indicating low or no risk of infection.

Risk map

Fig. 3 shows the risk map of *O. hupensis* snails infected with *S. japonicum* at 28 field sites in five provinces of PR China. The distribution of the histogram columns representing the positive rates of infection of *O. hupensis* snails in seven out of 28 monitoring sites, and the smoothing map obtained by the kriging method showing the geographical trend of the infection rate, indicated that the higher risk areas of schistosomiasis are located in Hubei and Hunan provinces, particularly areas along the Puyang lake, followed by the adjacent areas between the Hubei and Anhui provinces.

Table 2
Comparison of LAMP and nested-PCR assays for the detection of pooled sample of snails.

		No. (%) with nested-PCR		Total No. (%)
		+	−	
No. (%) with LAMP	+	4(14.3)	3(10.7)	7(25)
	−	0(0)	21(75)	21(75)
Total No.(%)		4(14.3)	24(85.7)	28(100)

Discussion

Schistosomiasis remains a serious health problem worldwide (Suzuki et al., 2006). It was identified as the second largest cause of morbidity and mortality associated with parasitic infections in endemic areas (Curtis and Minchella, 2000). In PR China, since the mid-1950s, schistosomiasis control activities have reduced the overall prevalence of human infection with *S. japonicum* to less than 10% (Zhou et al., 2005; Utzinger et al., 2005). However, schistosomiasis has not been completely eradicated. In 2010, the total area in PR China infested by snails was approximately 373,596 ha (Lei et al., 2011), an increase of 0.33% compared with that in 2009 (Hao et al., 2010). Traditionally, microscopic methods have been used to detect infected snails and this is the current mainstay. However, they are time consuming and can result in misdiagnosis in areas of low prevalence. While PCR methods, sensitive and specific, require training and expensive thermocycling equipment, making them difficult to apply in the field. To monitor the infection rate and distribution of infected snails effectively, we evaluated a new molecular detection tool based on the amplification of DNA by the LAMP method to detect *S. japonicum* DNA in *O. hupensis* snails.

Kumagai et al. (2010) reported that both LAMP and PCR assays could detect *S. japonicum* DNA very sensitively, even from 1 day after infection with miracidia. This indicated that LAMP and PCR assays are sensitive enough to detect of only the mature cercariae, but also the prepatent infections with sporocytes and germ balls. Quantities of prepatent infected snails are likely to exceed those of snails with mature cercariae. Sporocytes and germ balls are easily missed by traditional microscopic methods. Therefore, LAMP and PCR assays provide an appropriate method for detection of prepatent infections. Sandoval et al. (2006) reported that the sensitivity of PCR was 15 pg, whereas Xia et al. (2009) reported the detection limit of PCR was 0.8 pg. The LAMP method is more sensitive than the PCR method since the detection limit for an *S. japonicum* DNA template was 100 fg, indicating that the LAMP assay is sensitive enough to detect DNA at a low level of infections. When detecting the DNA in pooled samples, compared with nested-PCR the LAMP assay was more sensitive than the nested-PCR. Compared with PCR, the LAMP assay is rapid and the results can be achieved within 60 min without using thermocyclers. Using the FD dye, the amplification products can be assessed by the naked eye. The use of color inspection is preferable under field conditions. For large-scale screening in endemic areas, the current LAMP assay could be improved technically for application in field laboratories. Hamburger et al. (2013) addressed some technical points of LAMP assays for *S. haematobium* and *S. mansoni* in field laboratories. These measures included simplifying the DNA harvesting procedure based on snail digestion by NaOH and then blotting onto treated membranes, and using a ready-mix of LAMP reagents suitable for transportation at normal temperatures and storage in small refrigerator.

Kumagai et al. (2010) reported that LAMP could amplify specific DNA from a group of 100 normal snails artificially mixed with one infected snail. Here, we report the LAMP assay was practical to detect infection in pooled samples of field snails. Pooled samples have proved to be an effective practice in many research fields. Pooled samples can reduce the effects of biological variation, but

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006

Table 3
Detection of *Oncomelania hupensis* infection at 28 field sites in five endemic provinces in PR China.

Location code and name	Georeference East Longitude/North Latitude	No. examined	No. LAMP positive pools/pools	Proportion of LAMP positive pools (%)	Minimum positive rate (%)
1 Erli	103.66/30.13	300	0/6	0	0
2 Yonghong	112.83/29.03	300	0/6	0	0
3 Xingchun	118.49/31.20	300	0/6	0	0
4 Heini	112.99/29.12	300	2/6	33.33	0.67
5 Yachi	103.58/30.06	300	0/6	0	0
6 Zhiqi	104.24/30.02	300	0/6	0	0
7 Xima	112.57/30.00	300	4/6	66.67	1.33
8 Huangshan	116.84/30.49	300	2/6	33.33	0.67
9 Hongjia	112.81/28.43	300	2/6	33.33	0.67
10 Yangjing	112.07/30.33	300	2/6	33.33	0.67
11 Tuanzhou	112.80/29.33	300	1/6	16.67	0.33
12 Suliao	112.78/28.49	300	1/6	16.67	0.33
13 Jiangxin2	116.59/30.03	300	0/6	0	0
14 Jiangxin1	116.59/30.03	300	0/6	0	0
15 Qunying	117.96/31.09	300	0/6	0	0
16 Guanghui	117.75/30.94	200	0/6	0	0
17 Danghuangtun	112.40/30.36	300	0/6	0	0
18 Sanli	118.58/31.31	300	0/6	0	0
19 Shenyuan	112.28/30.08	300	0/6	0	0
20 Yueyuan	112.81/28.41	250	0/5	0	0
21 Liulin	112.72/28.51	300	0/6	0	0
22 Xintian	118.78/30.75	300	0/6	0	0
23 Yaogeng	118.44/31.20	300	0/6	0	0
24 Yongle	100.00/26.15	300	0/6	0	0
25 Baota	115.27/29.83	300	0/6	0	0
26 Dukou	114.94/29.69	300	0/6	0	0
27 Guifan	117.43/30.58	300	0/6	0	0
28 Meiwan	103.46/30.04	300	0/6	0	0

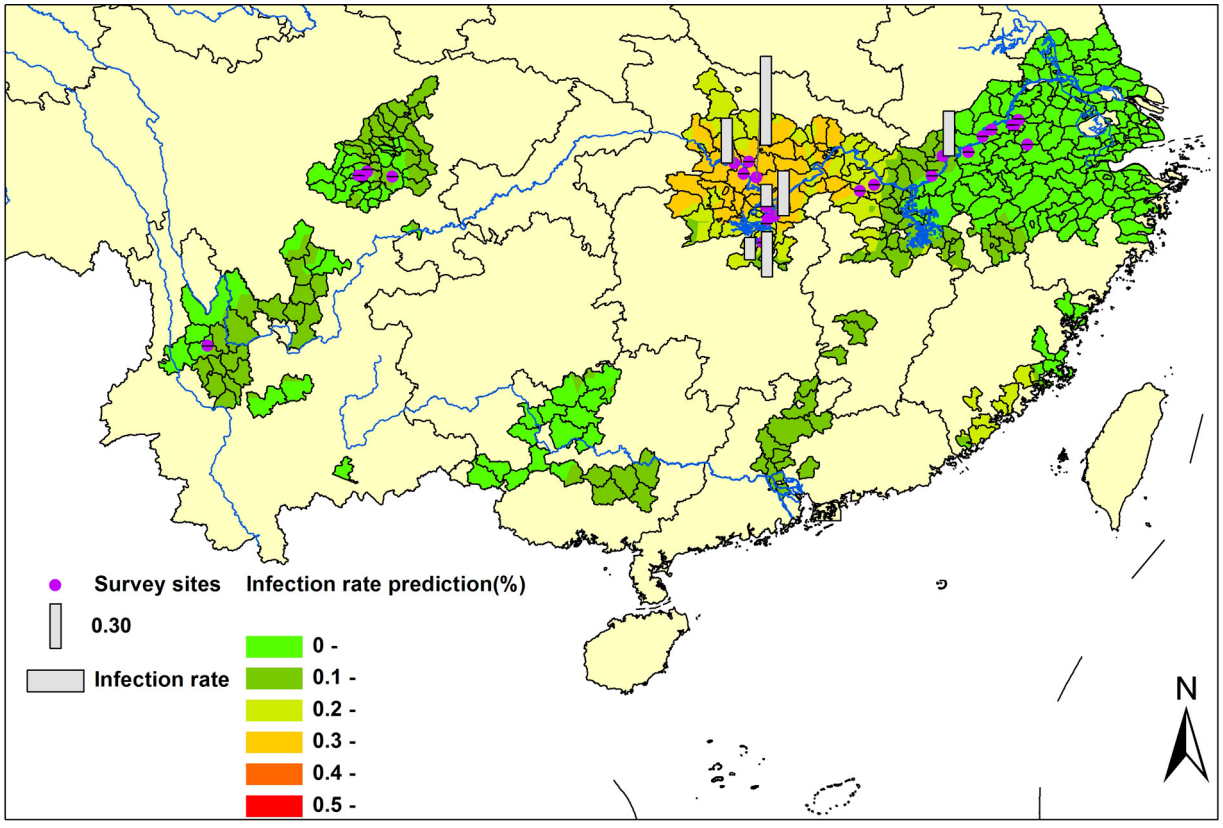

Fig. 3. Risk map showing *Oncomelania hupensis* snail habitats at 28 field sites in five endemic provinces in PR China. The height of each column represents grades of 0, >0.33%, >0.67% and >1.33%, respectively, according to the positive percentage of pooled samples detected by the LAMP assay. ArcMAP constructed the prediction smooth map.

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006

not the biological variation itself. Detection from pooled samples has opened the door for large-scale low-cost screening. Kendziorski et al. (2005) reported that pooling strategies resulted in cost savings of 53.3% (five samples per pool) and 44.0% (ten samples per pool) (Kendziorski et al., 2005; Zhang and Gant, 2005). Pooled samples for detecting *Chlamydia trachomatis* by PCR were found to be an accurate and cost-saving approach for diagnosis and large-scale prevalence studies in St Petersburg, Russia (Shipitsyna et al., 2007). In the present study, the established LAMP method using pooled samples proved useful for detecting schistosomal DNA for a large number of snails in endemic areas, and makes it possible to monitor low infection rates of snails in these areas.

The risk of schistosomiasis still exists in PR China and snail control remains a significant challenge in the field (Wang et al., 2009; McManus et al., 2010; Lv et al., 2013). In the present study, the risk map generated from pooled snail samples using LAMP assay indicated that in Xima and Yangjiang of Hubei Province; Heini, Hongjia, Tuanzhou and Suliao of Hunan Province; and Huangshan of Anhui Province contained the infected snails, which is an indication of the risk of schistosomiasis transmission. The smooth map of the infection risk integrated with the LAMP detection data in the endemic areas represents a faster approach to detect the high-risk area of schistosomiasis transmission (Chen et al., 2007; Sun et al., 2011). The results can be used to guide further local investigations and snail control activities, particularly to provide a quick response to any potential outbreaks in areas infested with *O. hupensis* snails. Moreover, the schistosomiasis control program in Hubei, Hunan and Anhui provinces had reached national infection control criteria in 2008 and progressed toward the elimination of schistosomiasis. Hence, less intensive schistosomiasis transmission and a more sporadic distribution of risk spots will be observed in those areas in the future (Spear et al., 2011; Zhou et al., 2011). By considering of the aforementioned facts, more sensitive approaches need to be developed in the surveillance system to respond to further decreases in the transmission intensity leading to the elimination of schistosomiasis. Our risk map indicated that control intervention should target the high-risk areas in endemic areas of Anhui, Hubei and Hunan provinces, especially in those areas along the Puyang lake. The methodology should also be incorporated into the routine surveillance system in PR China. Therefore, the risk map of infected snail distribution generated by LAMP from pooled samples could be considered a new tool to guide surveillance and response in high-risk areas, ultimately leading to the elimination of schistosomiasis japonica in PR China.

One limitation of the study was that the snail samples collected from field did not cover all endemic provinces, such as Jiangxi and Jiangsu provinces. Further study to expand the range of snail sample collection sites is warranted. This also implied that the risk map produced from the study could not represent the entire, current picture of the transmission situation in PR China. However, these results satisfied our research aims and are capable of being applied in future routine surveillance and response systems (Bergquist and Whittaker, 2012; Zhou et al., 2013).

Conclusions

The LAMP assay targeting 28S rDNA is a rapid and effective method to detect *S. japonicum* DNA from *O. hupensis* snails, especially when applied to pooled samples. This is the first report of monitoring infected snails from field sites of low endemicity areas by the LAMP assay using pooled samples. The use of pooled sampling provides a more efficient way to screen large numbers of samples compared with the previously reported PCR and LAMP methods. The risk map supported by the LAMP results could be adopted as an innovative tool to guide surveillance and response strategies in risk areas, ultimately leading to schistosomiasis elimination.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Tong QB performed the main experiments, and wrote the manuscript. Chen R, Zhang Y, Lou D, Kumagai T and Furushima-Shimogawara R prepared materials and joined the experiments. Yang GJ and Yang K produced the risk map and statistical analysis. Lu SH and Zhou XN conceived and designed the study, and drafted the manuscript. Wen LY and Nobuo O supervised the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the National S & T Major Program (Grant No. 2008ZX10004-011, 2012ZX10004-022), International S&T Cooperation Program of China (2013DFG32960), Zhejiang Science and Technology Project (2009F20036, 2012C33014), the Projects of Zhejiang Health Department (XKQ-009-003, XKQ-010-001, 11-ZC01), Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents, the Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (23659209), and Japan-China Medical Association, 2011.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2014.01.006.

References

Abbasi, I., King, C.H., Muchiri, E.M., Hamburger, J., 2010. Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by loop-mediated isothermal amplification: identification of infected snails from early prepatency. Am. J. Trop. Med. Hyg. 83, 427–432 [PubMed: 20682894].

Adams, E.R., Schoone, G.J., Ageed, A.F., Safi, S.E., HD, S., 2010. Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of *Leishmania* parasites in clinical samples. Am. J. Trop. Med. Hyg. 82, 591–596 [PubMed: 20348505].

Aonuma, H., Yoshimura, A., Perera, N., Shinzawa, N., Bando, H., Oshiro, S., Nelson, B., Fukumoto, S., Kanuka, H., 2009. Loop-mediated isothermal amplification applied to filarial parasites detection in the mosquito vectors: Dirofilariaimmitis as a study model. Parasites Vectors 2, 15 [PubMed: 19284882].

Bao, H., Wang, X., Zhao, Y., Sun, X., Li, Y., Xiong, Y., Chen, H., 2012. Development of a reverse transcription loop-mediated isothermal amplification method for the rapid detection of avian influenza virus subtype H7. J. Virol. Methods 179, 33–37 [PubMed: 21930158].

Bavia, M.E., Carneiro, D.D., Gurgel Hda, C., Madureira Filho, C., Barbosa, M.G., 2005. Remote sensing and geographic information systems and risk of American visceral leishmaniasis in Bahia, Brazil. Parassitologia 47, 165–169 [PubMed: 16044686].

Bergquist, R., Whittaker, M., 2012. Control of neglected tropical diseases in Asia Pacific: implications for health information priorities. Infect. Dis. Poverty 1, 3.

Collins, C., Xu, J., Tang, S., 2012. Schistosomiasis control and the health system in PR China. Infect. Dis. Poverty 1, 8.

Curtis, J., Minchella, D., 2000. Schistosome population genetic structure: when clumping worms is not just splitting hairs. Parasitol. Today 16, 68–71 [PubMed: 10652491].

Croft, S.L., Vivas, L., Brooker, S., 2003. Recent advances in research and control of malaria, leishmaniasis, trypanosomiasis and schistosomiasis. East Mediterr Health J. 9, 518–533 [PubMed: 15748049].

Chen, Z., Zhou, X.N., Yang, K., Wang, X.H., Yao, Z.Q., Wang, T.P., Yang, G.J., Yang, Y.J., Zhang, S.Q., Wang, J., Jia, T.W., Wu, X.H., 2007. Strategy formulation for schistosomiasis japonica control in different environmental settings supported by spatial analysis: a case study from China. Geospat. Health 1 (2), 223–231 [PubMed: 18686247].

Chen, R., Tong, Q., Zhang, Y., Lou, D., Kong, Q., Lv, S., Zhuo, M., Wen, L., Lu, S., 2011. Loop-mediated isothermal amplification: rapid detection of *Angiostrongylus cantonensis* infection in *Pomacea canaliculata*. Parasit. Vectors 4, 204 [PubMed: 22023992].

Dang, H., Zhu, R., Guo, J.G., 2006. Report of the result of national surveillance for schistosomiasis in China, 2005. Re Dai Bing Yu Ji Sheng Chong Xue 4 (4), 189–192.

De la Rocque, S., Michel, J.F., De Wispelaere, G., Cuisance, D., 2001. New tools for the study of animal trypanosomiasis in the Sudan: model-building of dangerous epidemiological passage by remote sensing geographic information systems. Parasite 8, 171–195 [PubMed: 11584749].

Gu, W., 1998. Estimation of vector prevalence by using pool sampling. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 16, 29–33 [PubMed: 12078204].

Gomes, L.I., Dos Santos Marques, L.H., Enk, M.J., de Oliveira, M.C., Coelho, P.M., Rabello, A., 2010. Development and evaluation of a sensitive PCR-ELISA system for detection of *schistosoma* infection in feces. PLoS Negl. Trop. Dis. 4, e664 [PubMed: 20421918].

Hirayama, H., Kageyama, S., Moriyasu, S., Sawai, K., Onoe, S., Takahashi, Y., Katagiri, S., Toen, K., Watanabe, K., Notomi, T., Yamashina, H., Matsuzaki, S., Minamihashi, A., 2004. Rapid sexing of bovine preimplantation embryos using loop-mediated isothermal amplification. Theriogenology 62, 887–896 [PubMed: 15251240].

Han, E.T., Watanabe, R., Sattabongkot, J., Khuntirat, B., Sirichaisinthop, J., Iriko, H., Jin, L., Takeo, S., Tsuboi, T., 2007. Detection of four *Plasmodium* species by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. J. Clin. Microbiol. 45, 2521–2528 [PubMed: 17567794].

Hamburger, J., Abbasi, I., Kariuki, C., Wanjala, A., Mzungu, E., Mungai, P., Muchiri, E., King, C.H., 2013. Evaluation of loop-mediated isothermal amplification suitable for molecular monitoring of schistosome-infected snails in field laboratories. Am. J. Trop. Med. Hyg. 88, 344–351 [PubMed: 23208875].

Hao, Y., Zheng, H., Zhu, R., Guo, J.G., Wu, X.H., Wang, L.Y., Chen, Z., Zhou, X.N., 2009. Schistosomiasis status in People's Republic of China in 2008. Chin. J. Schisto Control 21, 451–456.

Hao, Y., Zheng, H., Zhu, R., Guo, J.G., Wang, L.Y., Chen, Z., Zhou, X.N., 2010. Schistosomiasis situation in People's Republic of China in 2009. Chin. J. Schisto Control 22, 521–527.

Kendziorski, C., Irizarry, R.A., Chen, K.S., Haag, J.D., Gould, M.N., 2005. On the utility of pooling biological samples in microarray experiments. Proc. Natl. Acad. Sci. 102, 4252–4257 [PubMed: 15755808].

Kjetland, E.F., Hove, R.J., Gomo, E., Midzi, N., Gwanzura, L., Mason, P., Friis, H., Verweij, J.J., Gundersen, S.G., Ndhlovu, P.D., Mduluza, T., Van Lieshout, L., 2009. Schistosomiasis PCR in vaginal lavage as an indicator of genital *Schistosoma haematobium* infection in rural Zimbabwean women. Am. J. Trop. Med. Hyg. 81, 1050–1055 [PubMed: 19996436].

Kumagai, T., Furushima-Shimogawara, R., Ohmae, H., Wang, T.P., Lu, S., Chen, R., Wen, L., Ohta, N., 2010. Detection of early and single infections of *Schistosoma japonicum* in the intermediate host snail, *Oncomelania hupensis*, by PCR and Loop-Mediated Isothermal Amplification (LAMP) Assay. Am. J. Trop. Med. Hyg. 83, 542–548 [PubMed: 20810818].

Kong, Q.M., Lu, S.H., Tong, Q.B., Lou, D., Chen, R., Zheng, B., Kumagai, T., Wen, L.Y., Ohta, N., Zhou, X.N., 2012. Loop-mediated isothermal amplification (LAMP): early detection of *Toxoplasma gondii* infection in mice. Parasit. Vectors 5, 2 [PubMed: 22214421].

Lei, Z.L., Zheng, H., Zhang, L.J., Zhu, R., Guo, J.G., Li, S.Z., Wang, L.Y., Chen, Z., Zhou, X.N., 2011. Schistosomiasis status in People's Republic of China in 2010. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 23, 599–604 [PubMed: 22379811].

Li, Y.S., Sleigh, A.C., Ross, A.G., Li, Y., Williams, G.M., Forsyth, S.J., Tanner, M., McManus, D.P., 1999. A 2-year prospective study in China provides epidemiological evidence for resistance in humans to re-infection with *Schistosoma japonicum*. Ann. Trop. Med. Parasitol. 93, 629–642 [PubMed: 10707108].

Liang, S., Yang, C., Zhong, B., Qiu, D., 2006. Re-emerging schistosomiasis in hilly and mountainous areas of Sichuan, China. Bull. World Health Organ. 84, 139–144 [PubMed: 16501732].

Lier, T., Johansen, M., Hjelmevoll, S., Vennervald, B., Simonsen, G., 2008. Real-time PCR for detection of low intensity *Schistosoma japonicum* infections in a pig model. Acta Trop. 105, 74–80 [PubMed: 18036505].

Li, S.Z., Luz, A., Wang, X.H., Xu, L.L., Wang, Q., Qian, Y.J., Wu, X.H., Guo, J.G., Xia, G., Wang, L.Y., Zhou, X.N., 2009. Schistosomiasis in China: acute infections during 2005-2008. Chin. Med. J. (Engl). 122, 1009–1014 [PubMed: 19493433].

Lv, S., Tian, L.G., Liu, Q., Qian, M.B., Fu, Q., Steinmann, P., Chen, J.X., Yang, G.J., Yang, K., Zhou, X.N., 2013. Water-related parasitic diseases in China. Int. J. Environ. Res. Public Health 10, 1977–2016.

Mabey, D., Peeling, R.W., Ustianowski, A., Perkins, M.D., 2004. Diagnostics for the developing world. Nat. Rev. Microbiol. 2, 231–240 [PubMed: 15083158].

McManus, D.P., Gray, D.J., Li, Y., Feng, Z., Williams, G.M., Stewart, D., Rey-Ladino, J., Ross, A.G., 2010. Schistosomiasis in the People's Republic of China: the era of the Three Gorges Dam. Clin. Microbiol. Rev. 23 (2), 442–466 [PubMed: 20375361].

Noor, A.M., ElMardi, K.A., Abdelgader, T.M., Patil, A.P., Amine, A.A., Bakhiet, S., Mukhtar, M.M., Snow, R.W., 2012. Malaria risk mapping for control in the republic of Sudan. Am. J. Trop. Med. Hyg. 87 (6), 1012–1021 [PubMed: 23033400].

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28, E63 [PubMed: 10871386].

Njiru, Z.K., Ouma, J.O., Enyaru, J.C., AP, D., 2010. Loop-mediated Isothermal Amplification (LAMP) test for detection of *Trypanosoma evansi* strain B. Exp. Parasitol. 125, 196–201 [PubMed: 20109454].

Nkouawa, A., Sako, Y., Nakao, M., Nakaya, K., Ito, A., 2009. Loop mediated isothermal amplification method for differentiation and rapid detection of *Taenia* species. J. Clin. Microbiol. 47, 168–174 [PubMed: 19005142].

Nkouawa, A., Sako, Y., Li, T., Chen, X., Wandra, T., Swastika, I.K., Nakao, M., Yanagida, T., Nakaya, K., Qiu, D., Ito, A., 2010. Evaluation of a loop-mediated isothermal amplification method using fecal specimens for differential detection of *Taenia* species from humans. J Clin Microbiol 48, 3350–3352 [PubMed: 20631114].

Oliveira, L.M., Santos, H.L., Goncalves, M.M., Barreto, M.G., Peralta, J.M., 2010. Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity *Schistosoma mansoni* infection. Diagn. Microbiol. Infect. Dis. 68, 416–421 [PubMed: 20884153].

Omumbo, J.A., Noor, A.M., Fall, I.S., Snow, R.W., 2013. How well are malaria maps used to design and finance malaria control in Africa? PLoS One 8 (1), e53198 [PubMed: 23326398].

Poon, L.L., Wong, B.W., Ma, E.H., Chan, K.H., Chow, L.M., Abeyewickreme, W., Tangpukdee, N., Yuen, K.Y., Guan, Y., Looareesuwan, S., Peiris, J.S., 2006. Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat treated blood by loop-mediated isothermal amplification. Clin. Chem. 52, 303–306 [PubMed: 16339303].

Sandoval, N., Siles-Lucas, M., Pérez-Arellano, J.L., Carranza, C., Puente, S., López-Abán, J., Muro, A., 2006. A new PCR-based approach for the specific amplification of DNA from different *Schistosoma* species applicable to human urine samples. Parasitology 133, 581–587 [PubMed: 16834820].

Scholte, R.G., Carvalho, O.S., Malone, J.B., Utzinger, J., Vounatsou, P., 2012. Spatial distribution of *Biomphalaria* spp., the intermediate host snails of *Schistosoma mansoni*, in Brazil. Geospat. Health 6 (3), S95–S101 [PubMed: 23032289].

So, A.D., Ruiz-Esparza, Q., 2012. Technology innovation for infectious diseases in the developing world. Infect. Dis. Poverty 1, 2.

Spear, R.C., Seto, E.Y., Carlton, E.J., Liang, S., Remais, J.V., Zhong, B., Qiu, D., 2011. The challenge of effective surveillance in moving from low transmission to elimination of schistosomiasis in China. Int. J. Parasitol. 41 (12), 1243–1247 [PubMed: 21920366].

Steinmann, P., Keiser, J., Bos, R., Tanner, M., Utzinger, J., 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect. Dis. 6, 411–425 [PubMed: 16790382].

Suzuki, T., Osada, Y., Kumagai, T., Hamada, A., Okuzawa, E., Kanazawa, T., 2006. Early detection of *Schistosoma mansoni* infection by touchdown PCR in a mouse model. Parasitol. Int. 55, 213–218 [PubMed: 16822708].

Sun, L.P., Liang, Y.S., Wu, H.H., Tian, Z.X., Dai, J.R., Yang, K., Hong, Q.B., Zhou, X.N., Yang, G.J., 2011. A Google Earth-based surveillance system for schistosomiasis japonica implemented in the lower reaches of the Yangtze River, China. Parasit Vectors. 4, 223–231 [PubMed: 22117601].

Shipitsyna, E., Shalepo, K., Savicheva, A., Unemo, M., Domeika, M., 2007. Pooling samples: the key to sensitive, specific and cost-effective genetic diagnosis of *Chlamydia trachomatis* in low-resource countries. Acta Derm. Venereol. 87, 140–143 [PubMed: 17340020].

Standley, C.J., Adriko, M., Arinaitwe, M., Atuhaire, A., Kazibwe, F., Fenwick, A., Kabatereine, N.B., Stothard, J.R., 2010. Epidemiology and control of intestinal schistosomiasis on the Sesse Islands, Uganda: integrating malacology and parasitology to tailor local treatment recommendations. Parasit. Vectors 3, 64 [PubMed: 20663223].

Thekisoe, O.M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J., Inoue, I., 2007. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. Acta Trop. 102, 182–189 [PubMed: 17574198].

Takagi, H., Itoh, M., Kasai, S., Yahathugoda, T.C., Weerasooriya, M.V., Kimura, E., 2011. Development of loop-mediated isothermal amplification method for detecting *Wuchereria bancrofti* DNA in human blood and vector mosquitoes. Parasitol. Int. 60, 493–497 [PubMed: 21930238].

Takagi, H., Itoh, M., Islam, M.Z., Razzaque, A., Ekram, A.R., Hashighuchi, Y., Noiri, E.E.K., 2009. Sensitive, specific, and rapid detection of *Leishmania donovani* DNA by loop-mediated isothermal amplification. Am. J. Trop. Med. Hyg. 81, 578–582 [PubMed: 19815869].

Tong, Q.B., Lu, S.H., Wang, T.P., Chen, R., Lou, D., MM, Z., 2009. Detection of *Schistosoma japonicum* infection by nested-PCR. Acta Parasitol. Med. Entomol. Sin. 16, 203–207.

Tsegaw, T., Gadisa, E., Seid, A., Abera, A., Teshome, A., Mulugeta, A., Herrero, M., Argaw, D., Jorge, A., Aseffa, A., 2013. Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach. Geospat. Health 7 (2), 299–308 [PubMed: 23733292].

Utzinger, J., Zhou, X.N., Chen, M.G., Bergquist, R., 2005. Conquering schistosomiasis in China: the long march. Acta Trop. 96, 69–96 [PubMed: 16312039].

Wang, L.D., Chen, H.G., Guo, J.G., Zeng, X.J., Hong, X.L., Xiong, J.J., Wu, X.H., Wang, X.H., Wang, L.Y., Xia, G., Hao, Y., Chin, D.P., Zhou, X.N., 2009. A strategy to control transmission of *Schistosoma japonicum* in China. N. Engl. J. Med. 360 (2), 121–128 [PubMed: 19129526].

Xia, C.M., Rong, R., Lu, Z.X., Shi, C.J., Xu, J., Zhang, H.Q., Gong, W., Luo, W., 2009. *Schistosoma japonicum*: a PCR assay for the early detection and evaluation of treatment in a rabbit model. Exp. Parasitol. 121, 175–179 [PubMed: 19027005].

Xu, J., Rong, R., Zhang, H.Q., Shi, C.J., Zhu, X.Q., CM, X., 2010. Sensitive and rapid detection of *Schistosoma japonicum* DNA by loopmediated isothermal amplification (LAMP). Int. J. Parasitol. 40, 327–331 [PubMed: 19735662].

Yang, G.J., Tanner, M., Utzinger, J., Malone, J.B., Bergquist, R., Chan, E.Y., Gao, Q., Zhou, X.N., 2012. Malaria surveillance-response strategies in different transmission

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006

zones of the People's Republic of China: preparing for climate change. Malar. J. 11, 426 [PubMed: 23256579].

Zhang, H., Thekisoe, O.M., Aboge, G.O., Kyan, H., Yamagishi, J., Inoue, N., Nishikawa, Y., Zakimi, S., Xuan, X., 2009a. *Toxoplasma gondii*: sensitive and rapid detection of infection by loop-mediated isothermal amplification (LAMP) method. Exp. Parasitol. 122, 47–50 [PubMed: 19545521].

Zhang, L.J., Zhu, R., Dang, H., Guo, J.G., 2009b. Surveillance of schistosomiasis in China in 2008. Chin. J. Schisto Control. 21, 477–481.

Zhang, S.D., Gant, T.W., 2005. Effect of pooling samples on the efficiency of comparative studies using microarrays. Bioinformatics 21, 4378–4383 [PubMed: 16234321].

Zhou, X.N., Wang, L.Y., Chen, M.G., Wang, T.P., Guo, J.G., Wu, X.H., Jiang, Q.W., Zheng, J., Chen, X.Y., 2005. An economic evaluation of the national schistosomiasis control programme in China from 1992 to 2000. Acta Trop. 96, 255–265 [PubMed: 16154104].

Zhou, X.N., Lin, D.D., Wang, T.P., Chen, H.G., Guo, J.G., Liang, Y.S., Qiu, D.C., Dong, X.Q., Li, S.Z., 2011. Control strategy of schistosomiasis and key points in the 12th Five-Year Plan in China. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 23 (1), 1–4 [PubMed: 22164364].

Zhou, X.N., 2012. Prioritizing research for "One health-One world". Inf. Dis. Poverty 1, 1.

Zhou, X.N., Bergquist, R., Tanner, M., 2013. Elimination of tropical disease through surveillance and response. Inf. Dis. Poverty 2, 1.

Please cite this article in press as: Tong, Q.-B., et al., A new surveillance and response tool: Risk map of infected *Oncomelania hupensis* detected by Loop-mediated isothermal amplification (LAMP) from pooled samples. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.01.006