CHAPTER EIGHT

Control of eosinopilic meningitis caused by *Angiostrongylus cantonensis* in China

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Abstract

Rat lungworm *Angiostrongylus cantonensis* is the major infective agent of human eosinophilic meningitis (EM) in the world. The parasite was first noted in China in 1933. However, the public health importance was not realized until several EM outbreaks occurred recent years. Such disease is considered as emerging infectious disease in the People's Republic of China (P.R. China) since the major source of infection is invasive snail species, particularly *Pomacea* spp. National Institute of Parasitic Diseases (NIPD) initiated a systematic implementation research on this disease since 2003. Our researchers in NIPD developed the lung-microscopy for detecting *A. cantonensis* larvae in *Pomacea* snails and further accomplished the atlas of larval morphology by this method. We studied the determinants in infection, which helped the field collection of snails and improved the infection procedure in laboratory. Our researches promoted the promulgation of diagnosis criteria of angiostrongyliasis cantonensis by the Ministry of Health. We explored the molecular diversity of rat lungworm and its major snail host for development of source-tracing technique. The transmission modelling could provide the vulnerable area for surveillance. All the studies supported the surveillance system of EM caused by *A. cantonensis* in P.R. China. Such implementation research will provide a case study for control of emerging infectious diseases.

1. Introduction

The rat lungworm was first discovered from the pulmonary arteries of *Rattus rattus* and *R. norvegicus* in Guangzhou, China in 1933 (Chen, 1933) and named as *Pulmonema cantonensis* (Chen, 1935). The parasite was also reported as *Haemostrongylus ratti* in Taiwan in 1937 (Matsumoto, 1937; Yokogawa, 1937). Dougherty named rat lungworm as *Angiostrongylus cantonensis* (Dougherty, 1946). The first human case with rat lungworm infection was reported in Taiwan (Beaver and Rosen, 1964; Nomura and Lin, 1945). However, the importance of public health was not realized until 1960s (Alicata, 1962). Rat lungworm is now considered as the major cause of eosinophilic meningitis (EM) in tropic and subtropical regions (Wang et al., 2008). The main route of infection is ingesting terrestrial and freshwater mollusks containing the third-stage larvae of *A. cantonenis*. Infected lizard, frog, shrimps as well as contaminated vegetables could be source of infection (Fig. 1).

The first human case with discovery of larvae of *A. cantonensis* from cerebrospinal fluid in the mainland of the People's Republic of China (P.R. China) was reported in 1982 (He et al., 1984). Only few human cases of EM caused by *A. cantonensis* were reported before 1997. However, this disease occurred in many outbreaks in China due to the recent bio-invasion and geographical expansion of apple snail (*Pomacea* spp.) and African giant

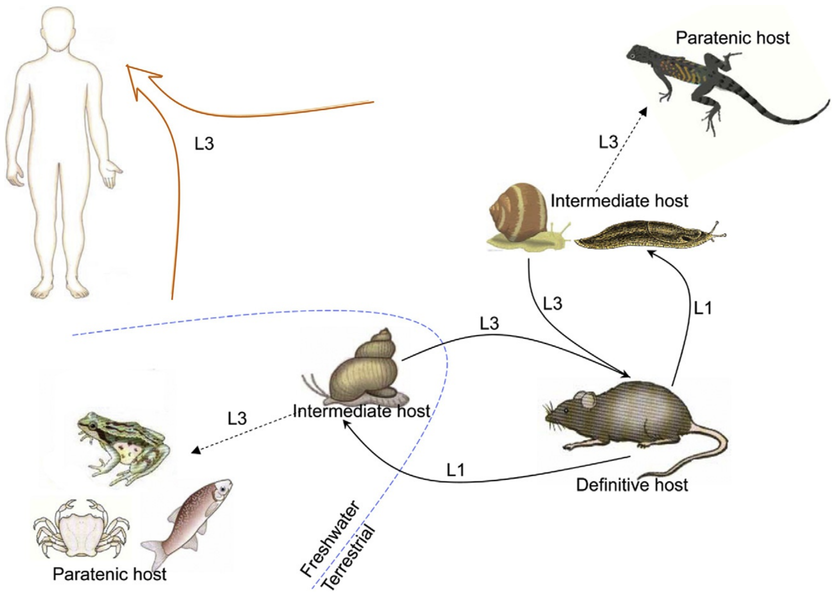

Fig. 1 Life cycle of *A. cantonensis*. L1: first-stage larva, L2: second-stage larva, L3: third-stage larva.

land snail (*Achatina fulica*) and hence considered as an emerging infectious disease (Lv et al., 2008, 2009b).

National Institute of Parasitic Diseases (NIPD), affiliated to Chinese Center for Disease Control and Prevention (China CDC), is the only national center for parasitic diseases control. Researchers from NIPD paid attention to EM caused by *A. cantonensis* as early as 1992 when the second human EM case in the mainland China was parasitologically confirmed in NIPD (Chang et al., 1997). In 1997 the first EM outbreak occurred in Wenzhou city located in the east coast area (Xue et al., 2000). The director of NIPD as the leader of expert committee set by Ministry of Health went to Wenzhou and urgently held a meeting to discuss the measures against the outbreak. Finally the source of infection was revealed and the invasive snail species, *Pomacea canaliculata*, was involved into that outbreak (Pan et al., 2002). The snail became a concern in public health for the first time.

NIPD conducted systematic research on *A. cantonensis* since 2003. Ministry of Science & Technology and Ministry of Health financially supported the research. In this chapter, we summarize the NIPD work pertaining to the research and control of EM caused by *A. cantonensis* in the mainland of P.R. China.

2. Research on life cycle

2.1 Larval morphology in snails

The morphology of *A. cantonensis* was described in many publications. However, most authors focused on the characteristics of adult and third-stage larvae (L3). Although the previous morphological description of L3 was helpful for confirmation of infection in mollusks, we indeed found some snails harbouring pre-third-stage larvae during field surveys, which implied an early infection. Therefore, we study the morphological evolution of *A. cantonensis* larvae in order to facilitate the identification of not only L3 but also second (L2) or first (L1) stage larvae in mollusks (Lv et al., 2009c).

We infected lab-breeding the freshwater snail *P. canaliculata* with the larvae of *A. cantonensis* isolated from fresh faeces of Sprague-Dawley (SD) rats and sacrificed two snails every 2 days to observe the change of larval morphology. We separated and opened the lung (left mantle cavity) of snails. The larval cysts on the lung wall were isolated and larvae were picked out from cysts. Morphology of the larvae was studied under a microscope. There were one and two complete sheaths surrounding L2 and L3, respectively. We found that the refractile granules filled the rear part of larval body at late first stage and second stage, but missed at early first stage and disappeared at third stage. We also observed the feature of larval movement at different stages in vitro. Early L1 obtained from fresh rat faeces actively moved with a coiled tail. When the early L1 invaded and migrate in snail, the coiled tail became straight. Late L1 was only able to shake the head mildly and the body became rigid. The L2 was almost static. L3 became active again and could be seen wriggling within sheaths. L3 normally broke through the sheaths after being isolated from tissue and was characterized by a mellifluous 'Q'-movement. The features mentioned above are helpful for identification of early infection and also facilitate the distinguishing them from free-living nematodes that are often observed to contaminate mollusc samples.

The atlas of larval morphology has been used in the first and second national survey as well as the surveillance in P.R. China. Meanwhile, the larval morphology is also used in some surveys in Brazil (Guerino et al., 2017; Maldonado et al., 2010; Thiengo et al., 2010), Colombia (Córdoba-R et al., 2017), Cuba (Vazquez and Sanchez, 2015), and USA (Qvarnstrom et al., 2013).

2.2 Determinants on infection and larval development

P. canaliculata could develop from a 1 mm egg to an 80 mm adult snail. We tested the susceptibility of *P. canaliculata* of different developmental stages to *A. cantonensis* in lab (Liu et al., 2005). We divided snails breeding at laboratory into four ranks (I–IV) according to the weight and exposed the snails to L1, respectively. Their mortality, infection rate, worm burden, and the size, development and distribution of larvae in snails were determined. The infection rate ranged from 76% to 100%. No significant difference was found among the groups. Snails at earlier developmental stage showed higher mortality. The heaviest worm burden and the largest number of snails harbouring more than 100 larvae were found in snails of rank III. In general the larvae in snails showed a synchronous development in the groups. The period before the L3 emergence and the peak time for L3 exhibited no significant difference among the four ranks. We concluded that the snails at different developmental stage showed similar susceptible to *A. cantonensis*. The snail age or weight is not the determinant of infection and development of *A. cantonensis*.

The developmental period of *A. cantonensis* larvae in snails could be considerably variable in different environment. *A. cantonensis* as well as the mollusc host are poikilothermal. Their developmental rate and thus population density relies on the environmental temperature. We determined the relationship between temperature and development of *A. cantonensis* larvae in *P. canaliculata* (Lv et al., 2006b). The threshold temperature for larval development is 15.04 °C, which means the *A. cantonensis* larvae cannot trigger development until the environmental temperature rise up to 15.04 °C. The thermal constant is 262.53 degree-days, which indicates that development from L1 to L3 needs an effective accumulated temperature of 262.53 degree-days. Therefore, increase in environmental temperature promotes the development and thus facilitates the transmission of *A. cantonensis*. Such parameters are useful in prediction of potential distribution range and the assessment of the impact of climate change on transmission of *A. cantonensis* (Kim et al., 2019; Lv et al., 2011).

Dormancy can facilitate the survival of *P. canaliculata* in adverse environment, such as low temperature and draught. We studied the influence of dormancy of snails on the development and infectivity of *A. cantonensis* (Liu et al., 2006). We found the period from L1 to L3 in dormant snails was shorter than that in the snails in aquarium under the same room temperature. We speculated that the lower water temperature probably decrease the growth of larvae. All L3 at various degree of activity recovered from snails in winter room conditions, including dormant snails and active snails in water, infected rats successfully. Although the weight of dormant snails decreased in winter, the survival rate was significantly higher than that of the snails in aquarium within 60 days. The findings from our study indicated that the freshwater snails *Pomacea* collected from field could be kept and brought to lab in dormant status.

Many mollusc species could serve as the intermediate host of *A. cantonensis* (Lv et al., 2008). However, few species play a role in transmission from snail to human. Three species of freshwater snails, i.e., *P. canaliculata*, *Cipangopaludina chinensis*, *Bellamya aeruginosa*, are commonly sold in food markets in P.R. China. We compared the compatibility of three snail species with *A. cantonensis* in lab (Lv et al., 2006a). We found that the mortality and infection rate were not related to exposure time and snail species. Worm burden of *P. canaliculata* was significantly higher than other two species. However, worm density (worm burden/snail weight) of *P. canaliculata* and *B. aeruginosa* was not significantly different, but higher than that of *C. chinensis*. In addition, the worm burden and worm density of *P. canaliculata* and *B. aeruginosa* depended on exposure time. All the three snail species could be infected by *A. cantonensis* in lab, but the compatibility of *P. canaliculata* is superior to the other two species. Since *C. chinensis* and *B. aeruginosa* are benthic, the possibility of contact with faeces of rats in field is less than *P. canaliculata* which living in shallow water or along bank of water body. Therefore, *P. canaliculata* is more important in transmission of *A. cantonensis*.

2.3 Molecular profile for infection in intermediate host

Pomacea spp. is one of few freshwater snails that play a role in transmission of rat lungworm. We study the change of gene expression post-infection with *A. cantonensis* to explore the potential molecular interaction between parasite and host (Yue, 2019). We collected snail 1 day, 10 day, 20 day post-infection. Meanwhile we collected control snail at the same intervals. We extract total RNA and then reverse-transcribed into cDNA. The cDNA were sequenced. A total of 983 genes were identified due to differential expression. Only 13 genes differentially expressed at all three stages. The verified genes were mostly involved in cell growth and metabolism, signal transduction, immune response and stress response, which indicated a complex molecular interface between host and parasite involved in infection. The down-regulated genes were more than the up-regulated.

G lysozyme and α-tubulin were demonstrated the key molecules in infection. We found the two genes of *Pomacea* spp. were down-regulated after exposure to *A. cantonensis*. We further study the expression profile in different organs or tissues by time. We found that G lysozyme gene was highly expressed in liver of both non-infected female and male snails, which significantly higher than that in kidneys, lung, digestive gut, and foot muscles (Yue et al., 2019b). However, α-tubulin gene showed the differential expression in different organs or tissues. The α-tubulin gene was highly expressed in liver of male and female snails. In addition, this gene was also highly expressed in lung and digestive gut of female snails.

In infected *Pomacea* snails, G lysozyme gene was significantly down-regulated in liver, kidneys, digestive gut, lung, and foot muscles at early and middle stage of infection, but the expression become normal in liver and kidneys at later stage (20 day post-infection). Digestive gut, lung, and foot muscles were sites of invasion by *A. cantonensis* larvae. Most larvae dwell in these organs. As an immune-related gene, the expression of G lysozyme gene was probably influenced by circulating antigens released from larval development. The larvae could develop to third-stage larvae surrounded by two sheathes approximately 3 weeks after infection. The third-stage larvae will maintain diapause for a long time in snail. Therefore, the decreasing circulating antigens in liver and kidneys cause increasing expression of G lysozyme gene.

The α-tubulin gene was normally down-regulated in all organs with an exception of food muscles at the middle and later stage (10 day and 20 day post-infection) (Yue et al., 2019a). Interestingly, such gene expression did not show different before and after exposure. Alpha-tubulin is a protein associated with cell growth. Our result indicated infection with *A. cantonensis* will influence the growth and development of snails.

3. Detection and diagnosis

3.1 Detection methods

Artificial digestion is the main method for detection of *A. cantonenis* larvae in mollusks. However, this method is time-costing and shows less effectiveness at low worm burden. PCR is a newly developed and sensitive method. However, sampling from a large-sized snail, such as *P. canaliculata*, lead to an uncertainty in detection result, since only part of tissue was submitted to DNA extraction. In addition, PCR is not accessible in some settings. The large-sized snails, *Pomacea* spp. and *A. fulica*, are the major source of

A. cantonensis infection. The special anatomical structure of their lung makes it possible to directly observe the larval cysts under a dissecting microscope (Lv et al., 2009c). To make sure the cyst is due to *A. cantonensis* larvae, we picked the cyst under the dissecting microscope and isolate the larvae. We identify the species of larvae according to the morphology and moving features mentioned above. We call this method as lung-microscopy. Compared to the conventional artificial digestion, lung-microscopy significantly save the time and showed the similar effectiveness (Liu et al., 2007). We also established the relationship between worm burden in lung tissue and that in other tissues. The result showed larvae burden in lung was positively correlated with that in other tissues. Therefore, we recommended this method to be applied in large-scale survey. The method is not only a routine method in surveillance of snail food for *A. cantonensis* but also was recommended in other countries (Córdoba-R et al., 2017; Giraldo et al., 2019; Solorzano Alava et al., 2014).

3.2 Diagnosis criteria

The outbreak of angiostrongyliasis occurred in Beijing in 2016 was the biggest one up to date, which involved 160 individuals. The outbreak implied that infections can occur in non-endemic area through the transportation of snail food. Since EM caused by *A. cantonensis* is an emerging infectious disease, the awareness and diagnosis capacity of physicians is limited. Therefore, a diagnosis guide is necessary for the clinics to look for the evidences to support the reliable diagnosis. Shortly after the outbreak in Beijing the Ministry of Health held a consultant conference. Diagnosis criteria is one of the most important topics. In fact, an empirical diagnosis criteria had been implemented during the Beijing outbreak (Yin et al., 2006). In the early 2008 another outbreak of EM caused by *A. cantonensis* occurred in Dali city, Yunnan province (Lv et al., 2009a). The authors conduct a survey on the patients and collected the epidemiological and clinic data of inpatients and outpatients. The patients mainly distributed in two hospitals. However, the diagnosis criteria were not identical. Since the discovery of parasite from human body is very difficult, a diagnosis should standardized to guide the physicians to find the most reliable evidence to support their diagnosis. NIPD took the task from the Ministry of Health to organize experts to draft the diagnosis criteria for EM due to *A. cantonensis*. We categorized diagnosis into suspected, clinically diagnosed, pathologically confirmed cases. The diagnosis criteria was eventually issued by Ministry of

Health in 2010 after several rounds of revisions (Zhang and Lv, 2010). We also propagated the criteria in national training course. It became the only official standard for diagnosis of EM due to *A. cantonensis* in China.

4. Epidemiology and surveillance of eosinopilic meningitis

4.1 Epidemiology based on systematic review

The first human case of angiostrongyliasis in the mainland of P.R. China was reported in 1984. Only three more cases were recorded before 1997 when the first outbreak occurred in Wenzhou city, Zhejiang province located in east coast. Some sporadic human cases were reported in the following years. The importance of public health was not realized until the large outbreak occurred in Beijing in 2006. There were 160 cases involved in that outbreak, which indicate EM caused by *A. cantonensis* could occur in a non-endemic area by transportation of snail food. In order to understand the epidemiology of *A. cantonensis* and EM in China, we performed a systematic review based on literature in 2006 (Lv et al., 2008). A total of 334 cases including four dead cases were identified and 86.5% cases were involved in 7 outbreaks. They distributed in 9 out of 31 provinces. The major source of infection was *P. canaliculata* and *A. fulica*. The rat lungworm was endemic in six provinces. We revealed the distribution of EM due to *A. cantonensis* in P.R. China first time. Such findings directly guided our first national survey on *A. cantonensis* with the focus on *P. canaliculata* and *A. fulica* and facilitated the determination of survey range.

4.2 First national survey in 2006–2007

Although some areas were confirmed endemic with *A. cantonensis* in south China (Lv et al., 2008), the current status remained unknown due to long time span and small scale surveys. In order to reveal the endemic range of *A. cantonensis*, the Ministry of Health decided to conduct a national survey soon after the Beijing outbreak. NIPD took this task and carried out the first national survey between 2006 and 2007 (Lv et al., 2009b). We determined the survey region based on potential distribution of *P. cantonensis* according to a degree-day model (Zhou et al., 2003). The sampling sites were selected by a method of geographical grid sampling. We collected terrestrial and freshwater mollusks as well as rats. All the specimens were brought back to lab for detection. In addition, we also sampled and investigated the snails in markets, restaurants and snail farms. Finally, 164 counties in 19 provinces were involved in this survey. We identified 59 counties in 7 provinces were endemic of *A. cantonensis*. The major intermediate hosts were *Pomacea* spp. and *Achatina fulica* in China. They were widely distributed in south China and the infection rate was 6.8% and 13.4%, respectively. The survey for the first time revealed the present distribution of *A. cantonensis* and two invasive snail species. The findings implied that *Pomacea* spp. and *Achatina fulica* as the vector played an important role in a local endemic infectious diseases, which highlighted the health importance of invasive species. The survey also provided the basic information for establishment of surveillance.

4.3 Second national survey in 2016–2018

Biological invasion and the impact are recognized in China. The Ministry of Science and Technology set a series of survey on invasive species, including vectors of pathogen. NIPD lead a team including seven institutes to conduct a national survey on *Pomacea* spp. (unpublished data). Based on the results of first national survey and the transmission modelling (Lv et al., 2011) we chose 49 counties. The survey indicated that the range of *Pomacea* has extended to the basin of Yangtze River. The snails were commonly found in Hunan, Hubei, Jiangxi, Zhejiang as well as Shanghai. We draw the map of *Pomacea* snail based on our two surveys and other published data (Fig. 2). Since the snail is an important intermediate host of *A. cantonensis*, the further survey on the infection should be push forward.

4.4 Survey in Southeast Asia

EM caused by rat lungworm is endemic in Southeast Asia, but little is known about its distribution in Laos, Cambodia and Vietnam. We performed a multicountry survey for *A. cantonensis* in these countries and estimated the prevalence in snails along the Mekong River and the east coast of Vietnam (Lv et al., 2018). We visited 34 villages and found *Pomacea* spp. in most of villages. A total of 1291 *Pomacea* snails were detected for larvae of rat lungworm. The overall prevalence of *A. cantonensis* was 2.5% with a highest prevalence of 16.2%. Our morphological study and DNA sequencing indicated that only *A. cantonensis* was endemic in the study area. The wide accessibility of *Pomacea* snails as well as infection with *A. cantonensis* indicates that this snail species could be used as an index for risk of EM outbreaks.

Freshwater snails are commonly food items in Southeast Asia and the Far East. According to our surveys in P.R. China and Southeast Asia *Pomacea*

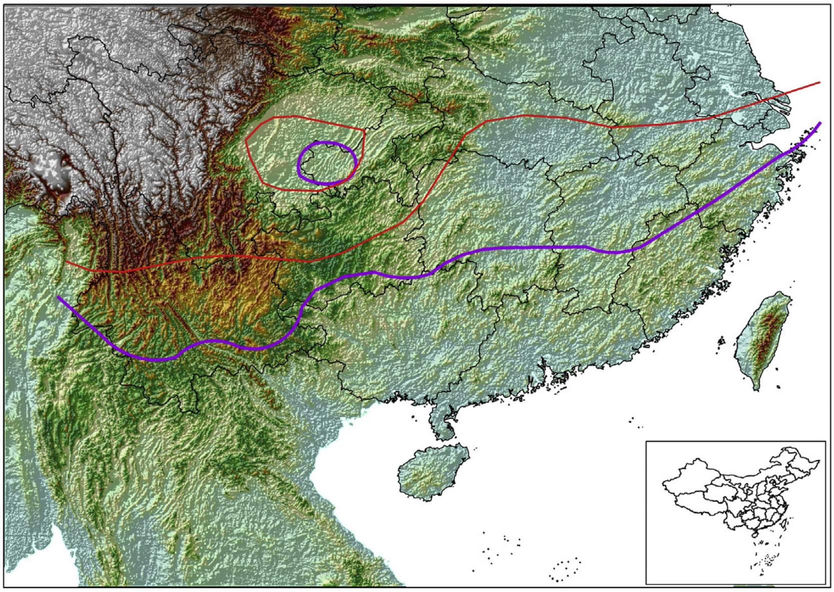

Fig. 2 Geographical range of *Pomacea* spp. in P.R. China. Purple line denotes the distribution of *Pomacea* snails by first national survey 2006–2007; red line denotes the distribution by second national survey and other public data source.

snails could be an indicator for surveillance for epidemicity of *A. cantonensis*. First, *Pomacea* snails have been widely spread and saw in food markets in Southeast Asia and the Far East (Joshi and Sebastian, 2006). Second, *Pomacea* snails are much easier to find than *Pila* and other freshwater snails since they lay highly visible bright pink eggs above the water surface on emergent hard surfaces. Third, the time for collecting *Pomacea* snails is not dependent on weather and light that influence the activity of land snails and slugs. Finally, a simple detection technique for *Angiostrongylus* in *Pomacea* has been developed (Liu et al., 2007; Lv et al., 2009c).

4.5 Surveillance system

After outbreaks in Beijing and Dali, the surveillance system of EM caused by *A. cantonensis* was established and implemented in Beijing, Yunnan, Fujian, Guangdong and Shanghai. Two parallel surveillances are done in the trial city. Physicians in the pilot hospitals identify the EM due to *A. cantonensis* according the diagnosis criteria issued by Ministry of Health and report cases through an internet-based reporting system to NIPD. On the other hand, the staff in local CDCs routinely collected and examined the snail samples (i.e. *Pomacea* spp., *Achatina fulica*, *Bellamya* spp. and *Cipangopaludina* spp.) from food markets and presented the data to NIPD. The hospitals and CDCs regularly exchange the information pertaining to the patients and surveillance results on snails. In the last 10 years, we identified 265 patients in pilot hospitals and also found an outbreak through this surveillance system. No parasitologically confirmed patient was reported. Infected snails were commonly found in markets in Fuzhou and Xiamen, Fujian province and Dali, Yunnan province. The results showed seasonality. In addition, we conducted training for the staff from pilot hospitals and CDCs every year and promoted the capacity of response to the outbreaks. However, we realized the efficacy of surveillance became weak since the EM due to *A. cantonensis* was rare in China after all. For instance, we found infected snails in markets in some cities, but recently few human cases were reported. The occurrence of EM due to *A. cantonensis* is dependent on the preparedness of snail food to some extent. We are considering to extent the disease to the surveillance of hypereosinophilic syndrome.

5. Population genetics

5.1 *Angiostrongylus cantonensis*

A. cantonensis and *A. costaricensis* are two known species of *Angiostrongylus* that can cause illness in human beings. We characterized their mitochondrial (mt) genomes (Lv et al., 2012). Their mt genomes were 13,497 and 13,585 bp, respectively, and represented the smallest in the class of Chromadorea so far. Each mt genome encodes 12 proteins, 22 transfer RNAs, and 2 ribosomal RNAs. All genes are located on the same strand. Nucleotide difference of the two mt genomes is 18.4%. To our knowledge we first characterized the mt genome of genus *Angiostrongylus*. The mt genomes of other *Angiostrongylus* species or stains were determined shortly (Aghazadeh et al., 2015; Cervena et al., 2019; Gasser et al., 2012; Yong et al., 2015, 2016). The information provided by complete mt genomes is useful to screen the biomarker for population genetic study.

P.R. China is the presumptive home range of the rat lungworm (Prociv et al., 2000). However, the genetic diversity was little known. We conducted a study of the genetic variation of *A. cantonensis* in P.R. China to deepen the current knowledge pertaining to its origin and global spread from a molecular perspective (Lv et al., 2017). The specimens used in this study were collected from 32 collection sites during first national survey 2006–2007 as well as following complementary investigations. We characterized the complete cox1 and nad1 genes of 130 specimens and obtained 357 nuclear DNA sequences containing two complete ITS (ITS1 and ITS2) and 5.8S rRNA of the same 130 samples. Only *A. cantonensis* was identified in our study. There were two major groups of *A. cantonensis* in P.R. China. The first group showed higher diversity and could be further divided into six distinct clades, while lower diversity was observed in the other group. The most common genotypes were Group Ia (47.7%) and Group II (22.3%).

We also compared the different gene markers and sequencing policies. Genotyping showed a striking difference between mitochondrial DNA and ITS. Since intragenomic variation of ITS sequences was found in a large proportion of the samples, we thought ITS was not ideal marker for population genetic study of *A. cantonensis*. In addition, we observed a considerable proportion of singlets in nad1 gene. It was probably caused by the sequencing strategy, i.e., clone sequencing. Therefore, clone sequencing cannot be recommended.

5.2 *Pomacea* spp.

Pomacea spp. is the most important source of *A. cantonensis* infection in China. Many EM outbreaks were attributed to the snail (Odermatt et al., 2010). In addition, the snail is also notorious for crop damage and ecological degradation (Joshi and Sebastian, 2006). We determine the genetic diversity of invasive snails (*Pomacea* spp.) to reveal their distribution pattern in P.R. China (Lv et al., 2013). We collected *Pomacea* snails in the first national survey on *A. cantonensis* (Lv et al., 2009b). A total of 544 snails from 54 sites were used for our study. We successfully obtained 521 cox1 sequences and identified 24 unique haplotypes, of which 6 haplotypes were commonly found in P.R. China. *P. canaliculata*, *P. insularum* and two cryptic groups were observed. The distribution of the 24 haplotypes across P.R. China shows a mosaic pattern, which indicated that multiple and secondary introductions occurred in P.R. China.

We also compared our haplotypes to the information available in GenBank. Globally, only 6 of 112 haplotypes of *P. canaliculata*, *P. insularum*, *P. dolioides*, *P. lineata* and *P. paludosa* are shared between introduced and native (South

America) snail populations. We categorized the 112 haplotypes into 16 clusters, 5 of which occur in mainland P.R. China. Three of the five clusters could be traced back to South America. The remaining two clusters were unique to P.R. China. Therefore, there is still a gap in knowledge of population genetic of *Pomacea* in South America.

5.3 *Achatina fulica*

A. fulica is a large-sized land snail species. The emergence in the mainland of P.R. China was noted in 1931 in a university campus located in south-east coast (Jarrett, 1931). The first national survey of *A. cantonensis* showed that the snail infested in Fujian, Guangdong, Guangxi, Hainan, Yunnan and Guizhou provinces (Lv et al., 2009b). According to Fontanilla (Fontanilla et al., 2014) the genetic variation of *A. fulica* outside East Africa and Indian Ocean islands was low. Indeed, the invasion history of *A. fulica* is well known (Mead, 1961). A bottleneck effect could be an explanation of low genetic diversity in Southeast Asia. We hence did not research the population genetics of *A. fulica* in P.R. China. Instead, we characterized the whole genome of *A. fulica* (Guo et al., 2019). The genome size of *A. fulica* was estimated 2.12 Gb with a high repeat content up to 71%. A total of 101.6 Gb genomic long-read data were generated and finally a genome of 1.85 Gb was assembled. We predicted 23,726 protein-coding genes in the whole genome, of which 96.3% genes were functionally annotated. To our knowledge, this was the first complete characterized genome of terrestrial mollusks to date. The chromosome sequence will provide a valuable genetic resource for research on environmental adaptation of the invasive snail as well as chromosome-level of evolution within mollusks. Since *A. fulica* played an important role of global spread of rat lungworm at early stage, global invasion history of *A. fulica* based on whole genome should be helpful in clarification of global transmission of *A. cantonensis*.

6. Transmission modelling

Climate change is a global concern and is imposing negative biotic impact. Vector- and snail-borne tropical diseases are being influenced by climate change. Rat lungworm is transmitted by poikilothermal mollusks. Only few freshwater snail species play an important role in transmission of *A. cantonensis*, which is partially explained by the low likelihood of contact between snails in the water body and infected rat faeces. *Pomacea* spp. is an exception due to their ecological characteristics. The snail was introduced into China in 1981 and has become the key intermediate host for *A. cantonensis*. Thus far, the snail has been recorded in 13 provinces of southern China (Lv et al., 2009b). We developed a biological model and assessed potential impacts of climate change on the distribution of *P. canaliculata* and hence the transmission of *A. cantonensis* (Lv et al., 2011). The transmission of *A. cantonensis* is dependent on both definitive and intermediate hosts. Therefore, the interaction between parasite and hosts as well as the dependence of hosts on environment were considered in our model. We identified the mean January temperature and snail generation intensity (generation number) as the key factors determining *P. canaliculata* distribution, while the generation intensity in the intermediate host, the longevity of *A. cantonensis*-infected rats and the dormant period of *P. canaliculata* are the major factors for *A. cantonensis* transmission. Our models predicted a remarked increase for the 'spread' regions of *Pomacea* snails and potential endemic area of *A. cantonensis* by 2030s. We also identified the vulnerable areas for *A. cantonensis* transmission, which help us to set the surveillance sites. Our modelling provided a case study pertaining the impact of climate change on parasite transmission (Cizauskas et al., 2017; Okamura and Feist, 2011).

7. Conclusions

Since 2003 we conducted a systematic implementation research on *A. cantonensis* and its hosts, covering field surveys, laboratory studies, modelling and surveillance. All the studies were performed for the establishment of surveillance system and disease control. Additionally, our researches supported five master and one PhD students and provided a postdoc position in last 15 years. A total of 45 peer-review articles were published. More than 1000 staff from local CDCs and hospitals was trained. Our research promoted the elevation of control capacity. The findings from our research were also instructive for other researches by other Chinese and abroad institutes. Such implementation research will provide a case study for control of emerging infectious diseases (Fig. 3).

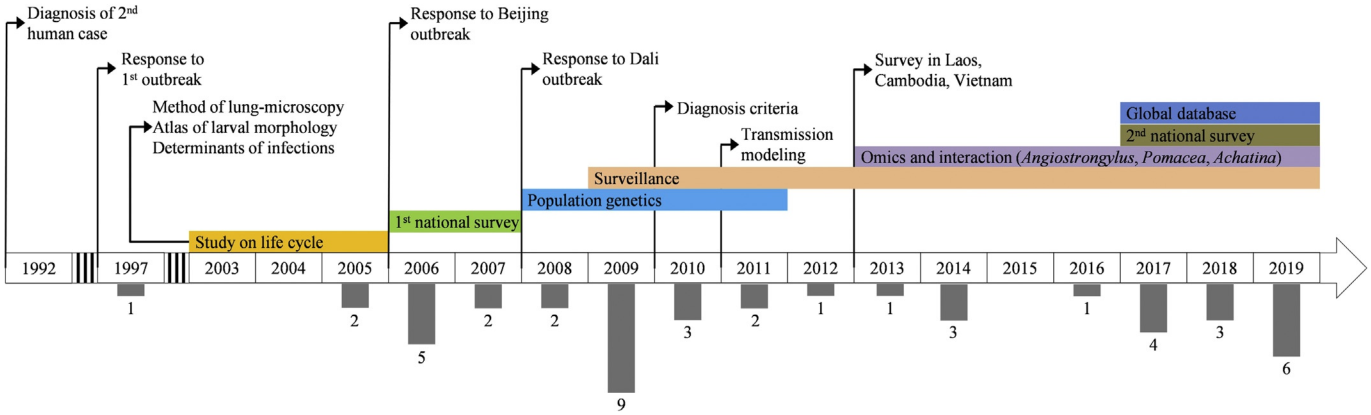

Fig. 3 Main outputs pertaining to *Angiostrongylus cantonensis* since 1992. Grey bar denote the number of publication by NIPD.

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