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Elimination du paludisme en Chine, évolution et défis de la transmission transfrontalière

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List of abbreviations

ACT	Artemisinin Combination Therapy
BRI	Silk Road Economic Belt and the 21st-Century Maritime Silk Road Initiative
CDC	Center for Disease Control and Prevention
Cirad	the French Agricultural Research Centre for International Development
CNM	National Center for Parasitology, Entomology and Malaria Control, Cambodia
DDT	Dichlorodiphenyltrichloroethane
DVS	Dominant Vector Species
E-2020	WHO identified 21 countries with the potential to eliminate malaria by the year 2020. These countries were known as "E-2020 countries"
GIS	Geographic Information System
GMEC	Global Malaria Eradication Campaign
GMEP	Global Malaria elimination plan
GMS	Greater Mekong Sub region
IRD	Institut de recherche pour le développement, France
IRS	Indoor Residual Spraying
KU	Kasetsart University, Thailand
LLIN	Long Lasting Insecticide mosquito Net
LULC	Land Use and Land Cover
MENLMR	Malaria Elimination Network in Lancang-Mekong Region
MMP	Mobile and Migrant Population
NIPD	National Institute of Parasitic Diseases, China CDC
PCR	Polymerase Chain Reaction
TDR	Special Programme for Research and Training in Tropical Diseases
WHA	World Health Assembly
WHO	World Health Organization

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Résumé

Le paludisme est présent en Chine depuis plus de 4000 ans et en 1949 la transmission de la maladie été observée dans 80% des comtés. Après plusieurs décennies d'efforts, le paludisme a considérablement diminué en Chine et est en voie d'élimination dans tout le pays. Parallèlement aux progrès importants réalisés dans l'élimination du paludisme, un certain nombre d'articles scientifiques ont été publiés sur les méthodes de contrôle et d'élimination du paludisme. Cependant, il y a toujours un certain manque d'informations sur les vecteurs du paludisme, ce qui pourrait gêner l'élaboration de stratégies de surveillance efficaces, ainsi que la certification par l'OMS.

En outre, une fois l'absence de cas autochtones établie, le principal risque pour une élimination durable du paludisme est la réintroduction de la maladie à partir de cas de l'étranger (paludisme importé). Avec le développement de la coopération internationale, un nombre croissant de cas de paludisme importés sont associés à des citoyens chinois revenant de pays touchés par la maladie. Des informations sur les caractéristiques de ces cas, sur les facteurs favorisant l'introduction et les voies d'introduction des cas de paludisme importés dans différentes régions de Chine fourniront aux décideurs politiques des données factuelles pour définir où et quand ils doivent effectuer les interventions. Cela permettra ensuite d'élaborer des directives efficaces pour l'évaluation des risques liés à la réintroduction du paludisme et d'affecter les ressources appropriées à la surveillance du paludisme.

En tant que pays comptant plus de 1,3 milliard d'habitants sur un territoire couvrant différentes zones climatiques, la Chine a tiré des leçons de la campagne d'élimination du paludisme qui pourraient servir de référence aux pays en voie d'élimination du paludisme. En outre, du fait des initiatives de la nouvelle route de la Soie, aussi appelée « Une ceinture, une route », proposées par le Président chinois, la Chine se voit impliquée plus que par le passé dans des collaborations bilatérales et multilatérales pour le contrôle / élimination du paludisme. Cette expérience acquise par la Chine sur la gestion du paludisme présente un intérêt certain pour les experts internationaux du paludisme, l'OMS et les parties prenantes dans les pays actuellement touchés par le paludisme.

Ce travail de Doctorat a en conséquence été conduit avec trois objectifs principaux :

1. Définir la répartition des vecteurs du paludisme dans le pays, leurs caractéristiques bionomiques, les mesures de contrôle et études associées, et fournir des données concrètes et factuelles pour l'évaluation des risques du paludisme avant et après son élimination.

2. Décrire les différences entre les caractéristiques spécifiques et communes du paludisme importé dans des environnements différents en Chine par le biais d'une analyse comparative et identifier les lacunes dans la surveillance et la réponse au paludisme importé dans chaque zone.

3. Présenter les progrès accomplis en matière d'élimination du paludisme en cartographiant les foyers de paludisme résiduels et la répartition des cas de paludisme en Chine, et partager les enseignements tirés de l'élimination du paludisme en Chine, au bénéfice des pays sur la voie de l'élimination de cette maladie.

Ce mémoire de thèse comprend cinq parties, l'état des connaissances sur le sujet présenté dans le premier chapitre, le contenu principal de l'étude dans les chapitres 2 à 4 et enfin une conclusion générale et les perspectives d'étude dans le chapitre 5.

La première partie de l'étude, montre que l'aire de répartition des principaux vecteurs du paludisme en Chine s'est réduite, notamment pour *Anopheles lesteri* (synonyme: *An. anthropophagus*) et *Anopheles dirus* s.l., ainsi que pour les deux principales espèces de vecteurs du paludisme, *An. dirus* et *An. baimaii,* qui ont presque disparu après plusieurs années de lutte antipaludique. *Anopheles sinensis,* qui était considéré comme moins efficace dans la transmission du paludisme, est en train de devenir l'espèce prédominante dans le sud-ouest de la Chine. En outre, les données d'échantillonnage sur le terrain indiquent la présence de vecteurs du paludisme hautement efficaces, comme *Anopheles minimus* et *An. harrisoni* à la frontière Sino-Birmane. De plus, les taux élevés de piqûres sur la population humaine, les fortes densités d'adultes et de larves et les taux de parité des femelles observés chez *An. sinensis* et *An. harrisoni*, révèlent une très grande réceptivité et un risque élevé de réintroduction du paludisme le long de la frontière Sino-Birmane. Par conséquent, pour parvenir à l'élimination du paludisme d'ici 2020, il est nécessaire d'évaluer et de suivre

les changements de comportement des espèces d'anophèles en Chine. Ces changements peuvent être dus à des changements environnementaux comme à la lutte antivectorielle. Il est donc important de réactualiser les outils de surveillance entomologique. Par exemple, le rôle des vecteurs secondaires du paludisme, comme *An. sinensis* dans le Yunnan, doit être surveillé de près. L'indice de réceptivité, qui représente la capacité d'une région donnée à présenter des conditions favorables pour la transmission du paludisme, devrait être adopté comme indicateur de surveillance pour évaluer le risque de réintroduction de cette maladie. Les études biologiques sur les moustiques anophèles, en particulier le comportement trophique, lors du repas sanguin et au repos, nécessitent des recherches plus approfondies. C'est particulièrement important dans les zones frontalières, où un pays a réussi à éliminer le paludisme, tandis que l'autre présente encore de la transmission du paludisme au niveau local. Les approches moléculaires de type PCR devraient être systématiquement mises en œuvre pour identifier les espèces d'*Anopheles* afin de mieux cerner leur rôle respectif dans la transmission du paludisme. Ceci est particulièrement pertinent pour les espèces jumelles telles que celles du complexe Minimus, mais également celles du complexe Dirus bien que ce dernier soit restreint à des zones limitées en Chine.

Dans la deuxième partie de l'étude, l'analyse comparative des cas importés de paludisme signalés dans des zones anciennement non-endémiques et dans d'anciennes zones endémiques en Chine a montré que toutes les zones anciennement non-endémiques signalaient désormais des cas importés de paludisme. Cependant, la plus grande proportion des cas importés provient des anciennes zones d'endémie. Les

caractéristiques démographiques du paludisme importé dépendent du pays d'expatriation des patients, des espèces de parasites, de la profession et du lieu d'origine des travailleurs. Les établissements de santé situés dans les anciennes zones d'endémie étaient plus performants que ceux des zones précédemment non-endémiques, sans doute grâce aux meilleures pratiques et à l'expérience du personnel médical des anciennes régions d'endémie. De l'information, des médicaments ainsi qu'un suivi doivent être fournis aux expatriés, tout en mettant en œuvre le renforcement des capacités et la formation continue à tous les niveaux du système de santé en Chine. La frontière entre la Chine et le Myanmar est un site très sensible pour l'introduction du paludisme qui subit des mouvements massifs de populations, légaux et illégaux. Cette région a donc été choisie comme site prioritaire d'étude dans ce chapitre. Un modèle de simulation stochastique a été introduit pour évaluer la corrélation entre la mobilité des populations et la vulnérabilité des communautés à la réintroduction du paludisme le long de cette frontière. De forts mouvements de population ont été observés avec différents risques d'exposition au paludisme dans les villages situés le long de la frontière Chine-Myanmar, lesquels étaient à leur tour associés à divers niveaux de vulnérabilité. Ceci souligne l'association du risque de paludisme avec la mobilité importante des populations à la frontière Sino-Birmane. Cette région devrait faire l'objet d'une attention toute particulière lors de la conception et la mise en œuvre de la stratégie nationale de surveillance du paludisme.

La troisième partie de l'étude porte sur l'analyse des progrès réalisés dans l'élimination du paludisme depuis 2010, année du lancement du programme

d’élimination du paludisme par la Chine. L’accent a été mis sur la cartographie des foyers de paludisme résiduels et la répartition des cas de paludisme. L'incidence du paludisme contracté localement a fortement diminué, parallèlement à la diminution concomitante des zones d'endémie palustre de 762 comtés signalant le paludisme en 2010 à seulement deux comtés adjacents aux zones frontalières (Province du Yunnan, Chine-Myanmar et Tibet, Chine-Inde) en 2017. Cette même année, la Chine n’a déclaré aucun cas de paludisme autochtone et ce pour la première fois. Le programme national d'élimination du paludisme a été mis en œuvre avec succès et la zone de paludisme endémique a considérablement diminué. La localisation des foyers résiduels et l’origine des cas de paludisme indiquent que les principaux défis pour l’élimination du paludisme en Chine sont la gestion des cas importés et la prévention de la réintroduction dans les zones frontalières, comme celle entre la Chine et le Myanmar. La collaboration internationale bilatérale ou multilatérale est en conséquence fortement préconisée, ce qui bénéficierait également à la réalisation des objectifs régionaux et mondiaux d’élimination du paludisme.

Le paludisme figure sur la liste du projet «Santé Chine 2030» publié par le Conseil des Affaires d'Etat de Chine en 2016. Cela garantit l'investissement durable requis. Suite à ce plan, une série de directives techniques pour la surveillance du paludisme et les interventions au stade post-élimination, ainsi que des protocoles pour prévenir le rétablissement de la transmission du paludisme, sont en cours d’élaboration pour une publication à court terme. Parallèlement, des mécanismes permettant de maintenir la capacité antipaludique du système de santé sont mis en place. Un concours technique

national de diagnostic et de test des maladies parasitaires est organisé chaque année pour les agents de santé des agences cliniques et des CDC régionaux Chinois. Ce concours est un moyen efficace de maintenir la sensibilisation du personnel de santé et l'efficacité du système de santé en matière de détection et de prévenir l'érosion des capacités qui mettrait en péril l'élimination du paludisme. Cependant, ce processus d'élimination est avant tout une entreprise internationale. Un large engagement et des investissements soutenus sont nécessaires avec le soutien de multiples partenaires internationaux. En 2013, le Président Chinois Xi Jinping a proposé l'initiative nationale connue sous le nom de nouvelle route de la soie ou 'One Belt-One Road » avec des propositions pour la coopération et le développement international. La coopération dans le domaine de la santé est l'un des éléments clés. La Chine a déjà été activement impliquée dans la gouvernance mondiale de la santé, mais un niveau de participation plus important est maintenant attendu, et l'expérience chinoise en matière d'élimination du paludisme sera certainement mise à profit dans le cadre de la nouvelle route de la soie. Plusieurs plateformes sont en cours de développement, telles que le Réseau d'élimination du paludisme dans la région du Lancang-Mékong (MENLMR) et le Programme de coopération Chine-Afrique. La sous-région du Grand Mékong (GMS) et l'Afrique sub-saharienne sont fortement touchées par le paludisme à cause de la résistance aux antipaludiques, du fort impact sur la population et du faible niveau de ressources. Ce sont donc des régions prioritaires pour des interventions ciblées permettant l'élimination du paludisme. En outre, ces pays sont confrontés à d'innombrables difficultés pour mener à bien leur programme d'élimination et ont

cruellement besoin de l'aide internationale pour combler le déficit de financement. Bien que la Chine ait appliqué un modèle efficace et réalisé des progrès significatifs en matière d'élimination du paludisme, le modèle et l'expérience chinois ne peuvent être directement appliqués dans ces pays. Une évaluation et des tests sur le terrain sont nécessaires pour estimer la faisabilité opérationnelle. Des zones pilotes ont été identifiées pour mettre en œuvre les projets de démonstration visant à évaluer le niveau de faisabilité soutenu conjointement par la Chine et les pays ciblés. Ces projets préliminaires fourniront des éléments factuels pour élaborer une stratégie et un modèle appropriés pour chaque pays afin d'atteindre l'objectif ultime d'élimination du paludisme.

En conclusion, la Chine a fait des progrès considérables dans le processus d'élimination du paludisme et est en bonne voie pour atteindre cet objectif en 2020. Le risque de réintroduction lié au grand nombre de cas importés et la présence dans le pays de vecteurs du paludisme hautement efficaces plaident pour un renforcement de la surveillance.

General Introduction

China launched the National Malaria Elimination Program in 2010. According to the WHO guideline of malaria elimination, a country or a region who applies for the malaria elimination certification should pay more attention to modifications of national malaria surveillance strategy. The strategy at the malaria pre-elimination and elimination stage should focus more on sensitive case-based surveillance system and should aim to the interruption of local transmission (1-3). Thus, detailed information about malaria vectors nationwide and the performance of malaria surveillance and response systems are priorities to be addressed through research (4-8). In addition, an evidence based national strategy is required, especially with respect to the assessment of the risk of malaria re-introduction (1, 9, 10).

After several decades of effort and the intensified approaches since the launch of malaria elimination program, malaria prevalence decreased drastically in China and is currently approaching elimination throughout the country. Together with the significant progress of malaria elimination, a number of scientific articles have been published on malaria control and elimination approaches (11-15). However, well-documented information on malaria vectors is still missing, could hinder the development of appropriate surveillance strategies and WHO certification.

In addition, after achieved zero indigenous case report, the major risk that could prevent sustained malaria elimination is the re-introduction of the disease from

imported malaria cases. Along with the development of international cooperation, there are an increasing number of imported malaria cases from Chinese citizens returning from malaria-affected countries (16-21). The information about the characteristics, drivers and route of introduction of imported malaria cases in different areas of China will provide evidence-based data to policy makers where and when they have to carry out the interventions. This will in turn allow them to develop efficient guidelines for risk-assessment of malaria re-introduction and for allocating appropriate resources to malaria surveillance.

As a country with over 1.3 billion population with a territory covering different climatic zones, China has learnt lessons during the malaria elimination campaign that could serve as references to the countries on the track to malaria elimination. Moreover, according to the Belt and Road initiatives, proposed by the Chinese President, China will participate more globally to governance related activities, including global health governance and bilateral, multi-lateral collaborations on malaria control/ elimination (22, 23). International malaria researchers, WHO and stakeholders in current malaria affected countries are interested to the future opportunity and potential fields that would involve Chinese expertise and contributions.

This PhD work was therefore designed to address the following three main objectives:

- **Objective One**, To summarize the nationwide distribution of malaria vectors, their bionomic characteristics, control measures and related studies, to

provide evidence-based data for malaria risk assessment at pre- and post-malaria elimination. It is also specifically focusing on the China-Myanmar border area, which is currently the most exposed region to malaria and which thus displays a high potential risk of malaria re-introduction.

- **Objective Two**. To describe the differences between the specific and common characteristics of imported malaria in areas with different environmental types in China through a comparative analysis, and to identify the gaps in the surveillance & response to imported malaria concerning each area. The China-Myanmar border is a hotspot for malaria introduction and is affected by massive movements of populations, both legal and illegal. It was therefore selected as a priority study site in this chapter. A stochastic simulation model was used to evaluate the correlation between population mobility and community vulnerability to malaria re-introduction at the China-Myanmar border.

- **Objective Three**. To present the malaria elimination progress through mapping the residual malaria foci and the distribution of malaria cases in China, and to share the lessons learned from malaria elimination in China benefiting countries on the way to malaria elimination.

The publications included in each chapter follow the objectives are listed below:

Chapter Two: present the study with objective one

Article 1. **Zhang SS**, Guo SH, Feng XY, Afelt A, Frutos R, Zhou SS, Manguin S.

Anopheles Vectors in mainland China while approaching malaria elimination[J]. Trends Parasitol, 2017, 33(11) : 889-900

Article 2. Feng XY, **Zhang SS**, Huang F, Zhang L, Feng J, et al. Biology, Bionomics and Molecular Biology of *Anopheles sinensis* Wiedemann 1828 (Diptera: *Culicidae*), Main Malaria Vector in China[J]. Front Microbiol, 2017, 8:1473.

Article 3. Chen TM, **Zhang SS**, Zhou SS, Wang XZ, Luo CH, et al. Receptivity to malaria in the China–Myanmar border in Yingjiang County, Yunnan Province, China [J]. Malar J, 2017, 16 (1): 478

Article 4. **Zhang SS**, Zhou SS, Zhou ZB, Chen TM, Wang XZ, et al. Monitoring of malaria vectors at China-Myanmar border while approaching malaria elimination [J]. Parasit Vectors, 2018, 11(1): 511

Chapter Three: present the study with objective two

Article 5. Chen TM, **Zhang SS**, Feng J, Xia ZG, Luo CH, et al. Mobile population dynamics and malaria vulnerability: a modelling study in the China-Myanmar border region of Yunnan Province, China [J]. Infect Dis Poverty, 2018, 7 (1): 36

Article 6. **Zhang SS**, Feng J, Zhang L, Ren X, Geooffroy E, et al. Imported malaria cases in former endemic and non-malaria endemic areas in China: are there differences in case profile and time to response?[J]. Infect Dis Poverty, 2019, 8 (1): 61

Chapter Four: present the study with objective three

Article 7. Hu T, Liu YB, **Zhang SS**, Xia ZG, Zhou SS, et al. Shrinking the malaria map in China: measuring the progress of the National Malaria Elimination Programme[J]. Infect Dis Poverty, 2016, 5 (1): 52

Article 8. Feng J, Tu H, Zhang L, **Zhang SS**, Jiang S, et al. Mapping transmission foci to eliminate malaria in the People's Republic of China, 2010–2015: a retrospective analysis [J]. BMC Infect Dis, 2018, 18(1):115

Article 9. **Zhang SS**, Zhang L, Feng J, Yin JH, Feng XY, et al. Malaria Elimination in the People's Republic of China: Current Progress, Challenges, and Prospects. In: Manguin S, Dev V, editors. Towards malaria elimination-a leap forward. London, United Kingdom: IntechOpen; 2018. p. 233–55.

Chapter 1. Background

General knowledge on malaria

Plasmodium

Plasmodium is a protozoan parasite under the Phylum Apicomplexa with an evolution from the Coccidian stem. *Plasmodium* belongs to the Sub-Order Haemosporidae that includes all apicomplexans that live within blood cells. Based on the presence of the pigment hemozoin and the method of asexual reproduction, the order is further split into four families. *Plasmodium* belongs to the family *Plasmodiidae* (24, 25). The genus *Plasmodium* consists of over 250 species, generally described based on their appearance in blood smears of infected vertebrates. These species have been categorized on the basis of their morphology and host range into 14 subgenera (24). Species of *Plasmodium* infecting monkeys and apes (the higher primates) with the exceptions of *P. falciparum* and *P. reichenowi* (which together make up the subgenus *Laverania*) are classified in the subgenus *Plasmodium*. Historically, four species of *Plasmodium* were recognized as causative agents of human malaria such as *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, but *Plasmodium knowlesi* was recently reported as the fifth human *Plasmodium* species, an important malaria agent in Southeast Asia (26-29) (Table 1). Occasionally, human beings can be infected either naturally or accidentally by several simian species such as *P. cynomolgi*, *P. cynomolgi bastianelli*, *P. brasilianum*, *P. schwetzi* and *P. inui* (30).

Table 1. The classification of human malaria pathogen of genus *Plasmodium* (31)

Domain	Eukaryota
Kingdom	Chromalveolata
Superphylum	Alveolata
Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporida
Sub-Order	Haemosporidiidea
Family	*Plasmodiidae*
Genus	*Plasmodia*
Sub-genus	*Plasmodium; Laverania*
Species	*P. falciparum* *P. malariae* *P. ovale* *P. vivax* *P. knowlesi**

**P. knowlesi* is a primary simian malaria parasite that can infect human beings

Species of *Plasmodium* are distributed globally wherever suitable hosts and vectors are found. Vectors, or definitive hosts, are most frequently mosquitoes of the genus *Anopheles*, but for avian malaria vectors belong to the sub-family *Culicinae* and for reptiles to the sub-family *Culicinae* or the genus *Phlebotomus* (25). Vertebrate hosts include reptiles, birds, and mammals. Charles Laveran first identified *Plasmodium* parasites in the late 19th century. Over the course of the 20th century, many other species were discovered in various hosts and classified (25, 32, 33).

1. Life cycle of *Plasmodium*

The life cycle of *Plasmodium* requires a female *Anopheles* mosquito as the definitive host and a human as the secondary host (**Figure 1**). *Plasmodium* parasites are transmitted through mosquito bites to the human host under the form called sporozoite. The sporozoite then travels through the blood vessels to the liver where it enters into hepatocytes for its asexual reproduction generating thousands of merozoites, also called **human liver stage** produced during the **exo-erythrocytic cycle**. These merozoites infect red blood cells and initiate a series of asexual multiplication cycles (**human blood stage** / **erythrocytic cycle**) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew. Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilized *Anopheles* female mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. Male and female gametocytes fuse and form an ookinete—a fertilized, motile zygote. The Ookinete crosses the midgut wall to form an oocyst that produces a large number of sporozoites that migrate to the mosquito salivary glands, ready to infect a new mammalian host (**Sporogonic Cycle**). The sporozoites in the saliva are injected through the skin when the mosquito takes a blood meal (34). Beside the mosquito bites, the plasmodium can also be transmitted by blood transfusion, this occasionally happened in both malaria endemic and non-endemic areas (35-38).

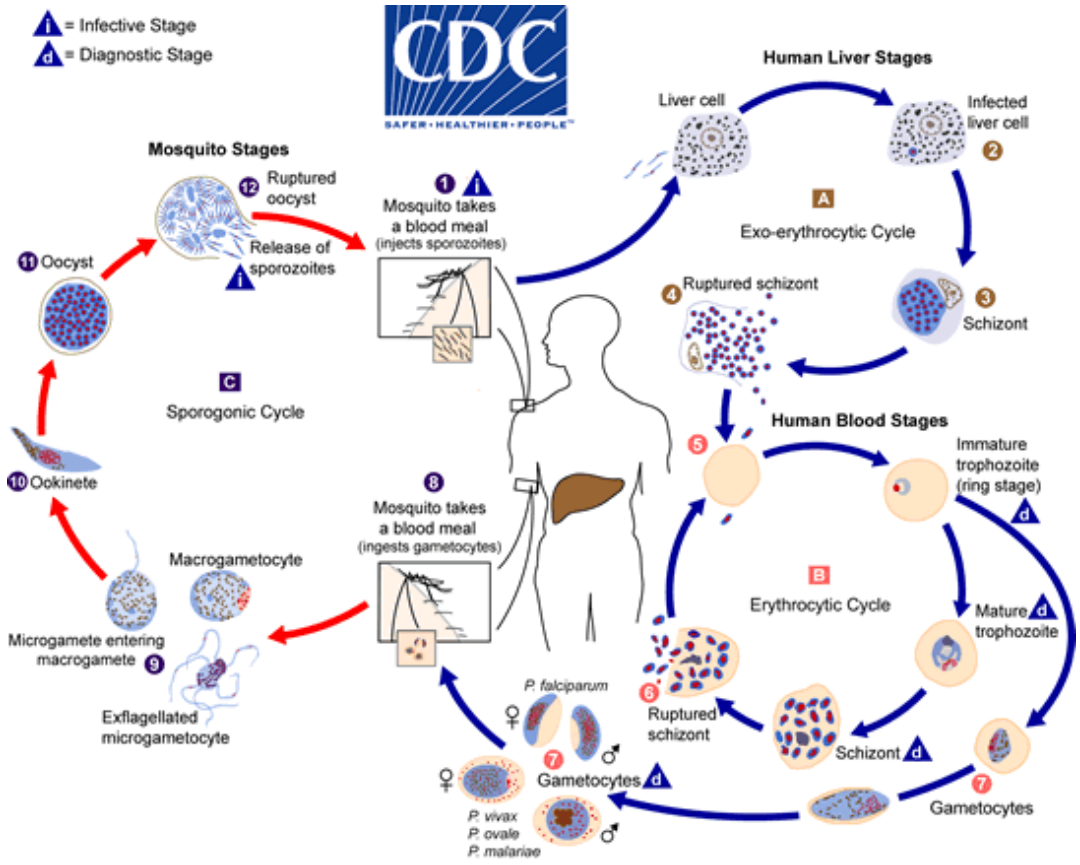

Figure 1. Life cycle of human *Plasmodium*

(https://www.cdc.gov/malaria/about/biology/index.html)

2. Phylogeography of *Plasmodium*

Recent studies of *Plasmodium* species using molecular methods have implied that the group phylogeny does not correspond perfectly to the established taxonomy (32, 39). Many *Plasmodium* species which are morphologically similar or infect the same hosts turned out to be only distantly related (32). In the 1990s, several studies sought to evaluate evolutionary relationships of *Plasmodium* species by comparing ribosomal RNA and a surface protein gene from various species. *P. falciparum* was found more closely related to avian parasites than to other parasites of primates (24, 25). However, later studies sampling more *Plasmodium* species found the parasites of mammals to

form a clade along with the genus Hepatocystis, while the parasites of birds or lizards appear to form a separate clade with relationships not following the subgenera (24, 39).

Estimates on when different *Plasmodium* lineages diverged have differed broadly. Estimates for the diversification of the sub-order Haemosporidae range from around 16.2 million to 100 million years ago (24). The inventory of the various species is still pending to be completed. All the parasites of primates belong to the genus *Plasmodium,* which evolved from parasites of reptiles. Garnham (40) has estimated the age of different members of the genera *Plasmodium* and *Laverania* based on the genealogy of primates (**Figure 2**).

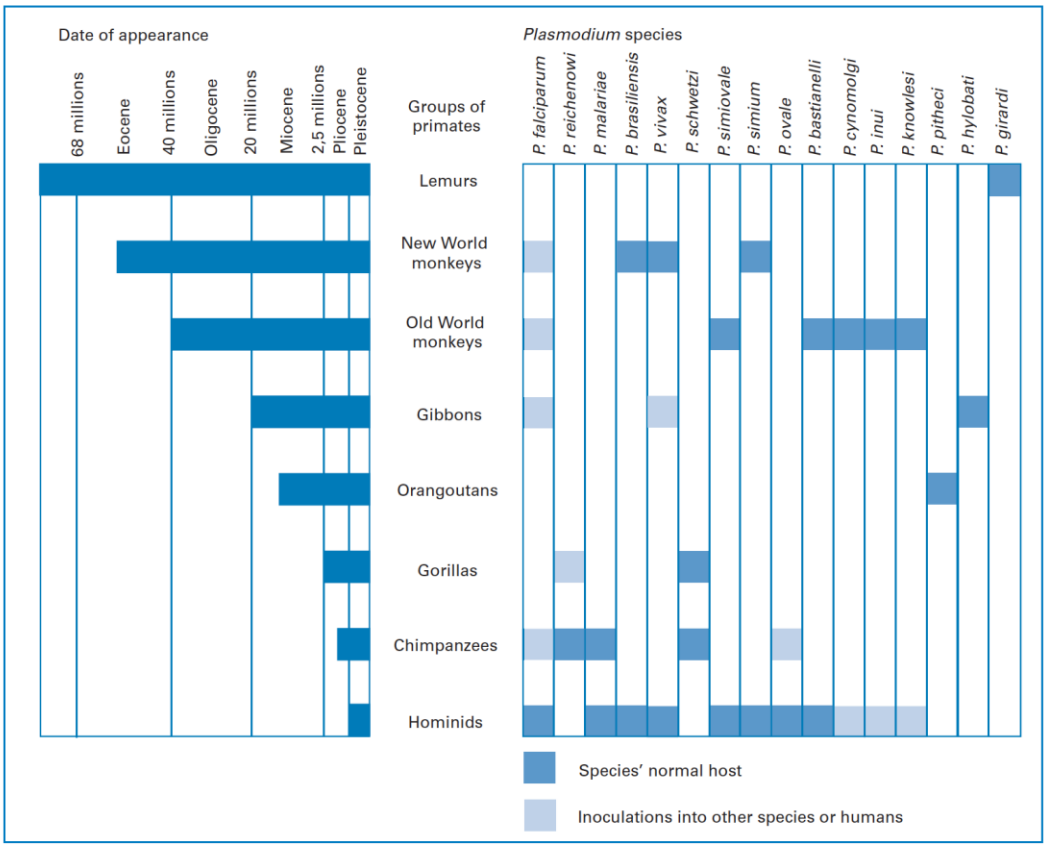

Figure 2 *Plasmodium* species associated with various groups of primates (25)

There has been a particular interest in dating the divergence of the human parasite *P. falciparum* from other *Plasmodium* lineages due to its medical importance. For decades, *P. falciparum* was known as closely related to *P. reichenowi* and most phylogenies recovered topologies of these two taxa distantly related to the other mammalian *Plasmodium* lineages (24, 39, 41-44). Recently, numerous studies have revealed that great apes (gorillas, chimpanzees, and bonobos) in western Africa are harboring communities of more diverse parasites than had previously been suspected and that many of these wild hosts possess lineages of *Plasmodium* that may be very closely related. (45-49). These works provided the information about the abundant switches between humans and wild hosts in the areas where they come into contact. However, attention must be paid when making conclusions based on samples isolated without blood films to confirm that transmission is occurring (24, 50). Nevertheless, data from these studies suggest that *P. falciparum* may be part of a more diverse group, even if it was considered as a well investigated parasite (24).

For *Plasmodium vivax*, another predominant human parasite, its origin has also been debated in the literatures with three potential hypotheses. ①Several species of New World monkeys are infected with a very similar parasite, known as *Plasmodium simium*. One hypothesis is that the parasite originated from South America and was then acquired by humans in post-Columbian times (51). ②Another hypothesis is that *P. vivax* arose in Africa, specifically in western and central human populations, where virtually all individuals lack the Duffy antigen on their red blood cell surfaces. Duffy

antigen is the only receptor known to be used by parasite to invade the red blood cell. Thus, it has been proposed that *P. vivax* co-evolved with African populations for longer than with other human populations (52-55). ③The preponderance of molecular phylogenetic data indicate that *P. vivax* is most closely related to three macaque parasites, *P. cynomolgi*, *P. knowlesi* and *P. inui*, pointing to an Asian origin for this parasite species (53, 56, 57). A broad study looking at isolates of *P. vivax* worldwide also showed strong support for an Asian origin, with migrations likely into Africa rather than the reverse (58).

3. Anti-malaria treatment and drug resistance

Antimalarial drugs have thousands years of history. The chronicle of the main antimalarial drugs discovery is summarized in Figure 3. The discovery of each main antimalarial drugs is always considered as a milestone in the human anti-malaria campaign, such as Quinine (59) and Chloroquine (60).

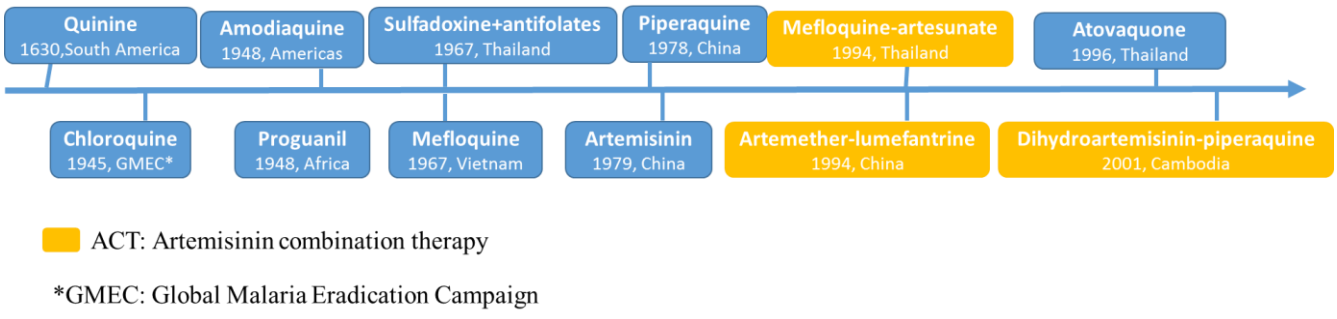

Figure 3. Chronicle of Antimalarial drug discovery (61)

All the drugs used for human malaria treatment are targeting the asexual trophozoite or/and schizont stages. Antifolates, primaquine (8‑aminoquinoline) and

atovaquone (naphthoquinone) also target liver-stage parasites (Figure 4). Endoperoxides (artemisinins) target all asexual and early sexual blood-stage parasites. Thus, Artemisinins is reported to block the ring stage and sexual stage that link to the infection of mosquito when it takes a blood meal (62). Primaquine is the only drug that targets latency in the liver to prevent the relapsing infection characteristic of *Plasmodium vivax* (63) (**Table 2**).

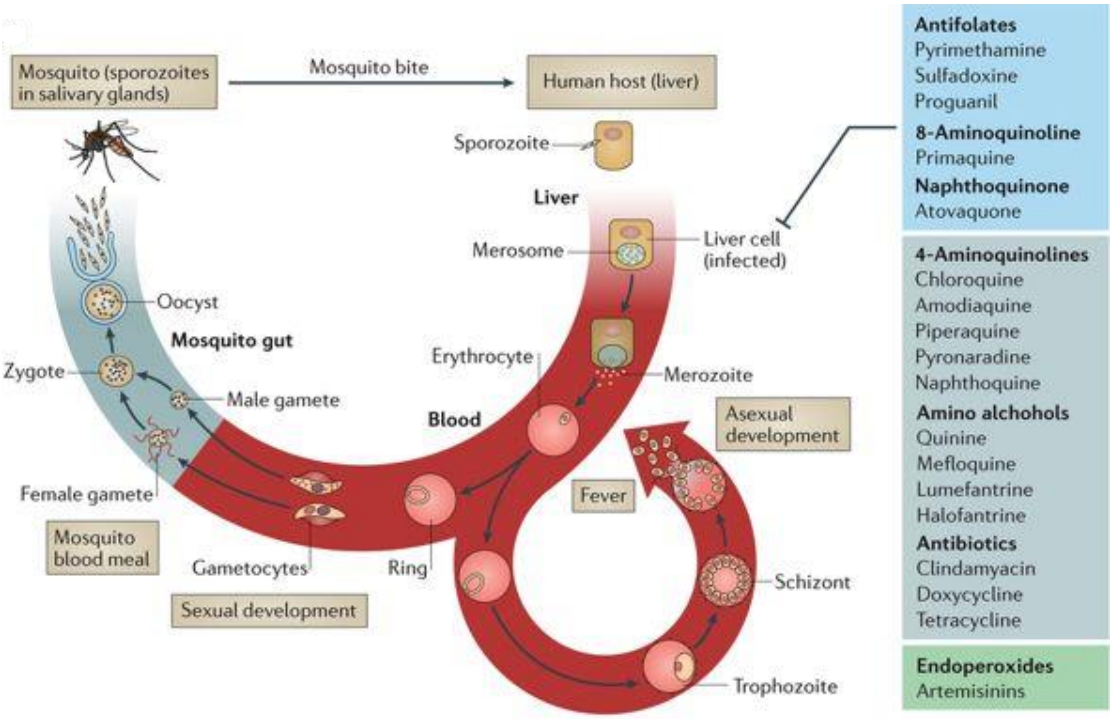

Figure 4. Life cycle of *Plasmodium* and anti-malarial target stages (63, 64)

Table 2. Antimalarial drugs and targeted parasitic stages (63)

Chemical class	Common name	Targeted parasite stages
Sesquiterpene lactone endoperoxides	Artemisinin Artesunate Artemether Dihydroartemisinin	All parasite stages
4‑Aminoquinolines	Chloroquine Amodiaquine Piperaquine Naphthoquine	Blood stages (trophozoite and schizont)
	Pyronaridine	Blood stages (ring, trophozoite and schizont)
Amino alcohols	Quinine	Blood stages (trophozoite and stages I to III gametocytes)
	Mefloquine Lumefantrine Halofantrine	Blood stages (trophozoite and schizont)
8‑Aminoquinoline	Primaquine	Blood (gametocyte) and liver (schizont) forms
Antifolates	Pyrimethamine	Blood and liver schizont and mosquito stage (oocysts)
	Sulfadoxine	Blood and liver schizont
	Proguanil	Blood stages (schizont and gametocyte) and liver schizont
Naphthoquinone	Atovaquone	Blood stages (schizont and gametocyte) and liver schizont
Antibiotics	Clindamycin Doxycycline Tetracycline	Blood stages

The emergence of the main antimalarial drug resistance has always been a major challenge for global malaria control programs. Thus, the surveillance on antimalarial drug efficacy and studies on drug resistance remain critical issues for malaria control and elimination, especially drug resistance of *P. falciparum* (65-68). A detailed map

showing the distribution of *P. falciparum* resistance to chloroquine, sulfadoxine–pyrimethamine and artemisinin in Southeast Asia and Africa was compiled by Haldar Kasturi, et al. based on published literature (49, 63, 69) (**Figure 5**). Artemisinin resistance is based on two criteria: parasite clearance and *PfKelch13* - associated mutation. Although the A578S mutation has been reported as also spreading outside the Mekong region, into Bangladesh and India, no *Pfkelch13* mutations associated with resistance have been confirmed yet (resistance is indicated at the Bangladesh–Myanmar border) (69-71). Moreover, an artemisinin resistant strain of *P. falciparum* has been isolated from a Chinese migrant worker coming back from Africa in 2017 (72). Resistance to chloroquine and sulfadoxine–pyrimethamine (but not artemisinin) has emerged in the Amazon Basin, South America (69, 73). Resistance of *P. vivax* to chloroquine is emerging in Latin America (74) (not shown in the figure). In Figure 5, each dot represents a region of emergence of drug resistance.

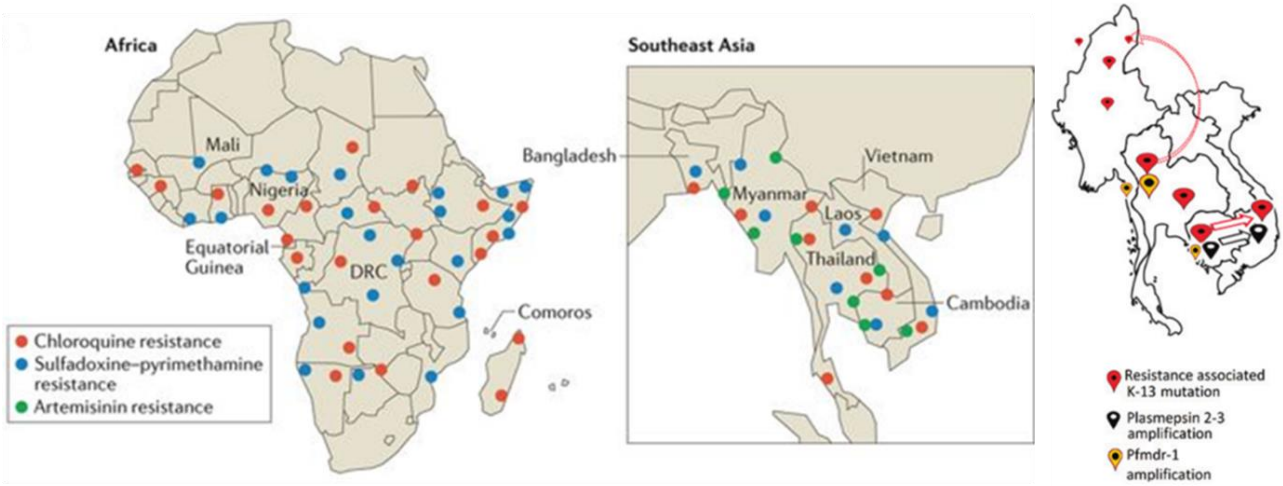

Figure 5 Distribution of anti-malaria drug resistance in Africa and Southeast Asia, updated in 2018 (63, 69, 75)

The emergence of antimalarial drug resistance requires the spontaneous arising of mutations or gene duplications conferring reduced drug susceptibility, which is then selected in the individual by the presence of antimalarial drug concentrations sufficient to kill or inhibit the growth of sensitive parasites, but allowing expansion of the resistant clone (67, 76). For the resistant parasite to be successful, the gene alterations conferring resistance should not deeply affect the parasite fitness (77, 78). Drug-resistant mutations can arise in the sexual parasite stages in the mosquito (where diploid and meiosis occur), in the pre-erythrocytic liver stages or in the asexual erythrocytic parasite stages, and there has been much debate on the most likely source (79). It seems that resistant parasites are most likely to emerge during high levels of asexual-stage parasitemia in patients with sub-therapeutic drug levels and, less likely, in the liver stages (79, 80). Antimalarial drugs will be more prone to resistance when use in monotherapy, when requiring a limited number of genetic events conferring a considerable level of resistance (e.g. atovaquone or mefloquine), and when their pharmacokinetic properties include a long terminal half-life translating into a long period of sub-therapeutic drug levels (e.g. piperaquine). Once resistance starts emerging, its transmission and spread are facilitated by the increased production of gametocytes in partial resistant strains, e.g. sulfadoxine–pyrimethamine (81).

Although the total number of circulating *P. falciparum* parasites and the number of spontaneous genetic events are much higher in high transmission settings in sub-Saharan Africa, history shows that antimalarial drug resistance is much more likely to emerge successfully in low transmission settings (67). In particular, Southeast Asia has

in the last decades been the hotspot for the emergence of *P. falciparum* resistance to chloroquine (82, 83), atovaquone (84), sulfadoxine–pyrimethamine (85, 86), mefloquine (87, 88), and more recently to artemisinins (89, 90) and piperaquine (69, 91, 92).

An important reason for this apparent brake on resistance emergence in regions with high stable transmission is host immunity, which can contribute substantially to parasite clearance of partial resistant parasite and makes that older children and adults can carry substantial numbers of parasites without causing illness, also refer to as asymptomatic cases (93, 94). Because these individuals will not seek treatment, the associated large asymptomatic reservoir dilutes the selective pressure provided by antimalarial drugs at the population level (78). In addition, in high transmission areas, patients have multiple-strain infections transmitted to the mosquito vector. Crossing over of genes during meiosis in the mosquito can then break up resistance and compensatory mutations, and this greater opportunity for recombination will result in increased parasite diversity and direct competition between different parasite strains, with less opportunity for resistant alleles to become fixed (95, 96). This is not the case in low transmission areas where multiple infections are much less common, infected individuals are less pre-immune, usually more prone to be symptomatic, and, as a consequence, to be treated with possible poor quality antimalarial drugs, incomplete treatment courses, or (artemisinin) monotherapies. Parasite population of *P. falciparum* in Southeast Asia are highly structured with high rates of parasite inbreeding; particular genetic background alleles seem to predispose to the development of resistance-causing

mutations through multistage processes in natural parasite populations (97, 98). Moreover, hemoglobinopathies (mainly HbAE or HbEE) and glucose-6-phosphate dehydrogenase deficiency (G6PDH), which are highly prevalent in Southeast Asian human populations, may have selected parasites less susceptible to oxidative stress, while most anti-malarial drugs currently in clinical use exert their activities, at least in part, by increasing oxidative stress in the parasitized erythrocytes (99).

Poor drug stewardship has been an important driver of antimalarial drug resistance, and in particular the emergence of Artemisinin resistance in Southeast Asia. In the early 1960s, pyrimethamine and later chloroquine were added to salt for consumption as a measure of malaria prophylaxis in Cambodia (100). Although artemisinin have been deployed as combination therapies in ACTs, unregulated Artemisinin or artesunate monotherapy has been available since the mid-1970s in the region. In most countries, including Cambodia where Artemisinin resistance was first recognized, the majority of patients obtain their antimalarial treatment through the private sector, which provided until recent years mainly artesunate monotherapy (101). A ban on Artemisinin monotherapies and deployment of fixed dose combinations for the majority of ACTs has been an important step forward. Counterfeited with no active ingredient or substandard drugs that contain less active ingredients than stated are additional sources of sub-therapeutic dosing of artemisinins, may also have contributed to the selection of resistant parasite strains (102). Moreover, it is possible that the different pharmacokinetic properties of artemisinins in subgroups of the population, such as pregnant women and children, have resulted in under dosing (103). It is thought that an

important driver of the rapid spread of resistance to sulfadoxine–pyrimethamine in Africa has been underdosing of the drug in children with *falciparum* malaria (104).

Artemisinin resistance in *P. falciparum* is considered as the key challenge to malaria elimination in the Great Mekong Sub region and represents an enormous threat to malaria treatment in Africa considering the increase in international cooperation (2, 105-108). Further study addressing the mechanism of resistance emergence and the dynamic of spread are in much need, as well as updated monitoring tools (69, 109, 110).

Anopheles malaria vectors

All vectors of mammalian *Plasmodium* belong to the *Culicidae* family, more specifically to the sub-family of *Anophelinae*. Three genera are recognized in the *Anophelinae* family, but only the genus *Anopheles* comprises malaria vectors, the other two genera, *Bironella* and *Chagasia* are not vectors of malaria agents (25, 111). Around 525 species are found under the *Anopheles* genus (25, 111-115). We will mostly focus on the *Anopheles* species that contribute to human malaria transmission in the following sections.

1. Life cycle of *Anopheles* mosquitoes

Anopheles mosquitoes are dipterous insects. They are holometabolous, i.e. they undergo complete metamorphosis with four different stages in their life cycle. Eggs, larvae and pupae are referred to as pre-adult stages, which are exclusively aquatic. The

adult or imago stage can fly and feed on host (**Figure 5**). The female lays 40-100 individual eggs per batch on the water surface. Larvae will hatch from eggs after 24 to 48 hours, depending on temperature. In contrast from *Aedes* and *Culex* larvae, *Anopheles* larvae has no siphon on the abdomen for breathing, giving the larva a characteristic position parallel to the water surface. Larvae feed on floating debris at the surface and grow in a discontinuous fashion from the 1st larval instar (L1) to the 2nd (L2), 3rd (L3) and 4th (L4), thus molting four times. The fourth molt gives rise to the pupal stage that in most cases lasts less than 48 hours. The pupa does not feed but undergoes massive morphological remodeling leading to metamorphosis and the emergence of the adult, which subsequently flies off. Adult males live for one to two weeks, while females live from three to four weeks in the tropics. In temperate areas, females may live longer as a result of the phenomenon of winter diapause (25). As soon as it emerges, the adult rests above the water for 10-24 hours while its cuticle and mouthparts harden and its wings are deployed. During this time, the reproductive parts of the male rotate through an angle of 180° to become functional. Depending on the species, mating occurs either in flight in the course of swarming, or on a support. Males have feathery antennae whereas females have glabrous antennae. Males mate several times during their lives but females usually mate only once and keep the sperm in their spermatheca. Females bite on vertebrates for blood meal mandatory for the maturation of their eggs. The biological cycle of *Anopheles* known as gonotrophic cycle includes ingesting a blood meal through bites a vertebrate host followed by digestion of the blood, maturation of oocytes, laying eggs and searching for another blood meal on a

new host (**Figure 6**). The duration of the gonotrophic cycle differs depending on species and temperature. Under tropical and sub-tropical climates, the cycle takes 48 to 72 hours. In cold or temperate climates, it may last up to one week and in winter time, it may be interrupted by a hibernation phase referred to as winter diapause (25). The gonotrophic cycle is always used to estimate life expectancy and the likelihood of *Anopheles* to become infected (30, 116).

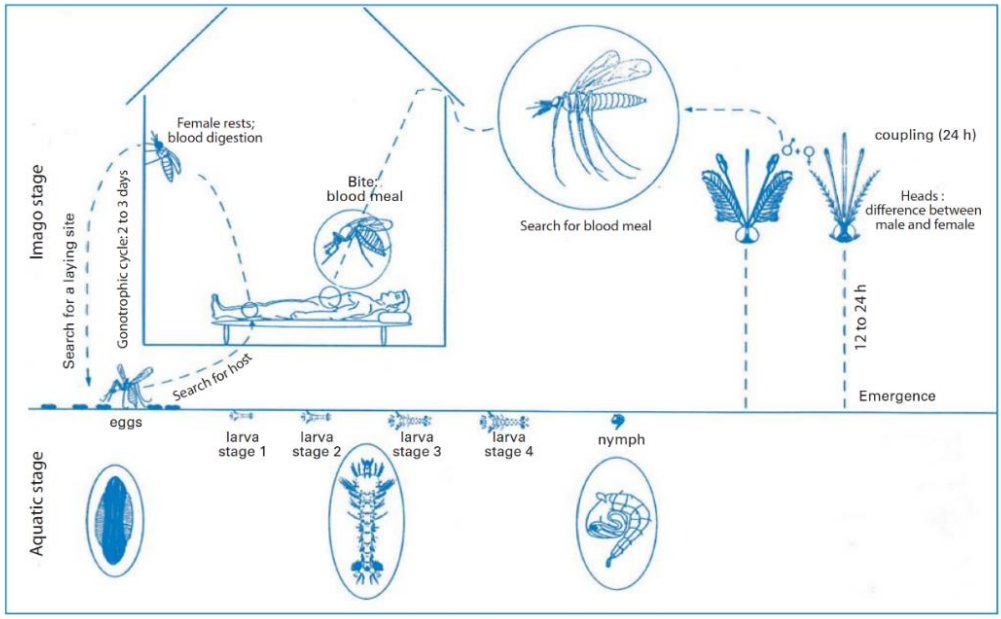

Figure 6. Biological cycle of *Anopheles* (25, 30)

2. Geographic distribution of major *Anopheles* vectors

There are around 525 species among which more than 50 nameless members of species complexes of *Anopheles* mosquitoes throughout the world (30, 117, 118). Among them, only 70 are able to transmit malaria pathogens and 41 are considered as major vectors of malaria under natural conditions (119-122). The discrimination between the terms of "major vector" and "secondary vector" is based on the overall role

played in malaria transmission, such as being a vector throughout the year *versus* sporadic, at the large geographical scale *versus* limited one, biting mainly humans (anthropophilic) *versus* attracted to animals (zoophilic). Major vectors are species playing a key role or making a major contribution to malaria transmission (e.g. sporozoite rate > 1%), whereas minor vectors are species playing or suspected to take a minor part in malaria transmission (sporozoite rate < 0.1%) (2). Most areas have several sympatric species of *Anopheles*, which are either regionally or locally distributed, but no *Anopheles* species has a worldwide distribution such as *Aedes aegypti* or *Culex quinquefasciatus*. The most efficient vector species, which predominate in Africa, are *An. gambiae*, *An. arabiensis*, and *An. funestus* (120). The most common vectors in Asia are *An. stephensi*, *An. minimus* s.l. and *An. dirus* s.l., while *An. darlingi* is the dominant malaria vector in Latin Americas (121-124).

According to the Global Dominant Vector Species (DVS) map (Figure 7) published in 2012, the Asian-Pacific region harbors 19 DVS, whereas only seven DVS are found in Africa (120, 123). The three 'primary' DVS are shown on the global map (Figure 7). Of the 19 species in the Asian-Pacific region, nine are now considered species complexes, whereas out of the seven African DVS, only *An. nili* is a confirmed species complex (the *An. gambiae* complex is not included, as specific individual members of the complex are categorized within these seven African DVS) (117, 125). The complexity of malaria vectors in the Asian-Pacific region is such that further investigation is needed, although scientists and entomologists have made considerable effort since World War II. Knowledge gaps also exist on categorizing vector behavior,

caused by the limitation of identification tools and techniques (117, 121, 126). Further investigation and routine surveillance are in need of updated tools and techniques such as PCR-based assays for species identification (127-129).

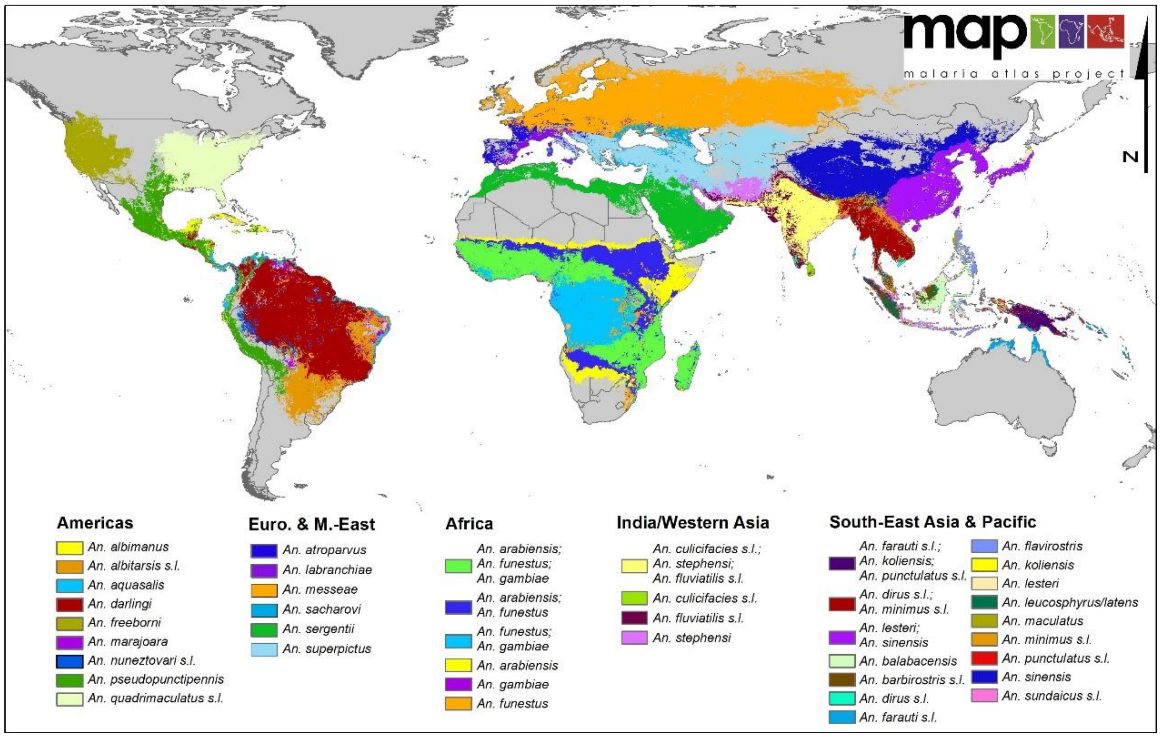

Figure 7. The global distribution of 34 DVS. (122)

s.l.: *sensu lato*, which means 'in the broad sense', is referring to species complex

3. Vector behavior and bionomics

Bionomics of *Anopheles* represent mandatory information to develop appropriate vector control strategies. The different species of *Anopheles* have a huge variety of bionomics, including in their biological traits, behaviors, and distribution, both at the larval and adult stages. Larval habitats cover a wide variety of water-bodies and wetlands as summarized in **Table 3**. The seasonal changes in flora can also affect the breeding habitats that may vary at different time of the year. For example, in rice

paddies, species as *An. minimus* complex thrives when fields are irrigated for planting, whereas once rice is growing, some other species seeking shade from emergent plants, such as *An. sinensis* in China, colonize rice paddies (130-132).

Table 3 Variety of *Anopheles* larval sites (25)

Type of water	Areas	*Anopheles* species & complex
Flowing stream or river	Africa	*An. nili*
Slow moving river and stream	Central Africa	*An. moucheti*
	Southeast Asia	*An. minimus*
	Latin America	*An. darlingi*
Residual pools	Asia	*An. culicifacies*
	Latin America	*An. pseudopunctipennis*
Stagnant and sunny waterbodies	Africa	*An. gambiae*
Wetlands with emergent plants	Africa	*An. funestus*
	Central America	*An. albimanus*
Woodland puddles	Southeast Asia	*An. dirus* *An. balabacensis*
Brackish water in coastal zones	Southeast Asia	*An. sundaicus*
	Africa	*An. melas* *An. merus*
	South America	*An. aquasalis*

At the adult stage, the main behaviors of *Anopheles* females are mating, biting and feeding on blood from vertebrates, and laying eggs. Males and females derive all their energy from nectar, whereas the female feed also on blood of vertebrates for egg maturation. Females take blood meals every two or three days. *Anopheles* is a night-active mosquito and biting on host happens from sunset (6 pm) to sunrise (6 am).

However, the biting time depends upon the species and even the local populations. Some species tend to bite in the early evening but several major vectors are active between 11 pm and 3 am, during sleeping time. The blood meal preference is also dependent on the species. The *Anopheles* species that prefer biting on humans are called anthropophilic whereas those biting animals are zoophilic. Exclusively zoophilic species are not considered as malaria vectors. Many species are polyphagous as they feed on both human and livestock, whichever is available. The shift in preferred feeding host of *Anopheles* species represents a challenge for malaria control managers. This was identified as one of the reasons for malaria re-emergence in central China (133). Another two terminologies related to blood feeding behavior are endophagic and exophagic, the former refers to the behavior of taking blood meal inside the house, the latter is referring to blood meals taken outside the house (25, 134). After taking a blood meal, the *Anopheles* mosquitoes rest inside the house throughout the gonotrophic cycle. This behavior is referred to as endophilic behavior (e.g. *An. minimus* and *An. lesteri*) (126, 132, 135). By contrast, some species like *An. dirus* prefer to leave the house immediately after feeding and rest outside to accomplish the gonotrophic cycle. These are exophilic species (25, 131, 136). During the gonotrophic cycle, the *Anopheles* oocytes grow to fill most of the abdominal cavity before the eggs are released. Simultaneously, the blood meal presents in the midgut is gradually digested. This dual maturation/digestion is called the trophogonic concordance. Once the oocytes are mature, the females lay eggs into aquatic breeding sites, and then search for another blood meal source.

Bionomics is also different among the sibling species within a complex. For instance, the Minimus complex, which includes one primary malaria vector species, is found in the hilly-forested areas of Asia (123). The complex contains three sibling species, *An. minimus* (formerly species A), *An. harrisoni* (formerly species C) and *An. yaeyamaensis* (formerly species E) (137-139). This latter species has a very restricted distribution, only being found in the Ryukyu Archipelago in southern Japan, where it was considered a major malaria vector before successful eradication of the disease in 1962 (117, 140). *Anopheles minimus* is a primary malaria vector across its geographic range, which covers much of Southeast Asia, while *An. harrisoni* is considered as a secondary vector in its patchy distribution, except in China (126, 141). *Anopheles minimus* is more ubiquitous than *An. harrisoni* allowing it to develop in various habitats, including dense canopy forests to open rice fields and therefore has a greater distribution. *Anopheles harrisoni* tends to be restricted to deforested agricultural sites, although adaptable to environmental changes such as cornfields (*versus* rice paddies) and turbid water (142, 143). However, its patchy distribution is mostly due to the lack of studies using PCR assay to differentiate both species that by default are recorded as *An. minimus*. The behavior of *Anopheles minimus* is highly variable and considered as an opportunistic vector, although this has been claimed to be a consequence of the species complex (142). The larvae of *An. minimus* complex are found in small streams with shade, slow-running and clear water. Females lay their eggs on partially shaded grassy margins (129, 144-146). Larvae have also been found in water containers in Hanoi in Vietnam and Yunnan Province in China (147-149). *Anopheles minimus* is an

opportunistic feeder, reported as mainly zoophilic in Thailand (150), while strongly anthropophilic in India (151). Host preference in blood feeding is mainly influenced by the availability of hosts like humans or domestic cattle (131, 152, 153). Biting habit is also variable and dependent on location, with reports of endophagic behavior in India, Thailand, China Yunnan and central Vietnam, but also exophagic behavior in Cambodia and northern Vietnam (131, 132, 146, 152, 153). The same is found for resting behavior, although there appears to be a large influence of the use of insecticide in indoor residual spraying (IRS) on resting location and population densities for this species (129, 154). Overall, *An. harrisoni* was reported more consistent in behavior, generally as exophagic, exophilic and zoophilic and tends to be a less dominant vector (129, 149, 155).

4. Dynamic of malaria transmission

Plasmodium parasites ingested by *Anopheles* mosquitoes will go through a development phase called sporogonic cycle. The period of incubation of the parasite into the vector, known as extrinsic incubation, is temperature-dependent. *P. falciparum* takes 8–11 days to complete the extrinsic incubation at an optimal ambient temperature of 28°C and 22 days at 20°C. The temperature of the mosquito gut equals that of its surroundings; a low environmental temperature therefore results in a longer development time of the parasite. *P. falciparum* is unable to develop below 19 °C while *P. vivax* can develop in mosquitoes at temperatures as low as 16°C; consequently *P. vivax* transmission is found in areas where the average temperature is too low for *P. falciparum* transmission. Due to this difference in temperature sensitivity, *P. falciparum*

is common in tropical regions while *P. vivax* prevails in both tropical and temperate to cold regions (25, 30, 156).

Transmission through blood transfusion, accidental needle stick, or needle sharing, leads to the transfer of asexual stages of the parasite. The incubation period of the disease is therefore much shorter than after natural transmission of sporozoites by mosquito bite. Transfusion of blood infected with *P. vivax* and *P. ovale* parasites does not lead to clinical relapse because pre-erythrocytic schizogony does not occur and hence the dormant hepatic forms are not produced. Transmission of malaria across the placenta from mother to fetus is diagnosed when parasitemia is found in the neonate within seven days after birth, or later if there has not been any other possibility of transmission to the neonate (by blood or mosquito bite). Despite the high prevalence of placental infection, congenital transmission of malaria is rare.

Malaria control and elimination

1. History of human malaria control

Malaria is originally known as Latin words “bad air” in ancient Roman times and still a major health threat to human. Malaria due to *Plasmodium* parasites has a very long history (157, 158). The history of the development of malaria control measures is parallel to that of the knowledge on malaria (25) (Table 4). The first records of humans fighting against malaria could be dated back to more than 4,000 years ago in China (11, 25, 135). The earliest evidence of a preoccupation with malaria control in western countries was recorded by Hippocrates in ancient Greece, who recommended that villages should be built near rivers but far from marshlands to avoid fever (25). The first active treatment against fever was based on *Cinchona* bark from which quinine was eventually extracted by Pelletier and Caventou in 1820 (159). The efficacy of this compound made it the drug of choice for curing fever and protecting both residents and travelers. In the Far East, *Artemisia annua,* known in Chinese as “Qinghao” has been in use in China for more than 2,000 years (160, 161). Vector control becomes an intervention for malaria control right after malaria was recognized to be transmitted by anopheline mosquitoes (162). A series of effective drugs, such as chloroquine, mefloquine and pyrimethamine, were introduced into anti-malarial treatment along with improved knowledge on the life cycle of *Plasmodium* (25, 163, 164). *Plasmodium* drug resistance and *Anopheles* insecticide resistance have been monitored and represent a challenge to malaria control and elimination.

Table 4. Chronology of key discovery on human malaria and anti-malaria interventions

Timeline	Discovery on malaria	Updated anti-malaria measures
1st millennium BC	Description of malaria clinical symptom and discussion on its pathogenesis in earliest Chinese medical classics (Huangdi Nei Jing)	
1st millennium BC		Anti-malaria activity with herb of *Artemisia annua* (Qinghao) recorded in China, *Zhouhou Beiji Fang* (The Handbook of Prescriptions for Emergencies)
5th century BC	Description of benign tertian and quartan fevers (Hippocrates)	
1600	Effect of quinquina bark to treat fever (Pedro Liva, Juan Lopez)	
1820	Isolation of quinine and quinidine (Pelletier & Caventou)	
1822		First use of Quinine as anti-malaria drug
1880	Observation and description of malaria parasite in human blood (Laveran)	
1891		Development of staining system for *Plasmodium* diagnosis (Romanovsky)
1895	Role as malaria vector of *Anopheles* mosquitoes with first description of oocysts (Ross)	
1899	Confirm malaria transmission mechanism (Grassi, Bastianelli, Bignami)	
1934	Discovery of chloroquine	
1936-1939	Discovery the insecticidal activity of DDT	
1943		Indoor spraying with DDT
1948	Description of pre-erythrocytic forms of *P. vivax*	

1952	Discovery of primaquine & pyrimethamine (Hichings)	
1953	*Anopheles* insecticide resistance (Belios, Livadas)	
1955		Launch of Global malaria Eradication Program (WHO)
1957	Anti-malaria drug resistance (*P. falciparum* to Chloroquine)	
1967		Launch of 523 projects in China, to find new drug for malaria treatment (165, 166)
1972	Discovery of Artemisinin (Tu Youyou)	
1985	Development of artemisinin derivatives	
2001		WHO recommended artemisinin1-based combination therapies (ACTs) as first-line treatment for uncomplicated *P. falciparum* malaria
2008-2009	Artemisinin or ACTs resistance observed in Thailand-Cambodia border	
2013		WHO launched Emergence Response to Artemisinin Resistance in Greater Mekong Sub region
2015		WHO issued Global Technical Strategy for Malaria 2016-2030 and Strategy for Malaria Elimination in the Greater Mekong Subregion (2015–2030), calling for acceleration of malaria elimination.
2016-2019	Algeria, Argentina, Armenia, Maldives, Paraguay, Sri Lanka and Kyrgyzstan were certificated as malaria-free; China reported zero indigenous case since 2017.	

2. Global malaria elimination progress and updated strategies

The World Health Organization is taking the lead on Global anti-malaria strategies development and revision in regards to the world malaria situation and recent research achievements. The Global Malaria Eradication Programme (GMEP) was launched by the 8th World Health Assembly (WHA) in 1955, with the following statement: "The Ultimate Goal of Malaria Control Programs Should Be the Eradication of the Disease" (167). After 14 years when it became apparent that global malaria eradication could not be achieved in the near future, the malaria eradication strategy was revised during the 22nd WHA, but it was not repealed (168). In Resolution 22.39, it was reaffirmed that "Global Eradication of Malaria Should Remain the Long Term Goal", but in areas where malaria elimination could not yet be achieved in a time-limited manner, "an initial period of malaria control should form a logical step towards the eventual launching of an eradication programme". Although malaria eradication remained the overarching objective of global malaria activities after the 1969 resolution, resources became focused on reducing the burden of disease in high-risk groups. Significant progress was made in reducing morbidity and mortality from malaria after access to several important malaria interventions, including Long-Lasting Insecticide Treated Nets (LLINs) and Artemisinin-Based Combination Therapies (ACTs), which improved the malaria situation worldwide (10).

Because of the intensification of anti-malaria interventions, especially in sub-

Saharan Africa where the disease burden is the highest, the malaria burden was reduced all over the world (2, 105, 169, 170). In some African countries displaying high malaria burdens, there was evidence of a significant decrease of malaria incidence and deaths among children and adults. A Few of countries in which the malaria burden was relatively low but persistent have eliminated malaria (Table 5). In 2007, the United Arab Emirates was certified by WHO as being malaria-free, then Morocco and Turkmenistan in 2010, Armenia in 2011, Maldives in 2015, Sri Lanka and Kyrgyzstan in 2016, Paraguay and Uzbekistan in 2018, and Algeria and Argentina in 2019 (171-173). In 2016, under the E-2020 initiative, WHO identified 21 countries as having the potential to eliminate malaria by 2020. They were selected based on an analysis on the trends of national malaria incidence between 2000 and 2014 and field assessment from WHO experts (174). Within these 21 countries, China and El Salvador have achieved zero indigenous malaria case report in 2017 for the first time (105, 175, 176).

Regarding the current progress of malaria elimination in the world (2, 169), WHO issued in 2017 an updated guideline for malaria elimination, entitled “A framework for Malaria Elimination” (1). It is reaffirmed that the certification of malaria elimination is the official recognition by WHO of a country’s malaria-free status. The criteria of application to WHO certificate for malaria elimination are: 1) local transmission of all human malaria parasites has been interrupted throughout the country, resulting in zero indigenous case for at least 3 consecutive years; and 2) a fully functional surveillance and response system that can prevent re-establishment of indigenous transmission must be in place (1). When meeting these criteria, a country can submit an official request

for certification to WHO. The certification of malaria elimination is managed by the WHO Global Malaria Programme. Following these criteria, at least 35 former malaria endemic countries have planned to achieve malaria elimination by 2030, including China, some countries in Southeast Asia and others in Africa (2, 106, 107).

Table 5. Countries and Territories officially certificated as malaria free by WHO (173)

WHO region	Name of country	Year official Certificated
Africa	Algeria	2019
	Mauritius	1973
	La Réunion (France)	1979
Eastern Mediterranean	Morocco	2010
	United Arab Emirates	2007
Europe	Armenia	2011
	Bosnia and Herzegovina	1973
	Bulgaria	1965
	Croatia	1973
	Cyprus	1967
	Hungary	1964
	Italy	1970
	Montenegro	1973
	Netherlands	1970
	The Republic of North Macedonia (former Yugoslav Republic of Macedonia)	1973
	Poland	1967
	Portugal	1973
	Romania	1967
	Serbia	1973
	Slovenia	1973
	Spain	1964
America	Argentina	2019
	Cuba	1973

	Dominica	1966
	Grenada	1962
	Jamaica	1966
	Paraguay	2018
	Saint Lucia	1962
	Trinidad and Tobago	1965
	United States of America	1970
Central Asia	Turkmenistan	2010
	Kyrgyzstan	2016
	Uzbekistan	2018
Asian Pacific	Australia	1981
	Singapore	1982
	Brunei Darussalam	1987
	Maldives	2015
	Sri Lanka	2016

3. Updated terminology of malaria elimination

In recent years, there has been a proliferation of new terms in relation to malaria and malaria elimination in scientific publications, media and technical reports, as well as terms with new or modified use and meaning. These changes stem from renewed global interest in malaria elimination and eradication, increasing access to scientific and technical information and faster translation of research findings into evidence-based policies. However, this proliferation has raised certain difficulties in communications, when some terms used to describe malaria interventions have different meanings in other public health programs or several similar terms have the same meaning. Hence, WHO updated the terminology, based on the document issued in 1963 and several WHO publications on malaria surveillance, control and elimination in the past 10 years

(1, 134, 177). We summarized some of the key terminologies, associated with this study as shown in alphabetical order (Table 6).

Table 6. Terminology associated within this study

Phrases/words	Description
Biting-capture, biting collection, human bait collection	Sampling of populations of mosquitoes and other haematophagous insects by capture when they bite on human bait or other hosts Note: Discouraged for ethical reasons, to prevent human exposure to risks of transmission of vector-borne diseases; human landing collection is the recommended alternative.
Breeding site, breeding place	Obsolete term for larval habitat: site at which developmental stages of mosquitoes (eggs, larvae, pupae) are found, including sites that appear to be ecologically suitable for particular species
Infection interval	Period elapsing from the time an individual is infected until he or she becomes infectious to others. In malaria, the infection interval is the period between the inoculation of a human being with sporozoites and the appearance of gametocytes potentially infective to mosquitoes. To be distinguished from incubation interval and incubation period.
Malaria elimination	Interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite species in a defined geographical area as a result of deliberate activities. Continued measures to prevent reestablishment of transmission are required. Note: The certification of malaria elimination in a country will require that local transmission is interrupted for all human malaria parasites.
Malaria eradication	Permanent reduction to zero of the worldwide incidence of infection caused by all human malaria parasite species as a result of deliberate activities. Interventions are no longer required once eradication has been achieved
Mass blood examination	Examination of the blood of all members of a unit of population, which may be repeated at certain intervals. Blood specimens are commonly obtained during house-to-house visits. Unlike other case-detection methods, mass blood examinations are used to detect all people harboring malaria parasites, even those who have no clinical symptoms;

	they thus supplement routine methods in problem areas and are useful for demonstrating the proportion of asymptomatic carriers present in the community examined. Mass blood examination forms part of case-detection activities and must be distinguished from malariometric surveys, which are carried out on a sampling basis in selected groups.
Outbreak	A case or a greater number of cases of locally transmitted infection than would be expected at a particular time and place. Note: The correct term is “epidemic”.
Outpatient register	List of patients seen in consultation at a health facility. A register may include the date of consultation, patient age, place of residence and presenting health complaint, tests performed and diagnosis
Phase, attack	In malaria eradication terminology, the phase during which antimalarial measures that can be used on a large scale for interrupting transmission are applied for total coverage of an operational area. This phase is sometimes called the period of total coverage spraying.
Phase, consolidation	In malaria eradication terminology, the phase that follows the attack phase. It is characterized by active, intense, complete surveillance, with the objective of eliminating any remaining infections and proving the eradication of malaria. It ends when the criteria for eradication have been met.
Phase, maintenance	In malaria eradication terminology, period that begins when the criteria for malaria eradication have been met in an operational area and will continue until worldwide eradication has been achieved. During this period, vigilance is exercised by the public health services to prevent the spread of malaria imported from across the borders of the area concerned.
Phase, preparatory	In malaria eradication terminology, the time devoted to preparation for attack operations. It ends when epidemiological and geographical reconnaissance in the operational area is completed, central and peripheral stations and essential services are established, staff are recruited and trained and logistics and reporting systems are organized.
Population, vulnerable	Groups of people who are particularly vulnerable to malaria infection in certain situations or contexts, such as mobile workers. Each country should define the populations that are particularly vulnerable in the epidemiological and social context.

Rate, malaria morbidity	Number of recorded clinical cases of malaria per unit of population over a certain period. The malaria morbidity rate is too imprecise to be of value in malaria eradication.
Rate, parasite	Percentage of people in a defined age group showing, on a given date, microscopically detectable parasites in peripheral blood. The parasite rate should always be defined in terms of the age group examined.
Receptivity	Receptivity of an ecosystem to transmission of malaria. Note: A receptive ecosystem should have features such as the presence of competent vectors, a suitable climate and, a susceptible population.
Re-establishment of transmission	Renewed presence of a measurable incidence of locally acquired malaria infection due to repeated cycles of mosquito-borne infections in an area in which transmission had been interrupted. Note: A minimum indication of possible reestablishment of transmission would be the occurrence of 3 or more indigenous malaria cases of the same species per year in the same focus, for three consecutive years.
Surveillance, active	A surveillance system in which public health workers seek reports on a regular basis from participants in the surveillance system, rather than waiting passively for the reports to be submitted
Surveillance, case-based	Each case is reported and investigated immediately and included in the weekly reporting system. *Note*: Surveillance in which all cases included in the regular reporting system are investigated
Surveillance, community-based	Surveillance in which notification starts at community level, usually reported by a community worker. It can be active (looking for cases) or passive (reporting cases). Community-based surveillance may be particularly useful during an epidemic and when syndromic case definitions can be used.
Surveillance, hospital-based	Surveillance in which notification starts with identification by a hospital of a patient with a particular disease or syndrome
Surveillance, passive	Surveillance in which reports are awaited and no attempt is made to seek reports actively from the participants in the system.
Surveillance, sentinel	Collection and use of data from a random or non-random sample of collecting sites as indicator data for the population as a whole, in order to identify cases of a disease early or to obtain indicative data about

	trends of a disease or health event that is not malaria specific.
Vectorial capacity	Number of new infections that the population of a given vector would induce per case per day at a given place and time, assuming that the human population is and remains fully susceptible to malaria.
Vector efficiency	Imprecise way of ranking vector species or populations as relatively more or less important in transmission. *Note*: More difficult to calculate than vectorial capacity
Vector potential	Species with vector competence and appreciable vectorial capacity
Vulnerability	The frequency of influx of infected individuals or groups and/or infective anopheline mosquitoes. Note: Also referred to as 'importation risk'. The term can also be applied to the introduction of drug resistance in a specific area.

Current distribution of malaria

Malaria is an infectious disease transmitted by *Anopheles* vectors. As a vector-borne disease, endemic areas are distributed according to the vector distribution, which is affected by environment and climate (30). Indigenous malaria has been recorded as far north as 64°N latitude (Archangel in the former USSR) and as far south as 32°S latitude (Cordoba in Argentina) (30). It has occurred in the Dead Sea area at 400 m below and at 2800 m above sea level in Cochabamba (Bolivia) (30). As mentioned in previous parts, malaria control and elimination have made progress worldwide in the past decades. Since 2000, millions of malaria deaths, especially among children under five years old, have been averted in malaria-endemic countries with the unprecedented global investment in the fight against this disease. The World Malaria Report issued by WHO in 2018 summarized the global status until 2017 towards the Global Technical Strategy of Malaria 2016–2030 targets for the year 2020 (105, 107, 169). In 2017, the

estimated number of reported malaria cases was 219 million in the world. Most of them occurred in the WHO African Region (200 million, 92%), followed by the WHO Southeast Asia Region with 5% of the total cases and 2% in the WHO Eastern Mediterranean Region (105). The WHO European region remains free of malaria, while China and El Salvador reported zero indigenous malaria in 2017. Fifteen countries in sub-Saharan Africa and India carried almost 80% of the global malaria burden. Five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%) (105).

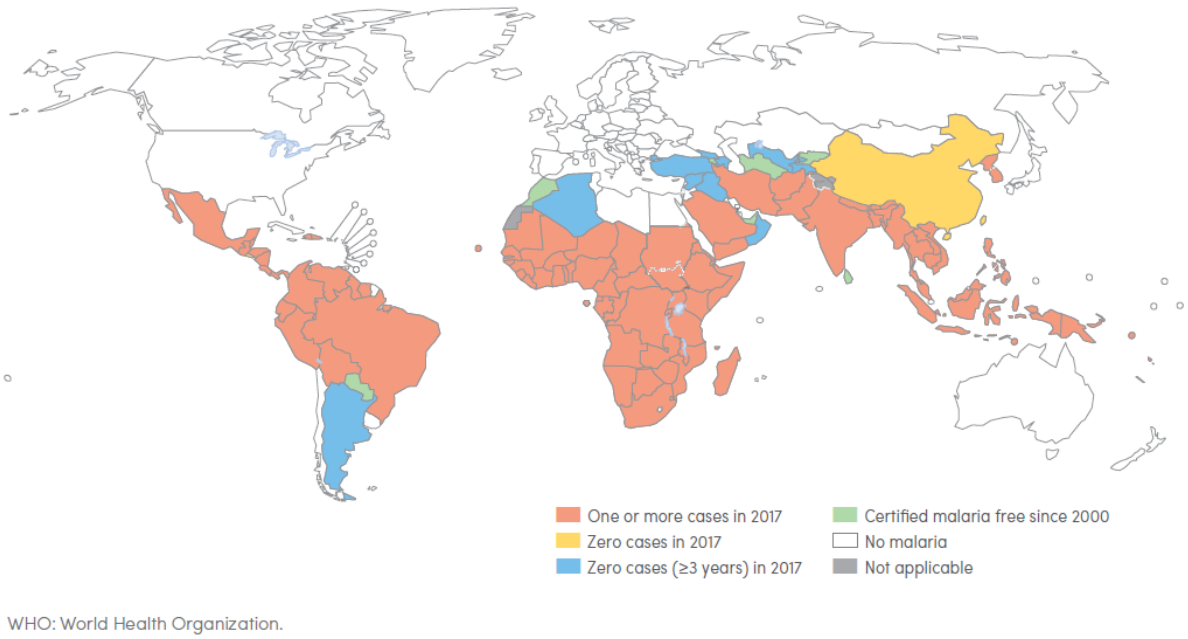

Figure 8. Distribution map of malaria in the world in 2017 (105)

Chapter 2 Malaria vectors in China at the pre-elimination stage

Introduction

Malaria has been endemic in China for more than 4,000 years and, in 1949, transmission occurred in 80% of the counties. After several decades of effort, malaria prevalence decreased drastically in China and is currently approaching elimination throughout the country. Together with the significant progress of malaria elimination, numerous scientific articles have been published on malaria control and elimination approaches. However, well-documented information on malaria vectors is still missing, which could hinder the development of appropriate surveillance strategies and WHO certification. This chapter is aiming at summarizing the nationwide distribution of malaria vectors, their bionomic characteristics, control measures and related studies, to provide evidence-based data for malaria risk assessment at pre- and post-malaria elimination. It is also specifically focusing on the China-Myanmar border area, which is currently the region most exposed to malaria and which thus displays a high potential risk of malaria re-introduction.

Articles displayed in Chapter 2

Article 1. **Zhang SS**, Guo SH, Feng XY, Afelt A, Frutos R, Zhou SS, Manguin S. *Anopheles* Vectors in mainland China while approaching malaria elimination[J]. Trends Parasitol, 2017, 33(11) : 889-900

Article 2. Feng XY, **Zhang SS**, Huang F, Zhang L, Feng J, et al. Biology, Bionomics and Molecular Biology of *Anopheles sinensis* Wiedemann 1828 (Diptera: *Culicidae*), Main Malaria Vector in China[J]. Front Microbiol, 2017, 8:1473.

Article 3. **Zhang SS**, Zhou SS, Zhou ZB, Chen TM, Wang XZ, et al. Monitoring of malaria vectors at China-Myanmar border while approaching malaria elimination [J]. Parasit Vectors, 2018, 11(1): 511

Article 4. Chen TM, **Zhang SS**, Zhou SS, Wang XZ, Luo CH, et al. Receptivity to malaria in the China–Myanmar border in Yingjiang County, Yunnan Province, China [J]. Malar J, 2017, 16 (1): 478

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REVIEWS

Review

Anopheles Vectors in Mainland China While Approaching Malaria Elimination

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China is approaching malaria elimination; however, well-documented information on malaria vectors is still missing, which could hinder the development of appropriate surveillance strategies and WHO certification. This review summarizes the nationwide distribution of malaria vectors, their bionomic characteristics, control measures, and related studies. After several years of effort, the area of distribution of the principal malaria vectors was reduced, in particular for *Anopheles lesteri* (synonym: *An. anthropophagus*) and *Anopheles dirus* s.l., which nearly disappeared from their former endemic regions. *Anopheles sinensis* is becoming the predominant species in southwestern China. The bionomic characteristics of these species have changed, and resistance to insecticides was reported. There is a need to update surveillance tools and investigate the role of secondary vectors in malaria transmission.

An Overview of Malaria in Mainland China

Malaria is one of the most important vector-borne diseases in the tropical and subtropical belt. Although significant progress has been made on malaria control in several countries, it is still on the list of top health threats to humans, causing 429 000 deaths worldwide in 2015 (WHO, World Malaria Report 2016Appendix A. Without a vaccine, **vector control** (see Glossary) is considered as a key intervention for malaria control and **elimination** [1]. Therefore, for implementing appropriate and targeted vector control strategies as malaria elimination approaches, it is crucial to better understand the distribution and bionomics of malaria vectors [2,3].

Malaria has been endemic in China for more than 4000 years, and in 1949, transmission occurred in 80% of the counties. After the establishment of the People's Republic of China, malaria prevalence decreased drastically from 1553.85/100 000 in 1960 to 1.1/100 000 in 2009 [4,5]. In 2010, the Chinese government launched the national malaria elimination program with the goal of reaching elimination by 2020 (Action plan of China malaria elimination (2010–2020)Appendix A. Since then, a number of articles have been published about malaria control and elimination in China [4,6,7], but, in comparison, information on malaria vectors in China has suffered from a lack of consideration. Moreover, the absence of information on malaria vectors is also a problem for policy makers and researchers to develop strategies for malaria surveillance, risk assessment, and other studies at the elimination stage [3,8,9].

Trends

Malaria is drastically declining in China, and the country is approaching malaria elimination.

The history and epidemiology of malaria in China have been well documented since 2010, but the involvement of malaria vectors has, by comparison, received less attention.

In China, areas with active malaria transmission, and the geographic distribution of its main vectors, were reduced, following the launch of the national malaria elimination program in 2010.

Changes to mosquito behavior, ecology, and insecticide susceptibility induced by environmental changes and control interventions should be further evaluated to secure malaria elimination in China by 2020.

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Malaria is transmitted by mosquitoes of the *Anopheles* genus, which comprises 60 species and/or complexes formally recognized in China. Of those, 14 are able to transmit human malaria parasites. Among these 14 species/complexes, the following eight taxa were reported as predominant malaria vectors in China: *An. sinensis*, *An. lesteri* (synonymy with *An. anthropophagus*), *Anopheles minimus* s.l. (including *Anopheles minimus* and *Anopheles harrisoni*), *An. dirus* s.l. (including *Anopheles dirus* and *Anopheles baimaii*), *Anopheles liangshanensis* (synonymy with *An. kunmingensis*), *Anopheles messeae*, *Anopheles sacharovi*, and *Anopheles pseudowillmori* [10–14]. However, after several decades of malaria control efforts, in addition to changes in land use and land cover, some of the *Anopheles* mosquitoes were no longer found or reported according to national malaria surveillance reports [15]. Only four species/complexes have been considered as predominant malaria vectors throughout the country since the beginning of the 21st century, that is, *An. sinensis*, *An. lesteri*, *An. minimus* s.l. and *An. dirus* s.l. Furthermore, *An. pseudowillmori* from the Maculatus Group has been considered as a potential malaria vector in Tibet since 2006 [16]. Hence, this review focuses mostly on these five latter taxa, and on their bionomics (ecology and behavior), distribution and control, during the period 2000–2016, as China approaches malaria elimination.

Geographic Distribution of Malaria Vectors

In mainland China, 24 provinces with previous local malaria transmission reported the presence of *Anopheles* mosquitoes (Figure 1A–E) [12,17]. However, after comprehensive malaria control measures and efforts, the **endemic areas** of *An. lesteri* or *An. dirus* s.l. were drastically reduced [18–22] (Figure 1B,C). Meanwhile, *An. minimus* s.l. was only reported in limited areas [18,23–26] (Figure 1D). These data fit well with malaria prevalence trends in the progress of elimination (Figure 2A–D) [4,27]. The elimination process has been very efficient since its implementation in 2010, as presented in Figure 2C,D.

Hyrcanus Group

Two species within the Hyrcanus Group were confirmed as malaria vectors in China, *An. sinensis* and *An. lesteri* (synonym: *An. anthropophagus*) [12,28,29]. *An. sinensis* is still the most widespread species distributed all over the country with records from northeast (Liaoning Province) to southwest (Yunnan Province) (Figure 1A). Moreover, ecologically suitable areas for *An. sinensis* were modelled, in prospective simulations, to expand along with climate change [30–32]. *An. lesteri* was considered as the **principal malaria vector** in the area ranging from 22°N to 33°N [17,29]. The most northern location where *An. lesteri* was identified is Liaoning Province (around 42°N) [33]. However, after several years of malaria control, as well as environmental changes, the distribution of *An. lesteri* is shrinking, and it even disappeared from some provinces, such as Fujian Province in the southeast and Yunnan Province in the southwest of China [21,34–36] (Figure 1B).

Dirus Complex

The Dirus Complex is now composed of eight sibling species throughout its geographic distribution [37]. Two are present in southern China (Figure 1C), that is, *An. dirus* (former *An. dirus* species A) reported in Hainan and *An. baimaii* (former *An. dirus* species D) collected in Yunnan below 23°N [14,38,39]. This complex is known to play a major role in malaria transmission in both China and southeast Asia [37,40]. Unfortunately, there is no information on their specific role in malaria transmission in China as molecular tools for species identification have not yet been implemented within the national malaria control program.

Minimus Complex

Based on molecular identification, the Minimus Complex consists of two sibling species, *An. minimus* and *An. harrisoni* [41,42]. *An. harrisoni,* named in 2007, was the former *An. minimus* C, whereas *An. minimus* was previously known as species A. In China, the literature referred

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almost exclusively to *An. minimus* s.l. (Figure 1D). Molecular identification was seldom processed to differentiate the two sibling species for malaria control programs, with few exceptions [41–46]. Regarding their distribution, *An. minimus* and *An. harrisoni* were reported as sympatric in Yunnan Province up to latitude 24.5°N [40,42,47]. In addition, *An. minimus* occurs eastward from Yunnan through southern Guangxi, Hainan, and Guangdong Provinces, whereas *An. harrisoni* occurs northward up to latitude 32.5°N to northern Guangxi, Guizhou, and Sichuan Provinces [42]. *An. minimus* was identified as the principal malaria vector throughout its distribution range [26,40,47–50], while *An. harrisoni* seems to play a secondary role in malaria transmission [42].

Maculatus Group

Out of the eight species of the Maculatus Group, four have previously been recorded in southern China, that is, *An. maculatus*, *An. pseudowillmori*, *An. willmori,* and *An. sawadwongporni* [12,13]. All four are known as malaria vectors in southeast Asia [48,49]. *An. pseudowillmori* has recently been identified as a potential malaria vector along two international border areas (Figure 1E), in Tibet (China–India border) [16,51], and in Yunnan (China–Myanmar border) [50,52,53]. Very limited information is available on the occurrence of these species in China, and more studies are required to define their specific role in malaria transmission.

Bionomics of Larval and Adult Stages

Bionomic traits, including trophic behavior, biting, and resting behaviors, and preferential breeding sites, are key indicators for monitoring the role of *Anopheles* mosquitoes in malaria transmission. Most larval ecological studies focused on four taxa, *An. sinensis*, *An. lesteri*, *An. minimus* s.l., and *An. dirus* s.l. These taxa share a common preference for shaded breeding sites, and in some areas they could be found sympatrically [13,17,33,54] (Table 1). *An. sinensis* and *An. minimus* s.l. displayed changes in both **host preference** and resting behavior. These changes were attributed to human intervention and environmental modification. *An. sinensis* could shift from zoophilic to **anthropophilic** behavior in areas where the number of cattle decreased [54–57]. The proportion of **endophilic** *An. minimus* s.l. diminished in areas with **indoor residual spraying (IRS)** [26,45,46], a trait also described in Central Vietnam [58]. However, very limited information was available on *An. pseudowillmori* for both larval and adult stages.

Vector Control Efforts and Challenges

Vector Control and Insecticide Resistance

Vector control is one of the key interventions to control and possibly eliminate malaria transmission [59]. China has developed its vector control strategies in line with WHO guidelines. These strategies were locally tailored, considering *Anopheles* taxa, the intensity of transmission, and species of *Plasmodium*. In Central China, where *An. sinensis* is the predominant vector and *Plasmodium vivax* is the predominant parasite, the use of door and window screens, as well as mosquito repellent, was recommended in addition to environmental reforming measures, such as intermittent irrigation in rice fields [11,17,29]. Chemical treatments, such as indoor residual spraying (IRS), **insecticide-treated nets (ITNs)** and **long-lasting insecticidal nets (LLINs)** were used mainly in southern China, in particular Yunnan and Hainan where *An. minimus* s.l. and *An. dirus* s.l. are the predominant vectors with both *Plasmodium falciparum* and *P. vivax* as the main parasites [17,29]. However, these chemical interventions were not conducted uniformly but only in selected foci and populations [17] (Action plan of China malaria elimination 2010–2020Appendix A. In addition, a biological vector-control approach based on *Bacillus thuringiensis* var. *israelensis* (Bti) was also explored in Henan and Hubei Provinces in Central China and was found to be effective against larvae of *An. sinensis* and *An. lesteri* [29,60,61].

Glossary

Anthropophilic: mosquitoes showing a preference for feeding on humans, even when nonhuman hosts are available.
Endemic area/region: an area in which there is an ongoing, measurable incidence of malaria infection and mosquito-borne transmission over a succession of years.
Endophagy: tendency of mosquitoes to blood-feed indoors. Antonym: exophagy.
Endophily: tendency of mosquitoes to rest indoors; usually quantified as the proportion resting indoors versus outdoors. Antonym: exophily.
Exophagy: tendency of mosquitoes to feed outdoors; usually quantified as the proportion of biting hosts outdoors versus indoors. Antonym: endophagy.
Exophily: tendency of mosquitoes to rest outdoors, usually quantified as the proportion resting outdoors and indoors. Antonym: endophily.
Host preference: tendency of female *Anopheles* mosquitoes to blood-feed on a specific host species.
Human landing catch: a method for collecting vectors as they land on human individuals. The purpose is to monitor exposure of the human population to vector populations and define the anthropophilic index of the vector species.
Indoor residual spraying (IRS): operational procedure and strategy for malaria vector control involving spraying indoor surfaces of dwellings with a residual insecticide to kill or repel endophilic mosquitoes.
Insecticidebioassay: in applied entomology, experimental testing of the biological effectiveness of an insecticide by deliberately exposing insects to it.
Insecticide resistance: property of mosquitoes to survive after an exposure to a standard dose of insecticide.
Insecticide-treated net (ITN): a mosquito net that has been treated by dipping it into a WHO-recommended insecticide formulation. To ensure its continued insecticidal effect, the net should be retreated periodically.
Long-lasting insecticidal net (LLIN): a factory-treated mosquito net made of material into which insecticide is incorporated or bound

Insecticide resistance is a key indicator in malaria surveillance and in the assessment of vector-control efficiency. It is commonly monitored by **insecticide bioassay**, either by determining LC_{50} (50% lethal concentration) or by using uniform diagnostic doses [62–65]. In China, the diagnostic dose was adopted as a common method in line with WHO recommendations but with modified concentrations depending on local cases of insecticide resistance [66]. A first set of recommendations was related to the test procedures for insecticide resistance monitoring in malaria vector mosquitoes [67]. A second set addressed the pesticide evaluation scheme (WHO Pesticide Evaluation Scheme, 2016Appendix A). The criteria used in China for each insecticide are reported in Table 2, and the geographic distribution of insecticide resistance in *An. sinensis* is summarized in Figure 1F. *An. sinensis* resistance to insecticides was monitored from north to south, covering the geographic distribution of the species in China [65] (Figure 1F). Resistance to organochlorine was reported in Hubei Province, as well as to dichlorodiphenyltrichloroethane (DDT) [68]. Resistance to organochlorine in this species was also reported in Liaoning and Jiangsu Provinces, and to pyrethroids, such as deltamethrin, in Jiangsu [69]. *An. lesteri and An. dirus* s.l. have been reported to be still susceptible to these insecticides [62,66]. Resistance to insecticides in *An. minimus* s.l. was monitored only in southern China, where this vector is present (Figure 1F). Although resistance to DDT was recorded in one area of Yunnan, in other regions, *An. minimus* was found to be still susceptible to the insecticides tested, including malathion, deltamethrin, and permethrin [62]. Limited information is available on *An. pseudowillmori*, but it suggests the absence of resistance at the genome level [70].

Although very little insecticide resistance has been reported in China, the ability of vectors to develop diverse resistance mechanisms to insecticides has been well documented worldwide [71,72]. The capacity of vectors to develop resistance to insecticides will undoubtedly pose a major obstacle to malaria control and elimination in China in the future, especially in the case of the widely distributed *An. sinensis* (Figure 1A).

Evolution of Mosquito Behavior

All front-line vector-control methods currently used in China (e.g., ITNs, IRS), as well as in other regions of the world, are based on the stereotypical view that vectors bite and rest primarily inside houses. This assumption is based on the early characterization of *Anopheles* behaviors of feeding and resting almost exclusively indoors [11]. However, even these endophilic species feed outside to some degree, and may do so increasingly in response to domestic insecticide interventions [73,74].

Documented examples of adaptable vector behaviors that could impact interventions were reviewed by Durnez and Coosemans in 2013 [75]. They, for instance, reported that the declining efficiency of ITNs and IRS was attributed to changes in mosquito behavior, such as host-species preferences (from anthropophilic to zoophilic) [58,76,77] and feeding preference (from **endophagy** to **exophagy**, or in the early evening when people are not protected in their houses or under bed nets) [74,78,79]. During the 1970s, several records of mosquitoes shifting from feeding inside to feeding outside, and from human to animal hosts, were reported in response to indoor insecticide use (DDT spraying and DDT-treated mosquito nets) [80,81]. It is still unknown whether these behavioral shifts were a consequence of phenotypic plasticity or evolutionary change within mosquito populations. Regardless of the mechanism, such behavioral plasticity limits contact between vectors and insecticides, thus diminishing the effectiveness of interventions [82,83]. With respect to China, no monitoring of behavioral change was conducted and no records are available.

Effect of Environmental Changes

Climate and environmental changes are driving the expansion of numerous vector species and the intensification of pathogen transmission in many locations [84]. Specific examples include

around the fibers. The net must retain its effective biological activity for at least 20 WHO standard washes under laboratory conditions, and 3 years of recommended use under field conditions.

Malaria elimination: interruption of local transmission (reduction to zero incidence) of a specified malaria parasite in a defined geographical area as a result of deliberate control activities. Continued measures to prevent re-establishment of transmission are required.

Principal or main vector: the species of *Anopheles* mainly responsible for transmitting malaria parasites at a regional scale or/and all year-round.

Secondary vector: species of *Anopheles* that plays a local or seasonal role in malaria transmission compared to the principal vector – although capable of maintaining malaria transmission at a reduced level.

Vector control: measures of any kind against malaria-transmitting mosquitoes, intended to limit their ability to transmit the disease.

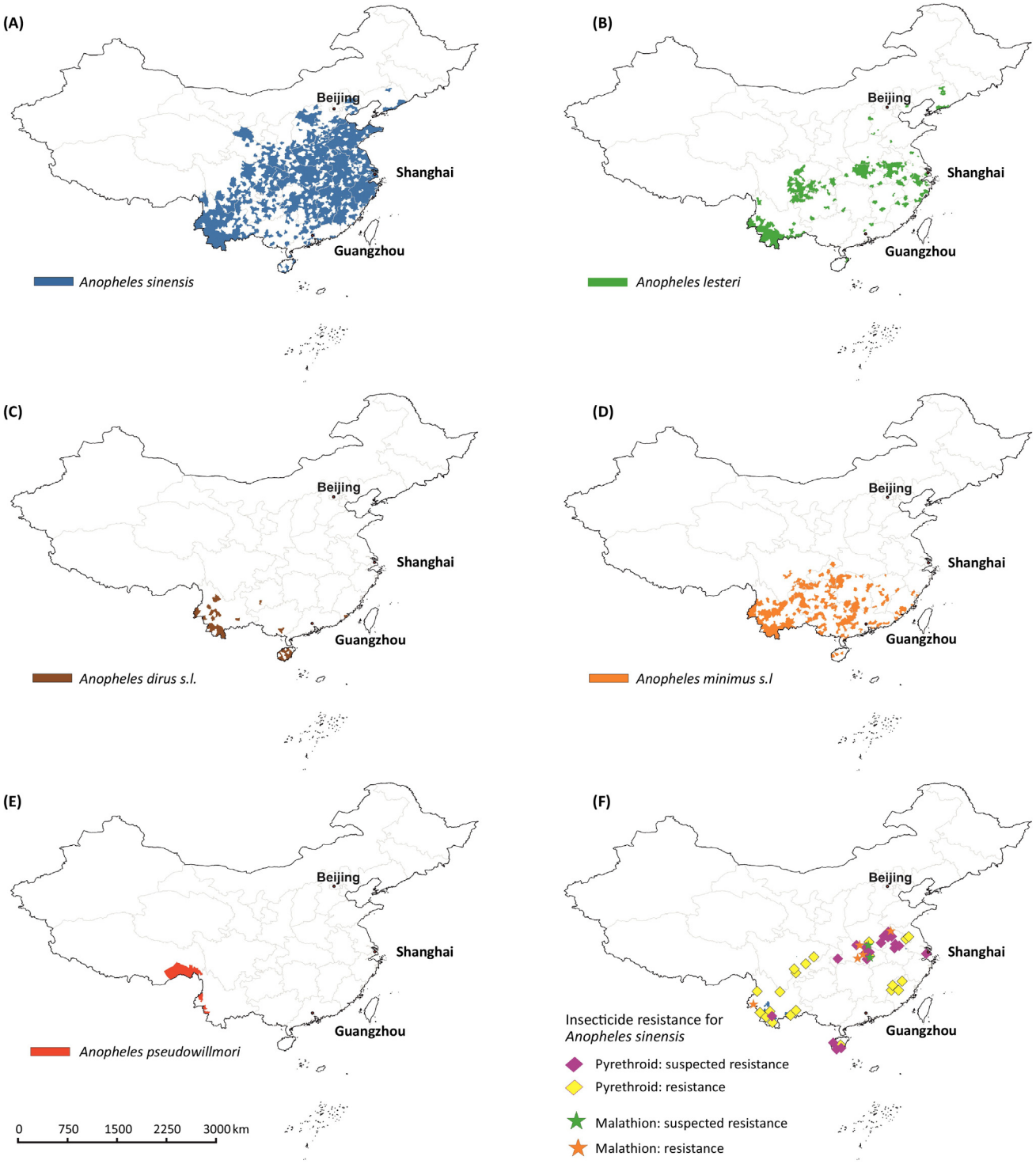

Figure 1. Geographic Distribution of Predominant Malaria Vectors and Their Resistance to Insecticide in China. Distribution of (A) *Anopheles sinensis*, (B) *Anopheles lesteri*, (C) *Anopheles dirus* s.l., (D) *Anopheles minimus* s.l., and (E) *Anopheles pseudowillmori*. (F) Resistance of *Anopheles sinensis* to insecticides. On the map, pyrethroid refers to deltamethrin and permethrin, the two major pyrethroid insecticides used in malaria vector control. Data based on literature search for the period 2000–2016.

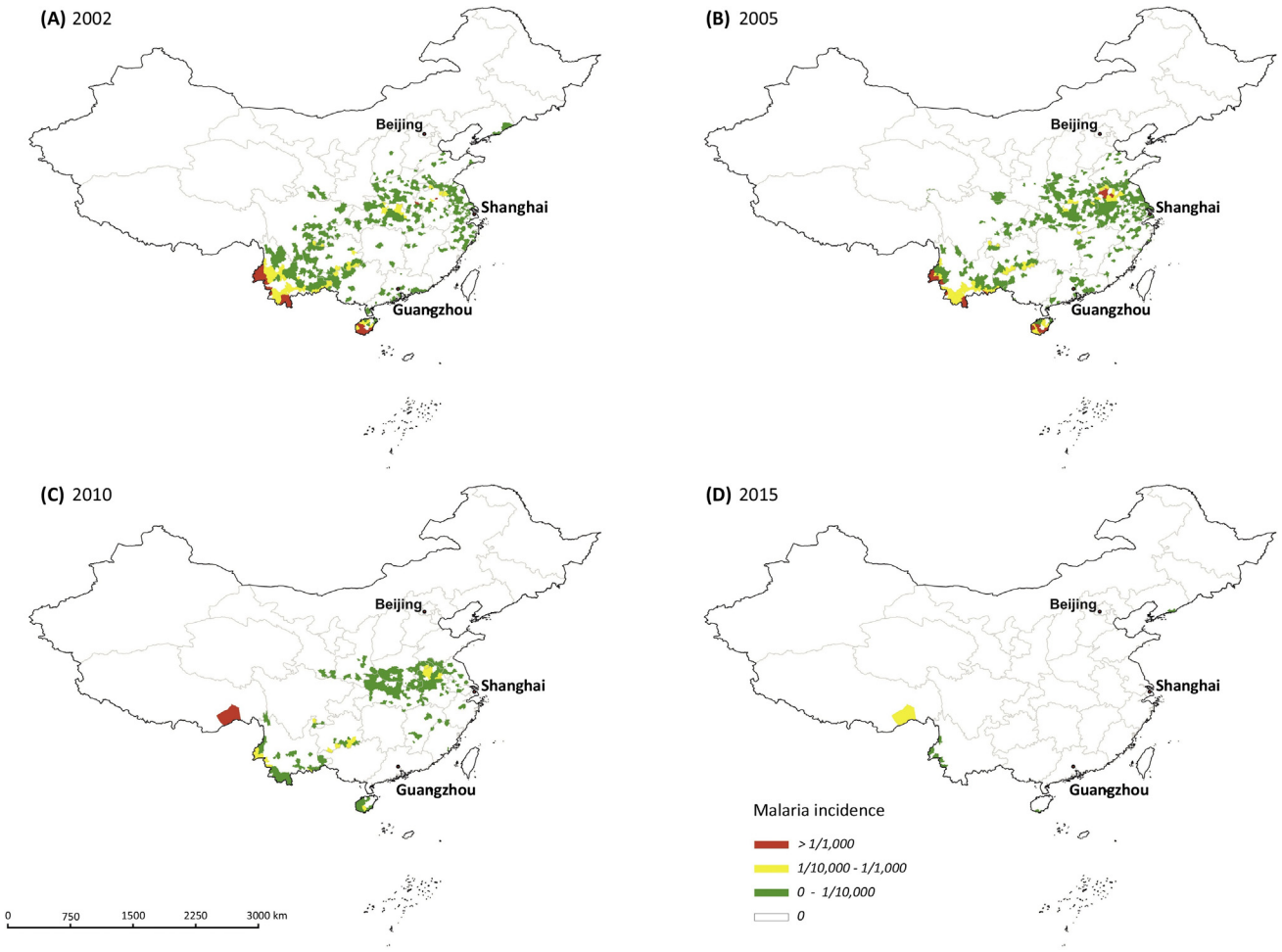

Figure 2. Evolution of Local Malaria Transmission in China from 2002 to 2015. (A) In 2002. (B) In 2005. (C) In 2010. (D) In 2015. Data from the national malaria annual report in China [27,102–105].

deforestation, which has prompted an increase in the human-biting rate of formerly zoophilic vectors in several parts of the tropics and the instigation of new malaria epidemics [85,86]. Historical and forecasted rises in temperature have also been involved in the spread of malaria into new habitats and regions [32,87]. Although climate change is suspected to also play a role in vector distribution in China, this is poorly documented. Only one work has investigated this issue in China and reported a correlation [32]. In this study, the potential impact of climate change on four dominant malaria vectors (*An. dirus* s.l., *An. minimus* s.l., *An. lesteri*, and *An. sinensis*) was assessed by species-distribution models. The environmentally suitable area (ESA), which incorporated the effect of land use and climate, was introduced as the indicator to predict the distribution of malaria vectors. The findings, established on simulation-based estimation, suggest that urbanization and global climate change would increase the ESA for *An. lesteri* and *An. sinensis*, while this increase would be limited for *An. dirus* and *An. minimus* because bioclimatic variables overwhelmed land use variables for these two species [32]. Mitigating against the detrimental impacts of environmental change on malaria transmission will be particularly difficult when public health goals conflict with economic development: for example, the removal of livestock from the landscape caused the formerly zoophilic *An. sinensis* to switch its feeding target from livestock to humans. Such change was considered

Table 1. Bionomics of Malaria Vectors in China

Species	Breeding sites				Resting behavior	Biting behavior			Seasonal fluctuation (peak season of abundance)	Refs
	Preferred	Second option	Third option	Not suitable		Place	Preferred blood source	Peak time of blood-seeking activity at night		
Anopheles sinensis	Rice field	Canal, ditch	Pond	Fishpond	Exophily[a]	Endophagy[a]	Zoophily (1st option) Anthropophily (2nd option)	8 p.m.–9 p.m.[b] 5 a.m.–6 a.m.[b]	July–August	[55,56,98,99,106–109]
Anopheles lesteri	Heliophobic, canal, ditch	Rice field	Filter well	Fishpond	Endophily[a]	Endophagy	Anthropophily[c]	1 a.m.–2 a.m.	August–September	[11,12,20,33,110,111]
Anopheles dirus s.l.	Heliophobic stream in forest	Pit with water	Footprint of cattle	–	Exophily	Endophagy and exophagy[a]	Anthropophily	11 p.m.–1 a.m.	June–July	[11,12,17,18,50,112]
Anopheles minimus s.l.	Heliophobic stream	Canal, ditch	Rice field	Big pool	Endophily	Endophagy	Zoophily/ Anthropophily[d]	10 p.m.–12 p.m.	June–July and September–October April–June[d]	[13,26,45,46,113]
Anopheles pseudowillmori[e]	Rice field, pond, ditch, *etc*	–	–	–	Exophily	Endophagy	Semi-zoophily and semi-anthropophily	11 p.m.–2 a.m.	–[e]	[12,16,50,51,53,114,115]

[a]See Glossary.
[b]There are two peaks during the night. The first peak appears at the first hour after sunset, and the second peak 1 hour before sunrise. The exact time is not the same from east to west because of the time difference.
[c]It has been reported to be zoophilic in Southern China, for example, Hainan [20], Guangdong [110].
[d]Reported especially in Hainan.
[e]*Anopheles pseudowillmori* was reported only in Yunnan[53] and Linzhi Districts, Tibet[16], although it is considered as the potential malaria vector in these areas (especially in Tibet). Its involvement in malaria transmission is still under investigation.

Table 2. Concentration (CO%) and Time of Exposure (min) to Monitor Resistance to Four Insecticides, According to Chinese or WHO Guidelines

Insecticide	China		WHO		Refs
	CO (%)	Time (min)	CO (%)	Time (min)	
DDT	4	60	4	60	(i) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, WHO 2016, pp.15–16, http://www.who.int/malaria/publications/atoz/9789241511575/en/; (ii) WHO Pesticide Evaluation Scheme, 2016, http://www.who.int/whopes/resistance/en/Diagnostic_concentrations.pdf?ua=1 [62,66]
Malathion	3.2	60	5.0	60	
Deltamethrin	0.01	30	0.05	30	
Permethrin	0.1	30	0.75	30	

as one of the key factors of vivax malaria re-emergence in the central part of China in 2006–2008 [7,57,88]. A similar situation was reported for *An. aquasalis* in the Demerara River estuary of Guyana [89]. Irrigation and dam constructions have also been linked to an increase in malaria risk, although the nature of the effect varies substantially between epidemiological, entomological, and socioeconomic settings [90,91]. While environmental changes enabling poverty reduction are essential to economic development, sustaining malaria elimination will require a clearer mechanistic understanding of the impacts of both vector control and concurrent changes in natural resource management and land use [92].

Facing the Challenges

Updating Knowledge on Malaria Vectors

To manage the challenges related to malaria elimination mentioned above, it is crucial to have updated knowledge on malaria vectors, especially on accurate species identification. This is particularly important when considering that most malaria vectors belong to complexes in which sibling species are morphologically indistinguishable. Ecological characteristics are also key elements to capture in order to implement appropriate control interventions. It is therefore necessary to upgrade the tools for entomological surveillance according to bionomic changes of malaria vectors. Recent publications on the distribution and bionomics of malaria vectors worldwide are good examples of the global knowledge on *Anopheles* mosquitoes [40,47,93–96]. These reviews also updated information on malaria vectors in China, but there are still several knowledge gaps that must be filled. These include, in particular, the influence of behavioral changes of the main vectors on malaria transmission, and the precise distribution and role of sibling species, as well as **secondary vectors**. The focus given to main malaria vectors until recent years has left the secondary ones in the shade, and a real effort should now be given to these secondary vectors in China, and more widely in southeast Asia, as their involvement in malaria transmission must be better framed and analyzed. In addition, there is a need for a nationwide map of the distribution of current malaria vectors based on high-quality surveillance data, including molecular identification of sibling species and the GPS locations of mosquito-collecting points, as done in Thailand for instance [94]. This will be essential for both malaria elimination certification by WHO (WHO: *Eliminating Malaria*, 2016, pp. 22–23Appendix A) and the development of a surveillance strategy at the postmalaria elimination stage.

Updating Surveillance Tools

Entomological survey is the only way to get pertinent information on malaria vectors and to monitor their behavior and their role in malaria transmission. However, the methodology and tools used today, such as light trap, **human landing catch**, cow bait collection, and larval collection, are labor-intensive and time-consuming. In addition, the study sites selected for these investigations are in need of a precise method, which will provide data-based criteria other than experience-based criteria. In this regard, remote sensing, geographic information

system, and spatial analysis approaches represent good candidates. Such approaches have been developed since the late 1980s with the objective of establishing host–vector–parasite relationship models allowing for more precise spatiotemporal surveillance of vectors and disease. They were carried out to map the geographic distribution of *Anopheles* mosquitoes in endemic malaria areas such as Belize, Mexico, French Guiana, Brazil, and China [97–101], providing good examples for efficient vector surveillance in further studies.

Concerning the emergence of insecticide resistance, which is a dynamic process, assessing its impact on the efficacy of interventions is an essential but difficult task. In China, although the national malaria surveillance program adopted the monitoring of insecticide resistance since the beginning of the program in 2005, data have been collected from selected fixed sentinel sites. Hence, the surveillance data could only present the status of mosquito populations in limited areas. In a country as large as China, both environmental and socioeconomic developments are highly diverse. Moreover, data sharing on resistance and insecticide use between national health and agriculture departments does not really take place. Data availability and interoperability are still largely missing elements in insecticide resistance management and need to be considered with more attention in future studies. In addition, malaria foci in China, as well as in southeast Asia, are mainly spread along international borders. Their elimination will gain in efficacy if a task force is created with partners and malaria managers from neighboring countries, working in coordination to prevent malaria transmission.

Concluding Remarks

Since the launch of the national malaria elimination program in 2010, malaria prevalence and the distribution zones of the main malaria vectors in mainland China have decreased. However, to achieve malaria elimination by 2020, there is a need to evaluate and follow-up on the behavioral changes of *Anopheles* species in China, driven by both environmental changes and control interventions, and to update the tools for entomological surveillance (see Outstanding Questions). For instance, the role of secondary malaria vectors, like *An. sinensis* in Yunnan, should be monitored with close scrutiny. *An. sinensis,* which settles in rice fields, may represent a major risk for malaria elimination. With *An. sinensis* being an important vector locally, agriculture, and thus the key sector of food production, may also turn into a threat for malaria elimination. A crucial issue for the coming 5 years will therefore be to monitor possible vector replacement but also to investigate potential competition between key societal and economic sectors, such as public health and agriculture, to avoid the occurrence of competitive trends. These studies must bring information to national and international policy makers. They must develop national and international guidelines and decisions on the proper actions to take in order to prevent sectorial competition and to ensure the successful implementation of malaria elimination. Another risk to consider and assess is the reintroduction at postmalaria elimination phase, and proper monitoring and preventive actions will be required. The index of receptivity, which represents the capacity of a given area to be favorable to malaria transmission, should be adopted as an indicator for malaria surveillance. The ecological behavior, such as trophic behavior, biting, and resting behaviors, are in need of further investigation and routine monitoring to assess the potential risk of reintroduction. This is particularly important along international border areas, where one country has achieved malaria elimination while the other neighbor still has local malaria transmission. Molecular techniques, such as PCR, should be routinely implemented to identify vector species and populations and to investigate their respective role in malaria transmission, especially for sibling species such as those of the Minimus and Dirus Complexes.

Outstanding Questions

What is the distribution of the main malaria vectors in China, after several years of malaria control efforts?

What is the current insecticide resistance status of the main malaria vectors in China?

Did environmental change and/or control efforts alter the bionomics of malaria vectors in China?

Do the changes in bionomic characteristics of the main malaria vectors challenge the elimination process? How could we manage these challenges?

What is the role of secondary malaria vectors on malaria transmission in China?

Do we have any of the right tools to monitor the shift in malaria vectors with respect to both population density and bionomics?

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Resources

[i]www.who.int/malaria/publications/world-malaria-report-2016/report/en/

[ii]www.moh.gov.cn/mohbgt/s10788/201005/47529.shtml

[iii]www.nhfpc.gov.cn/zwgk/wtwj/201304/15a4cc7a40b0452191fe409590ca99d8.shtml

[iv]www.who.int/whopes/resistance/en/Diagnostic_concentrations.pdf?ua=1

[v]www.who.int/malaria/publications/atoz/eliminating-malaria/en/

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REVIEW
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Biology, Bionomics and Molecular Biology of *Anopheles sinensis* Wiedemann 1828 (Diptera: Culicidae), Main Malaria Vector in China

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China has set a goal to eliminate all malaria in the country by 2020, but it is unclear if current understanding of malaria vectors and transmission is sufficient to achieve this objective. *Anopheles sinensis* is the most widespread malaria vector specie in China, which is also responsible for vivax malaria outbreak in central China. We reviewed literature from 1954 to 2016 on *An. sinensis* with emphasis on biology, bionomics, and molecular biology. A total of 538 references were relevant and included. *An. sienesis* occurs in 29 Chinese provinces. Temperature can affect most life-history parameters. Most *An. sinensis* are zoophilic, but sometimes they are facultatively anthropophilic. Sporozoite analysis demonstrated *An. sinensis* efficacy on *Plasmodium vivax* transmission. *An. sinensis* was not stringently refractory to *P. falciparum* under experimental conditions, however, sporozoite was not found in salivary glands of field collected *An. sinensis*. The literature on *An. sienesis* biology and bionomics was abundant, but molecular studies, such as gene functions and mechanisms, were limited. Only 12 molecules (genes, proteins or enzymes) have been studied. In addition, there were considerable untapped omics resources for potential vector control tools. Existing information on *An. sienesis* could serve as a baseline for advanced research on biology, bionomics and genetics relevant to vector control strategies.

Keywords: *Anopheles sinensis*, biology, bionomics, gene, protein, molecule, China, vector

Abbreviations: aa, amino acid; *Ae. Aegypti, Aedes aegypti; An. Gambiae, Anopheles gambia; An. Sinensis, Anopheles sinensis;* AQP, aquaporin; CCEs, carboxylesterases; cDNA, complementary DNA CPF; CPF, cuticular protein family; *Cu. Fatigans, Culex fatigans;* EIR, entomological inoculation rate; GFATM, The Global Fund to Fight AIDS, Tuberculosis and Malaria; GO, gene ontology; GSTs, glutathione-*S*-transferases; HSP40, heat shock protein 40; IRE-BP1, Iron responsive element binding protein 1; IRS, indoor residual spraying; ITS2, Internal transcribed spacer 2 capacity; JEV, Japanese encephalitis virus; KEGG, Kyoto Encyclopedia of Genes and Genomes; LLINs, long-lasting insecticidal nets; miRNAs, microRNAs; NGS, Next Generation Sequencing; nt, nucleotide; OBP, odorant-binding proteins; ORF, open reading frame; *P. f, Plasmodium falciparum; P. v, Plasmodium vivax;* PCR, polymerase chain reaction; sRNAs, small RNAs; SRPN14, serine protease inhibitor 14; TH, tyrosine hydroxylase; UTR, Untranslated Regions; VC, vector competence; WHO, World Health Organization.

INTRODUCTION

Malaria was once epidemic in China and disease levels were high. However, a significant decline of malaria incidence has occurred with reported cases declining from > 9 million cases in the 1960s to only 3078 cases in 2014 (Tang, 2010; Wang, 2014; Li et al., 2015). In 2010, the Chinese Government launched Malaria Elimination Program with a goal to eliminate malaria in the entire country by 2020 (Feng et al., 2014).

Four Anopheline species, *Anopheles sinensis*, *Anopheles anthropophagus*, *Anopheles minimus*, and *Anopheles dirus,* are considered main vectors for malaria transmission in China. Among these, *An. sinensis* is the most widely distributed species (Zhu et al., 2013). It is the most important malaria vector in flatlands, especially in the paddy planting regions. *An. sinensis* is considered to be a competent vector for *Plasmodium vivax* malaria since it is the only major vector in central China where *P. vivax* is prevalent, locally transmitted, and where several malaria epidemics have occurred (Zhou et al., 2007). Besides malaria, *An. sinensis* can also transmit lymphatic filariasis (Reid, 1968), JEV and *Rickettsia felis* (Scherer et al., 1959; Zhang et al., 2014).

The distribution, habitat, feeding behavior, and host selection of *An. sinensis* in China has been extensively studied. Ma (1954) published the first biology study of *An. sinensis* and since then, there have been many reports on its biology or bionomics. This information has contributed to the success of malaria control programs. Larval reduction by drainage, and filling, and IRS have been the main malaria control measures. The primary intervention measures for malaria elimination in China continue to target the adult vector by IRS or LLINs (Liu and Liu, 2010).

Interactions between vector and parasite are important in malaria transmission dynamics. Identification of molecules involved in multifaceted developmental cycles of parasites within the vector and the related mechanisms accounting for survival and proliferation can provide attractive targets to interfere in the disease transmission (Sreenivasamurthy et al., 2013). However, many of the molecules and mechanisms in *An. sinensis* are still remain unknown. Understanding the underlying details of the vector-pathogen interaction would underpin the prevention and control of parasitic diseases.

Although there are many studies on *An. sinensis* distribution, bionomics and molecular study in China, the information was notably dispersed in the literature. So, current studies were systematically reviewed. The objective of the present study was to review the biology, bionomics and molecules of *An. sinensis* in China. This could provide insights for development of novel mosquito control strategies and increase the effectiveness of the vector control interventions in elimination campaign.

METHODS

An electronic search of peer-reviewed scientific and medical literature published between January 1954 and September 2016 in Chinese and English was conducted using PubMed (MEDLINE), CNKI (China National Knowledge Infrastructure), VIP (Chongqing VIP Database), and CSPD (China Science Periodical Database, Wan Fang) and Web of Science databases. Gray literature and programmatic documents were also searched using Google, Google Scholar and other search engines using the same search terms.

The following search terms (or their Chinese equivalents) were used: *Anopheles sinensis*, distribution, biology, bionomics, molecule, ecology and China. The decision tree for the inclusion or exclusion of articles is shown in **Figure 1**. Only publications reporting biology, bionomics, and molecules of *An. sinensis* from China were included. Articles submitting a report on morphology, development, reproductive, life cycle, vector competence, larval and adult ecology, vector capacity, molecules involved in physiology and pathology were included. Studies involving insecticides were excluded because it would be the focus of a future review. These data were extracted and processed through a series of rigorous checking procedures before classification into a database. All results were initially reviewed for mosquito bionomics (larval and adult ecology), biology and related molecular studies based on the title and abstract. Relevant publications were further reviewed using the full text to determine whether the data focused on distribution, bionomics, epidemiology, vector competence, or identified molecules.

RESULTS

Figure 1 illustrates the search results. The initial search strategy generated 1469 records. After first round review of titles and abstracts, a total of 824 articles were amalgamated for review. After removing 44 duplicates, 722 relevant publications were further reviewed based on the full text to find out whether primary or secondary data on the biology, bionomics, molecules. Finally, a total of 538 papers and reports met these criteria. Selected articles were saved in Endnote and their characteristics corresponding to the criteria manually entered into Microsoft Excel for ongoing data management. From these articles, we assessed risk of bias for included studies but did not exclude studies on the basis. The outcomes of analysis by study area are outlined below.

BIOLOGY

Morphology

Anopheles sinensis Wiedemann (1828) is a member of the *Anopheles* hyrcanus species group. Adults are morphologically distinguished from sibling species by the presence of four pale bands on the palpi, a fringe spot at vein 5.2, a tuft of dark scales on the clypeus on each side in the female (**Figure 2**) and a T-type speckle on ventral aspect (Feng and Zhang, 1962; Yang et al., 1994). Morphometric and morphological characteristics of *An. sinensis* eggs were studied using scanning electron microscopy (Zhang et al., 1982, 1984; Li D. et al., 2010). Eggs of *An. sinensis* were black (newly laid eggs were white and blackened about an

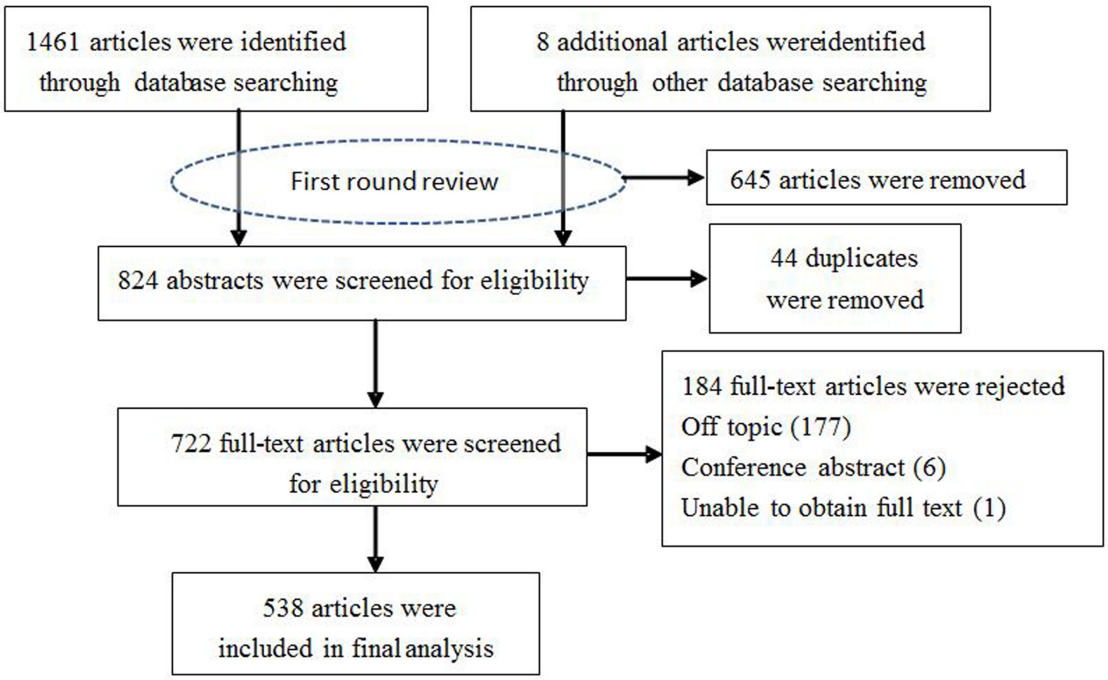

FIGURE 1 | Flow diagram of the selection procedure used for the systematic review of accessible articles.

hour later (Liu, 1986). They were boat-shaped in lateral, view with a mean length of 0.5mm. Floats present on sides of the egg surface and these had 7∼10 ribs.

Development (Egg to Adult)

Like all mosquitoes, *An. sinensis* has four life stages: egg, larva, pupa, and adult. *An. sinensis* female lay individual egg on water surface. According to the studies, the number of eggs laid ranges from dozens to hundreds (Lu, 1984; Li et al., 1994; Qu et al., 2000i; Zhong, 2000; Zhong and Tan, 2000). The number of eggs laid were affected by the season (Qu et al., 2000i), temperature (Zhong, 2000; Zhong and Tan, 2000), blood resources (Su and Su, 1989), and experimental factors (Luo et al., 1988; Li and Tang, 2010). Luo et al. (1988) noted that the number of eggs laid in cow sheds was greater than in human dwellings and speculated this might be an adaption related to blood preference. Lu (1984) observed that most female laid eggs during entire night, and were prone to oviposit between dusk and dawn (usually from 7 pm to 5 am). The result was consistent with studies in other provinces of China (Liu, 1986; Xiang, 1988; Qu et al., 2000d,i). Generally the female adults began to lay eggs in around 3 days (2.57–3 days) after blood feeding, and its peaks started from 5 to 16 days (Fengwen et al., 1988; Qu et al., 2000a) in July and August (Qu et al., 2000d,i). Interestingly, in *An. gambiae s.s.*, Christiansenjucht et al. (2015) observed temperature had effect on the egg laying, the number of eggs laid was highest and lowest when adults were kept at 27°C and 31°C respectively. Differences were also observed among the successive blood meals in times to egg laying and hatching, number of eggs laid, and chances of feeding and egg laying.

Embryogenesis and hatching have been described in detail by different research teams (Wang et al., 1985; Li et al., 1994; Shen et al., 1995; Lai et al., 1995; Qu et al., 2000e). Generally it takes around 2.07–2.88 days (mean) or more for full embryonic development depending on the temperature and environment (Fengwen et al., 1988; Qu et al., 2000a; Zhong, 2000; Zhong and Tan, 2000). Hu et al. (1986) observed that the optimal temperature was between 25 and 28°C. At 25–30°C, *An. sinensis* hatched at 2.9 d after oviposition. At 19°C and 22°C, hatch took 6.4 and 6.7 days respectively, and below 16°C, embryonic development of the *An. sinensis* cannot be completed. Hatching rates ranged from 63.68 to 90.88% (Wang et al., 1985; Li et al., 1994; Lai et al., 1995; Shen et al., 1995; Qu et al., 2000e) in different provinces in China. Hatching rates were lower under natural conditions compared to under experimental conditions, and there could be diapause in embryogenesis due to the low temperature (Zhao and Zhen, 1997). Larval breeding environments largely depended on the sites where females laid their eggs, and the oviposition locations were not stringent. The environment had no effect on the sex ratio (Zhou et al., 1988). The sex ratio was very close to 1:1 which meant that the natural quantity of males and females was equal.

The life cycle duration from the egg to the adult was a popular research topic. Eight studies between 1984 and 2000, indicated that the average duration of the life cycle ranged from 14 to 20 days (Wang et al., 1985; Zhu, 1989; Xu L. et al., 1991; Xu R. et al., 1991; Lai et al., 1995; Shen et al., 1995; Qu et al., 2000d,e). The development time also varied in different studies conducted in different regions of China. The longest time was 20.9 days in the Guizhou province (Wang et al., 1985), and the shortest

FIGURE 2 | Typical morphological characteristics of *An. sinensis*. **(A)** A pale fringe spot at vein 5.2. **(B)** Four pale bands on the palpi. **(C)** Patch of pale scales at midcoxa. **(D)** Apical pale bands at hindtarsomeres.

time was 13.74 days occurred in the Henan province (Qu et al., 2000d). The time required for the development of *An. sinensis* was temperature dependent. Wang et al. (2010) documented a shortened duration as the temperature increased from 16 to 31°C. The life cycle duration were 30.7, 23.3, 15.5, 13.5, and 12.5 days at 19°C, 22°C, 25°C, 28°C, and 31°C respectively. *An. sinensis* can complete their development at temperatures as low as 16°C (Sun et al., 1994), but cannot develop successfully at temperatures > 31°C. In contrast, *An. gambiae s.s.* development was fastest between 28 and 32°C; adults did not emerged at <18°C or >34°C (Kirby and Lindsay, 2009). Development time from egg to adult was also largely temperature-dependent.

After completion of larval development, the pupation rate was generally high except in one study. Shen et al. (1995) documented a pupation rate of only 21.28–33.68%. Li et al. (1994) found that the larvae stage needed 8.16 days to develop and the mean duration for female larvae (8.54 ± 1.49) was longer than for males (7.78 ± 0.96). The larval stage fed on organic debris and microorganisms in the water including bacteria, protozoa, pollen grains, and fungal spores (Yuan et al., 1988). The distribution of young larvae in the water was similar in different rice field locations (Chen, 1990). The strong clustering habit of larvae weakened as they were growing, spreading from the center to around (Chen et al., 1990). Larval growth was influenced by many factors such as food types, food quantity, and larval density, etc. (Dong and Liu, 1990). Xu D. et al. (1988) indicated that development would be reduced if the larval density was too great.

Larvae of *An. sinensis* can successfully develop under a wide range of temperatures, whereas low winter temperature (usually after October in China) restricts the development of *An. sinensis* (Ding et al., 1991; Wu et al., 2005). In addition, Lai et al. (1995) noted that the time required for larvae development correlated with the time required for embryogenesis and hatching. If the time required for embryogenesis and hatching increased, the length of time required for larval development would decrease accordingly to keep the life cycle duration in balance of about 14 days, and vice versa (Lai et al., 1995).

The larval molted their exoskeleton four times before becoming pupae. *An. sinensis* remain in the pupal phase for about 2 days until the adults emerge and disperse to seek blood or nectar (Pan et al., 1984; Wang et al., 1985; Fengwen et al., 1988; Xu L. et al., 1988; Xu R. et al., 1991; Shen et al., 1995; Qu et al., 2000a). The eclosion rate from all the studies was

higher than the hatching rate and the pupation rate (Shen et al., 1995; Zhong and Tan, 2000), indicating that death in premature stage mostly occurred during the larval stage. Compared to the high mortality during the larval stage, survival was higher in the pupal stage and during eclosion (Qu et al., 2000g,h). Like other mosquito species, the *An. sinensis* males often emerge first and form swarms, they cannot copulate until the genitalia rotate 180° which occurs in about 1 day. Females emerge subsequently, and then enter swarms to copulate in the air (Zhu, 1989).

An. sinensis is holometabolous insect and has four different developmental stages. The developmental parameters, such as number of eggs laid, hatching rate, pupation rate, and duration of the life cycle, can influence malaria transmission. A detailed understanding of *An. sinensis* biology pertaining to development could help in generating novel control strategies. Investigation of *An. sinensis* development may reveal mosquito-specific adaptations and could provide stage-specific targets for mosquito-borne infectious disease control.

Longevity

The life expectancy of *An. sinensis* under natural condition was 5–7 days (Xu et al., 1987; Zhang et al., 1990; Qu et al., 2000e), which was shorter than the values measured under experimental conditions (Zhou et al., 1995) in which the constant nectar and blood were available. Female and male *An. sinensis* from different locations in China exhibited a significant difference in life expectancy. Qu et al. (2000a) observed average female and male *An. sinensis* life expectancies of 13.83 and 8.33 days, and maximum longevity was 32 and 14 days respectively (Qu et al., 2000f,i). Zhou et al. (1995) observed mean female and male life expectancies of 21.63 and 17.51 days, and maximum longevity was 51 and 46 days respectively. The results of the studies on *An. sinensis* life expectancy between female and male in different provinces indicated that the life expectancy of females exceeded that of males. In addition, temperature had a significant influence on the life expectancy of adult mosquitoes (Zhang et al., 1990; Xu L. et al., 1991). The average life expectancy in July was longer than that in August because the temperature in August was often higher than that in July (Qu et al., 2000d). In addition, Hu et al. (1988) showed that the average life expectancy was 48.6, 27.4, 23, 20.6, 14.8, and 11.1 days at 16°C, 19°C, 22°C, 25°C, 28°C, and 31°C. The longest life expectancy was at 16°C where individuals had very low metabolic rates (Pan et al., 1984; Hu et al., 1986).

Gonotrophic Cycle

The mean gonotrophic cycle length for *An. sinensis* was 2.5 days (Liu, 1986; Xu et al., 1987, 1990; Wang et al., 1990; Li et al., 1994; Shi et al., 1996; Qu et al., 2000i). The shortest gonotrophic cycle for *An. sinensis* was 48 h (Wang et al., 1990) in Shanghai, while the longest was 2.65 days (Xu et al., 1987) in Fujian Province. The gonotrophic cycle for the different strains (Shanghai, Zhengzhou and Fujian strains) were similar (Qu et al., 2000b), but varied slightly related to the month (Zhang et al., 1990; Qu et al., 2000d), blood source (Su and Su, 1989), temperature (Xu and Zhang, 1988) and sunlight (Sun et al., 1987).

Hibernation

Generally, *An. sinensis* begin to appear in late April or May and disappear in October. When the temperature related to the about 10°C, *An. sinensis* tended to seek sheltered places for hibernation. On the basis of studies on the hibernation of *An. sinensis* (Ma, 1954), together with studies conducted in Zhejiang, Henna, Hubei, and Jiangsu provinces etc. (Liu and Chen, 1959; You et al., 1964; Xue et al., 1990), *An. sinensis* hibernates in the adult stage. Both male and female adults were caught in cow sheds, mountain caves, and cellars. The mosquitoes caught in hibernation sites were found to be nulliparous and have sperm in the spermathecae. Much body fat accumulated during the winter months. In northern China, *An. sinensis* females hibernated in sheltered places from the end of October onward (Anonymous, 1973b; Chen et al., 1979). When the weather warmed the following spring, the hibernating mosquitos became active and fly off for the new life cycle (Anonymous, 1979).

Flight Dispersal

Anopheles sinensis has limited dispersal with most adults staying close to their larval sites or habitats. Dispersal distances are generally less than 1 km, but longer passive dispersal by planes, ships, or other human devices could occur. There were relatively few studies on adult flight range or dispersal. Liu et al. (2011) studied the dispersal range of *An. sinensis* using a mark-release-recapture technique. They marked 3000 wild *An. sinensis* and recaptured mosquitos for ten successive days using light traps. The recapture rate was very low and most marked *An. sinensis* were recaptured within a 100 m radius of the release site. The maximum flight distance was 400 m.

BIONOMICS

Distribution

Anopheles sinensis has been recorded from Afghanistan, Myanmar, Cambodia, China, Indonesia, India, Japan, Korea, Malaysia, Singapore, Thailand, and Vietnam (Reid, 1953). It is distributed in 29 provinces within China and all regions except Xinjiang and Qinghai provinces (Lu et al., 1997). Xu and Feng (1975) published the first national distribution of the *Anopheles hyrcanus complex* in China including *An. sinensis*. The report primarily consisted of a brief survey of egg, larval, pupa and adult stages around the surveyed sites during 1962–1965. The results were limited to a short description of the main bionomic characteristics.

In the present review, an *An. sinensis* distribution map was produced by overlaying occurrence data from 332 source collected reports. *An. sinensis* was the mosquito taxon most commonly found and identified in China. However, this study did not document an extension to its previous distribution range. *An. sinensis* were found in 29 provinces and regions (**Figure 3A**) consistent with historical records. According to the analysis, *An. sinensis* has been recorded both on mainland (Chen, 1990; Pan et al., 2012) and on island areas (**Figure 3B**) (Zhang et al., 2001), and it was widespread across the plain areas and mountainous areas but more abundant in plain areas (Huang et al., 2015). *An.*

sinensis was collected at 270 up to 2,300 m in elevation, but is generally restricted to elevations of 300–500 m (Liu et al., 1989). A more accurate, or a lower level distribution map is currently unavailable and the data currently consist of heterogeneous records that were not made on a national scale.

Seasonal Population Fluctuation

In almost all areas in China, *An. sinensis* was found from July to December with a population peak in August. The population density of *An. sinensis* in cow sheds had a June peak in most places with a second small peak in late August or September. In Heilongjiang, Jilin, Liaoning and Xizang, *An. sinensis* was recorded only in August and September after which no specimens were found due to cold weather. In some warmer provinces like Yunnan, active *An. sinensis* were present in December but in relatively small numbers. In general, the collection data showed the seasonal abundance of *An. sinensis* fluctuated monthly (temperature-driven model) at various study sites during different study periods. *An. sinensis* emerged in April, developed into large populations from July to December with a peak in August.

Habitats

Larval Habitats

Anopheles sinensis larvae were found in diverse habitats. Examples of major habitats include rice-fields and ponds (Liu et al., 1962). The larvae were also collected in irrigation channels, abandoned wells, ground pools, and pools beside rivers, marshes, stream margins, ditches, seepages, shallow ponds, sumps, hoof footprints, and wheel tracks. The larvae have also been found in polluted pools and cesspits (Cai, 1959). Environmental factors associated with larval habitats have been studied. The available data (Anonymous, 1973b; You and Xu, 1979; Chen, 1990) observed that the breeding sites of *An. sinensis* were located within 50–200 m from irrigation wells, human or livestock settings, and the larvae were most often found in water bodies with abundant aquatic vegetation. Physicochemical properties tests of habitats showed that *An. sinensis* larvae had high adaptability to variable water quality (Wang, 1979).

Indoor and Outdoor Habitats

The preferred indoor habitats (resting habits) for *An. sinensis* were mainly cow sheds, pigpens, sheepfolds and human dwellings (Cai, 1959; Ouyang et al., 1992; Yi et al., 1998), especially when they are seeking hosts for blood meals. About 61% of resting *An. sinensis* was captured from cow sheds, 37.7% from human dwellings, and 0.74% from spare houses (Cai, 1959). A report from Jaingsu province found that the average number of *An. sinensis* in livestock sheds was 49 times greater than that in human dwellings (Gao et al., 2004). Both studies found a higher tendency for resting mainly in cow sheds, although other resting places can be selected.

Outdoor habitats for *An. sinensis* were mainly grass or leaves growing near or along rice fields and streams when they are not actively seeking hosts or oviposition habitats. Typical plants were rice near permanent water or subject to regular irrigation, or sweet potatoes, vegetables, shrubs and weeds around human residences and livestock sheds. Other potential outdoor resting sites were soil cave and ravines with damp, dark and humid environments (He et al., 1962). Most *An. sinensis* rested at heights of 5–15 cm above the ground on the trunk of a plant or under leaves.

The habitats for *An. sinensis* varied among the different regions, and were under the influence of the local biotope. There was evidence for various habitats with diverse geography. For instance, *An. sinensis* on Hainan Island tended to rest on the grass or other nearby vegetation after a blood meal and disperse before dawn, perhaps seeking more secluded places to rest. *An. sinensis* in Guangdong, Guangxi, and Shanghai were more likely to stay in structure for a period after feeding, usually a human house or cow shed (Liu, 1962). This difference may be the result of different natural environments in these regions. For example, Hainan Island has a tropical climate and dense vegetation. In contrast, there is relatively less vegetation in other regions but they have more buildings with solid walls. The perennial impact of the external environment may have altered feeding habits.

The factors affecting *An. sinensis* habitat were more complicated. Despite clear evidence that the longitude and latitude have an influence on *An. sinensis* habitats, other factors such as temperature, rainfall, human activities are also involved. Human and animal activity seemed to play a critical role in habitat selection. Local residents sleeping outdoors, degree of vegetation cover, and grazing habits of livestock were all important in habitat preferences of *An. sinensis*.

Biting Habits and Feeding Preferences

Female *An. sinensis* fed throughout the night but were most active from sunset to midnight. Peaking feeding activity apparently occurred at different times depending on locality and habitats. Cai (1959) showed that *An. sinensis* in human dwellings fed from dusk to dawn, with two peaks one between 8:00 pm and 9:00 pm and another at 1:00 am. In cow sheds, only one peak occurred at 8:00 pm. Chen et al. (1979) observed peak activity in Henan province occurring between 8:00 pm to 9:00 pm. Peak activity in Zhejiang province was from 9:00 pm to 10:00 pm (Zhang et al., 1990). Similar peak activity has been recorded in several other areas of China and during different time periods. Considering that these data were somewhat dated, we examined the most recent studies conducted between 2013 and 2014 in Hunan Province (Zhu et al., 2015) and in 2012 in four counties in Yunnan Province (Zhang et al., 2015). By contrast, although the results differed in the bait method, selected localities, weather conditions and other factors, the similar peak activity, either one peak after the sunset (between 8:00 pm and 10:00 pm) or with a second peak before dawn (between 1:00 am and 2:00 am) could be observed in different localities.

Under normal circumstances, *An. sinensis* females are facultative feeders but relatively more zoophilic. *An. sinensis* preferred to feed on livestock in the presence of both humans and their preferred animal hosts (buffalo and cattle), and they were prone to be found inside livestock corrals. Proportion tests on blood meals to detect feeding preferences revealed that most *An. sinensis* were zoophilic. However, the females also fed on the blood of whatever vertebrates are available in the

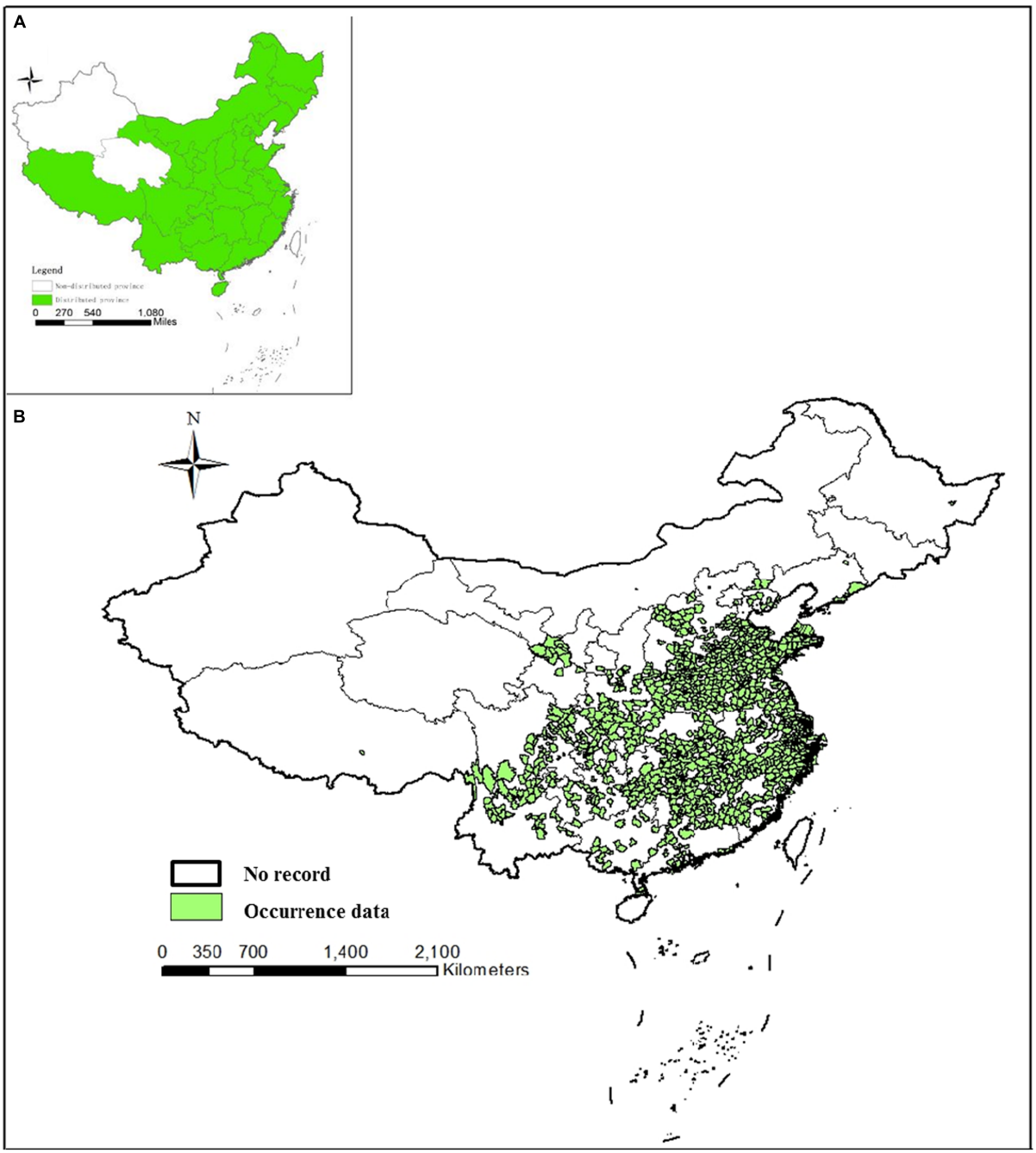

FIGURE 3 | Distribution maps of *An. sinensis* in China. **(A)** Distribution of *An. sinensis* at province level. **(B)** Distribution of *An. sinensis* at county level based on occurrence data. Points have been georeferenced and digitized from publication maps using current departmental base map of China.

vicinity, and sometimes they readily fed on humans. *An. sinensis* mostly feed on large animals such as cows, buffalos, pigs, horses, donkeys, mules, and goats. Among them, cows were the most attractive animal. Two studies (Malaria Group, 1984; Qian et al., 1984) between 1979 and 1981 showed that the human blood proportion was 3.52 and 2.60% respectively in captured *An. sinensis*. Hu et al. (1988) also found a low percentage of human blood (2.90%) in *An. sinensis* compared with cow blood. So *An. sinensis*is is facultatively anthropophilic but prefers to be zoophilic.

Vector Competence

Malaria parasites must undergo development within the mosquito before they are infectious to humans. *An. sinensis* is a *P. vivax* malaria vector in China, Indonesia, Japan, and South Korea (Rueda et al., 2010). In China, Wang et al.

(1982) and Qu et al. (2000c) studied *P. vivax* development in *An. sinensis*. Within 15–20 min after mosquito ingestion, the male (microgametocytes) and female (macrogametocytes) gametocytes could be detected in the mosquito's midgut. The fertilization event produced a zygote, then developed into an ookinete in as short as 50 min and fully developed within 26 h. The ookinete traversed the peritrophic membrane of the midgut, crossing the midgut epithelium, entered the basil lamina, and formed an oocyst in 48 h. Over a period of 3–7 days, after oocyst maturation was completed, the oocyst ruptured to release multiple immature sporozoites into the haemolymph. The sporozoites then migrated to the salivary glands in 7–8 days. The *Plasmodium vivax* cycle begins again when the female mosquito takes a blood meal, injecting the sporozoites from its salivary glands into the human bloodstream.

Vector competence is an indication of mosquitoes ability to obtain a disease agent (microorganism, such as parasite, arbovirus etc.) from a reservoir host and then transmit the infectious agent to another susceptible host (Min et al., 2002). Vector competence for malaria is evaluated by the susceptibility of Anopheline species to malaria parasites and the ability to transmit a susceptible host (Beier, 1998). Generally, malaria vector competence is determined through either observation of sporozoites in the salivary glands of field-caught mosquitoes or infection experiments using laboratory reared mosquitoes. *An. sinensis* natural infection has been extensively studied (Anonymous, 1973a, 1975; Gou et al., 1998; Zhou et al., 2005) and had sporozoite rates ranging from 0.00 to 0.33% in the salivary glands under microscope. A null sporozoite rate was detected in Hunan, Guangxi, and Shanghai (Malaria Group, 1984; Wang et al., 1987, 1990), a 0.16% rate was found in Pucheng county, Fujian province (Xu et al., 1990), and 0.33% in Xuzhou, Jiangsu province (Qiu and Zhao, 1978). *An. sinensis* is a confirmed malaria vector in China and has been reported naturally infected with malaria parasites in many provinces. However, the results of published studies by dissecting the salivary gland of field *An. sinensis* only reported the positive sporozoite rates, but failed to identify the Plasmodium species until the sporozoite ELISA kit was applied.

Experimental infection of *An. sinensis* against *P. vivax* has been done in different provinces at different times. Hu et al. (1981) found that *An. sinensis* oocyst rates (23.66%) were significantly higher than sporozoite rates (6.21%) in Shandong province and determined that 25–28°C was the best temperature range for *P. vivax* sporozoite multiplication within *An. sinensis*. Wang et al. (1982) found much higher *P. vivax* oocyst rates (38.10–95.90%) and sporozoite rates (21.90–50.80%) under experimental condition both in two *An. sinensis* strains in Henan province. Xiangkun et al. (1991) observed the highest *P. vivax* oocyst rates and sporozoite rates in the *An. sinensis* Guizhou strain (81.22–100.00%; 86.36–100.00%) and the Shanghai strain (85.71–100.00%; 91.67–100.00%) under experimental infection respectively. These findings suggest that there can be a large difference in the *An. sinensis* experimental infection rate depending on both the strain of mosquito and the parasite.

The susceptibility of different *An. sinensis* strains to a single *P. vivax* strain, together with the susceptibility of a single *An. sinensis* strain to different *P. vivax* strains was studied by Zheng et al. (1986), Xu and Ye (1987), and Zhu (1989). Xu et al. (1987) reported that the susceptibilities of Shanghai and Guangxi *An. sinensis* strains against the Guangxi *P. vivax* strain were low (0.00% and 12.3%), but significantly higher against Hainan *P. vivax* strain (36.40% and 43.60%). Zhu (1989) observed that the susceptibility of the Simao *An. sinensis* strain against *P. vivax* was significantly lower compared to the Shanghai *An. sinensis* strain. In contrast, Zheng et al. (1986) reported similar sporozoite rates in four different *An. sinensis* strains (Changsha, Shanghai, Zhengzhou, and Wuhan strain) against two *P. vivax* strains. Vector competence may vary among different *An. sinensis* strains for different *P. vivax* strains but the reasons for this are not clear.

In addition to the mosquito infection experiments, the researchers also investigated the correlation of infection rate with the patient status (Shi et al., 1983b; Qu et al., 2000c), parasite density in donor blood (Zheng et al., 1986), long incubation period strains (Xu and Ye, 1987), course of the disease (Zheng et al., 1986; Xu and Ye, 1987), and relapse or recrudesce (Zhu, 1993; Zhu et al., 2007, 2013), etc. Shi et al. (1983b) showed that *An. sinensis* sporozoite rate of the long incubation period *P. vivax* strain was higher (61.0%) than that in short incubation period *P. vivax* strain (33.3%), and infection rate of *An. sinensis* was correlated with the density and sex ratio of gametocytes. Li et al. (1998) reported that infection rate of *An. sinensis* for *P. vivax* under experimental conditions increased with parasite density and stage of the disease. *An. sinensis* showed a high infection rate in relapse or recrudesce malaria cases. Li et al. (1998) and Zhu et al. (2007) both reported infection rate and oocyst rates of *An. sinensis* to *P. vivax*. The blood resources from patients in the fever stage were lower than those in non-fever stage patients. Interestingly, Zhu et al. (2007) found that sporozoite rates were higher in the *An. sinensis* group fed on fever stage patients in the same study. However, in another study, Zhu et al. (2013) reported that the gametocytemia, asexual parasitemia, and ratio of macrogametocytes to microgametocytes, did not correlate with either oocyst or sporozoite infection, while in the oocyst-positive mosquitoes, there was a correlation between gametocytemia and the average oocyst number.

Anopheles sinensis was refractory to *P. falciparum* in Thailand (Rongsriyam et al., 1998). How about this situation in China? *An. sinensis* susceptibility to *P. vivax* versus *P. falciparum* in Guangxi province showed average infection rates of 59.80% (oocyst rate) and 12.80% (sporozoite rate) compared to no infection with *P. falciparum* (Xu et al., 1983). However, studies conducted by Shi et al. (1983a) and Liu et al. (1984) indicated that *An. sinensis* could be infected with *P. falciparum* but at a relatively low rate. Shi et al. (1983a) found that *P. falciparum* oocyst rates ranged from 2.14 to 8.6%, and sporozoite rates ranged from 0.00 to 3.54%. Liu et al. (1984) observed an average *P. falciparum* oocyst rate of 11.30% and average sporozoite rate of 3.00%. These provide evidence that *An. sinensis* in China is not totally refractory to *P. falciparum*. Vector competence of *An. sinensis* is thought to be significantly higher for *P. vivax* compared to *P. falciparum*.

In summary, *An. sinensis* infection experiments showed variable sporozoite infection rates with *P. vivax* (6.21–100.00%) and *P. falciparum* (0.00–3.54%). The presence of a natural infection sporozoite rate together with the relatively high value of both oocyst and sporozoite rates under laboratory infection experiments supports the potentially high vector competence for *P. vivax*. Although *An. sinensis* could be infected by *P. falciparum* in some studies, dissection of salivary glands in field *An. sinensis* failed to reveal the presence of *P. falciparum* sporozoites. These data support the conclusion that *An. sinensis* is not a vector for *P. falciparum* malaria in China.

Vectorial Capacity

Vectorial capacity is a measurement of the efficiency of vector-borne disease transmission. In addition to competence, vector capacity includes a variety of other essential traits, i.e., host preferences, biting rate, mosquito population density, longevity, and etc. The malaria transmission intensity can be characterized as the vectorial capacity (C) formulated by Macdonald (1957) and Garrett-Jones (1964), which is defined as:

$$C = \frac{m\alpha^2 p^n}{-\ln(p)}$$

where *ma* is the human biting rate (the number of female mosquitoes per person per night); *a* is the human-biting frequency per day (daily probability of a human host being bit by a vector, which is indirectly estimated as the human blood index divided by duration of gonotrophic cycle; *n* is the length of the sporogonic cycle; *p* is the daily survival rate of the vector.

In China, the *An. sinensis* vector capacity was measured and compared by investigating of *An. sinensis* at different times and in different provinces (**Table 1**). The first and most recent reports of *An. sinensis* capacity were published in 1983 and 2015, the results showed that VC was 0.24 and 0.15 respectively (Song, 2015). The vector capacities of *An. sinensis* varied from north to south in China, and were correlated with the season (Yao et al., 2002). Pan et al. (2008) founded that the vector capacity of *An. sinensis* in Huizhou and Zhuhai, Guangdong province was 0.25 and 0.66, respectively. Zhao et al. (1996) and Yu et al. (2014) reported that the vector capacities of *An. sinensis* were 0.01 and 0.15 in Sichuan and Shandong province (north), respectively. Differences observed in the vectorial capacity of *An. sinensis* may be due to variation in ecological habits or genetic background in different localities. The overall low vectorial capacity of *An. sinensis* may be diminished by tendencies for zoophilic feeding behavior in some study sites. In addition, vectorial capacity of *An. sinensis* could also serve as an indicator for annual parasite index (API) or malaria prevalence (Yang and Tian, 1994; Liu et al., 1995; Qian et al., 1996).

Vectorial capacity includes a number of factors. Human blood index (HBI) and human biting rate (*ma*) are important indicators for vector capacity, and would determine the capacity to transmit malaria protozoa. Two studies reported that the vector capacity of *An. sinensis* was highest in July or August corresponding to the highest human biting rate (Station, 1985; Yao et al., 2002). Another study conducted by Qian et al. (1996) compared the vector capacity of *An. sinensis* between the 1970s and the 1990s, and found a significant decrease both in human biting rate and vector capacity. In other studies conducted in different provinces a positive correlation between the HBI, human biting rate and vector capacity (Liu et al., 1995; Zhang X. et al., 2006).

Other indicators such as duration of the gonotrophic cycle (GC), length of the sporogonic cycle (n), and the daily survival rate of the vector (*p*) generally have stable values corresponding to the mosquito species. In different studies, the gonotrophic cycle of *An. sinensis* in China was 2.5–3.7 days, the length of the sporogonic cycle was 8.5–14.8 days, and the daily survival rate of the vector was 0.77–0.91. Given that the *P. vivax* incubation time in *An. sinensis* was of 9–14 days, *An. sinensis* would require two to three blood meals before becoming infectious agents. In addition, calculation of daily survival rate requires introduction of another parameter–the parity rate. Studies by Gao et al. (2004) found the mean parity rates of *An. sinensis* samples collected over years from Hunan province to be 42.86%. The parity rates varied in different habitats, months, indoor and outdoor environments, daytime and night, as well as before midnight and after midnight (Wang, 2013). This variation may be attributed to numbers of emerging mosquito, the mortality rate of larvae and sampling methods (Qian et al., 1984).

The vectorial capacity measures efficiency of pathogen transmission, but EIR is a measure of malaria transmission intensity. It is usually interpreted as the number of infectious bites per human during a season or annually (usually annually) and is referred as a more direct way to measure transmission risk. However, although studies (Anonymous, 1975; Liu et al., 1984; Liu et al., 1986; Zhang et al., 1989) have provided estimates of EIR values ranging from 0.000031 to 0.24 (**Table 1**), which is conventionally computed by taking the product of biting rate multiplied by the sporozoite rate. The data still made it difficult to investigate the accuracy and precision of EIR because sporozoite rates were exceedingly low (<0.19%), while appropriate values ranging from 1 to 20% are generally suggested for EIR determination in Africa.

MOLECULAR BIOLOGY

Molecules Revealed by Genome and Transcriptome

Scientists have unveiled the genomes of two *An. sinensis* strains from Korea[1] and China (Zhou et al., 2014). The genome of *An. sinensis* (China strain) was fully sequenced in 2014. *An. sinensis* has six chromosomes representing two three-chromosome sets. The genome size was estimated 220.78 Mb, coding for 16, 766 genes. Approximately 14% of the putative genes were orthologous with genes in 235 known biological pathways. The genome had 3, 972 gene clusters containing 11,300 genes that were common to the genomes of three previously sequenced mosquito species, *An. gambiae*, *An. aegypti*, and *Cu. quinquefasciatus*. Gene orthology prediction revealed a total of 4,727 orthologous genes were shared among the mosquitoes (Zhou et al., 2014). Analysis of

[1]https://www.ncbi.nlm.nih.gov/assembly/GCA_000472065.2/

these orthologous genes revealed the most gene-enriched domain and family were peptidase, while KEGG pathway revealed that genes were most enriched in metabolic pathways. The results indicate that functions such as feeding behavior are central to mosquito biology. In addition to protein-coding genes, 41 microRNA, 348 tRNA and 2017 rRNA genes have also been identified.

A comprehensive reference transcriptome of *An. sinensis* sampled from different developmental stages of egg, larva, pupa, and adult was sequenced by Chen et al. (2014). Approximately 51.6 million clean reads were obtained, and these were assembled into 38,504 unigenes. Among them, 98.4% (37,884/38,504) of unigenes could be mapped onto the *An. sinensis* reference genome, and 69% (26,650) of the unigenes could be annotated with known biological functions. In addition, a total of 8,057 expressed sequence tags (ESTs) were assigned to GO and KEGG annotation. A large number of ESTs were restricted to metabolic pathways, biosynthesis of secondary metabolites, and microbial metabolism. The study also found that 2,131 ESTs were differentially expressed between deltamethrin resistant and deltamethrin susceptible mosquitoes collected from the same field site. Further, more than 2,400 microsatellite markers have also been identified (Zhu et al., 2014). These studies would definitely enhance knowledge on the *An. sinensis* and lay an

TABLE 1 | Vectorial capacity (VC) and entomological inoculation rate (EIR) of *An. sinensis* in China.

Reference	Location	*ma*	HBI	GC	*a* (HBI/GC)	*n*	*p*	*C*	SR (%)	EIR (*ma** SR*coefficient)
Anonymous, 1975	Shangqiu, Henan	13.80	NA	3.00	NA	10.50	0.85	NA	0.09	0.24
Liu et al., 1983	Zhejiang	6.90	NA	NA	NA	NA	0.85	0.24	NA	NA
Liu et al., 1984	Shucheng, Anhui	NA	NA	NA	NA	NA	NA	NA	0.19	0.00
Qian et al., 1984	Pixian, Jiangsu	NA	0.10	2.50	0.04	NA	0.86–0.88	NA	NA	NA
Station, 1985	Guidong, Hunan	0–77.25	NA	NA	NA	NA	NA	0–2.03	NA	NA
Hu, 1986	Yunnan	NA	NA	NA	NA	NA	NA	0.01	NA	NA
Liu et al., 1986	Junlian, Sichuan	0.41	0.05	2.50	0.02	10.00	0.88	0.02	0.00	0.00
Wang et al., 1987	Gunagxi	0.03–2.02	NA	NA	0.02–0.05	NA	NA	0.00–0.26	NA	NA
Xu et al., 1987	Fujian	8.10	0.08	NA	0.03	9.80	NA	0.19	NA	NA
Mo et al., 1988	Gunagxi	13.60	0.30	NA	NA	NA	NA	0.08	0.19	NA
		0.80	0.02					0.02	0.00	
Xu L. et al., 1988	Fujian	8.10	NA	NA	0.03	9.80	NA	0.28	NA	NA
Zhang et al., 1989	Yunnan	1.40	0.05	2.50	0.02	NA	0.87	0.03	0.00	0.00
Wang et al., 1990	Shanghai	9.14	0.06	2.00	0.03	NA	0.86	0.35	0.00	NA
Xu et al., 1990	Fujian	8.10	NA	2.50	0.03	9.80	0.84	0.26	0.16	NA
Ouyang et al., 1992	Hunan	NA	NA	NA	NA	NA	NA	0–2.7	NA	NA
Yang et al., 1994	Yanhe, Guizhou	32.90–76.50	NA	3.00	0.04	NA	NA	0.42–14.94	NA	NA
Liu et al., 1995	Ganyu, Jiangsu	0.25–2.38	NA	NA	0.00–0.03	NA	0.85–0.90	0.00–0.12	NA	NA
Nie et al., 1996	Longli, Guizhou	2.50	0.02	NA	0.01	14.51	0.82	0.01	NA	NA
		1.31	0.02		0.01	14.82	0.85	0.01		
Shi et al., 1996	Deqing, Zhejiang	NA	0.04	2.50	0.02	8.50	0.87	0.04	NA	NA
Zhao et al., 1996	Jining, Shandong	4.07	0.13	2.50	0.05	10.00	0.82	0.15	NA	NA
Zhang and Zhang, 1997	Tengchong, Yunan	1.33	0.03	2.50	0.01	NA	0.78	0.00	0.00	NA
Lang et al., 2000	Taizhou, Zhejiang	3.08	NA	3.67	0.01	11.39	0.79	0.01	NA	NA
Feng et al., 2002	Qionglai, Sichuan	0.12	NA	NA	0.02	NA	NA	0.01	NA	NA
Yao et al., 2002	Haining, Zhejiang	9.85	NA	3.70	0.01	9.50	0.81	0.08	NA	NA
Gao et al., 2004	Changzhou, Jiangsu	NA	0.00	2.00	NA	NA	NA	0.00	NA	NA
Luo et al., 2006	Yiwu, Zhejiang	15.36	NA	3.67	0.02	8.54	0.87	0.73	NA	NA
Zhang X. et al., 2006	Wenzhou, Zhejiang	7.24	0.06	NA	0.02	NA	NA	0.22	NA	NA
Wang et al., 2007	Mengcheng, Anhui	11.19	0.18	2.50	0.07	9.79	0.83	0.15	NA	NA
Li et al., 2008	Fenghua, Zhengjiang	2.87	0.04	2.50	0.02	10.84	0.91	0.18	NA	NA
Pan et al., 2008	Huizhou, Guangdong	5.01	0.48	NA	0.13	NA	NA	0.25	NA	NA
	Zhuhai, Guangdong	4.31	0.44		0.12			0.66		
Pan et al., 2012	Huaiyuan, Anhui	6.10	0.67	2.50	0.27	10.00	NA	0.77	NA	NA
	Yongcheng, Henan	5.90	0.64	2.50	0.26	10.00		0.55		
Yang et al., 2013	Shuangliu, Sichuan	1.93	0.05	3.50	NA	NA	NA	0.02	NA	NA
Yu et al., 2014	Chengdu, Sichuan	1.20	0.05	3.50	NA	NA	0.85	0.01	NA	NA
Song, 2015	Xiangshan, Zhejiang	2.64	NA	3.67	0.03	10.41	0.88	0.12	NA	NA

ma, Human biting rate; HBI, Human blood index; GC, Gonotrophic cycle; SR, Sporozoite rate; EIR, entomological inoculation rate; NA, not available.

important foundation for further functional analysis so as to provide new tools for future malaria vectors control.

Molecules Identified by Experiments

A compendium of molecules are involved the interaction of the Plasmodium parasites within their host vectors (Sreenivasamurthy et al., 2013), however, little is known about the analogous process in *An. sinensis*. The first gene of *An. sinensis* was studied by Zhi et al. (2002) who attempted to clone the defensin gene (immune gene) from the main mosquito vectors of China. Sequence analysis showed that the amplified fragments from *An. sinensis* were homologous to the reported defensin sequence of *An. gambiae*. Since then, newer research has focused on cloning the defensin gene, prokaryotic expression and the product activity (Yan et al., 2005; Zheng et al., 2005). The complete defensin gene was cloned by Zhang Y.J. et al. (2006). It has a total length of 2,256 bp, including the 5′ and 3′ UTR fractions and two exons separated by an 85 bp intron. The entire cDNA sequence of *An. sinensis* defensin gene was 324 bp; its ORF encoded 107 amino acids, and mature peptide had 40 amino acids residues. Then, another immune gene, partial SRPN14 gene of *An. sinensis*, was identified and characterized (Feng et al., 2015). The SRPN14 gene was found to be located on 2L: 23C of salivary gland chromosomes of *An. sinensis* by *in situ* hybridization, which had 77% (nt) and 88% (aa) similarities with *An. gambiae*. Several mosquito proteins have been involved in regulating the maturation of malaria parasites. For example, HSP40 was isolated from *P. yoelii* infected *An. sinensis* and its full-length cDNA of 1,159 bp with an ORF of 1,014 bp, encoding 337 amino acids was amplified (Li Y. et al., 2010). An analysis of gene expression throughout development has been conducted for *An. gambiae* (Strode et al., 2006). In *An. sinensis*, several important homologous genes have been identified (**Table 2**). Liu B.Q. et al. (2016) found that there were four CPF (cuticular protein family) gene families in *An. sinensis* genome. Analysis of these CPFs in *An. sinensis* revealed that the full-length cDNA sequences ranged from 531 to 1,001 bp and coded for 148 to 345 amino acids. These AsCPFs contained a 44-amino-acid conserved region and a C-terminal region, which were secretory proteins with signal peptide sequences except for AsCPF2 that was a non-secretory protein and lacked a signal peptide sequence but contained a transmembrane region. Qiao et al. (2016) studied on the role of expression and regulation of TH (the initial enzyme in the melanin pathway) on specific physiological processes during mosquito development by silencing of AsTH. Significant disruption of cuticle in experiments strongly suggested that TH was essential for pupae tanning and immunity in *Anopheles*.

In addition, AQPs (water channels) are integral membrane proteins in biological cells. Some AQPs were abundantly expressed in Malpighian tubules of *An. gambiae*, and reduced expression could increase mosquito survival in dry environments (Liu K. et al., 2016). Tang et al. (2012) identified a full-length cDNA of AQP from *An. sinensis*, which consisted of 762 bp coding for 253 amino acids, with a predicted molecular mass of 63.2 kD. The AsAQP shared 76 and 78% identities with AQPs of *Cu. quinquefasciatus* and *Ae. aegypti* AQPs, respectively. To analyze the differential expression of iron responsive element binding protein genes in different strains of *An. sinensis,* Wang et al. (2015) identified partial sequences of IRE_BP1 by two-dimensional electrophoresis followed by corresponding PCR applications. They found that the IRE_BP1 gene expression in resistant *An. sinensis* strains was 9.42 times than that of susceptible strains by fluorescence quantitative PCR. The IRE_BP1 may be useful as a resistance control target and gene detection indicator.

The recently published *An. sinensis* genome and transcriptome provide an opportunity for advanced study of gene products. Based on the comparison between OBP conserved motifs with *An. gambiae*, He et al. (2016) identified 64 putative odorant-binding protein genes (OBP) in the genome of *An. sinensis*. The authors also investigated motif conservation, gene structure, genomic organization and classification. *An. sinensis* OBP genes were classified into three subfamilies, some genes might have originated from a single gene through a series of historic duplication events. Zhou et al. (2015) found 174 detoxification genes by analyzing *An. sinensis* genome, including 93 cytochrome P450s (P450s), 31 GSTs, and 50 choline/carboxylesterases (CCEs). Based on a combination analysis of available *An. sinensis* transcriptome, several candidate genes overexpressed in a deltamethrin-resistant strain were identified as belonging to the CYP4 or CYP6 family of P450s and the Delta GST class. Che et al. (2014) identified CYP6P5 gene in *An. sinensis* and analyzed its bioinformatics characteristics. The entire sequence was 1, 583 bp long with an ORF of 1, 527 bp, encoding 508 amino acids. Phylogenetic and similarity analyses of amino acid sequences showed that *An. sinensis* CYP6P5 had the closest phylogenetic relationship to the CYP6P5s of *An. funestus* and *An. gambiae*, with similarity values of 89.4 and 89.0%, respectively.

Although, the current literature on molecular biology was systematically reviewed, significant gaps in knowledge of *An. sinensis* basic biology remain. For instance, recent advances in molecular biology have resulted in development of a genetically modified mosquito species for the purpose of population control (Lounibos, 2004; Christophides, 2005). More inspiringly, mosquito gut microbiota have emerged as a novel target to modulate homeostasis in greater depth and to develop new paradigms for disease transmission control (Rodgers et al., 2017). Therefore, studies on vectors and their molecular biology remain important to develop possible applications for more effective vector control.

DISCUSSION

This report updates available knowledge on *An. sinensis* and characterizes biology, bionomics and molecules research in China through an extensive review gathered in the country from 1954 to 2016. Most of the literatures on biology and bionomics were during the early time of high incidence periods in China. In contrast, there was little recent entomology research focused on the ecology and life history study in recent years. Research conducted before 1970 was generally limited and sporadic.

Entomological research increased following two events. One event occurred in 1985 and it was the start of a nationwide malaria control program activated by two decades of outbreak and pandemic transmission between 1960 and 1979 (Yin et al., 2014). Another event started in 2003 after GFATM began to support malaria control campaign in the People's Republic of China (Wang et al., 2014). The first molecular study on *An. sinensis* was published in 2002, but there are still few publications on gene functions and molecular mechanisms, despite a large numbers of molecules being found using omics tools.

A detailed summary of biology information on *An. sinensis* was presented and represents the accumulation of many years' studies in China. Before molecular tools were developed, the identification of *An. sinensis* and its sibling species depended on morphological characteristics (Reidenbach et al., 2009), which were sometimes undependable and likely to misidentify sibling species. For instance, a narrow-ovate form of *An. sinensis* was first described as a new species but later identified as *Anopheles lesteri* (Makhawi et al., 2013). PCR assays based on molecular markers have been developed to accurately distinguish in this species complex (Fanello et al., 2002) in 1990s. *An. sinensis* has been successfully identified from *Anopheles hyrcanus* complex based on specific DNA nucleotide differences in the sequences of the second internal transcribed spacer (ITS2) of the ribosomal DNA (rDNA) (Ma and Xu, 2005). Morphological characteristics together with nucleotide differences have enabled accurate identification of *An. sinensis* from *Anopheles hyrcanus* complex, which is essential for more precise knowledge of this species.

Anopheles sinensis has a complex life cycle. Current knowledge of the *An. sinensis* life cycle is basically complete and sustained by extensive field investigations and laboratory rearing experiments. Most egg laying of *An. sinensis* occurs at the night following digestion of a blood meal, however, exceptions to this behavior have also been noted. For example, egg laying is sometimes observed in the daytime (Yusheng and Xingbang, 1958). The timing and amounts of oviposition depend on local conditions, blood-feeding, and the season. Various experimental factors, such as photoperiod, substrate and bacteria, were also involved (Zhao et al., 1991; Pan et al., 1993; Li and Tang, 2010). Oviposition time and location may have important consequences for vector population dynamics and epidemiology.

Knowledge of the different stages of *An. sinensis* should enhance the efficacy of control strategies. *An. sinensis* larvae lie horizontally at the water surface and feed on microorganisms, algae, protozoa, and organic detritus. The rate of larval development is temperature dependent and correlated to the time required for embryogenesis and hatch. The pupa stage usually lasted about 2 days, and this may be extended or shortened related to temperature. The survival rate of *An. sinensis* pupae was highest compared to the other development stages. There was a difference between male and female sexual maturity at the time of emergence. Males usually emerge 1∼2 days before the females. *An. sinensis* has a limited flight range of 1 km; the species tend to stay near their breeding sites. Marked-release-recapture experiments indicate that they normally do not migrate long distances.

Studies on male and female *An. sinensis* life expectancy among different geographical strains indicated that male life expectancy was shorter than female. These results were consistent with the results of Suleman's and Suman's on *Cu. quinquefasciatus*. Shorter male life span may be a kind of biological rule for male and female mosquitoes. High temperatures and low humidity can also reduce *An. sinensis* longevity while sufficient food supply can extend the longevity, a result that is especially obvious under experimental conditions. *An. sinensis* overwinter as adult females. Usually, the females use the lipid reserves for overwintering survival in protected shelters when the temperature is less than 10°C.

Several studies described the complexity of *P. vivax* development in *An. sinensis*. The *P. vivax* gametocytes completed fertilization soon after mosquito blood-feeding on an infected host. They transformed to ookinetes and oocysts, and began to invade the salivary glands at 7∼8 days. Under experimental

TABLE 2 | dentified published molecules of *An. sinensis* in China.

Molecules	Accession number	Functions involved	Reference
Defensin	DQ002892	Immunity	Zhi et al., 2002; Yan et al., 2005; Zheng et al., 2005; Zhang Y.J. et al., 2006
Aquaporin (AQP)	NA	Biological process	Tang et al., 2012
CYP6P5	KF358704	NA	Che et al., 2014
CYP4G17	NA	NA	Yan et al., 2015
SRPN14	NA	Immunity	Feng et al., 2015
Detoxification supergene families	NA	Insecticide resistance	Zhou et al., 2015
Haem oxygenase (HO-1)	NA	NA	Zhi et al., 2015
Tyrosine hydroxylase (TH)	NA	Immunity/Biological process	Qiao et al., 2016
Odorant-binding protein (OBP)	NA	Biological process	Qin et al., 2014; He et al., 2016
Iron responsive element binding protein 1 (IRE_BP1)	NA	Insecticide resistance	Wang et al., 2015
Cuticular protein family (CPF)	NA	Biological process	Liu B.Q. et al., 2016
Heat shock protein 40 (HSP40)	HM013840	Immunity/molecular chaperones	Li Y. et al., 2010

NA, not available.

conditions, the sporozoite rates were lower than the oocyst rates (Wang et al., 1982; Qu et al., 2000c). Sporozoite rates were positively correlated with oocyst rates, and different *An. sinensis* strains showed different sporozoite rates and oocyst rates (Wang et al., 1982). The mean duration of the gonotrophic cycle for *An. sinensis* was 2.6 days. Assuming a *P. vivax* incubation time in mosquitoes of 7–8 days, *An. sinensis* would require three to four blood meals before being capable of transmitting sporozoites.

In the context of malaria elimination, the value of understanding the bionomics of *An. sinensis* and its role on malaria transmission is critical for malaria reduction. Distribution maps of *An. sinensis* in China were produced by combining current knowledge of *An. sinensis* distribution, and occurrence data from open access, and published papers. The occurrence data resulted in the collation of *An. sinensis* occurrence from 343 records across 29 provinces. The map was limited by the available data and bias sampling on original data acquisition. The maps can still serve as an accurate representation of the ranges of *An. sinensis* in China. However, to increase knowledge of the biology and bionomics of the mosquitoe in China, it is important to extend entomological surveys to unexplored northern regions which may represent its northern distribution boundary. A better understanding of the full distribution range of *An. sinensis* and maps which could predict the distribution trend by combining climate change models is highly desirable.

Field studies on larval presence of in water bodies indicate that *An. sinensis* can select a wide range of water bodies in which to lay their eggs. These studies implied that larvae habitats were heterogeneous in form, space, and physical features. Indoor and outdoor habitats for adult *An. sinensis* were also very heterogeneous. This could be partially explained by the differences in the investigation periods and the methods used in *An. sinensis* collection, such as larval collection tools, light traps, and human baited trap.

Adult *An. sinensis* have been incriminated as a natural and experimental malaria (*P. vivax*) vector in China, Indonesia, Japan, and South Korea (Ree, 2005). The known published data on *An. sinensis* host selection in China indicated that the degree of anthropophily of *An. sinensis* was low, and it was generally concluded that *An. sinensis* is not an effective malaria vector, especially in northern China. However, since 1960, uninterrupted malaria cases have occurred in central China, where *An. sinensis* is the only vector in most areas, thus it is difficult to explain why *An. sinensis*is appears to be an effective malaria vector in these areas. According to a study in Henan province, *An. sinensis* not only fed on human blood, but the biting frequency was higher and natural infection of *P. vavix* was also present. The evidence indicated that *An. sinensis* served as the major vector for endemic malaria in these regions. This suggests that malaria transmission competence differs from south to north, and this is also supported by variable vector capacity. It was concluded that the vivax malaria transmission ability of *An. sinensis* has probably been underestimated in central China (Pan et al., 2012). In addition, the competence of *An. sinensis* (laboratory strain) to *P. vivax* was similar to *An. anthropophagus* when evaluated by a membrane feeding assay under experimental conditions (Zhu et al., 2013). The overall low vectorial capacity of *An. sinensis* may be related to tendencies for zoophilic feeding behavior in some study sites. Interestingly, the enhancement in vector capacity of *An. sinensis* was attributed to the local resident habits and the decline in the number of large livestock leading to the reduction of the traditional biological barriers (Pan et al., 2012). Vector capacity of *An. sinensis* can also depend on other factors, such as the larval environment (Moller-Jacobs et al., 2014).

Different *An. sinensis* populations in China exhibit variability in morphology, chromosomes, ecology, vector capacity, mitochondrial DNA, and even entire genomic DNA composition (Feng et al., 2017). Understanding the molecular and genetic mechanisms that determine variability in transmission efficiency and mosquito susceptibility could aid in the development of novel vector control strategies. A draft genome and transcriptome are currently available and many genes, including small RNA genes have also been identified. However, only 12 genes have been experimentally studied. This is a huge knowledge gap compared to the detailed analysis of variation in gene expression throughout development or related to pathogen infections which have been studied in other mosquito species. Approximately 25% of the genes showed a complex pattern of changes in gene expression during the different life stages of *An. gambiae* (Strode et al., 2006), and a total of 94 molecules pertaining to parasite infection have been validated (Sreenivasamurthy et al., 2013). Attention also should be focused on the new research directions such as gut-microbiome–parasite interactions, genetics of mosquito behavior, epigenetics and non-coding RNA. Identified molecules will provide useful tools for further functional analysis of the genetic, ecological and immune aspects. Determining the interactions and employing these as efficient resources for malaria intervention will require more research.

As China moves toward malaria elimination, it will be necessary to continuously update and summarize *An. sinensis* vector research. Climate and environmental landscapes continue to change and appropriate entomological surveillance and evaluation will alert researchers to biological and bionomic changes. The value of applying new tools generated from molecular studies of *An. sinensis* malaria transmission could be highly significant.

CONCLUSION

This review provides current information on biology, bionomics, and molecules relevant to *An. sinensis* in China. Traditional research has provided a wealth of information on *An. sinensis* biology and bionomics. However, there is a lack of quantitative information required to characterize mechanisms of physiology and developmental biology and interactions with parasites. Future studies should fill these knowledge gaps. An integrated understanding of biology, bionomics, and molecules may yield more effective control strategies to facilitate malaria elimination in China.

AUTHOR CONTRIBUTIONS

XF, ShaZ, WH, and ShuZ were involved in the conception of the idea. XF and ShaZ conducted the literature searches. XF and WH participated in writing the final manuscript. ShuZ, FH, LZ, JF, HZ, and ZX reviewed and analyzed the documents. All authors read and approved the final manuscript.

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Malaria Journal

RESEARCH

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Receptivity to malaria in the China–Myanmar border in Yingjiang County, Yunnan Province, China

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Abstract

Background: The re-establishment of malaria has become an important public health issue in and out of China, and receptivity to this disease is key to its re-emergence. Yingjiang is one of the few counties with locally acquired malaria cases in the China–Myanmar border in China. This study aimed to understand receptivity to malaria in Yingjiang County, China, from June to October 2016.

Methods: Light-traps were employed to capture the mosquitoes in 17 villages in eight towns which were categorized into four elevation levels: level 1, 0–599 m; level 2, 600–1199 m; level 3, 1200–1799 m; and level 4, > 1800 m. Species richness, diversity, dominance and evenness were used to picture the community structure. Similarity in species composition was compared between different elevation levels. Data of seasonal abundance of mosquitoes, human biting rate, density of light-trap-captured adult mosquitoes and larvae, parous rate, and height distribution (density) of *Anopheles minimus* and *Anopheles sinensis* were collected in two towns (Na Bang and Ping Yuan) each month from June to October, 2016.

Results: Over the study period, 10,053 *Anopheles* mosquitoes were collected from the eight towns, and 15 *Anopheles* species were identified, the most-common of which were *An. sinensis* (75.4%), *Anopheles kunmingensis* (15.6%), and *An. minimus* (3.5%). *Anopheles minimus* was the major malaria vector in low-elevation areas (< 600 m, i.e., Na Bang town), and *An. sinensis* in medium-elevation areas (600–1200 m, i.e., Ping Yuan town). In Na Bang, the peak human-biting rate of *An. minimus* at the inner and outer sites of the village occurred in June and August 2016, with 5/bait/night and 15/bait/night, respectively. In Ping Yuan, the peak human-biting rate of *An. sinensis* was in August, with 9/bait/night at the inner site and 21/bait/night at the outer site. The two towns exhibited seasonal abundance with high density of the two adult vectors: The peak density of *An. minimus* was in June and that of *An. sinensis* was in August. Meanwhile, the peak larval density of *An. minimus* was in July, but that of *An. sinensis* decreased during the investigation season; the slightly acidic water suited the growth of these vectors. The parous rates of *An. sinensis* and *An. minimus* were 90.46 and 93.33%, respectively.

Conclusions: The *Anopheles* community was spread across different elevation levels. Its structure was complex and stable during the entire epidemic season in low-elevation areas at the border. The high human-biting rates, adult and larval densities, and parous rates of the two *Anopheles* vectors reveal an exceedingly high receptivity to malaria in the China–Myanmar border in Yingjiang County.

Keywords: Receptivity, Community structure, *Anopheles* community, Malaria transmission, Re-establishment, China–Myanmar border

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Background

Malaria remains a significant public health problem, especially in Africa and Southeast Asia. Owing to the inception of the World Health Organization (WHO)'s Mekong Malaria Programme a decade ago, the annual malaria incidence and mortality have declined continuously in the Greater Mekong Subregion (GMS) [1–3]. However, among the GMS nations, Myanmar has the heaviest disease burden of malaria and is one of the most threatening foci of malaria in Southeast Asia [4, 5]. The border of Kachin State in Myanmar has a high incidence and mortality rate of malaria [6]. It is thus crucial to assess the risk of malaria re-establishment in this border to allow the relevant departments in the region to develop optimal elimination strategies, since China and the GMS counties aim at malaria elimination by 2020 and 2030, respectively [7].

The infectivity-receptivity-vulnerability framework is an important method to assess the risk of malaria re-establishment in many countries [8–12]. The framework defines receptivity as the presence, distribution, seasonal abundance and bionomics of the potential vector [8, 9, 12]. Control of malaria transitions depends on integrated actions [7], and according to "A framework for malaria elimination" announced by the WHO, receptivity is a key point to malaria re-emergence [13]. Considering the high cost of measuring receptivity in an area, it is systematically difficult to obtain first-hand data on receptivity in the China–Myanmar border.

In China, malaria is being rapidly eliminated [7, 14, 15], which has been mainly attributed to malaria control in the China–Myanmar border in Yunnan Province. In 2014 and 2015, Yingjiang County was one of the few counties to report malaria transmission. In 2016, it was the only county to report locally acquired malaria cases in the border. Therefore, it is specifically important to determine receptivity to malaria in this county.

In Southeast Asia, including China and Myanmar, deforestation and cultivation of cash crops (such as banana, rubber, and maize) constitute the most important environmental changes in rural areas [16–18]. For example, field investigation and interview of the local primary public health care provider revealed that the main crop of these regions was rice, which occupied approximately 2 million square kilometres in Na Bang town, Yingjiang County, before 2005. After 2005, banana was grown as the main crop in these regions. Until early 2016, the area of banana production had increased to > 3 million square kilometres, and no rice crops were left. These changes may have led to alterations in the population density, life history [19], and behaviour of vectors such as laying eggs [20]. This change in ecotope in the China–Myanmar border may have resulted to change in receptivity to malaria in the region.

The main malaria vectors in the China–Myanmar border are *Anopheles minimus* and *Anopheles sinensis* [5, 19, 21, 22]; the major vectors in China are *An. sinensis*, *Anopheles lesteri*, *Anopheles dirus*, and *An. minimus* [23, 24]. In recent years, *An. minimus* in these areas has become the focus of research. Several studies investigated the ecological features of malaria vectors, including species composition and population dynamics, density, human blood index, proportion of sporozoites, and environmental factors (e.g., land use and land cover changes) [6, 18, 19, 21]. These studies provided valuable information for generating targeted intervention strategies for malaria control and elimination along the border areas. However, the community structure of *Anopheles* mosquitoes at different elevations in the border remains unknown. Moreover, the seasonal receptivity in the county, especially the seasonality of the human-biting rate and larval density, has not been well investigated in recent years.

Therefore, this study aimed to collect mosquitoes from 17 villages in eight towns in Yingjiang County at different elevations; analyse the community structure by species richness, diversity, dominance, and evenness [6, 25–32]; and examine receptivity to major malaria vectors (*An. minimus* and *An. sinensis*) in the China–Myanmar border.

Methods

Study area

Yingjiang County (24°24′ to 25°20′N, 97°31′ to 98°16′E), located in the west of Yunnan Province, has a population of > 0.3 million, includes 15 towns and 103 villages, and has a boundary line of 214.6 km. Its climate is warm and humid at low altitudes and cold at high altitudes. The main cash crops are rice, banana, coffee, sugarcane, and maize. Buffalo, yellow cattle, pigs, and dogs are also common. This variety in climate, ecology, and environment makes the county favourable for malaria vectors.

Two towns (Na Bang and Ping Yuan) in the county were selected as sentinel sites from June to October 2016. Na Bang, bordering on Myanmar and located west of the county, has a boundary line of 20.5 km and has nine villages, with a total population of 1751. The main cash crop is banana. The town has a tropical-subtropical climate and a low elevation, with the lowest elevation of 210 m. The average annual temperature is 22.7 °C, and the average annual precipitation is 2655 mm. Ping Yuan, the capital town of the county, has a population of 53.5 thousand and has 85 villages. Its main cash crop is rice, and its elevation is 937 m.

In this study, 17 villages in eight towns were included and categorized into four levels according to the elevation of the study sites (Fig. 1): level 1, 0–599 m; level 2, 600–1199 m; level 3, 1200–1799 m; and level 4, > 1800 m (Table 1). In May and October 2016, a cross-sectional

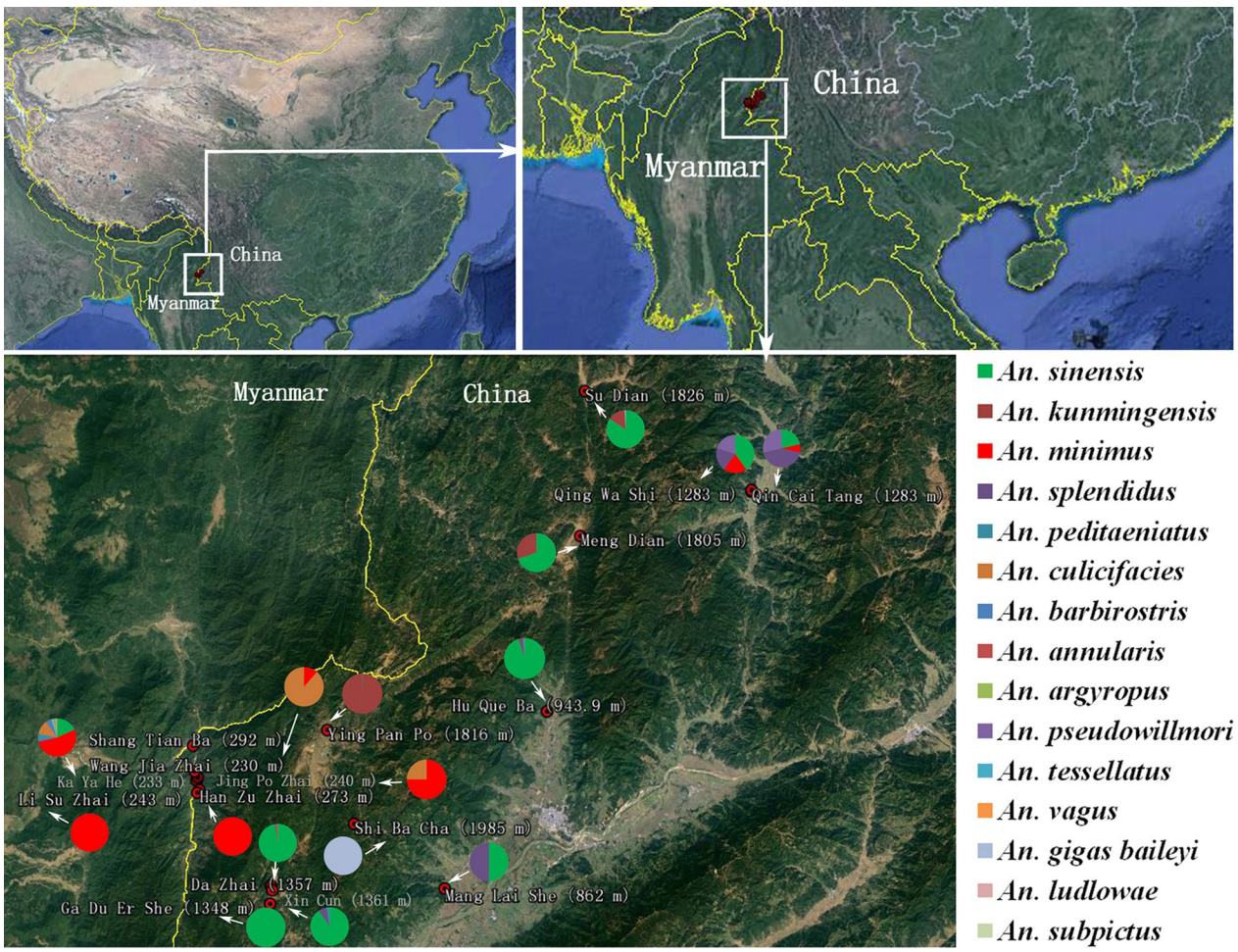

Fig. 1 Locations of study sites and a pie-chart showing *Anopheles* distribution (percentage) in Yingjiang County. (No *Anopheles* mosquito was captured in Shang Tian Ba.)

study was conducted on the community structure of *Anopheles* mosquitoes in the 17 villages. To determine the seasonal abundance of mosquitoes, the human-biting rate (*ma*) [19], density of light-trap-captured adult mosquitoes and larvae, parous rate [20], and height distribution (density) of *An. minimus* and *An. sinensis* were investigated in Na Bang and Ping Yuan each month from June to October 2016 (Table 2).

Mosquito collection and species identification

Centers for Disease Control and Prevention (CDC) light-traps without bait were used to capture mosquitoes. After transport to the laboratory, the mosquitoes were morphologically separated as *Anopheles*, *Culex*, *Aedes*, and other subfamilies or genera [6]. *Anopheles* mosquitoes were further morphologically sorted according to their species [6, 21]. After identifying the samples, each mosquito was kept in a cryogenic vial (Corning Inc., NY, USA) using 75% alcohol and stored in a − 20 °C freezer immediately to prepare for DNA extraction and identification using multiplex polymerase chain reaction (PCR). The DNA of *An. minimus* groups and the Hyrcanus Group were extracted from legs or wings of each mosquito for further species confirmation [33, 34].

Human-biting rate

In each sentinel town, two survey sites (inner and outer) in each village were used for surveillance of the human-biting rate of *An. minimus* and *An. sinensis*. Although the human-landing catch (HLC) is the gold standard for monitoring mosquitoes that bite humans [35–37], it is labour intensive, cumbersome, and hazardous and requires intense supervision [38]. Alternatively, the human-baited double-net (HDN) trap is a simple and cheap method to estimate the human-biting rate outdoors without exposing collectors to vector bites [39]; and so far the best-performing trap, with similar efficiency to HLC. In this study, the human-biting rate of *An.*

Table 1 Global positioning system information of selected survey sites at different elevations

Elevation levels	Town	Village	Latitude	Longitude
1 (0 m ~)	Na Bang	Ka Ya He	24.72171	97.569687
		Jing Po Zhai	24.724932	97.570903
		Wang Jia Zhai	24.7302	97.567
		Han Zu Zhai	24.713553	97.573415
		Li Su Zhai	24.710657	97.571707
		Shang Tian Ba	24.753889	97.563333
2 (600 m ~)	Ping Yuan	Hu Que Ba	24.809265	97.924073
	Tai Ping	Mang Lai She	24.639444	97.832944
3 (1200 m ~)	Zhi Na	Qing Wa Shi	25.02815	98.117844
		Qin Cai Tang	25.026906	98.118117
	Tong Bi Guan	Xin Cun	24.631647	97.657962
		Ga Du Er She	24.614827	97.65857
		Da Zhai	24.627342	97.659831
4 (1800 m ~)	Tai Ping	Shi Ba Cha	24.694127	97.738692
	Meng Nong	Meng Dian	24.97327	97.945571
	Su Dian	Su Dian	25.108227	97.939872
	Xi Ma	Ying Pan Po	24.777837	97.703559

Table 2 Height distribution of malaria vectors in selected towns in Yingjiang County

Elevation levels	Town	Number of villages	Number of light-trap nights
1 (0 m ~)	Na Bang	6	92
2 (600 m ~)	Ping Yuan	1	77
	Tai Ping	1	4
3 (1200 m ~)	Zhi Na	2	5
	Tong Bi Guan	3	4
4 (1800 m ~)	Tai Ping	1	3
	Meng Nong	1	2
	Su Dian	1	2
	Xi Ma	1	2
Total		17	191

minimus and *An. sinensis* was monitored for 10 nights in four houses using the HDN trap from June to October 2016. One local volunteer was employed to rest inside a small bed net and was consequently fully protected from mosquitoes for the whole night's duration. A larger bed net was hung over the smaller net and raised 30 cm above the ground. Both nets were protected from the elements by plastic-sheeting roof, but were not treated with any insecticide. One specialized person captured the mosquitoes from inside and outside the larger bed net per hour from 2000 to 0700 h. The species were then identified in the laboratory, and the number of captured mosquitoes was recorded at each survey site to calculate the human-biting rate.

Seasonal abundance of adult mosquitoes

The vectors in human bedrooms and cattle shelters were captured using CDC light-traps from June to October 2016, and the seasonal abundance in terms of density (per light-trap per night) of *An. minimus* and *An. sinensis* was calculated accordingly. In each village, four light-traps were hung separately in two human bedrooms and two cattle shelters per night, from 2000 to 0700 h of the next day. In each month, vectors were captured twice on two nights at the same place.

Density of larvae

All kinds of breeding sites (bogs, slow-flowing water bodies, rice paddies, pools, and ditches) of the two vectors were surveyed in the two towns each month during the survey season. Standard dippers with approximately 500 mL volume were used to collect larvae from the water bodies [40]. Ten dips of water were taken to determine the presence of anophelines. If anophelines were present, the larvae in the 10 dips were collected in a small bottle with some water. The bottles were then numbered and transported to the laboratory to count the number of first-, second-, third-, and fourth-instar larvae and pupae of the two vectors [41]. The species of late third- and fourth-instar anopheline larvae were identified under a microscope using commonly accepted guidelines [42]. The identified larvae were preserved in a cryogenic vial (Corning Inc.) containing 75% alcohol for further identification by PCR. The density of larvae (per 10 dips) was calculated accordingly. Additionally, the pH value and location of the breeding sites were surveyed to analyse the relationship between these factors and the density of larvae.

Parous rate

Landing collections were performed by collecting *An. minimus* and *An. sinensis* in cattle shelters in Na Bang and Ping Yuan each month, from 2130 to 2200 h per night. Mosquitoes were collected by four collectors using an aspirator. The collected mosquitoes were transported to the laboratory of Yingjiang CDC, where they were killed using chloroform and dissected using minute dissection needles to collect their ovaries. The ovaries were separated from the other internal organs (including the Malpighian tubules and stomach) and teased apart at approximately 40× magnification through a dissection microscope to confirm whether the mosquitoes had laid eggs. The parous rate was calculated accordingly.

Height distribution

The height of each site in the 17 villages of the eight towns, where a light-trap was hung, was recorded using a handset global positioning system (Garmin International Inc., Olathe, KS, USA) to analyse the relationship between the height and the density of the two vectors. The lowest elevation was 210 m in Na Bang town, and the highest was approximately 2000 m in Shi Ba Cha village in Tai Ping town. As mentioned, the towns were divided into four levels. Besides the two sentinel towns, six towns were selected at different elevation levels. The same mosquito-capturing method was adopted as the one used for investigating the seasonal abundance of adult mosquitoes. Thereafter, the captured mosquitoes were transported to the laboratory to confirm whether they were the target vectors.

Data analysis

Species richness of *Anopheles* mosquitoes was measured using the index *N,* which represents the number of species [6]. Species dominance was measured by the Berger–Parker dominance index *d,* which was equal to the fraction of a species with a majority proportion in the study site or area [6]. Species diversity and evenness were evaluated by three indices—Simpson diversity index *D*, Shannon diversity index *H*, and evenness index *E* [25–32]. Similarity among different elevation levels was measured using the Morisita–Horn similarity index *C* [32–43]. The indices *D* and *H* were calculated from the proportion of each species; *E,* also known as Shannon's equitability, was calculated by dividing *H* by richness; and *C* was calculated by the number of individuals of each species and the total number of mosquitoes [43]. These indices were represented by the following equations:

$$D = 1 - \sum_{n=1}^{N} p_i^2$$

$$H = -\sum_{n=1}^{N} p_i \ln p_i$$

$$E = \frac{H}{\ln N}$$

$$C = \frac{2\sum n_{1i} n_{2i}}{(\lambda_1 + \lambda_2) M_1 M_2}, \quad \lambda_i = \frac{\sum n_{ji}^2}{M_j^2}$$

where N is the richness index, p_i is the proportion of a species that belongs to the ith species, n_{ji} is the number of individuals of a species i in an area j, and M_j is the number of individuals in an area j.

In the cross-sectional study, the light-trap density (females/trap/night), *N*, *D*, *H*, *d*, and *E* were evaluated to determine the community structure of *Anopheles* mosquitoes in the 17 villages, and *C* was used to measure the similarity among different elevation areas. The community-structure indicators were used to examine the population dynamics at the two surveillance sites.

Microsoft Excel 2010 (Microsoft Corp., USA) was employed to represent the data. Data analysis was performed using SPSS 13.0 software. Differences between larvae and pH value of water were calculated using the Pearson correlation test and Chi square test. Differences between population density and height were calculated using the Pearson correlation test and curve fitting of the statistical model with observed data.

Results

Community structure and population dynamics of *Anopheles* mosquitoes

Over the study period, 191 trap nights were conducted, and 56,834 mosquitoes were collected in 17 villages. The majority of captured mosquitoes were *Culex* (45,180, 79.5%), followed by *Anopheles* (10,053, 17.7%), *Aedes* (1430, 2.5%), and other subfamilies or genera (171, 0.3%). Fifteen *Anopheles* species were identified and observed in the samples: *An. sinensis* (75.4%), *Anopheles kunmingensis* (15.6%), and *An. minimus* (3.5%), followed by 12 other *Anopheles* species (5.5%) (Table 3). The *Anopheles* distribution in each village is shown in Fig. 1.

The area with a level 1 elevation had the lowest *Anopheles* density (6.82 females/trap/night) and dominance index ($d = 0.51$), but the highest Simpson diversity index ($D = 0.68$), Shannon diversity index ($H = 1.47$), and evenness index ($E = 0.67$) (Table 4). Furthermore, the richness index ($N = 9$) in such area was lower than that of a level 2 area, but higher than those of level 3 and 4 areas. A level 2 area had the highest species richness index ($N = 11$) and dominance index ($d = 0.95$), but the lowest diversity indices *D* (0.09) and *H* (0.24) and evenness index *E* (0.10). Compared with a level 1 area, level 3 and 4 areas had lower *N*, *D*, *H* and *E* indices but higher *d* index. Among all *Anopheles* species, *An. minimus*, *An. sinensis*, and *An. kunmingensis* showed the highest proportion in areas of elevation levels 1, 2/3, and 4, respectively.

Similarity analysis showed that the species composition of level 2 and 3 areas had the highest similarity (Morisita–Horn index $C = 0.999$), but any other two level areas showed low similarities (Morisita–Horn index *C* range, 0.059–0.274) (Table 5). The results of the two surveillance sites showed that the major *Anopheles* species in Na Bang was *An. minimus*, followed by *An. sinensis* (Table 6). In Na Bang, the pooled density of the entire study season was 6.82 females/trap/night, with a peak of

Table 3 *Anopheles* species composition by elevation and pooled across study sites and study period

Species	Composition by elevation				Pooled	
	0 m ~	600 m ~	1200 m ~	1800 m ~	n	%
An. sinensis	16.91	95.29	91.99	17.96	7579	75.39
An. kunmingensis	0.00	0.00	0.00	81.58	1572	15.64
An. minimus	51.20	0.36	0.89	0.00	351	3.49
An. splendidus	0.64	3.07	4.09	0.00	240	2.39
An. culicifacies	16.43	0.01	0.00	0.00	104	1.03
An. peditaeniatus	5.74	0.91	0.71	0.05	104	1.03
An. barbirostris	4.63	0.01	0.00	0.00	30	0.30
An. annularis	0.00	0.19	0.89	0.00	18	0.18
An. argyropus	1.44	0.07	0.00	0.00	14	0.14
An. pseudowillmori	0.00	0.06	1.42	0.00	12	0.12
An. tessellatus	1.59	0.00	0.00	0.00	10	0.10
An. vagus	1.44	0.00	0.00	0.00	9	0.09
An. gigas baileyi	0.00	0.00	0.00	0.42	8	0.08
An. ludlowae	0.00	0.01	0.00	0.00	1	0.01
An. subpictus	0.00	0.01	0.00	0.00	1	0.01

Table 4 Population density and community structure of *Anopheles* mosquitoes at each elevation level

Elevation (m)	Density (f/t/n)	*N*	Diversity index		*d*	*E*
			D	*H*		
0 ~	6.82	9	0.68	1.47	0.51	0.67
600 ~	85.64	11	0.09	0.24	0.95	0.10
1200 ~	62.44	6	0.15	0.39	0.92	0.22
1800 ~	214.11	4	0.30	0.50	0.82	0.36

f/t/n, females/trap/night; *N*, species richness; *D*, Simpson diversity index; *H*, Shannon diversity index; *d*, dominance index; *E*, evenness index

Table 5 Similarity in species composition between different elevations

	0 m ~	600 m ~	1200 m ~	1800 m ~
0 m ~	1			
600 m ~	0.266	1		
1200 m ~	0.274	0.999	1	
1800 m ~	0.059	0.213	0.214	1

13.06 females/trap/night in June. Moreover, Na Bang had the highest *N* (8), *D* (0.75), *H* (1.66), and *E* (0.8) indices in September, but the highest *d* (0.67) index in June. All indicators of community structure in Na Bang showed a low variation during the season. In contrast, a large variation in these parameters was observed in Ping Yuan. The pooled density of the entire study season was 89.99 females/trap/night, with a peak of 244.60 females/trap/night in August. Additionally, the highest *N* (9), *D* (0.69), and *H* (1.46) indices were observed in October, but the highest *d* (0.98) index was observed in August, and the highest *E* (0.70) index in May (Fig. 2).

Human-biting rate

The human-biting rate of *An. minimus* was 1.4/bait/night at the inner survey site, but 5.2/bait/night at the outer survey site in Na Bang from June to October 2016. Meanwhile, the human-biting rate of *An. sinensis* was 0/bait/night in Na Bang, irrespective of the location. At the inner site, the peak human-biting rate of *An. minimus* was in June, with 5/bait/night (Table 7). However, at the outer site, although the human-biting rate of *An. minimus* was 9/bait/night in June, the peak was 15/bait/night (Fig. 3). *Anopheles minimus* was more likely to attack at 0100 and 0400 h at the inner site, but only at 0400 h at the outer site (Fig. 3).

In Ping Yuan, the human-biting rate of *An. minimus* was 0/bait/night, irrespective of the location. However, the human-biting rate of *An. sinensis* was 2.6/bait/night

Table 6 *Anopheles* species composition by month

Town	Species	May	June	July	August	September	October	Pooled	
								n	%
NB	*An. minimus*	33.33	66.99	63.89	65.25	8.54	17.86	321	51.20
	An. sinensis	5.56	19.62	10.19	5.93	42.68	16.07	106	16.91
	An. culicifacies	61.11	6.70	12.04	12.71	7.32	39.29	103	16.43
	An. peditaeniatus	0.00	1.91	0.00	7.63	13.41	21.43	36	5.74
	An. barbirostris	0.00	2.87	6.48	1.69	15.85	1.79	29	4.63
	An. tessellatus	0.00	1.44	1.85	3.39	1.22	0.00	10	1.59
	An. vagus	0.00	0.00	3.70	3.39	1.22	0.00	9	1.44
	An. argyropus	0.00	0.00	0.00	0.00	9.76	1.79	9	1.44
	An. splendidus	0.00	0.48	1.85	0.00	0.00	1.79	4	0.64
PY	*An. sinensis*	54.55	94.60	97.23	97.75	94.37	45.95	6606	95.34
	An. splendidus	36.36	4.01	1.19	1.10	4.35	29.73	209	3.02
	An. peditaeniatus	0.00	0.00	0.00	1.02	1.07	10.81	63	0.91
	An. minimus	6.82	0.77	0.13	0.13	0.14	4.05	25	0.36
	An. annularis	2.27	0.15	1.19	0.00	0.00	0.00	13	0.19
	An. argyropus	0.00	0.00	0.26	0.00	0.07	2.70	5	0.07
	An. pseudowillmori	0.00	0.31	0.00	0.00	0.00	2.70	4	0.06
	An. barbirostris	0.00	0.00	0.00	0.00	0.00	1.35	1	0.01
	An. ludlowae	0.00	0.00	0.00	0.00	0.00	1.35	1	0.01
	An. culicifacies	0.00	0.00	0.00	0.00	0.00	1.35	1	0.01
	An. subpictus	0.00	0.15	0.00	0.00	0.00	0.00	1	0.01

NB Na Bang, *PY* Ping Yuan

at the inner site and 4.6/bait/night at the outer site. The peak human-biting rate of *An. sinensis* was in August, with 9/bait/night at the inner site and 21/bait/night at the outer site (Fig. 3). *Anopheles sinensis* was more likely to attack at 2100 and 2400 h at the inner site and 2200 and 2400 h at the outer site (Fig. 3).

Seasonal abundance of adult mosquitoes

In Na Bang, the major vector was *An. minimus*. Its peak density was observed in human bedrooms in May (5 females/trap/night) and in cattle shelter in June (13.25 females/trap/night). There were two peaks (June and September) of *An. sinensis* in cattle shelters in the town, with densities of 4.5 females/trap/night and 4.25 females/trap/night, respectively. However, in human bedrooms, the density of *An. sinensis* decreased from May to October.

In Ping Yuan, the major vector was *An. sinensis*, the peak density of which was found in cattle shelters in August (422 females/trap/night) and in human bedrooms in September (140.25 females/trap/night). However, the density of *An. minimus* was low, and the seasonality of this vector was not evident in the town (Fig. 4).

Density of larvae

The seasonality of *An. minimus* larvae was evident in Na Bang, with a density of 7.5/10 dips. Meanwhile, the peaks of *An. sinensis*—in Na Bang were in June and September, with densities of 5.5/10 dips and 4.8/10 dips, respectively. Ping Yuan had a lower density of both vectors, and the density of *An. sinensis* decreased during the investigation season. No larva of *An. minimus* was detected in Ping Yuan during the investigation (Fig. 5).

A total of 87 samples were collected from different water bodies. There was a significant difference between the larvae of *An. minimus* and pH of the water samples surveyed ($\chi^2 = 4.721$, $P = 0.030$; Table 8). In contrast, no significant difference existed between the larvae of *An. sinensis* and pH value of the water samples surveyed ($\chi^2 = 0.001$, $P = 0.976$). However, the density of the larvae was negatively correlated with the pH value; the correlation coefficient ($r = -0.297$, $P = 0.005$) was calculated by the Pearson correlation test. Water samples with low pH showed a high density of *An. sinensis* larvae (Fig. 6).

Parous rate

In this study, 283 *An. sinensis* mosquitoes captured in cattle shelters were dissected, among which 256 (90.46; 95% confidence interval, 87.04–93.88%) had laid eggs. Fifteen *An. minimus* mosquitoes captured in cattle shelters were also dissected, among which 14 (93.33; 95% confidence interval, 80.26–100.00%) had laid eggs.

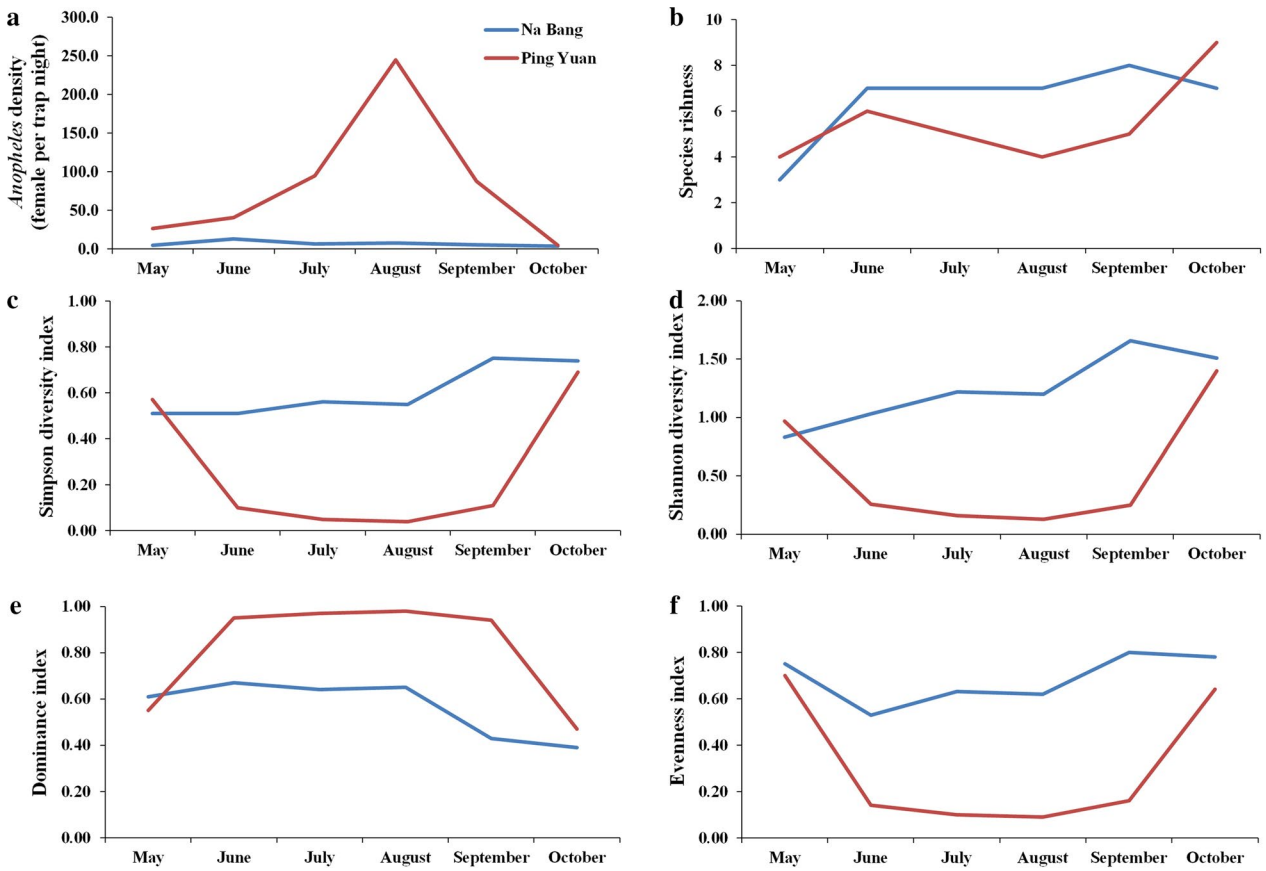

Fig. 2 Population dynamics of *Anopheles* mosquitoes at two study sites in Yingjiang County, May–October 2016. **a** Pooled population density (females/trap/night) of all *Anopheles* species. **b** Species richness. **c** Simpson diversity index. **d** Shannon diversity index. **e** Dominance index. **f** Evenness index

Table 7 Human-biting rate of *An. minimus* and *An. sinensis* in Na Bang and Ping Yuan from June to October 2016

Towns	Location	Number of bait	Number of night	Number of *An. minimus*	Number of *An. sinensis*	*ma* of *An. minimus* (per bait per night)	*ma* of *An. sinensis* (per bait per night)
Na Bang	Inner village	1	5	7	0	1.4	0
	Outer village	1	5	26	0	5.2	0
Ping Yuan	Inner village	1	5	0	13	0	2.6
	Outer village	1	5	0	23	0	4.6

Height distribution

191 trap nights were performed to capture mosquitoes in eight towns (Table 2). Analysis of data revealed that the density of *An. minimus* decreased with an increase in area elevation, irrespective of the location of collection (human bedroom: $r = -0.441$, $P = 0.000$; livestock building: $r = -0.297$, $P = 0.003$). A linear model may represent the relationship between the density and height. After analysing the data of 90 densities of captured *An. minimus* and the related height values, the model in human bedrooms was $y = 2.009 - 0.02x$ ($R^2 = 0.194$, $P = 0.000$), where y and x indicate the density of *An. minimus* and elevation of the area, respectively. After analysing the data of 101 densities of captured *An. minimus* and the related height values, the model in cattle shelters was $y = 6.147 - 0.005x$ ($R^2 = 0.088$, $P = 0.003$).

The density of *An. sinensis* increased with an elevation < 1200 m but decreased with > 1200 m (Table 9). A quadratic model could be used to represent the relationship between the density and height. Analysis of 90

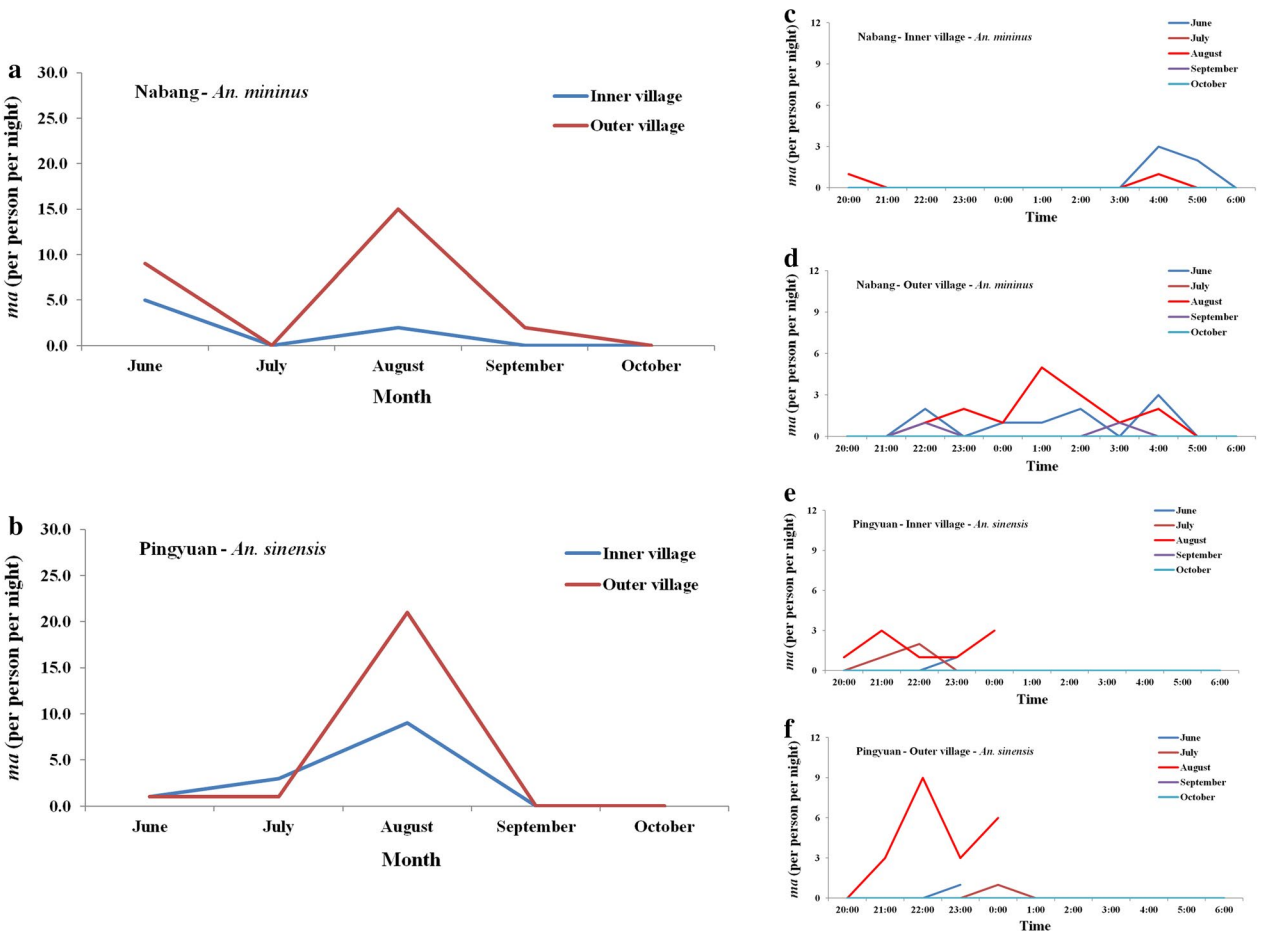

Fig. 3 Seasonality of human-biting rate of *Anopheles minimus* and *Anopheles sinensis* in two surveillance sites. **a** Human-biting rate of *An. minimus* at the inner and outer village sites of Na Bang from June to October 2016. **b** Human-biting rate of *An. sinensis* at the inner and outer village sites of Ping Yuan from June to October 2016. **c** Human-biting rate (per hour) of *An. minimus* at the inner village site of Na Bang. **d** Human-biting rate (per hour) of *An. minimus* at the outer village site of Na Bang. **e** Human-biting rate (per hour) of *An. sinensis* at the inner village site of Ping Yuan. **f** Human-biting rate (per hour) of *An. sinensis* at the outer village site of Ping Yuan. Data on the human-biting rate in Ping Yuan were only collected at 23:00 h in June and 24:00 h in August because of intense rainfall on those nights

densities of captured *An. sinensis* and the related height values showed that the model in human bedrooms was $y = -21.017 + 0.09x + 0.000029x^2$ ($R^2 = 0.199$, $P = 0.000$), where y and x indicate the density of *An. sinensis* and height, respectively. Analysing 101 light-trap-captured densities of *An. sinensis* and the related height values also revealed the following model in cattle shelters: $y = -77.444 + 0.373x + 0.000177x^2$ ($R^2 = 0.100$, $P = 0.006$).

Discussion

Although some studies have focused on community structure and receptivity to malaria in the China–Myanmar border (especially in the low-elevation areas) [6, 18, 21, 22], this study focused on other aspects to better understand *Anopheles* ecological features. Firstly, this study investigated the *Anopheles* distribution and found different community structure models at different elevation levels. The relationship between *An. minimus* density and elevation fitted well on a linear equation with one unknown model, but that between *An. sinensis* density and elevation fitted well on a quadratic equation with one unknown model. Secondly, this study found that high density, human-biting rate, and parous rate may lead to high receptivity to malaria in the border area. Finally, the slightly acidic water suited the growth of the two vectors.

The results of this study showed that the community structure of *Anopheles* was highly complex in areas below an elevation of 600 m. In these areas, the diversity indices D and H and the evenness index E were the highest, and the species richness index was also high up

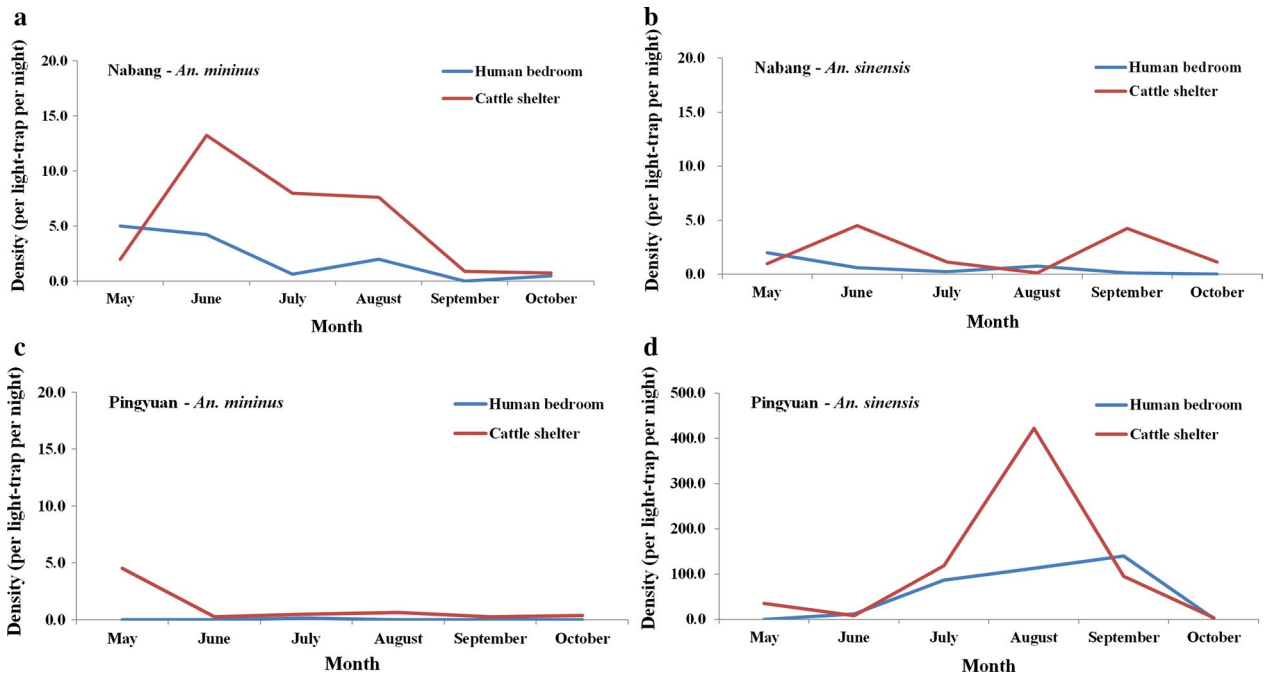

Fig. 4 Seasonality of trap-captured *Anopheles minimus* and *Anopheles sinensis* at the two surveillance sites. **a** Density of *An. minimus* in human bedrooms and cattle shelters in Na Bang. **b** Density of *An. sinensis* in human bedrooms and cattle shelters in Na Bang. **c** Density of *An. minimus* in human bedrooms and cattle shelters in Ping Yuan. **d** Density of *An. sinensis* in human bedrooms and cattle shelters in Ping Yuan

to 9. Although *An. minimus* was the major malaria vector in these areas, it was only 51% of the total *Anopheles* mosquitoes, which made the dominance index to be lowest among the four elevation level. Additionally, the proportion of *An. sinensis* and *Anopheles culicifacies* was as high as 16%. These results were slightly different with those of Yu et al. and Wang et al. [6, 21]. They reported that the first three predominant *Anopheles* species were *An. minimus*, *An. maculatus*, and *An. culicifacies*, with *An. sinensis* only accounting for < 4% [6]. These differences might be due to the different study years, changes in main cash crops, and different types of mosquito capture sites. Until early 2016, banana totally replaced rice and become the dominant cash crop in Na Bang. Furthermore, while Yu et al. and Wang et al. captured adult mosquitoes in human bedrooms, we captured mosquitoes in both human bedrooms and cattle shelters. *Anopheles minimus* belongs to four high-transmission-potential vectors in China, the rest being *An. sinensis*, *An. lesteri*, and *An. dirus* [23, 24]. *Anopheles sinensis* is a major malaria vector in China, especially northern China [23, 24], India [44, 45], Sri Lanka [46], and Iran [47]. The results of the cross-seasonal surveillance showed that the community structure was stable during the study season in the China–Myanmar border. Therefore, choosing the specific vector-control measures was more difficult in this region than in other elevation level because different targeted control measures were based on different ecological features.

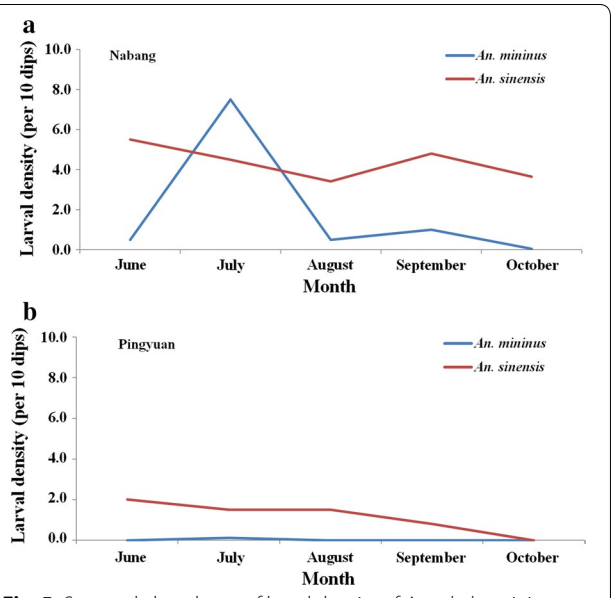

Fig. 5 Seasonal abundance of larval density of *Anopheles minimus* and *Anopheles sinensis* at two surveillance sites. **a** Larval density in Na Bang. **b** Larval density in Ping Yuan

The dominant species in the *Anopheles* community was *An. sinensis* at an elevation of 600–1800 m, with a

Table 8 Relationship between pH value and larvae of the two vectors

	pH < 7.0	pH > 7.0	Total
An. minimus	40	47	87
Positive	9	3	12
Negative	31	44	75
An. sinensis	40	47	87
Positive	20	26	46
Negative	20	21	41

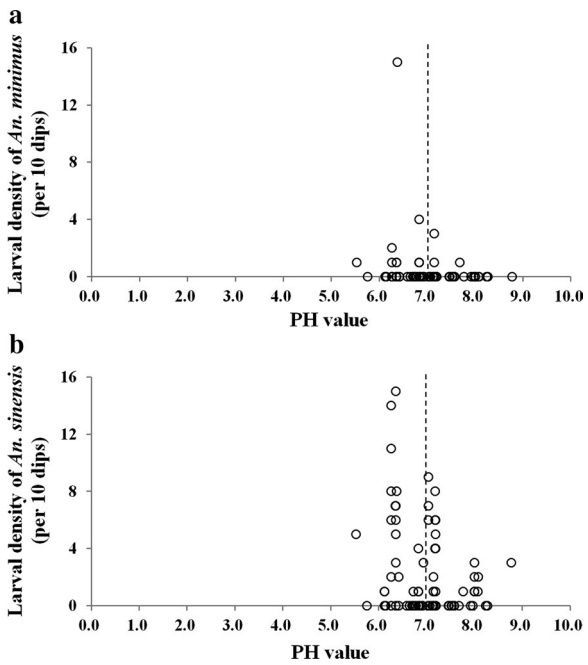

Fig. 6 pH value and larval density of *Anopheles minimus* and *Anopheles sinensis* in Yingjiang County. **a** *An. minimus*. **b** *An. sinensis*

dominance index > 0.9, indicating absolute predominance of the vector in the area. The results of the cross-seasonal surveillance in Ping Yuan showed that the density of *Anopheles* mosquitoes peaked from June to September and > 94% of them were *An. sinensis*, indicating low diversity and evenness during that period. The highest similarity was observed between areas with elevations of 600–1199 and 1200–1799 m, suggesting that these two areas could be combined into one area, with the target vector to control being *An. sinensis*. Although *An. sinensis* prefers biting animals such as cattle or water buffalo over humans [48, 49], its extremely high density in the area could lead to a high probability of malaria transmission. Latest research using membrane feeding assay under laboratory conditions demonstrated that the susceptibility of *An. sinensis* to *Plasmodium vivax* is similar to that of *Anopheles anthropophagus* [24]. In addition, *P. vivax* is a major parasite of malaria in the China–Myanmar border [50, 51]. Therefore, specific vector-control countermeasures aimed at *An. sinensis* should be strengthened in the region in case of the re-establishment of malaria.

High elevation of > 1800 m was correlated with low species richness, diversity, and evenness in the area. *Anopheles kunmingensis* was the major *Anopheles* mosquito in the high-elevation areas (> 1800 m). One study reported *An. kunmingensis* as the main malaria vector based on its indoor abundance, relatively high human-biting rate, and the finding of a sporozoite-positive specimen during a peak malaria season in Tengchong County, Yunnan Province, China [52]. However, the role of mosquitoes in transmission of malaria, especially in susceptibility to *Plasmodium* and receptivity to malaria, remains uncertain. Therefore, further research on *Anopheles* mosquitos is required to determine the integrated aspects of malaria transmission.

Table 9 Density of *An. minimus* and *An. sinensis* at different elevations

	Elevation (m)	Number of light-traps	Number of *An. minimus*	Density of *An. minimus* (per light-trap per night)	Number of *An. sinensis*	Density of *An. sinensis* (per light-trap per night)
Human bedroom	0 ~	46	76	1.65	16	0.35
	600 ~	40	1	0.03	1387	34.68
	1200 ~	3	0	0.00	293	97.67
	1800 ~	1	0	0.00	0	0.00
	Total	90	77	0.86	1696	18.84
Cattle shelter	0 ~	46	252	5.48	89	1.93
	600 ~	41	24	0.59	5223	127.39
	1200 ~	12	5	0.42	570	47.50
	1800 ~	2	0	0.00	0	0.00
	Total	101	281	2.78	5882	58.24

The results of this study further revealed that the human-biting rate of *An. minimus* was remarkably high in Na Bang, with the highest rate of 15 females/person/night in August. In the same month, the human-biting rate in Ping Yuan was 21 females/person/night. In addition, *An. minimus* was more likely to attack people after midnight, while *An. sinensis* before midnight. These findings necessitate the increased use of countermeasures such as bed nets and mosquito repellents for preventing vector bites from May to September, especially in August.

The seasonal abundance of *An. minimus* was significantly higher than that reported by Wang et al. [6] in Na Bang in 2012–2013. The densities in cattle shelters were higher than those in human bedrooms. *Anopheles minimus* preferred areas at low elevation and tropical areas and showed a high density in cattle shelters in June, while *An. sinensis* preferred a medium elevation and showed a high density in August. In areas of low elevation, the conditions in June and September were more suitable to the vector, although the density in these areas was < 5 females/trap/night. Moreover the seasonal peak of *An. minimus* larvae occurred in July, while that of *An. sinensis* larva occurred in August. Therefore, more mosquito-control measures such as pesticide sprays should be used in this region before June.

The parous rate of *An. sinensis* and *An. minimus* was 90.46% and 93.33%, respectively. If the duration from eclosion to laying eggs is 2.5 days, the daily survival probability of *An. sinensis* and *An. minimus* will be 96.07% and 97.28%, respectively, according to the function $p = M^{1/X}$, where p, M, and X refer to the daily survival probability, the parous rate, and the duration from eclosion to laying eggs, respectively. According to the MacDonald model [53], vectorial capacity (*VCAP*) [54–57], which is the indicator of receptivity to malaria, can be presented by the function $VCAP = ma \times a \times p^n \times 1/(-\ln p)$, where a and n indicate the vector biting rate and the parasite's extrinsic incubation period that is affected by ambient temperatures, respectively. Therefore, a higher human-biting rate and ratio of vectors having laid eggs lead to a higher *VCAP*. In this study, these two parameters of *An. sinensis* and *An. minimus* in Yingjiang County showed exceedingly high values.

Due to several complicating factors, malaria in the China–Myanmar border might threaten the elimination of malaria in China [5]. Yingjiang County harbours several ethnic minorities, and their subsistence activities associated with forest areas, such as logging, banana or rubber planting, and living in planting areas during the farming season or entire year, are likely to increase the risk of infection [58]. Under conditions of high receptivity and potential exposure of the local people, if imported malaria cases occur in the county without timely and effective control, the probability of re-establishment will be extremely high. Consequently, specified vector-control countermeasures should be strengthened in these areas in case of the re-establishment of malaria, which might affect the progress of malaria elimination in China, and more public health programmes should focus on controlling malaria transmission in the China–Myanmar border region to better achieve malaria elimination in China.

Limitation

The seasonality of species composition, density, *ma*, and parous rate was only investigated in two towns. Thus, more surveys are necessary across the four elevation levels to investigate the integrated aspects of receptivity to malaria in the China–Myanmar border.

Conclusions

This study showed that the community structure of *Anopheles* was complex and stable during the entire epidemic season at low elevation areas in the China–Myanmar border in Yingjiang County, China. The highest similarities in vector features were observed in areas with elevations of 600–1199 and 1200–1799 m. These areas of medium elevation showed significant seasonality in the community structure (such as density, diversity, dominance, and richness). Meanwhile, the community structure was relatively simple in areas of elevations > 1800 m compared with other areas. Based on the high human-biting rate, adult and larval density, and parous rate of the two vectors, receptivity to malaria was exceedingly high in the China–Myanmar border in Yingjiang County. These findings can provide insights into the epidemiology of malaria as well as direct and quantified evidence to draw up vector control strategies and promote progress of malaria elimination in China.

Abbreviations

WHO: World Health Organization; GMS: Greater Mekong Subregion; CDC: Centers for Disease Control and Prevention; PCR: polymerase chain reaction; HLC: human-landing catch; HDN: human-baited double-net; VCAP: vectorial capacity.

Authors' contributions

SSZ and TC conceived the study. TC, SZ, XW, CL, XZ, XG, ZL, XS, HZ, and HT collected the data. TC, SZ, and XW analysed the data. SSZ and TC wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Monitoring of malaria vectors at the China-Myanmar border while approaching malaria elimination

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Abstract

Background: Tengchong County was one of the counties located at the China-Myanmar border with high malaria incidence in the previous decades. As the pilot county for malaria elimination at the border area, Tengchong County is aiming to be the first county to achieve malaria elimination goal. A cross-sectional entomological survey was carried out to evaluate the feasibility of elimination approach and assess the receptivity of malaria reintroduction.

Methods: Light traps associated with live baits were used to investigate the abundance of adult mosquitoes in nine villages in Tengchong County. Light traps were set to collect adult mosquitoes in both human houses and cowsheds from dusk till dawn in each site.

Results: A total of 4948 adult *Anopheles* mosquitoes were collected from May to December in two villages. Of the mosquitoes were captured, 24.2% were in human houses and 75.8% in cowsheds. The peak of abundance occurred in July for *An. sinensis* and in September-October for *An. minimus* (*s.l.*) Ten *Anopheles* species were collected, the most prevalent being *An. sinensis* (50.3%), *An. peditaeniatus* (31.6%) and *An. minimus* (*s.l.*) (15.8%), contributing to 97.6% of the sample. Potential breeding sites were also investigated and a total of 407 larvae were collected, with *An. sinensis* (50.1%) and *An. minimus* (*s.l.*) (46.2%) as predominant species. Ponds and rice fields were the two preferred breeding sites for *Anopheles* mosquitoes; however, the difference between the number of adults and larvae captured suggest other breeding sites might exist. Both *An. sinensis* and *An. minimus* (*s.l.*) were found zoophilic with human blood index as 0.21 and 0.26, respectively. No *Plasmodium* positive *Anopheles* specimens were found by PCR among 4,000 trapped mosquitoes.

Conclusions: Although no indigenous malaria cases have been reported in Tengchong County since 2013, there is still a risk from the presence of vectors in the context of human population movements from neighboring malaria endemic areas. The presence of *An. sinensis*, associated to rice fields, is particularly worrying. Sustained entomological surveillance is strongly suggested even after malaria elimination certification.

Keywords: Malaria vector, China-Myanmar border, Malaria elimination, Ecological traits, Receptivity

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Introduction

Malaria is the deadliest vector-borne disease in tropical and subtropical areas, with a number of confirmed cases estimated at 216 million with 445,000 deaths in 2016 [1]. Out of 91 countries and territories with malaria transmission in 2016, 44 reported less than 10,000 cases and 21 are approaching malaria elimination, including China. Significant progress on malaria control has been made during the past decades and China is now aiming to achieve malaria elimination by 2020 [2, 3]. However, malaria control in international border areas is considered a challenge, especially at the China-Myanmar border in Yunnan Province [4–6]. Most of indigenous malaria cases (up to 90%) and malaria cases imported from Southeast Asia within China have been reported along this border since 2013 [7–11].

Tengchong County (TCC) is located in the southwest of Yunnan Province at the China-Myanmar border (Fig. 1). Because of the diversity of malaria vectors and large population movements across the border, the number of malaria cases reported in TCC was the highest for the whole country in previous years [6, 12]. Hence, TCC was designated in 2012 as the pilot county for malaria elimination at border areas with the objective of being the first border county to achieve malaria elimination. This status was officially granted in 2015 and no locally transmitted cases were observed since then. However, imported cases from neighboring Myanmar were recorded [8, 13, 14]. To investigate the feasibility of malaria elimination and assess the risk of reintroduction of malaria in TCC, a series of studies and analyses on epidemiology were carried out and published recently [8, 14]. These studies emphasized the risk of reintroduction of malaria in TCC due to population movement across the border into putative receptive areas. However, malaria vectors are the key drivers for malaria transmission and reintroduction [15–17]. We therefore investigated the presence of primary and secondary vectors in TCC through a cross-sectional survey.

Methods

Study sites

TCC covers an area of 5693 km^2 with a population of 6.68 million inhabitants and an international borderline with Myanmar of 148 km (98°05'E–98°45'E, 24°38'N–25°52'N). Mountains cover 84% of the territory with a maximum elevation of 3780 m (Fig. 2a). The altitude decreases from northwest to southeast with the lowest point at 930 m (Fig. 2a). The annual average rainfall is 1531 mm and the relative humidity is 77%, displaying the typical characteristics of a subtropical monsoon climate. The annual average temperature is 15 °C, decreasing to 0 °C during winter (January). The rainy season lasts from June to September. Nine villages located predominantly along the main regional river and with an elevation between 1032–1655 m were investigated (Fig. 2a, Table 1). These villages were chosen because they displayed the highest number of imported cases and the highest population movements across the international border with Myanmar, which is located 70 km away [5, 6, 8, 14, 18]. Land use and land cover (LULC) in TCC displayed two main features: (i) either predominantly cropland landscape; or (ii) predominantly forest mixed with grasslands (Fig. 2b). Study sites were selected also in relation to urbanized area, either: (i) at the edge of village area; (ii) close to

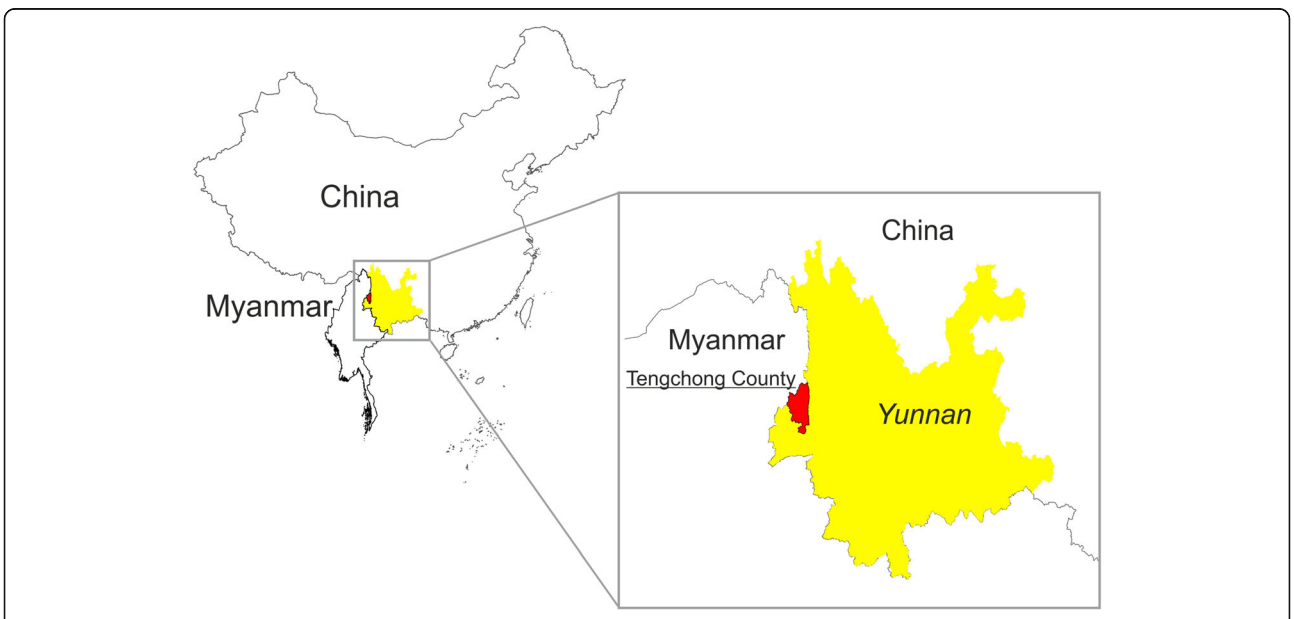

Fig. 1 Location of Tengchong County in the China-Myanmar border area

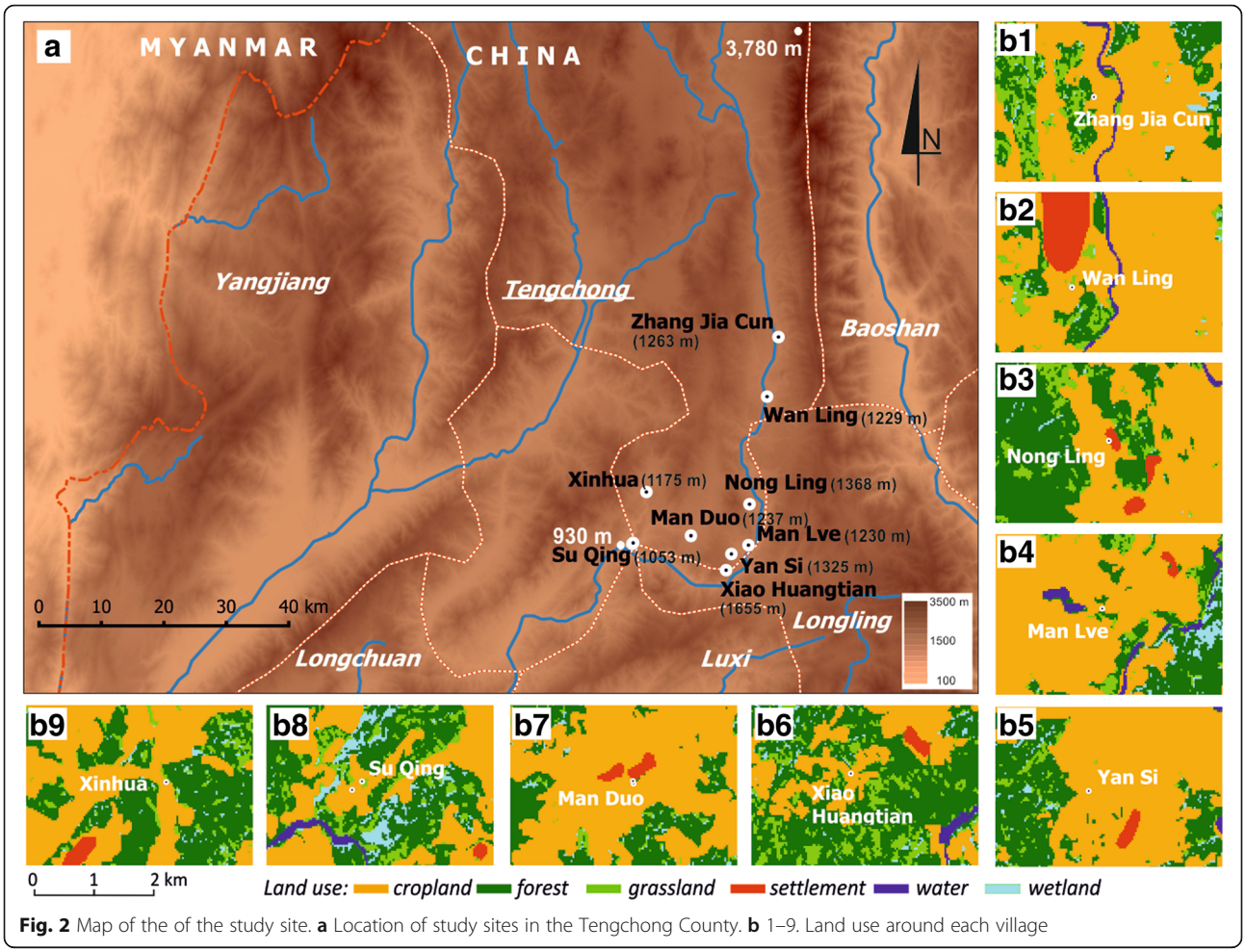

Fig. 2 Map of the of the study site. **a** Location of study sites in the Tengchong County. **b** 1–9. Land use around each village

village; or (iii) away from urbanized area (Table 1, Fig. 2b). Two sites were located close to the dense forest, Yan Si and Xinhua (Table 1, Fig. 2b5, b9), whereas five sites were next to fragmented forest, mixed with grasslands: Zhang Jia Cun; Nong Ling; Man Lve; Xiao Huangtian; and Su Qing (Table 1, Fig. 2b1, b3, b4, b6, b8). The last two sites, i.e. Wan Ling and Man Duo, were located in cropland areas nearby urbanized zones (Table 1, Fig. 2b2, b7).

Mosquito collection and species identification

Adult mosquitoes

The collection of adult mosquitoes was conducted from May to December 2015 in two villages, Man Lve and

Table 1 Sampling sites with village names, location, altitude, type of landscape and forest, and distance to urbanized area

Village	Township	Latitude (°N)	Longitude (°E)	Altitude (m)	Landscape	Type of forest	Urbanized area[a]
Xiao Huangtian	Tuan Tian	24.648424	98.611341	1655	Forest, grassland	Fragmented	Away
Yan Si	Tuan Tian	24.668723	98.619704	1325	Cropland	Dense	Away
Man Lve	Tuan Tian	24.679774	98.646722	1230	Cropland	Fragmented	Away
Nong Ling	Tuan Tian	24.730656	98.648392	1368	Forest, grassland	Fragmented	Edge
Wan Ling	Wu He	24.865358	98.676102	1229	Cropland	Away from fragm. forest	Close
Zhang Jia Cun	Mang Bang	24.939534	98.694098	1270	Cropland	Fragmented	Away
Man Duo	Pu Chuan	24.691051	98.555936	1232	Cropland	Away from fragm. forest	Edge
Su Qing	Xin Hua	24.681433	98.462895	1032	Forest, grassland	Fragmented	Away
Xinhua	Xin Hua	24.745937	98.485421	1175	Forest, grassland	Dense	Away

[a]Urbanized area located (i) away, (ii) at the edge or (iii) close to villages
Abbreviation: fragm., fragmented forest

Nong Ling, which are located 3 km apart and characterized by a difference in elevation and surrounded by forest, croplands and grasslands (Table 1, Fig. 2). Light traps were set up to collect adult mosquitoes in both human houses and cowsheds from sunset to sunrise (times varied depending on the season). Humans under bed nets and cattle were used as biological baits for mosquito collection using light traps in houses and cowsheds, respectively. These human and animal-occupied structures were selected in each village at differing distance from farmlands (farmlands are always located around the village), i.e. close, mid-distance and far from the farmland. Sampling was conducted for two nights every month, one night at the beginning of the month and the other at the end. The same sampling method was implemented in an additional sampling campaign to investigate the *Anopheles* diversity in October 2015 over seven additional villages bringing the overall sampling area to a total of nine villages (Table 1, Fig. 2). The same sampling effort was implemented and the same number of sampling sites was considered in all locations. Trapped mosquitoes were killed by chloroform, counted and identified according to morphological criteria [19].

Mosquito larvae

All potential *Anopheles* breeding sites (stream, rice field, small pool, canal, ditch, etc.) around the selected villages were investigated for larvae. The hand dipper sampling method was used to collect larvae (500 ml per dip, 10 dips for each waterbody) [20]. The morphological identification of specimens was only conducted for fourth-instar larvae under light microscope. Larvae under the fourth-instar were only counted but not identified. Pupae were kept until adult emergence in order to conduct morphological and molecular identifications. Both adult and larval specimens were preserved in ethanol for further PCR analysis. A series of multiplex PCR assays based on rDNA internal transcribed spacer 2 (ITS2) and D3 domain of *28S* rDNA sequences were run to identify the sibling species of the *An. minimus*, *An. culicifacies* and *An. fluviatilis* complexes and *An. maculatus* group [21–24].

Entomological data

Monthly abundance data of each *Anopheles* species were aggregated to analyze seasonal fluctuations. The resting behavior and breeding preference of adult mosquitoes were explored by analyzing the adult and larval composition in each study place. The adult population density for each *Anopheles* species was calculated as the number of females per trap per night (f/t/n). The overall (pooled) *Anopheles* density was calculated by summing captured individuals of all *Anopheles* species. Species richness was measured by the number of species and the indices described below. Generally, species diversity is an indicator of the wellbeing of an ecosystem [25]. Simpson's diversity index (D) is often used to quantify the biodiversity of an ecosystem. It takes into account the number of species present, as well as the abundance of each species. The value of this index ranges between 0 and 1, 0 representing the absence of diversity and 1 representing infinite diversity. Shannon-Wiener's index (H) takes into account individuals of each species to assess the species richness. The larger the value of the H index, the higher the diversity. The Evenness index (E) represents the equitability of populations [26]. The larger the value of the E index, the higher the equitability.

The indices were calculated as follows:

Simpson's diversity index: $D = 1-\sum_{i=1}^{N} p_i^2$

Shannon-Wiener's index: $H = -\sum pi \times \ln pi$

where pi is the fraction of a species which belongs to the i-th species and N is the number of species ($pi = N_i/N$).

Evenness index: $E = H/\ln S$

where H is the Shannon-Wiener's diversity index and S is the total number of species observed in a given place.

Detection of *Plasmodium* spp. in mosquitoes and blood source identification

Captured mosquitoes were dissected into different segments under light microscope. The head and thorax were separated for *Plasmodium* spp. test while the abdomen was used for blood-meal identification. DNA extraction was conducted with QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the supplier. PCR tests for blood source identification were conducted as previously described [27–29]. Primers are presented in Table 2.

Assessment of the parous rate

Mosquitoes were collected by six collectors using an aspirator in different locations, including human houses and cowsheds (Fig. 3). The collected mosquitoes were transported to the laboratory, where they were killed by chloroform and dissected with minute dissection needles for ovarian examination. Ovaries were separated from the other internal organs (including the Malpighian tubules and stomach) and teased apart on slides with deionized water. The slides were checked under light microscope at 10×–40× magnification to confirm whether the mosquitoes were parous or nulliparous.

Geographical data

Administrative spatial data were obtained from the GADM database of Global Administrative Areas (http://www.gadm.org). The relief model was prepared using SRTM 90m digital elevation data v4.1 [30]. Land cover data were obtained from the GlobeLand30 service operated by the National Geomatics Center of China [31]. Data were initially produced in 2010 and updated in 2014.

Table 2 Primers for *Plasmodium* spp. and blood meal identification

No.	Primer name	Sequence (5'-3')	Product size (bp)
Test for blood meal identification			
1	Human blood	GGCTTACTTCTCTTCATTCTCTCCT	334
2	Pig blood	CCTCGCAGCCGTACATCTC	453
3	Cow blood	CATCGGCACAAATTTAGTCG	561
4	Dog blood	GGAATTGTACTATTATTCGCAACCAT	680
5	UNREV	GGTTGTCCTCCAATTCATGTTA	–
Test for *Plasmodium* spp.			
1	Pf1	CCTGCATTAACATCATTATATGGTACATCT	273
2	Pf2	GATTAACATTCTTGATGAAGTAATGATAATACCTT	
3	Pv1	AAGTGTTGTATGGGCTCATCATATG	290
4	Pv2	CAAAATGGAAATGAGCGATTACAT	

Abbreviation: UNREV, Universal reversal primer

Fig. 3 Aerial view of Man Lve (top) and Nong Ling (bottom) with land use and locations of the sampling sites

Images used for GlobeLand30 (GLC30) classification were multispectral images with a 30-meter resolution. Six classes of land cover were displayed: crop land; forest; grassland; wetland; water bodies; and human settlements. Climate description was made using global climate data (Tutiempo Network). Data mapping was performed with Quantum GIS, version 2.8.2.

Results

Species richness and diversity

The diversity of species measured by the Simpson's diversity index (D) and the Shannon-Wiener's index (H), both for human houses and cowsheds, was at the highest in Man Lve and Nong Ling at the beginning of the fall, i.e. September-October, although the trend was already visible in August (Table 3). The diversity, both in terms of number of species and number of individuals per species, was at the highest during September and October and similar for both locations although indices were displaying some differing trends. Diversity for both species and number of individuals was slightly higher in cowsheds than human houses in Man Lve whereas it was higher in human houses than cowsheds in Nong Ling (Table 3). Evenness followed a similar trend, indicating thus equitability of populations along with the increasing the number of species.

Variation of adult mosquito incidence over time and space in Man Lve

The collection of adult mosquitoes in the village of Man Lve (Fig. 3) was conducted in two different types of shelters: human houses (Fig. 4a) and cowsheds (Fig. 4b) from May to December 2015. A total of 511 adult mosquitoes were collected inside houses over eight months

Table 3 *Anopheles* species richness and diversity per month, location and village

Month	Species richness[a]		Simpson's diversity index (D)		Shannon-Wiener's index (H)		Evenness index (E)	
	Human house	Cowshed	Human house	Cowshed	Human house	Cowshed	Human house	Cowshed
Man Lve								
May	2	3	0.43	0.52	0.52	0.79	0.90	0.72
June	3	4	0.18	0.46	0.46	0.83	0.35	0.60
July	6	4	0.40	0.52	0.52	0.92	0.40	0.67
August	4	6	0.56	0.60	0.60	1.09	0.73	0.61
September	4	5	0.54	0.68	0.68	1.18	0.70	0.73
October	3	4	0.36	0.62	0.62	1.07	0.57	0.77
November	2	4	0.50	0.42	0.42	0.84	1.00	0.60
December	1	1	0	0	0	0	0	–
Nongling								
May	1	1	0	0	0	0	–	–
June	1	3	0	0.12	0	0.28	–	0.25
July	5	4	0.29	0.37	0.59	0.67	0.37	0.49
August	5	3	0.51	0.48	0.90	0.70	0.56	0.64
September	4	3	0.66	0.53	1.13	0.89	0.82	0.81
October	4	5	0.66	0.52	1.15	0.95	0.83	0.59
November	0	0	–	–	–	–	–	–
December	0	0	–	–	–	–	–	–
Cumulative data								
May	2	3	0.35	0.51	0.54	0.77	0.77	0.70
June	3	4	0.13	0.40	0.30	0.75	0.27	0.54
July	6	4	0.36	0.48	0.69	0.85	0.39	0.61
August	6	6	0.53	0.57	1.00	0.98	0.56	0.54
September	5	5	0.64	0.65	1.13	1.10	0.70	0.68
October	4	6	0.63	0.62	1.02	1.08	0.74	0.60
November	2	4	0.50	0.42	0.69	0.84	1.00	0.60
December	1	1	0	0	0	0	0	0

[a]Species richness is defined by the number of species captured

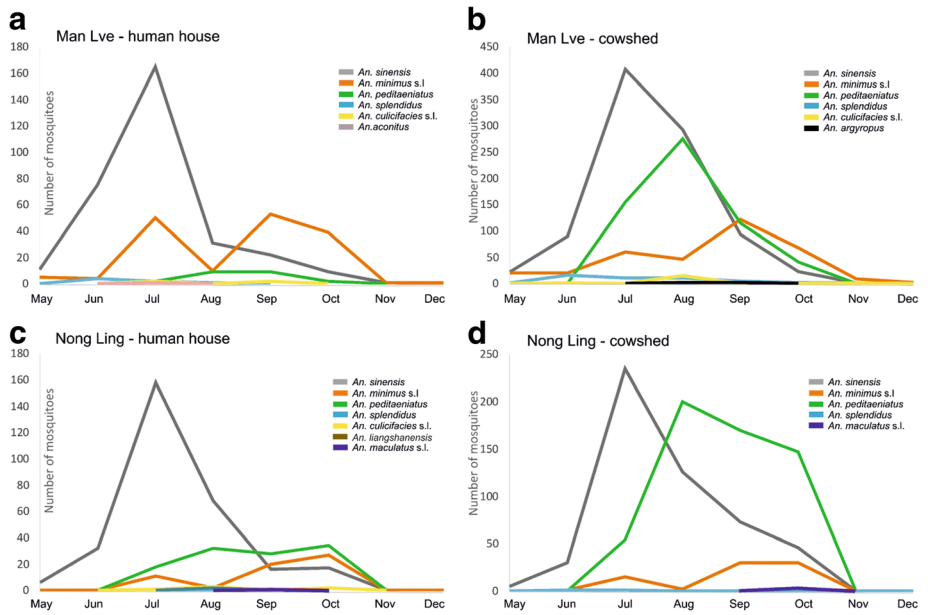

Fig. 4 Distribution and seasonal fluctuation of captured *Anopheles* taxa in two study sites, Man Lve (**a**, **b**) and and Nong Ling (**c**, **d**) in human house (**a**, **c**) and cowshed (**b**, **d**)

(Fig. 4a). The most prevalent species were *An. sinensis* (61.4%, 314/511), *An. minimus* (*s.l.*) (31.9%, 163/511) and to a lower extent *An. peditaeniatus* (4.3%, 22/511). Three other species were collected, i.e. *An. splendidus* (1.4%, 7/511), *An. culicifacies* (*s.l.*) (0.8%, 4/511) and *An. aconitus* (0.2%, 1/511), but in very limited numbers. *Anopheles sinensis* was mostly present from June to August with a peak in July but remained present until November (Fig. 4). *Anopheles minimus* (*s.l.*) was less prevalent but displayed a bimodal curve with two peaks in July and September. *Anopheles peditaeniatus* was recorded only from July to October with a maximum plateau in August and September. The survey of mosquito prevalence in cowsheds yielded a slightly different pattern (Fig. 4b). The first difference was the total number of mosquitoes collected in cowsheds, which was almost four-fold higher than in houses, the ratio between cowsheds and houses was 3.77 (1930/511). The same three species were the most prevalent, i.e. *An. sinensis*, *An. minimus* (*s.l.*) and *An. peditaeniatus*, but with different ratios than in human houses. *Anopheles sinensis* was still the most prevalent (48.1%, 929/1930), followed by *An. peditaeniatus* (30.4%, 586/1930) and *An. minimus* (*s.l.*) (18.0%, 347/1930). Three more species were detected in cowsheds at a lower extent: *An. splendidus* (2.4%, 47/1930), *An. culicifacies* (*s.l.*) (0.9%, 17/1930) and *An. argyropus* (0.2%, 4/1930). The main three species were recorded for the same period as in human houses. *An. sinensis* was mostly present from June to September with a peak in July; *An. peditaeniatus* was recorded from July to October, with a peak in August, and *An. minimus* (*s.l.*) displayed the same bimodal curve with peaks in July and September (Fig. 4b). *Anopheles liangshanensis* and *An. maculatus* (*s.l.*) were not found in Man Lve.

Variation of adult mosquito incidence over time and space in Nong Ling

Adult mosquitoes were collected in the village of Nong Ling (Fig. 3) over the same period and in similar places, i.e. houses (Fig. 4c) and cowsheds (Fig. 4d), as in Man Lve. The overall number of individuals captured in houses (n = 479) was in the same range as in Man Lve, with the same three main species, i.e. *An. sinensis* (62.0%, 297/479), *An. peditaeniatus* (23.4%, 112/479), and *An. minimus* (*s.l.*) (12.5%, 60/479). Unlike in houses in Man Lve, *An. peditaeniatus* was the second most prevalent species. The main difference was the population size in cowsheds: 22 in Man Lve and 112 in Nong Ling (Fig. 4). Light traps in cowsheds in Nong Ling yielded a higher number of captured adults (n = 1169), although less than in Man Lve. The ratio between cowsheds and houses in Nong Ling was only 2.4 (1169/479). The most prevalent species in cowsheds was *An. peditaeniatus* (48.8%, 571/1169) followed by *An. sinensis* (44.1%, 515/1169) and *An. minimus* (*s.l.*) (6.7%, 78/1169) (Fig. 4d). The bimodal curve of *An. minimus* (*s.l.*) displayed a plateau covering September and October. Beside these three dominant species, four more species were also collected such as *An. culicifacies* (*s.l.*) (1.3%, 6/479), *An. liangshanensis* (0.4%, 2/479), *An. maculatus* (*s.l.*) and *An. splendidus* (0.2%, 1/479) in human houses. *Anopheles aconitus* and *An. argyropus*, rare in Man Lve, were not found in Nong Ling.

Overall analysis of the prevalence of adult *Anopheles* mosquitoes

When considering the cumulated data in Man Lve and Nong Ling, the most frequent species were *An. sinensis* (50.3%, *n* = 2055), *An. peditaeniatus* (31.6%, *n* = 1291) and *An. minimus* (*s.l.*) (15.8%, *n* = 648). They contributed for 97.7% of the total *Anopheles* mosquitoes collected. *An. sinensis* was the predominant species in both human houses (61.7%, 611/990) and cowsheds (46.6%, 1444/3099). However, *An. minimus* (*s.l.*) was the second largest mosquito species in human houses (22.5%, 223/990). The PCR analysis of the 647 specimens of *An. minimus* (*s.l.*) indicated that 64.8% (419/647) were *An. harrisoni* (former *An. minimus* species C [32]) and 35.2% (228/647) were *An. minimus* (former *An. minimus* species A) (Table 4). One specimen, initially identified as *An. fluviatilis* by morphological identification, was confirmed as *An. harrisoni* by PCR (Table 4). Four specimens of the Maculatus Group were also confirmed as *An. maculatus* by PCR assay (Table 4). The study was extended in October 2015 to seven additional villages in the close vicinity of Man Lve and Nong Ling with the same methods (Fig. 2) to analyze the diversity of *Anopheles* species during a period of higher diversity. The same number of sampling points and same sampling efforts were applied in all the villages. A total of 859 adults were collected during this month over the 9 villages considered. Mosquitoes collected from human houses made up only 20.1% (173/859), while 79.9% were isolated from cowsheds (686/859) (Fig. 5). The number of collected mosquitoes were the highest (> 100 specimens) in four villages: Zhang Jia Cun (*n* = 117); Yan Si (*n* = 123); Man Lve (*n* = 184); and Nong Ling (*n* = 306). The most frequent species in the 9 villages were also *An. sinensis* (23.5%, 202/859), *An. minimus* (*s.l.*) (34.9%, 300/859) and *An. peditaeniatus* (30.2%, 259/859). *Anopheles sinensis* was found in all the sites investigated and *An. minimus* (*s.l.*) in all but one site, Man Duo (Fig. 6). Conversely, *An. peditaeniatus* was found in five out of nine sites only and was highly present in only three sites, Man Lve, Nong Ling and Man Duo (Fig. 6).

Detection of *Plasmodium* spp. in mosquitoes and parous rate

No *Plasmodium* parasite was detected in any captured mosquitoes (Table 5). Out of 295 mosquitoes captured and dissected for parous status, 101 were *An. sinensis* and 194 were *An. minimus* (*s.l.*) With respect to *An. sinensis*, 88 individuals (87.1%) were parous while 180 (92.8%) *An. minimus* (*s.l.*) mosquitoes were also found parous.

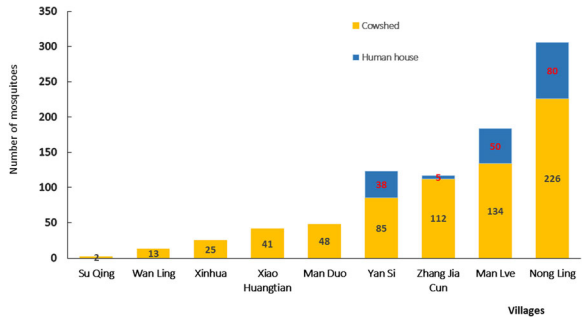

Fig. 5 Relative distribution of *Anopheles* mosquitoes in human houses and cowsheds in the nine study villages in October 2015

Blood meal identification

A total of 300 blood samples from trapped mosquitoes were tested. *An. sinensis* was found more zoophilic (27.3%) than *An. minimus* (*s.l.*) (10.7%). *Anopheles minimus* (*s.l.*) displayed more mixed blood meals, either animal/animal (13.1%) or animal/human (14.3%) than *An. sinensis* (3% and 9.1%, respectively) (Table 6). The human blood indices for *An. sinensis* and *An. minimus* (*s.l.*) are 0.21 (28/132) and 0.26 (44/168), respectively.

Distribution of larvae in potential breeding sites

A total of 407 mosquito larvae were collected in Man Lve and Nong Ling by hand-dipper sampling from four types of habitats such as pond (man-made), pool, ditch and rice field (Table 7). Four *Anopheles* species were identified among the fourth instar larvae and pupae collected (208 specimens in total), i.e. *An. sinensis*, *An. minimus* (*s.l.*), *An. culicifacies* (*s.l.*) and *An. peditaeniatus*. *Anopheles sinensis* (51.0%, 106/208) and *An. minimus* (*s.l.*) (46.2%, 96/208) were the predominant species (Table 7). Ponds (57.2%, 233/407) and rice fields (28.3%, 115/407) were the two preferred breeding sites for the *Anopheles* mosquitoes collected (Table 7).

Discussion

This study is part of the malaria surveillance activities associated to malaria elimination in China, particularly

Table 4 Molecular identification of sibling species

PCR results/Morphological results	*An. minimus* *n* (%)	*An. harrisoni* *n* (%)	*An. maculatus* *n*	Sub-total *n*
An. minimus (*s.l.*)	228 (35.2)	419 (64.8)	–	647
An. fluviatilis	0	1	–	1
An. maculatus (*s.l.*)	–	–	4	4

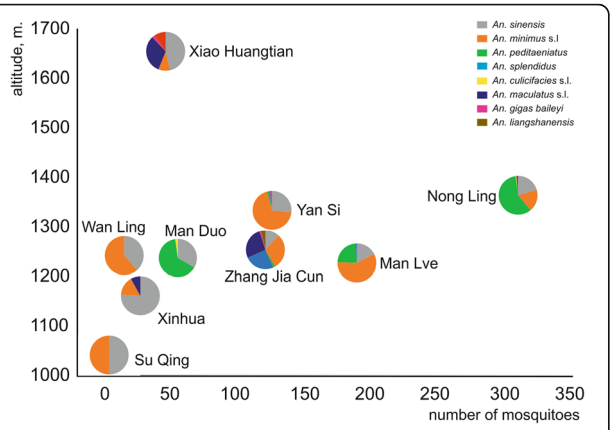

Fig. 6 Prevalence of captured *Anopheles* mosquitoes per village according to altitude (m)

for risk assessment of malaria reintroduction. China has made significant progress on malaria elimination since 2010, and has achieved zero report of indigenous malaria cases within the whole country in 2017 [33, 34]. These achievements were attributed to the promotion of the 1-3-7 approach [35, 36], in which "1-3" are mostly focused on timely case reporting and verification, while "7" is meant to assess the risk of transmission based on entomological information. The latter is considered as an important component of malaria surveillance and response at the elimination and post-elimination stage [37, 38]. The receptivity indicators are thus important for entomological surveillance, in particular in the China-Myanmar border area where a high diversity of *Anopheles* mosquitoes is occurring [37–41].

Tengchong County (TCC) is one of the major malaria endemic counties in Yunnan Province with both *Plasmodium falciparum* and *P. vivax* being transmitted by several *Anopheles* species. The climate and environment are suitable for propagation of malaria vectors and the high cross-border mobility of populations are conditions that favor malaria transmission [5, 6, 12, 14, 18]. However, after several years of malaria control effort, TCC has successfully decreased the incidence of malaria from 35.4/10,000 in 2006 to 2.09/10,000 in 2014 with no indigenous case reported since 2013 [8, 42]. After 2010, the year when malaria elimination program was launched, most malaria cases (40.6%), mainly imported ones, were reported from southern townships within TCC.

Table 5 Detection of *Plasmodium* in captured mosquitoes. All test results were negative

Anopheles spp.	Total number	Trapped place	
		House	Cowshed
An. sinensis	1243	314	929
An. minimus (*s.l.*)	510	163	347
An.peditaeniatus	608	22	586
An. sinensis	812	297	515
An. minimus (*s.l.*)	138	60	78
An. peditaeniatus	683	112	571
An. maculatus (*s.l.*)	4	1	3
An. liangshanensis	2	2	0
Total	4000	971	3029

Four *Anopheles* species or complexes were previously recorded as predominant malaria vector in TCC, i.e. *An. minimus* (*s.l.*), *An. dirus* (*s.l.*), *An. sinensis* and *An. liangshanensis* (syn. *An. kunmingensis*) [43, 44]. The latter was considered the primary vector of *P. falciparum* malaria with a local transmission in TCC at high altitude (> 1700 m), due to its greater susceptibility to *P. falciparum* compared to *P. vivax* [45]. However, both malaria control interventions (such as LLIN/ITN, IRS) and reduction of rice field surface at high altitude have decreased the density of *An. liangshanensis* populations in line with the number of local *P. falciparum* malaria cases [42, 46]. *Anopheles dirus* was found neither at the adult nor larval stage in this study. This indicates that the population density of *An. dirus* has decreased and might now play a negligible role in malaria transmission. Similar results were reported in neighboring counties [40] and in Hainan Province [47] where *An. dirus* initially present as the primary malaria vector has disappeared [39, 43]. The absence of *An. dirus* might not only be due to vector control activities (particularly the use of LLIN/ITN) but also to the destruction of breeding sites such as forests to develop plantations of cash crops.

In this study, *An. sinensis* was found to be the predominant species in both human houses and cowsheds. This differs significantly from previous reports from neighboring counties [48], where *An. minimus* (*s.l.*) was the predominant taxon. This is particularly important because *An. sinensis* displays specific traits making it a potential threat for malaria elimination. First, *An. sinensis* is associated with rice fields [39] and there is no possibility to eliminate this type of breeding sites. Secondly, *An. sinensis* has been reported as resistant to insecticides such as pyrethroids and Malathion [39, 49]. Thirdly, blood-meal analysis showed that *An. sinensis* displayed a similar tropism to humans as *An. minimus* (*s.l.*). These traits, combined with the predominant abundance of *An. sinensis* in TCC, are major concerns for the success and sustainability of malaria elimination. The exact role of *An. sinensis* in malaria transmission in TCC and, more widely in Yunnan Province, should then be thoroughly investigated. Moreover, regular movements of populations across the China-Myanmar border, owing to the existence of endemic malaria in Myanmar [5, 6], increase the risk of malaria vulnerability in TCC and Yunnan through transmission by *An. sinensis*. This threat must thus be further assessed and modeled while scenarios of risk management

Table 6 Blood meal identification sources in *Anopheles sinensis* and *An. minimus* (*s.l.*)

Species/ complex	Blood source, *n* (%)			Mix, *n* (%)		Total
	Pig	Cow	Human	Pig & cow mix	Pig or cow & human mix	
An. sinensis	64 (48.5)	36 (27.3)	16 (12.1)	4 (3.0)	12 (9.1)	132
An. minimus (*s.l.*)	84 (50.0)	18 (10.7)	20 (11.9)	22 (13.1)	24 (14.3)	168

must be developed. Furthermore, *An. minimus* (*s.l.*), another malaria vector in TCC, was consistently found in this study with two peaks of density during the year. A large part of the *An. minimus* (*s.l.*) population (64.8%) belonged to *An. harrisoni.* This could explain the lower local malaria transmission in TCC since *An. harrisoni* was reported to be more exophagic and zoophilic than *An. minimus*, its sibling species [40, 50–52]. Furthermore, *An. harrisoni* was also reported to be more adaptable to the environmental changes and flexible in its trophic behavior than *An. minimus* [51, 53–55], which could be a challenge to vector control strategies and entomological surveillance. For instance, the shift of species between *An. minimus* and *An. harrisoni* was reported to be a consequence of vector control measures in Southeast Asia [56]. Comprehensive vector control measures including LLIN/ITN and IRS were indeed conducted in TCC over the last decade [12]. The composition of *An. minimus* (*s.l.*) found in this study may have resulted from vector control measures, as well as environmental changes increasing the proportions of *An. harrisoni versus An. minimus*. Unfortunately, PCR techniques for identification of the *Anopheles* complexes were not used in previous routine surveillance and there is thus a lack of detailed information about the initial distribution of *An. minimus* (*s.l.*) [39]. It is therefore not possible to formally conclude what impacted the current composition of *An. minimus* (*s.l.*) This indicates that more detailed integrative analyses should be conducted to better understand the mechanisms involved in the dynamic of vector populations. A closer attention should also be brought to PCR species identification, an approach to be implemented in routine surveillance at malaria elimination stage and post-elimination stage. Despite the decrease of population density of *An. minimus* (*s.l.*) in TCC, the ecological behavior such as resting or blood-seeking behavior was found to be similar as previously described prior to population decrease [57]. Furthermore, a potential synergistic action of *An. sinensis* and *An. minimus* (*s.l.*) in potentiating malaria transmission should not be ignored. Owing to its zoophilic diet preference and considering the lack of competence for transmission of malaria parasites, *An. peditaeniatus* was not previously reported as a malaria vector [19]. However, the presence of *P. falciparum* in one specimen of *An. peditaeniatus* was recently confirmed by ELISA in Indonesia [58]. Since *An. peditaeniatus* was the second largest population of adult mosquitoes found in this study, further investigation should thus be conducted to monitor the risk of malaria transmission by this species in TCC. Another aspect to consider is the discrepancy between the number of adults and larvae of *An. peditaeniatus* captured. Only three fouth-instar larvae of *An. peditaeniatus* were identified out of 199 *Anopheles* larvae indicating a very low prevalence in all the breeding sites investigated. Conversely, there is a high prevalence of adults in cowsheds. This suggests that the actual breeding sites of this species were most likely missed. It is therefore essential to investigate thoroughly all possible breeding sites for *An. peditaeniatus*.

Environmental factors should also be considered when conducting entomological survey. *An. peditaeniatus* was found in large number in only three localities out of nine and at low level in two more. The two main vectors, *An. sinensis* and *An. minimus* (*s.l.*), were present in all localities and in all but one, respectively. Nevertheless, the main difference is the species richness and the number of individuals between human houses and cowsheds, which might be linked to *Anopheles* blood preference or more favorable living conditions. Although some predominant *Anopheles* species are known as zoophilic, a reduction of livestock may favor malaria re-emergence as some species are quite ubiquitous like *An. sinensis* [59]. Furthermore, models have shown that zoophilic mosquitoes can also play a significant role in the transmission of malaria to humans [60].

Table 7 Abundance of *Anopheles* taxa found in larval sampling in Man Lve and Nong Ling villages

Site	I-III instar	IV instar and pupa, *n* (%)					Total
		An. sinensis	*An. minimus* (*s.l.*)	*An. culicifacies* (*s.l.*)	*An. peditaeniatus*	Sub-total	
Pond	137	64 (66.67)	31 (32.29)	0 (0)	1 (1.04)	96	233
Pool	11	5 (45.46)	4 (36.36)	2 (18.18)	0 (0)	11	22
Ditch	12	1 (4.00)	23 (92.00)	0 (0)	1 (4.00)	25	37
Rice field	39	36 (47.36)	38 (50.00)	1 (1.32)	1 (1.32)	76	115
Total	199	106 (50.96)	96 (46.16)	3 (1.44)	3 (1.44)	208	407

Although no *Plasmodium*-infected mosquito was found, the high parous rate of *An. sinensis* (87.1%) and *An. minimus* (*s.l.*) (92.8%) suggests a high daily survival probability [61]. Continuous entomological surveillance and vector control measures are highly recommended even after TCC had officially achieved malaria elimination. Further research should be addressed such as: (i) Investigation of seasonal dynamics of the vectors through the implementation of a weather-based statistical dynamic and climate change model; (ii) Development of a distribution/predictive map of *An. minimus* complex and *An. sinensis* across TCC and the border areas; (iii) Evaluation of the length of the possible transmission season for *P. falciparum* and *P. vivax*; and (iv) Evaluation of the vectorial capacity of *An. minimus*, *An. harrisoni* and *An. sinensis*.

Conclusions

TCC was granted the malaria elimination certificate by Yunnan provincial authorities in 2016. However, considering the increasing mobility of the local populations, the border location with Myanmar, the positive vector competence of local *Anopheles* populations, and the risk posed by secondary vectors and insecticide resistance, further efforts should be devoted to surveillance, monitoring and development of scenarios for timely response to imported malaria cases. A specific attention should be paid to local environments and variation of vector prevalence when developing scenarios. Large-scale analysis might not be accurate and reliable enough. Precise actions from both local CDC and national program at this border area will be essential for the success of sustainable malaria elimination.

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Availability of data and materials

The data supporting the conclusions of this article are provided within the article. The datasets used and/or analyzed during the current study are available from the corresponding author upon a request.

Authors' contributions

SSZ, SSZ, ZBZ, XZW and XNZ conceived the study. SSZ, ZBZ, XZW, WQS, WKJ and XSL collected the data in the field. SSZ and AA analyzed the data. SSZ, SSZ, SM, RF and AA wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The volunteers who participated the mosquito trappings were the local CDC staffs. The informed consent were acknowledged by them before the work started as this was also part of their job responsibilities.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Conclusion

According to the results reported in this Chapter, the area of distribution in China of the principal malaria vectors was reduced, in particular for *Anopheles lesteri* (synonym: *An. anthropophagus*) and *Anopheles dirus* s.l., including the two main malaria vector species, *An. dirus* and *An. baimaii*, which nearly disappeared after several years of malaria control effort. *Anopheles sinensis*, which was previously reported to be less efficient for malaria transmission, is becoming the predominant species in Southwestern China. Besides, the field sampling results indicated the existence of high efficient malaria vectors, e.g. *Anopheles minimus* and *An. harrisoni* at the China-Myanmar border. In addition, elevated human-biting rates, high adult and larval densities, and important parous rates were found in both *An. sinensis* and *An. harrisoni*, which reveal a very high receptivity and risk of malaria re-introduction along the China–Myanmar border.

Therefore, to achieve malaria elimination by 2020, there is a need to evaluate and follow-up the behavioral changes of *Anopheles* species in China. These changes may be driven by both environmental changes and control interventions, and to update the tools for entomological surveillance. For instance, the role of secondary malaria vectors, like *An. sinensis* in Yunnan, should be monitored with close scrutiny. The index of receptivity, which represents the capacity of a given area to be favorable to malaria transmission, should be adopted as an indicator for malaria surveillance to evaluate the risk of malaria re-introduction. The biological studies of *Anopheles* mosquitoes,

especially the trophic, biting and resting behaviors, are in need of further investigation. This is particularly important along international border areas, where one country has achieved malaria elimination while the other neighbor still has local malaria transmission. Molecular techniques, such as PCR assays, should be routinely implemented to identify *Anopheles* species in order to better apprehend their respective role in malaria transmission and target the proper vector species during control programs. This is particularly pertinent for sibling species such as those of the Minimus Complex but also the Dirus Complex although restricted to limited areas in China.

Chapter 3 Malaria surveillance and response towards to malaria elimination

Introduction

Although China has achieved zero indigenous case in 2017, with the development of international cooperation, there is an increasing number of imported malaria cases caused by Chinese citizens returning from malaria-affected countries. The analysis of the characteristics, drivers and route of introduction in different areas of China has provided evidence-based data to policy makers where and when they have to carry out the interventions. This in turn allows them to develop efficient guidelines for risk-assessment of malaria re-introduction and for allocating appropriate resources. In this chapter, the comparative analysis of imported malaria cases reported from former non-endemic areas and former endemic areas in China was undertaken to describe the differences between the specific and common characteristics of imported malaria in these two environmental types of situations. Besides, the study was also intended to identify the gaps in the surveillance and response to imported malaria in relation to each area. The China-Myanmar border is a hotspot for malaria introduction and is affected by massive movements of populations, both legal and illegal. It was therefore selected as a priority study site in this chapter. A stochastic simulation model was introduced to evaluate the correlation between population mobility and community vulnerability to malaria re-introduction at the China-Myanmar border. This will provide a potential indicator for malaria re-introduction and for surveillance.

Articles displayed in Chapter 3

Article 5. Chen TM, **Zhang SS**, Feng J, Xia ZG, Luo CH, et al. Mobile population dynamics and malaria vulnerability: a modelling study in the China-Myanmar border region of Yunnan Province, China [J]. Infect Dis Poverty, 2018, 7 (1): 36

Article 6. **Zhang SS**, Feng J, Zhang L, Ren X, Geooffroy E, et al. Imported malaria cases in former endemic and non-malaria endemic areas in China: are there differences in case profile and time to response?[J]. Infect Dis Poverty, 2019, 8 (1): 61

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Mobile population dynamics and malaria vulnerability: a modelling study in the China-Myanmar border region of Yunnan Province, China

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Abstract

Background: The China-Myanmar border region presents a great challenge in malaria elimination in China, and it is essential to understand the relationship between malaria vulnerability and population mobility in this region.

Methods: A community-based, cross-sectional survey was performed in five villages of Yingjiang county during September 2016. Finger-prick blood samples were obtained to identify asymptomatic infections, and imported cases were identified in each village (between January 2013 and September 2016). A stochastic simulation model (SSM) was used to test the relationship between population mobility and malaria vulnerability, according to the mechanisms of malaria importation.

Results: Thirty-two imported cases were identified in the five villages, with a 4-year average of 1 case/year (range: 0–5 cases/year). No parasites were detected in the 353 blood samples from 2016. The median density of malaria vulnerability was 0.012 (range: 0.000–0.033). The average proportion of mobile members of the study population was 32.56% (range: 28.38–71.95%). Most mobile individuals lived indoors at night with mosquito protection. The SSM model fit the investigated data ($\chi^2 = 0.487$, $P = 0.485$). The average probability of infection in the members of the population that moved to Myanmar was 0.011 (range: 0.0048–0.1585). The values for simulated vulnerability increased with greater population mobility in each village.

Conclusions: A high proportion of population mobility was associated with greater malaria vulnerability in the China-Myanmar border region. Mobile population-specific measures should be used to decrease the risk of malaria re-establishment in China.

Keywords: Malaria, Importation, Vulnerability, Mobile population, Individual-based model

Background

Globalization and international population migration have caused imported malaria cases to become the predominant threat to the Chinese malaria elimination program [1, 2]. One major challenge is cross-border malaria transmission, which is a particular concern in the China-Myanmar border region [3–5]. Yingjiang is a county in the Yunnan Province, located at the China-Myanmar border; this region had the majority of national indigenous malaria cases reported in previous years. Therefore, this is a critical region to assess the risk of malaria re-establishment.

In addition to receptivity, malaria vulnerability is considered a major characteristic for risk assessment of malaria re-establishment [6–8]. According to the World Health Organization (WHO) framework for malaria elimination, malaria vulnerability is defined as either the probability of malaria parasite importation into a country or area, or the frequency of the influx of infected individuals, groups,

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and/or infective anopheline mosquitoes. However, since it is difficult to quantify the importation of infective mosquitoes, imported cases or asymptomatic infections are generally used to quantify vulnerability [6, 7, 9, 10].

Many researchers have found that vulnerability is related to population mobility [2, 3], and preventing the infection of a mobile population in a malaria-endemic area can effectively reduce the importation rate. Thus, it is important to understand the relationship between malaria vulnerability and characteristics of mobile populations. These characteristics include the proportion of mobile individuals in a population of a given area, exposure risk, and the frequency and duration of population movement. Mathematical models are frequently used to quantify a study population's characteristics, but these models may not always be based on traditional epidemiological methods. The stochastic individual-based model (IBM) and an ordinary differential equation model are commonly used in the quantification process [11–19]. The IBM is also used to assess the risk of malaria establishment [20, 21], although no studies have used these models to examine population mobility and malaria in the China-Myanmar border region. Therefore, by adapting some key components from the IBM model (i.e., simulation based on individuals using a random function), we developed a stochastic simulation model (SSM) using community-based, cross-sectional data to evaluate population mobility and its effect on malaria vulnerability in Yingjiang county.

Methods

Study setting

A community-based, cross-sectional survey was used to obtain data from five villages (Jing Po Zhai, Ka Ya He, Xin Cun, Zhuan Po Zhai, and Hu Que. Ba) in Yingjiang county of China (western Yunnan Province) (Fig. 1), which is 1 of 18 counties located at the China-Myanmar border. This county shares a 214.6 km border with the Myanmar state of Kachin. The population of Yingjiang county is 307 960 individuals, with cross-border trade, logging, mining, and plantation activities being common. The basic characteristics of the five selected villages are shown in Table 1.

Data collection

The epidemiological survey was performed during September 2016, which is the peak month for local malaria transmission. Before the survey, a pre-survey of four households was conducted in Hu Que. Ba to adjust the previously developed questionnaire and to improve the planning of the survey. There are 170 households located in the five villages, and an area sampling method that included all households was adopted to collect the basic information for each village and its residents. The basic information for each village was collected by interviewing the primary public health provider, and included the village name, terrain, average temperature, rainfall, main crops, number of households, and number of permanent residents (Additional file 1). The information for each individual was collected by interviewing people in each household, and one adult who could provide complete responses for all household members or visitors was interviewed to complete the standardized questionnaire (Additional file 2). Before the survey, the primary public health providers were asked to give the local residents a notice that included the interview date and survey objective, in order to ensure that one adult was at home during the survey. The questionnaire included all family members' demographic information (age, sex, occupation, education), temporary emigrant information (country, frequency of movement, duration of stay), temporary immigrant information (country, frequency of movement, duration of stay), and categorical exposure risk level (living indoors at night with protection, living indoors at night without protection, living outdoors at night with protection, and living outdoors at night without protection). Protection was defined as the use of a screen door or window, repellent, and/or bed nets, including normal bed nets, long-lasting insecticidal nets, or insecticide-treated nets.

An emigrant was defined as someone who had moved away from the selected village during the previous year. An immigrant was defined as someone who had moved from another place (e.g., Myanmar) into the selected village during the previous year. The mobile population was defined as individuals who had resided in at-risk areas for >1 night during the previous year, based on the malaria transmission route, although individuals were excluded from the mobile portion of the study population if they performed many daytime border crossings. The proportion of the mobile population was defined as the mobile population divided by the number of permanent residents.

All individuals, including mobile individuals who were residing in the village during the survey, were selected for a serosurvey. Finger-prick blood samples were obtained after each individual provided written informed consent. An 18S rRNA nested polymerase chain reaction (PCR) test and real-time PCR were used to detect *Plasmodium* spp. using the finger-prick blood samples [22, 23].

Case definition and classification

Data from malaria cases in the studied villages were reported to the web-based National Notifiable Infectious Disease Reporting Information System, with cases between 2013 and 2016 used in the analysis. Data included sex, age, date of illness onset, *Plasmodium* spp., and imported or indigenous case status. Malaria cases were classified as clinically diagnosed or laboratory-confirmed cases, which were

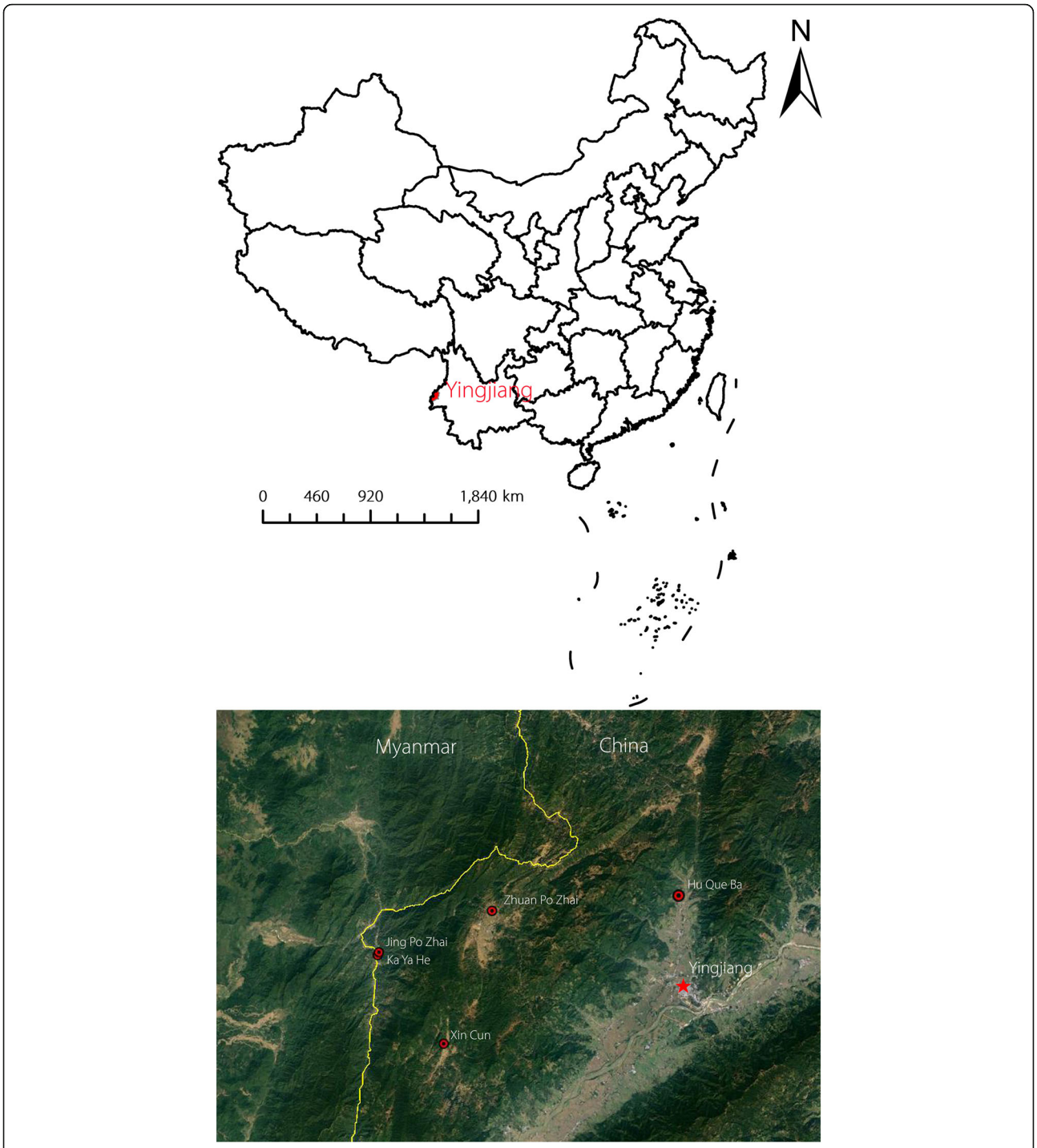

Fig. 1 Location of Yingjiang County as well as the five selected villages

both considered eligible for this study. Clinically diagnosed cases were defined as patients with malaria-like symptoms who had lived in or recently travelled to areas with known malaria transmission. Laboratory-confirmed cases were defined as clinically diagnosed cases with positive results from microscopy evaluation for malaria parasites, rapid diagnostic tests, and/or PCR tests [24].

For case classification especially the identification of indigenous or imported cases, there was a step-by-step protocol utilized that was based on dominated specific species, clear seasonality in China, and history of travel. If the case was confirmed as non *P. vivax* in the reference laboratory system, it would be classified as an imported case since only *P. vivax* transmission occurs in

Table 1 Epidemiological features of the mobile population and basic information regarding the five selected villages at the China-Myanmar border

	Jing Po Zhai	Ka Ya He	Xin Cun	Zhuan Po Zhai	Hu Que Ba
Terrain	Hilly areas	River valley	Hilly areas	Mountain	Plain
Average temperature (°C)	22	22	16	14	18
Rainfall (mm)	2500	2550	2300	2600	2200
Main crops	Banana	Banana	Rice	Rice	Rice
Number of households	39	22	24	53	32
Number of permanent residents	146	86	82	107	74
Number of mobile population	46	28	59	36	21
Gender (Male/Female)	22/24	13/15	28/31	16/20	9/12
Age (Years)					
0–10	5	3	4	3	2
11–20	5	1	6	3	2
21–30	20	8	14	6	7
31–40	7	7	17	6	4
41–50	2	2	7	8	4
51–60	4	3	10	6	1
> 60	3	4	1	4	1
Immigrant	4	2	0	6	3
Area 1	4	0	0	0	0
Myanmar	4	0	0	0	0
Area 2	0	0	0	0	0
Area 3	0	2	0	6	2
China-Myanmar border areas in China	0	2	0	6	2
Area 4	0	0	0	0	1
Transmission interruption areas in China	0	0	0	0	1
Emigrant	42	26	59	30	18
Area 1	23	8	46	3	8
Myanmar	23	8	46	3	7
The Myanmar-Thailand border region	0	0	0	0	1
Area 2	0	0	0	0	0
Area 3	12	6	6	17	1
China-Myanmar border areas in China	12	6	6	17	1
Area 4	7	12	7	10	9
Transmission interruption areas in China	7	12	7	10	9

China, except around the Yunnan border region. However, classification would be more complicated if the case was diagnosed as a *P. vivax*: based on the individual case investigation, if the onset occurred in the non-transmission season, it would be mostly classified as an imported case without the history of infection or an old infection with the history of infection, while if the onset occurred in the transmission season, it would be carefully classified as an imported case if an individual had a history of travel to malaria-endemic areas within 1 month after returning to China, which would otherwise be classified as an indigenous case. For some special cases, such as cases reported from the Yunnan border region and cases without clear evidence to be identified as old or new infections, they will be discussed and classified by an expert group which was established by the National Health and Family Planning Commission (NHFPC) of the People's Republic of China. Sometimes we also employed genotyping for case classification in the reference laboratory system, especially for the identification of non-vector-borne transmissions, such as infection by blood transfusion.

Calculation of vulnerability

Vulnerability is calculated using the following equations:

$$V = n_a + n_I \quad (1)$$

$$D_V = \frac{V}{N} \quad (2)$$

In these equations, V refers to vulnerability, n_a refers to the number of asymptomatic infections, n_I refers to reported imported cases, D_V refers to the density of vulnerability, and N refers to the number of inhabitants. In this study, n_a was estimated using the finger-prick blood samples that were collected during the cross-sectional survey. To avoid selection bias, n_I was estimated using the 4-year average for all imported cases from each village between January 2013 and September 2016.

Model establishment

The stochastic simulation model (SSM) model was developed to simulate the relationship between population mobility and malaria vulnerability according the mechanism of malaria importation (Fig. 2). In the model, we assumed that malaria vulnerability could be affected by the proportion of the population that is mobile, the epidemic status of the areas for temporary immigration and emigration, the risks of exposure to malarial vectors, the efficacy of any protection measures, the duration of exposure, and the frequency of movement. The model used the following equations:

$$D = \sum_{j=1}^{4} \left(M_j \times T_j \times E_j \times p_j \times q \times (1-e) \right) \quad (3)$$

$$M = \frac{M_{im} + M_{em}}{N} \quad (4)$$

$$T = f \times d \quad (5)$$

In the model, D is the density of imported cases (imported cases divided by total inhabitants), M is the mobile population proportion, M_{im} is the number of temporary immigrants, M_{em} is the number of temporary emigrants, N is the number of inhabitants, T is the total duration of exposure, f is the frequency of movement, d is the duration of each movement, E is the exposure risk, p is the probability of infection, and e is the efficacy of protection. We assumed that the probability of infection for mobile individuals who lived indoors at night should be multiplied by a protection coefficient (q; $0 < q < 1$). The probability of infection (p) depends on the malaria situation in areas 1–4, which we defined as intense transmission ($j = 1$; ≥5 cases/1000 population), pre-elimination ($j = 2$; 1–4.9 cases/1000 population), elimination ($j = 3$; < 1 case/1000 population), and malaria-free ($j = 4$; no cases) based on the WHO World Malaria Report 2015. These areas included counties or global areas. For example, Myanmar may be included in area 1, and some villages in China where malaria is locally transmitted may be classified into area 3. To simulate the stochastic process for p, a random function [$f(x)$ = random (0, 1)] was used with the condition that if $f(x) > p \times q \times (1 - e)$, the individual would be considered infected. All other individuals were considered uninfected.

Parameter estimation and simulation methods

Among the 9 parameters in the SSM model (M_{im}, M_{em}, N, f, d, E, p, q, and e), data regarding M_{im}, M_{em}, N, f, d, and E were obtained from the epidemiological survey. Data regarding the other 3 parameters (p, q, and e) were

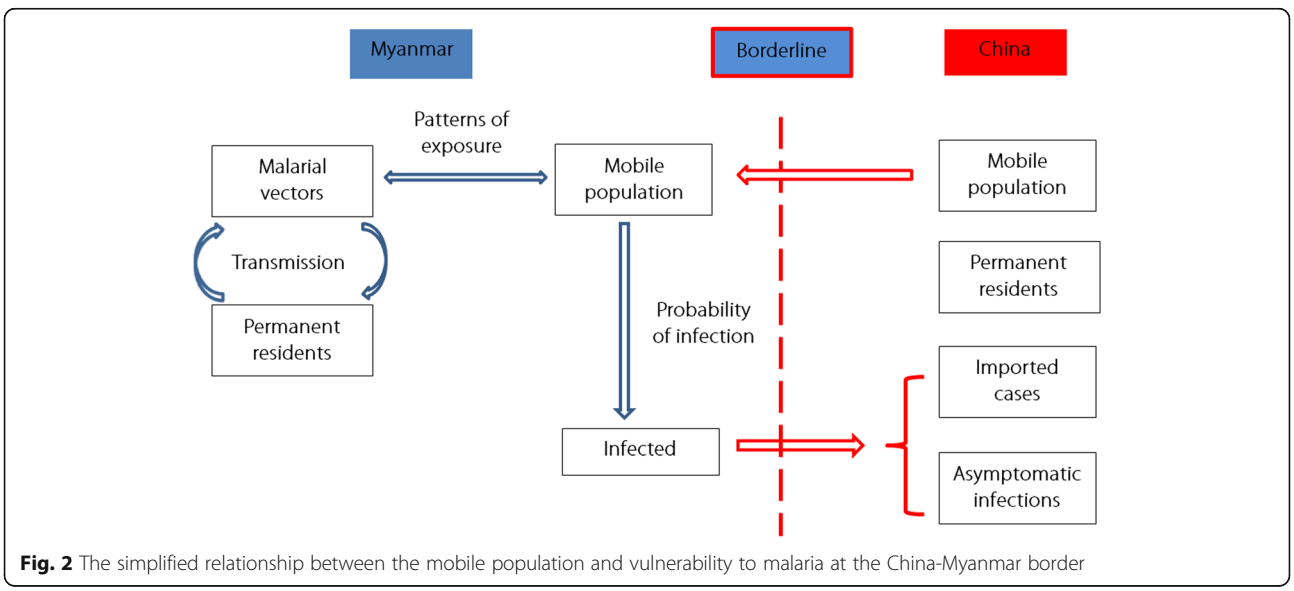

Fig. 2 The simplified relationship between the mobile population and vulnerability to malaria at the China-Myanmar border

obtained using a model fitted with the vulnerability data from the selected villages. In the model fitting, the simulated D values were compared to the malaria vulnerability values (density of imported cases and asymptomatic infections) by calibrating each parameter until the chi-square test revealed no significant difference ($P > 0.05$).

To explore the relationship between malaria vulnerability and the proportion of the mobile population, the SSM model was simulated 1000 times using different M values (10%, 20%, …, 100%). This approach allowed us to obtain the simulated densities of imported infections based on different M values for each village.

Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA) was used to run the SSM model. SPSS 13.0 (IBM Corp., Armonk, NY, USA) was used to perform the chi-square test and the Fisher's exact test.

Results

Basic village information and the mobile populations

The five study villages were located in warm and rainy areas with variable terrain. The main crops produced by all five villages were banana and rice. The median number of permanent residents was 86 (range: 72–146), and 92.11% of the mobile population were temporary emigrants (Table 1). Among the immigrants, 66.67% moved from area 3 (the China-Myanmar border region in China), 26.67% moved from area 1 (Myanmar), and 6.67% moved from area 4 (an area with transmission interruption in China). In contrast, among the emigrants, 50.29% moved to area 1 (Myanmar or the Myanmar-Thailand border region), 25.71% moved to area 4 (an area with transmission interruption in China), and 24.00% moved to area 3 (the China-Myanmar border region in China). The differences in the geographical distributions of temporary emigrants were significant in the five villages, based on Pearson's chi-square test ($\chi^2 = 56.667$, $P < 0.001$). The male mobile population was slightly larger than the female mobile population, although this difference was not significant in the five villages ($\chi^2 = 0.225$, $P = 0.994$). Most mobile individuals were 20–39-years-old, although no significant differences were observed in the age distributions for the five villages (Fisher's exact test, $P = 0.312$).

Malaria vulnerabilities in the five villages

During 2013–2016, 32 imported cases were reported in the five villages, with a 4-year average of one imported case per year (range: 0–5 cases). These cases predominantly involved *Plasmodium vivax* (93.75%) and generally involved male individuals (59.38%), although the cases were generally distributed equally among the seven age groups. The highest proportion of cases (50.00%) was detected in 2015. Fisher's exact test revealed no significant differences among the five villages in their distributions of species ($P = 1.000$), sex ($P = 0.651$), age ($P = 0.571$), or temporal distribution ($P = 0.233$), which are all shown in Table 2. There was no significant difference between the reported cases in 2016 and the adjusted 4-year average number of cases ($\chi^2 = 3.580$, $P = 0.466$).

No parasites were detected using PCR in the 353 blood samples from 2016. The median density of malaria vulnerability was 0.012 (range: 0.000–0.033) (Table 2).

Parameter estimation and model fitting

The epidemiological survey revealed high proportions of reported mobile populations in each village (median: 32.56%, range: 28.38–71.95%). All reported temporary immigrants lived indoors at night with protection. Among the reported temporary emigrants, 78.29% lived indoors at night with protection, 1.14% lived indoors at night without protection, and 20.57% lived outdoors at night with protection. Most villages had similar patterns of exposure, except Xin Cun Village, where 78.26% of reported temporary emigrants lived outdoors at night with protection (Additional file 3). The average reported exposure time for the majority of the mobile population was < 5 months. The average reported frequency of movement was < 3 times in Jing Po Zhai, Ka Ya He, Zhuan Po Zhai, and Hu Que. Ba. However, relatively high values of both reported exposure time (11 months) and reported movement frequency (13 times) were observed in Xin Cun (Additional file 3).

The results of the model fitting revealed that the SSM model fit the reported data ($\chi^2 = 0.487$, $P = 0.485$). The median probabilities of infection were 0.011 in area 1 (range: 0.0048–0.1585) and 0.003 in area 3 (range: 0.0021–0.0038). The efficacy of protection was 20% and the protection coefficient (p) for living indoors at night was 0.95 (Additional file 3), which indicated that only 20% of protection against infection was associated with using protection measures (e.g., a screen door or window, repellent, normal bed nets, long-lasting insecticidal nets, or insecticide-treated nets), and that living indoors only provided 5% of protection against infection (vs. living outdoors at night).

Simulated malaria vulnerabilities using different mobile population proportions

Figure 3 shows that although the density of imported cases increased with the proportion of mobile individuals in a given population, each village had different vulnerability values. The highest simulated malaria vulnerabilities were observed in Jing Po Zhai, which was followed by Ka Ya He, Xin Cun, Hu Que. Ba, and Zhuan Po Zhai.

At medium ($0.1 < M < 0.3$) or high ($0.3 \leq M < 0.5$) rates of migration from medium p_1 areas ($p_1 < 0.1$; Hu Que Ba

Table 2 Data and model for estimating vulnerability to malaria in the five selected villages

	Jing Po Zhai	Ka Ya He	Xin Cun	Zhuan Po Zhai	Hu Que Ba
Number of reported imported cases (2013–2016)	19	4	8	0	1
Species of malaria					
P. vivax	17	4	8	0	1
P. falciparum	2	0	0	0	0
Gender					
Male	10	2	6	0	1
Female	9	2	2	0	0
Age					
0–10	5	0	0	0	0
11–20	1	0	1	0	0
21–30	5	1	1	0	1
31–40	3	1	1	0	0
41–50	2	0	3	0	0
51–60	2	1	2	0	0
> 60	1	1	0	0	0
Year					
2013	0	0	0	0	0
2014	4	0	1	0	1
2015	9	4	3	0	0
2016	6	0	4	0	0
Four-year average reported imported cases (per year)	4.75	1.00	2.00	0.00	0.25
Number of blood samples collected	77	43	50	112	71
Number of tested asymptomatic infections	0	0	0	0	0
Density of vulnerability to malaria	0.03253	0.01163	0.02439	0.00000	0.00338
Simulated vulnerability to malaria	0.03248	0.01162	0.02438	0.00049	0.00338

and Zhuan Po Zhai), we found that malaria vulnerability increased slowly with the mobile population proportion. At exceedingly high ($M \geq 0.7$) rates of migration from medium p_1 areas ($p_1 < 0.1$; Xin Cun), or high ($0.3 \leq M < 0.5$) rates of migration from high p_1 areas ($0.1 \leq p_1 < 0.15$; Ka Ya He), we found that malaria vulnerability increased with the mobile population proportion. At high ($0.3 \leq M < 0.5$) rates of migration from exceedingly high p_1 areas ($p_1 > 0.15$; Jing Po Zhai), we found that malaria vulnerability increased dramatically with the mobile population proportion (Fig. 3).

Based on an average simulated M value of 10%, the imported case densities were 2.6/1000 population for area 1 (95% *CI*: 0.1–10.4/1000 population), 0.5/1000 population for area 3 (95% *CI*: 0.1–0.6/1000 population), and a total density of 3.2/1000 population (95% *CI*: 0.2–10.9/1000 population). The imported cases increased with an increasing proportion of mobile individuals in the study population (Fig. 4). Based on a simulated M value of 100%, the imported case densities increased to 30.5/1000 population for area 1 (95% *CI*: 0.4–103.1/1000 population), 1.9/1000 population for area 3 (95% *CI*: 0.7–5.7/1000 population), and a total density of 32.4/1000 population (95% *CI*: 1.6–107.4/1000 population).

Discussion

Progress towards eliminating malaria in China has recently reduced the malaria transmission rate in Yunnan Province [25–27]. However, Yingjiang's location in a warm and rainy area at the China-Myanmar border is associated with high malaria receptivity. Thus, malaria vulnerability has become a key factor for malaria re-establishment in this region. The present study revealed a high mobile population proportion in this county, with most mobile individuals temporarily moving to Myanmar. Fortunately, most mobile individuals lived indoors with protection, which may have reduced their risk of infection. However, our results indicate that living indoors only provided 5% of protection against infection (vs. living outdoors at night). The low efficacy of living indoors might be related to the several-hours period between sunset and actually going to sleep, as vectors

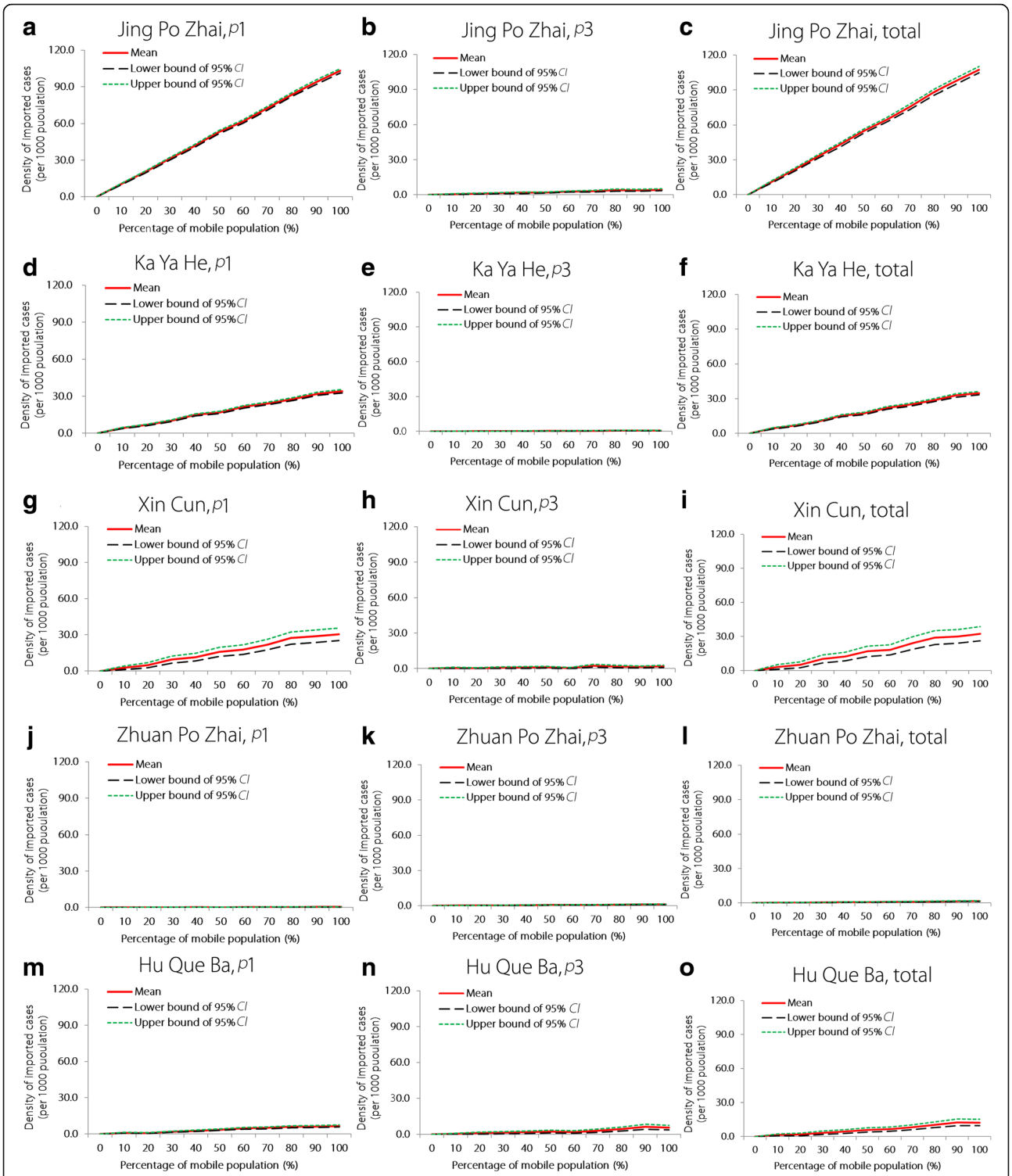

Fig. 3 Simulated density of imported cases caused by varying proportions of mobility in five villages of Yingjiang county, China. Panels **a**-**o** represent the simulated density of imported cases in the five villages under the conditions of p_1, p_3, and the total probability, respectively. p_1 indicates the probability of infection because of immigration or emigration from areas with the most intense transmission (≥5 cases per 1000 population). p_3 indicates the probability of infection because of immigration or emigration from malaria elimination areas where the incidence is < 1 case per 1000 population. The total probability of infection is calculated based on immigration or emigration from all areas

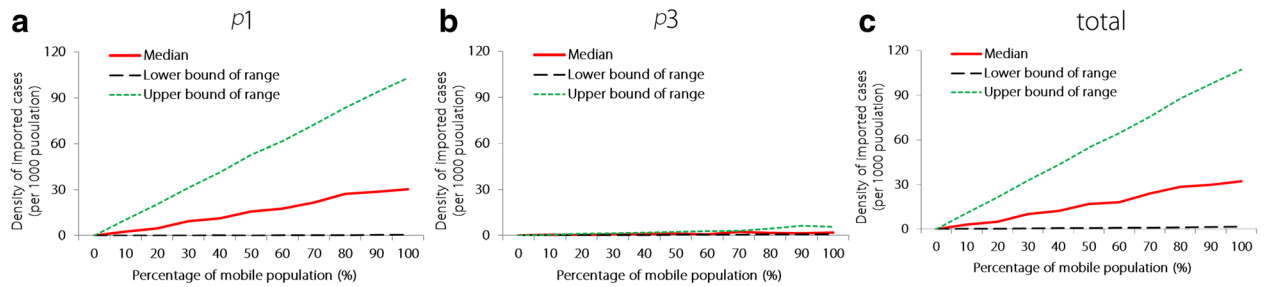

Fig. 4 The median and range values for the simulated densities of imported cases caused by varying proportions of mobility. **a**, p_1. **b**, p_3. **c**, total. p_1 indicates the probability of infection because of immigration or emigration from areas with the most intense transmission (≥5 cases per 1000 population). p_3 indicates the probability of infection because of immigration or emigration from malaria elimination areas where the incidence is < 1 case per 1000 population. The total probability of infection is calculated based on immigration or emigration from all areas

could enter the house during this period; it is also possible that unclosed or leaky windows and doors contributed throughout the entire night. Nevertheless, the precise mechanism remains unclear, and additional research is needed to better understand why living indoors is associated with low efficacy. Our results also indicate that only 20% protection against infection was associated with using protection measures (e.g., a screen door or window, repellent, normal bed nets, long-lasting insecticidal nets, or insecticide-treated nets). The low efficacy of protection measures might be related to inappropriate use, and the period between sunset and going to sleep might be a critical time for infection. Countermeasures may be needed to decrease the infection of mobile people during this time. Another reason might be that people tend to over-report their use of bed nets and to underreport both their migration behaviors and living outdoors at night. This reporting bias from mobile populations might lead to an underestimation of the efficacy of living indoors and protection measures.

The high proportion of mobile individuals in the study population was associated with greater malaria vulnerability, which might increase the likelihood of malaria re-establishment in Yingjiang county. In addition, the model that was used for analysis fit the reported data, which indicates that the SSM model can be used to simulate the relationship between mobile population proportion and malaria vulnerability. Interestingly, we observed different infection probabilities among the mobile populations of the five villages, with the greatest probability observed in area 1. These differences might be related to the heterogeneous distribution of malaria transmission in Myanmar. Thus, the mobile populations of Jing Po Zhai, Ka Ya He, and Xin Cun might have moved to high transmission areas, while the mobile populations of Zhuan Po Zhai and Hu Que. Ba might have moved to low transmission areas in Myanmar.

Most mobile individuals lived indoors at night with protection, although the efficacy of protection and rate of sleeping indoors was low during their stays in Myanmar. Furthermore, we detected high values for the mobile population proportion and malaria vulnerability in the China-Myanmar border region. Moreover, the SSM model predicted a linear relationship between mobile population proportion and malaria vulnerability. Therefore, to reduce the risk of malaria re-establishment in the border regions of China, we recommend introducing mobile population-specific measures, such as health education to reduce malarial vector exposure and blood screening with ultrasensitive reverse transcription PCR to identify asymptomatic infections when mobile people return to China [28].

The present study is limited in that it only evaluated PCR-based data regarding asymptomatic infections from a single community-based, cross-sectional survey in 2016, and it is probable that asymptomatic infections were not detected during 2013–2015 when PCR was not used. Therefore, the regional vulnerability might be underestimated by using the 4-year average for imported cases, although we believe that this would only have a minor effect on our findings. It is important to match temporal behaviors with infection data from the same period, and the present cross-sectional study collected mobile population data from the previous year to ensure that it matched the imported case data from 2016. However, we found that no imported cases were reported in three villages (Table 2). Thus, to avoid selection bias by using only a single year for analysis, the data were adjusted using the 4-year average case numbers from each village during 2013–2016. After the adjustment, we found that SSM model fit the data and there was no significant difference between the reported cases in 2016 and the adjusted 4-year average number of cases, which indicates that the 4-year average is appropriate for use in the model.

The simulated results of our study are based on the assumptions of the SSM model. In this model, the independent variables included the proportion of mobile individuals in the study population, the epidemic status of the regions

that have temporary immigration and emigration, the risk of exposure to malarial vectors, the efficacy of any protection measures, the duration of exposure, and the frequency of movement. However, there might be other independent variables or residual errors that should be considered in the model, and additional research is needed to more precisely explain the mechanism of malaria vulnerability.

Another limitation is the possibility of bias in linking *P. vivax* to migration patterns. For example, the high frequency of cross-border movement of mobile individuals and the long latent period of *P. vivax* infection [29] can make it difficult to determine when the *P. vivax* infection was acquired. Fortunately, China has developed a step-by-step protocol for case classification and a 5-level case confirmation network. The foundation of the network is comprised of each hospital and clinic that detects and reports malaria cases. Next, the county's Center for Disease Control and Prevention (CDC) implements an epidemiological investigation to categorize the case as imported or indigenous. The municipal CDC then checks the information that was reported by the county CDC, and each case is finally verified by a provincial reference laboratory after being reported by the local public health institute. The final confirmation of each malaria case is approved by the NHFPC expert group. This process and the step-by-step protocol ensure that each malaria case is diagnosed and categorized correctly, and minimizes the likelihood of diagnostic bias in China.

Conclusions

This community-based, cross-sectional study was performed to develop an SSM model that simulates mobile population dynamics and malaria vulnerability in the China-Myanmar border region. High population mobility was observed with different epidemiological characteristics and exposure patterns, which were associated with varying levels of vulnerability in the studied villages. Thus, the SSM model could be used as a tool to quantify the linear relationship between vulnerability and mobile populations, and it may be useful for assessing the risk of malaria re-establishment in China.

Additional files

Additional file 1 Registration Form for Village Profile in Yingjiang County. (DOCX 27 kb)

Additional file 2 Questionnaire for Household Survey in Yingjiang County. (DOCX 29 kb)

Additional file 3 Parameter definitions and values for the five selected villages. (DOCX 42 kb)

Abbreviations

CDC: Center for Disease Control and Prevention; IBM: Individual-based model; NHFPC: National Health and Family Planning Commission; PCR: Polymerase chain reaction; SSM: Stochastic simulation model; WHO: World Health Organization

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Availability of data and materials

Please contact author for data requests.

Authors' contributions

SSZ, TC, and SZ designed research; TC, SZ, JF, ZX, CL, XZ, XG, ZL, and HZ collected the data; SSZ, TC, and SZ conceived the experiments, TC and SZ conducted the experiments and analyzed the results; TC and SSZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was reviewed and approved by the ethical committee of the National Institute of Parasitic Diseases, Chinese Centre for Disease Control and Prevention.

Competing interests

The authors declare that they have no competing interests.

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Infectious Diseases of Poverty

RESEARCH ARTICLE Open Access

Imported malaria cases in former endemic and non-malaria endemic areas in China: are there differences in case profile and time to response?

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Abstract

Background: China has achieved zero indigenous malaria case report in 2017. However, along with the increasing of international cooperation development, there is an increasing number of imported malaria cases from Chinese nationals returning from malaria-affected countries. Previous studies have focused on malaria endemic areas in China. There is thus limited information on non-endemic areas in China, especially on the performance of malaria surveillance and response in health facilities.

Methods: A comparative retrospective study was carried out based on routine malaria surveillance data collected from 2013 to 2017. All imported malaria cases reported within the mainland of China were included. Variables used in the comparative analysis between cases in former endemic and former non-endemic areas, included age, gender and occupation, destination of overseas travel, *Plasmodium* species and patient health outcome. Monthly aggregated data was used to compare seasonal and spatial characteristics. Geographical distribution and spatial-temporal aggregation analyses were conducted. Time to diagnosis and report, method of diagnosis, and level of reporting/diagnosing health facilities were used to assess performance of health facilities.

Results: A total of 16 733 malaria cases, out of which 90 were fatal, were recorded in 31 provinces. The majority of cases (96.2%) were reported from former malaria endemic areas while 3.8% were reported from former non-malaria endemic areas. Patients in the age class from 19 to 59 years and males made the highest proportion of cases in both areas. There were significant differences between occupational categories in the two areas ($P < 0.001$). In former endemic areas, the largest proportion of cases was among outdoor workers (80%). Two peaks (June, January) and three peaks (June, September and January) were found in former endemic and former non-endemic areas, respectively. Time between the onset of symptoms and diagnosis at clinics was significantly different between the two areas at different level of health facilities ($P < 0.05$).

(Continued on next page)

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Conclusions: All the former non-endemic areas are now reporting imported malaria cases. However, the largest proportion of imported cases is still reported from former endemic areas. Health facilities in former endemic areas outperformed those in former non-endemic areas. Information, treatment, and surveillance must be provided for expatriates while capacity building and continuous training must be implemented at health facilities in China.

Keywords: Imported malaria, Non-malaria endemic area, Malaria endemic area, Surveillance and response, Health facilities performance

Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the five official working languages of the United Nations.

Background

According to the 2018 World Malaria Report, 219 million malaria cases and 435 000 associated deaths were reported globally in 2017 [1]. Malaria control efforts across China have led to the decrease of both morbidity and mortality over the past 60 years, from about 30 million cases each year in 1950 to about 7000 cases in 2010 [2–8]. Following implementation of the National Malaria Elimination Program (NMEP) in 2010, which aims to eliminate local transmission by 2020, local malaria transmission steadily declined throughout the country and achieved the goal of zero indigenous malaria case report in 2017 [2, 5, 6, 9]. By contrast, the number of malaria cases reported around the world consistently increased to 219 million in 2017 from 216 million in 2016 and 212 million in 2015. The rise in malaria morbidity in African and South-East Asian countries is substantial with countries displaying more than 20% increase [1, 10–12].

Within the mainland China, thousands of imported cases are still reported every year with a minimal decline in the past 5 years [6, 7, 13, 14]. These cases clearly pose a risk of re-introduction with important public health implications highlighted by policy makers and researchers [5, 13, 15, 16]. With the launch of the Belt and Road Initiative in 2013, international cooperation and international travel of Chinese nationals to malaria-affected countries, particularly in sub-Saharan Africa have increased [13, 15]. The seasonal characteristics of the imported cases differ from indigenous cases [13, 15] while the geographic distribution has also changed as imported malaria cases are now occurring in both former endemic and former non-endemic areas. Furthermore, the species of *Plasmodium* involved have shifted from only *Plasmodium falciparum* and *P. vivax* for the previous locally transmitted cases to four human *Plasmodium* spp. (including *P. malariae* and *P. ovale*) among imported cases [13].

Prior to 2015, studies only focused on the global national performance of health facilities or of those in former endemic areas only [17]. However, some preliminary studies have found significant differences on the malaria diagnosis capacities within China between health facilities in former malaria endemic and former non-endemic areas [18, 19]. Health workers in former malaria endemic areas had better knowledge of malaria epidemiology and malaria diagnosis than those from former non-endemic areas [19, 20]. Misdiagnosis of malaria cases may delay appropriate treatment and negatively impact health outcomes, and may lead to re-introduction of malaria, undermining the progress made through the malaria elimination campaign [21, 22]. These issues warrant an investigation on the characteristics of imported malaria cases and the performance of the health system. This study thus aims at comparing the profile of malaria cases reported in China from 2013 to 2017, time to response and capacity of response of health facilities in former endemic and former non-endemic areas.

Material and methods

Definitions

Former endemic areas

Historically, 24 provinces in mainland China were considered as malaria endemic areas with suitable environmental conditions for malaria vectors and local malaria transmission [23].

Non-endemic areas

The areas display no suitable environmental conditions for malaria vector breeding and no local transmission of malaria was previously reported. The requirement for surveillance and response to malaria cases at county and township level was different in former endemic and former non-endemic areas [17, 23].

Imported cases

According to the WHO malaria terminology, an imported case corresponds to a patient who acquired malaria infection outside the area where it is diagnosed [24]. Since there is no routine laboratory test to identify an "imported" case, the determination is achieved by investigation of patients' travel history to malaria endemic areas through epidemiological survey.

Data source and data collection

Variables used in the comparison of the demographic characteristics of reported imported malaria cases between former endemic and former non-endemic areas included the following: age, gender, occupation, destination of overseas travel, *Plasmodium* species and patient health outcome. To compare the seasonal and spatial characteristics of imported malaria cases from 2013 to 2017, their number was aggregated by month and plotted based on area classification. Finally, to compare the performance of malaria case identification and diagnosis, we created two duration variables using date of onset, date of diagnosis and date of report, together with other variables reflecting the method of diagnosis and the level of the reporting/diagnosis health facility for each case. According to the Chinese Law on Prevention and Treatment of Infectious Diseases (CLPTID) and to International Heath Regulation (IHR), malaria is a notifiable infectious disease. Health facilities at each of the administrative level are required to report every case within 24 h after diagnosis to the Chinese Infectious Disease Report System (CIDRS), a web-based reporting system for individual cases and data management for notifiable infectious diseases. All imported malaria cases reported in CIDRS between 2013 and 2017 from all the health facilities within the mainland of China (excluding Hong Kong, Macau and Taiwan) were included in the analysis. Information on individual cases in this study was obtained from CIDRS, which includes general demographic data, diagnosis data, treatment data and epidemiological data. Data used for this study was routinely collected as part of NEMP from 2013 to 2017.

Geographic and statistical analysis

The geographical distribution and the spatial-temporal aggregation analysis were performed using ArcGIS 10.0 (Esri Inc., Redlands, CA, USA). The comparative analysis between variables from former malaria endemic and former non-endemic areas was conducted with *t* tests and *Chi-square* tests using SPSS (version 25, IBM Corp, Armonk, NY, USA). The level of significance was set at $P < 0.05$.

Results

Demographic characteristics

A total of 16 733 malaria cases were reported from 31 provinces in the mainland China from 2013 to 2017 with 90 (0.54%) related deaths. Demographic and geographic characteristics of the imported malaria cases are shown in Table 1. The majority of cases, $n = 16\,090$ (96.2%), were reported from former malaria endemic areas while 643 (3.8%) cases were reported from former non-endemic areas. The age group ranging from 19 to 59 years and males made the overwhelming proportion of cases in both former endemic and former non-endemic areas (Table 1). There were significant differences between occupational categories of imported malaria cases in former endemic and former non-endemic areas ($P < 0.001$). In former endemic areas, the largest proportion of cases were outdoor workers (80%), with indoor workers making up to 10% of cases while the final 10% were unclearly identified (Table 1). Conversely, cases recorded in former non-endemic areas corresponded to indoor workers (39%) more than to outdoor workers (29%) while the occupation of 32% of cases was undetermined.

Epidemiological characteristics

Two peaks, i.e. June and January (Fig. 1a) and three peaks, i.e. June, September and January (Fig. 1b) were observed in former endemic and former non-endemic areas, respectively. Imported cases in former endemic areas were clustered in the Eastern coastal region and in the Southwestern border area, whereas cases were scattered in former non-endemic areas (Fig. 2). The destination of overseas travel of imported cases reported from former endemic and former non-endemic areas were found to be significantly different ($P < 0.001$). The imported cases reported in former non-endemic areas were primarily coming from Africa (94%), while a significant number of cases reported in former endemic areas were from Southeast Asia (19%) in addition to Africa (80%) (Table 1). Few cases were from Oceania in both endemic (1%) and former non-endemic areas (3%). With respect to the *Plasmodium* species, *P. falciparum* (75%) was the predominant species in former non-endemic areas, whereas there was a larger proportion of *P. vivax* in former endemic areas (*P. vivax* 24%, *P. falciparum* 64%). The proportion of *P. malariae* was almost the same in former non-endemic and former endemic areas (2%), while a larger proportion of *P. ovale* was reported in former endemic areas (8%) than in former non-endemic areas (2%). More cases were reported as "undiagnosed/missing diagnosis information" in former non-endemic areas than in former endemic areas (10% vs 0%) (Table 1).

Performance of health facilities

The duration between onset and diagnosis at admission was significantly different both between former endemic and former non-endemic areas and between different levels of health facilities ($P < 0.05$) (Table 2). The significant difference between health facilities in former endemic and former non-endemic areas was found in the time required from case diagnosis to case reporting ($P < 0.001$) (Table 2). No significant difference was found in the method of diagnosis between former endemic and former non-endemic areas. Nearly all cases

Table 1 Demographic characteristics of imported malaria cases in China, 2013–2017

Demographic Characteristics	Number of cases reported				*P* value
	Former malaria endemic areas		Non malaria endemic areas		
	Number	Proportion (%)	Number	Proportion (%)	
Total Cases	16 090		643		
Age					0.8
< 5 years	62	< 1	0	0	
5–18 years	240	1	6	1	
19–59 years	15 569	97	623	97	
≥ 60 years	219	1	12	2	
Gender					0.2
Male	15 172	94	598	93	
Female	918	6	45	7	
Occupation					< 0.001
Outdoor workers[a]	12 370	80	180	29	
Indoor workers[b]	1613	10	246	39	
Unclear[c]	1532	10	197	32	
Missing[d]	575	–	20	–	
Destination of overseas travel					< 0.001
Africa	12 436	80	475	94	
Southeast Asia/South Asia	3011	19	16	3	
South America	24	0	0	0	
Oceania	137	1	15	3	
Other: West/East Asia	4	0	0	0	
Missing[d]	478	–	137	–	
Plasmodium species					< 0.001
P. vivax	3928	24	64	10	
P. falciparum	10 278	64	481	75	
P. malariae	300	2	11	2	
P. ovale	1297	8	16	2	
P. falciparum + *P. ovale*	105	1	1	0	
P. falciparum + *P. vivax*	146	1	3	0	
Undiagnosed/missing	36	0	67	10	
Fatal outcome					
Death reported	76	0	14	2	–

-: Not applicable

[a]Outdoor workers: persons whose activity is mostly conducted outside. This includes Architectural engineers, Construction workers, Farmers, Fishermen, Overseas migrant worker (Expatriate Chinese nationals), Open mine workers, Sailors/Truck drivers, Field engineers, Herdsmen, Militaries/Soldiers, etc.

[b]Indoor workers: work mostly indoor, including: Businessmen, Caterers, Interpreters, Medical staff, Office workers, Teachers, Actors, Flight attendants, Baby-sitters, Middlemen, Cooks, Diplomats, Financial staff, Journalists, Underground mine workers, Prisoners (although not a "worker" per se, a prisoner is officially classified as an indoor worker since his/her time is spent indoor), Researchers, Waiters, etc.

[c]Unclear: the risk exposure cannot clearly be estimated. Children, Retirees, self-employees, Students, Unemployed people, Sportsmen and Sportswomen, Tourists, etc.

[d]missing data were not included into statistical analysis

were laboratory confirmed (99.6% in former endemic and 95.8% in former non-endemic). However, the majority of cases were diagnosed at the prefecture level (68%) in former non-endemic areas whereas in former endemic areas 41% of cases were diagnosed at the county level and 45% at the prefecture level (Table 3).

Discussion

The main feature in this analysis is the overwhelming presence of Africa as a travel destination among patients infected with malaria. Travelers to Africa represent 80% of patients from former endemic areas and 94% of patients from former non-endemic areas. The top ten

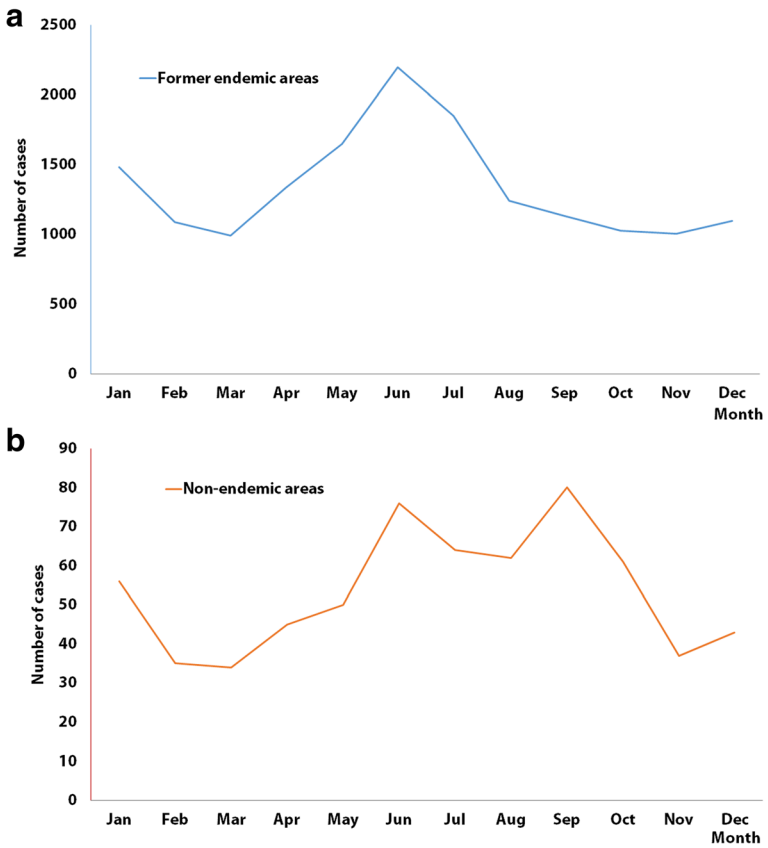

Fig. 1 Seasonal dynamic of imported malaria cases in former endemic and non-endemic areas in China, aggregated 2013–2017. **a** Seasonal dynamic in former endemic areas. **b** Seasonal dynamic in non-endemic areas

African countries found as the original infection of these imported cases were Angola, Nigeria, Democratic of Republic Congo, Chad, Uganda, Equatorial Guinea, Guinea, Cameroon, Sudan and Tanzania. Patients are almost exclusively men in the professionally-active class of age (19 to 59 years). The vast majority of patients are Chinese nationals went abroad to work on international projects and coming back home. This reflects the international involvement of Chinese companies in Africa. The overwhelming presence of this socio-professional class among malaria patients also matches the location of cases in major cities from the East coast [13, 15, 25]. Indeed, this correlates with the presence of air transportation hubs and labor export companies mainly in major cities on the East coast (http://femhzs.mofcom.gov.cn/fecpmvc/pages/fem/corp_ml_list2.jsp). Travel patterns, air network distribution, trade connection and malaria situation in the visited countries are features commonly considered to influence the risk of malaria introduction [26–28]. China is a country with a history of malaria endemicity who is now on the way to malaria elimination. Environmental conditions and efficient vectors are thus present and the risk of reestablishment following introduction is possible.

This study shows that travel to Africa for work may be the most important driver of imported malaria within China and the biggest risk for re-introduction. However, comparing former endemic and former non-endemic areas in China provides a more detailed view of the dynamic. A different pattern is observed between former endemic and former non-endemic areas. In former non-endemic areas, the introduction is due almost exclusively to workers coming back from Africa but the cases are equally distributed between outdoor workers and indoor workers. Indoor workers are not likely to be exposed to malaria vectors, which have a nocturnal behavior, during indoor day-time working hours. Nevertheless, they are as much affected as outdoor workers during the night time. The main cause of infection seems therefore to be the long presence in an endemic country and exposure to malaria vectors during everyday life, especially at night, rather than exposure due to occupation which occurs at day, and indoors for half of the reported cases. This also makes sense considering that malaria vectors are mostly nocturnal mosquitoes when occupations usually occur at day time. In former endemic areas there is a high concentration of outdoor workers (80%). There is no environmental reason to explain this difference.

Fig. 2 Geographic distribution of imported malaria cases in former endemic and non-endemic areas in China, 2013–2017

Workers from former endemic areas are exposed to the same conditions in Africa as workers from former non-endemic areas. An explanation might be that the typology of work for travelers differs between those coming from former endemic and former non-endemic areas, more outdoor workers coming from the former and more inside workers coming from the latter. Another main difference can be observed. In former non-endemic areas patients are lmost exclusively traveling to Africa (94%) whereas in former endemic areas only 80% are working in Africa and 19% are working in Southeast/South Asia. The most plausible reason for this difference is that some of former endemic areas are located along the Southern Chinese border and have thus established partnership with Southeast/South Asian countries with a tradition of expatriate workers and cross-border movements of populations [29–31]. It is also very likely that the typology of work might be more oriented in these former endemic areas towards outside occupations. The difference in the peaks of malaria observed between the two kinds of areas in China might also be related to this difference in proximity and to different patterns of the migrant population, such as the frequency of labor dispatching, holiday celebrations, local farming system, etc. [15, 25, 32–34]. The additional peak in September–October in former endemic areas can thus be attributed to the easier conditions of traveling from Southeast/South Asia. African countries are a lot more distant making traveling more difficult and expensive and rotations clearly defined in duration.

Table 2 Duration between onset of malaria symptoms and diagnosis and diagnosis and reporting in China, 2013–2017

	Number of cases	Former malaria endemic areas		Number of cases	Non malaria endemic areas		*P* value
		Median	IQR[a]		Median	IQR[a]	
Time from onset to diagnosis							
Total	15 965	3.54	(1.63–6.63)	643	4.42	(2.42–10.46)	0.03
Township	1023	2.75	(1.67–4.67)	1	0.42	(0.42–0.42)	
County	6568	2.71	(1.42–5.46)	20	1.96	(0.74–5.21)	
Prefecture and above	7270	4.38	(2.33–7.83)	438	4.42	(2.46–9.47)	
Private hospital	232	3.48	(1.59–5.53)	4	3.58	(2.7–6.06)	
Provincial	804	4.48	(2.38–8.66)	177	4.63	(2.44–13.46)	
POE[b]	68	2.65	(0.64–5.67)	3	1.58	–	
Time from diagnosis to report							
Total	15 965	0.05	(0.00–0.5)	643	0.07	(0.00–0.73)	
Township	1023	0.06	(−0.46–0.25)	1	0.96	(0.96–0.96)	
County	6568	0.07	(0.00–0.58)	20	−0.4	(− 0.58–0.13)	
Prefecture and above	7270	0.05	(−0.46–0.56)	438	0.49	(0.04–0.92)	
Private hospital	232	0.16	(0.05–0.69)	4	0.37	(−0.52–0.84)	
Provincial	804	−0.5	(−0.67–0.33)	177	−0.54	(− 0.63–-0.38)	
POE[b]	68	−0.5	(−0.63–0.42)	3	−0.58	–	

-Not applicable
[a]IQR: Interquartile range;
[b]POE: Point of Entry at the customs

Another main difference observed between former endemic areas and former non-endemic areas is the efficiency of reaction of health facilitates when admitting a case of malaria. The differing performance of health facilities in reporting and diagnosing malaria between former endemic and former non-endemic areas and depending on heath facility levels is clearly highlighting the need for strengthening the training of staff in malaria case detection, diagnosis and treatment. Fast detection and reporting were performed equally efficiently in health centers at the township/county and prefecture levels in former endemic areas whereas this achievement was encountered only at the prefecture level in former non-endemic areas. This might well be a consequence of the NMEP strategy to focus on capacity building towards county and community level facilities in former endemic areas. The capacity of malaria diagnosis and treatment in health facilities are key factors to efficiently implement detection, surveillance and response, especially at malaria elimination stage [35, 36]. Timely case detection

Table 3 Comparison between method of diagnosis and level of reporting/diagnosis facility, China, 2013–2017

	Former malaria endemic areas				*P* value
	Number	Proportion (%)	Number	Proportion (%)	
Method of diagnosis					0.9
Laboratory confirmed[a]	16 021	99.6	616	95.8	
Clinical	69	0.4	27	4.2	
Level of reporting & diagnosis health facility					< 0.001
Township	1023	6	1	< 1	
County	6568	41	20	3	
Prefecture	7270	45	438	68	
Private hospital	232	1	4	< 1	
Provincial	804	5	177	27.5	
POE[b]	68	< 1	3	< 1	
Missing	125	< 1	0	0	

[a]Diagnosis confirmed by Laboratory test which include Rapid Diagnosis Test (RDT), Polymerase Chain Reaction (PCR), Microscopy
[b]POE: Point of entry, screen test at customs;

and treatment will help to prevent the re-introduction of malaria in former endemic areas and reduce the occurrence of fatal issues [15, 27, 37]. There is thus an urgent need of intensive capacity building and training for the township/county health centers. Nevertheless, continuous capacity building must be implemented in former endemic areas in order to maintain the level of competence.

With the development of international cooperation, exemplified by the Belt and Road Initiatives, the main source of malaria infection and the main risk for malaria elimination are linked essentially to expatriate workers coming back from Africa and to a lower extent from Southeast/South Asia. This risk must be tackled at two levels. At the upstream level, there is a clear need to better equip expatriates with malaria prevention information and tools, such as risk exposure prevention, information on common symptoms, treatment options, before travelling to malaria endemic areas. This must be completed with the availability of appropriate antimalaria drugs [38]. A last aspect to consider at this level is the establishment of detection centers and detection campaigns on site in Africa by the companies employing expatriate workers. This should be preferably extended also to Southeast/South Asian countries. At the downstream level, there is a need for intensive and continuous capacity building for health centers in order to maintain the capacity of fast detection, an essential element for managing the risk of malaria introduction [36, 39].

There are limitations to this study relating to data quality and availability of data. Data availability was dependent on the recording by health facility staff. Missing data and unclear coding made up to 3% of occupation data. Detailed information on movements of populations, i.e. travel frequency, purpose of travel, etc., was not recorded. Standardized forms should thus be developed in order to record additional. However, this study was important as it addressed the situation of imported malaria and the health system performance in former endemic areas but more importantly in former non-endemic areas in China, which was rarely conducted before, previous works focusing mostly on endemic areas. Additionally, researchers adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting on observational research and the Reporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement for studies using routinely collected programmatic data [40, 41].

China has achieved zero indigenous case report in 2017 and is on the way to eliminate malaria by 2020 as planned [23]. However, together with the open-up policy and increase of international cooperation, imported malaria cases are now commonly reported across the country [8, 13, 15]. Further studies should therefore focus on the cross-border transmission, surveillance and response in major cities with detailed social and economic data. These studies should bring recommendations for proper control in areas massively affected by imported malaria.

Conclusions

Imported malaria was found to be more widely distributed in China from 2013 to 2017 than expected. All former non-endemic areas are now reporting imported malaria cases. However, the largest proportion of reports of imported cases is still coming from former endemic areas. The demographic characteristics of imported malaria depends upon the country of expatriation, species composition of parasites, occupation and place of origin of workers. Health facilities in former endemic areas outperformed those in former non-endemic areas, suggesting that targeted training for health staff in former non-endemic areas should be a priority along with proper information of expatriates and availability of drugs and detection on site in foreign countries.

Additional file

Additional file 1: Multilingual abstracts in the five official working languages of the United Nations. (PDF 448 kb)

Abbreviations

CIDRS: Chinese Infectious Disease Report System; CLPTID: Chinese Law on Prevention and Treatment of Infectious Diseases; IHR: International Heath Regulation; NMEP: National Malaria Elimination Program; RECORD: The REporting of studies Conducted using Observational Routinely collected health Data; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

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Authors' contributions

SSZ (Shao-Sen Zhang), SSZ (Shui-Sen Zhou) and EG designed the study and develop the first draft of manuscript, SSZ (Shao-Sen Zhang), JF, LZ, XR and EG carried out the data analysis, SSZ (Shui-Sen Zhou), SM, RF contributed to the revision of the manuscript. All authors have read and agreed to submit this manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study has been approved by the Ethical Review Committee of Chinese Center for Disease Control and Preventive, No. 201817.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Conclusion

This chapter presented the epidemiological characteristics of imported malaria cases reported from 2013 to 2017 in China and evaluated the risk of re-introduction in regards of population mobility at border areas.

The results of the two publications presented in this chapter show that all former non-endemic areas are now reporting imported malaria cases in China. However, the largest proportion of imported cases is still reported in former endemic areas. The demographic characteristics of imported malaria depend upon the country of expatriation, species of parasites, occupation and place of origin of workers. Health facilities in former endemic areas outperformed those in former non-endemic areas, most likely due to a better practice and experience of the medical staff in the former endemic regions. Information, treatment and surveillance must be provided for expatriates while capacity building and continuous training must be implemented at all levels of health facility in China.

Regarding the malaria risk linked to mobility of population at the China-Myanmar border, a particular attention must be paid when designing and implementing the national malaria surveillance strategy. High population mobility was observed with different epidemiological characteristics of exposure patterns in the villages along the China-Myanmar border, which were in turn associated with varying levels of vulnerability. Stochastic simulation model could be used as a tool to quantify the linear relationship between vulnerability and mobile populations, and it may be useful for evaluating the risk of malaria re-introduction in China.

Chapter 4 Malaria elimination in China: progress and perspectives

Introduction

In China, the malaria elimination program was launched in 2010 with the objective to eliminate this disease by 2020. Large-scale malaria control and elimination actions have been conducted with significant success since inception of the nationwide program. The incidence of locally acquired malaria has declined sharply along with the concomitant decrease of malaria-endemic areas from 762 counties reporting malaria in 2010 to just two counties adjacent to border areas (Yunnan Province: China-Myanmar and Tibet, China-India) in 2016. The year 2017 was the first year without reporting any autochthonous malaria case throughout the country. Hence, this chapter aims to present the malaria elimination progress through mapping the residual malaria foci and the distribution of malaria cases in China, and to share the lessons learned from malaria elimination in China benefiting countries on the way to malaria elimination.

Article displayed in Chapter 4

Article 7. Hu T, Liu YB, **Zhang SS**, Xia ZG, Zhou SS, et al. Shrinking the malaria map in China: measuring the progress of the National Malaria Elimination Programme[J]. Infect Dis Poverty, 2016, 5 (1): 52

Article 8. Feng J, Tu H, Zhang L, **Zhang SS**, Jiang S, et al. Mapping transmission foci to eliminate malaria in the People's Republic of China, 2010–2015: a retrospective analysis [J]. BMC Infect Dis, 2018, 18(1):115

Article 9. **Zhang SS**, Zhang L, Feng J, Yin JH, Feng XY, et al. Malaria Elimination in the People's Republic of China: Current Progress, Challenges, and Prospects. In: Manguin S, Dev V, editors. Towards malaria elimination-a leap forward. London, United Kingdom: IntechOpen; 2018. p. 233–55.

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Infectious Diseases of Poverty

RESEARCH ARTICLE Open Access

Shrinking the malaria map in China: measuring the progress of the National Malaria Elimination Programme

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Abstract

Background: Remarkable progress has been made towards the elimination of malaria in China since the National Malaria Elimination Programme (NMEP) was launched in 2010. The incidence of locally-acquired malaria cases has declined rapidly and endemic areas have also dramatically shrunk. In total, 3 078 malaria cases were reported in 2014, but only 56 cases were indigenous. In order to further promote the elimination programme, we reviewed the progress of and experiences associated with malaria elimination in China, and identified the challenges and priorities for the next stage of the programme.

Methods: Data were collected from the web-based China Information System for Disease Control and Prevention, and the China Annual Report of Malaria Elimination. The progress towards the elimination of malaria from 2010 to 2014 was measured.

Results: During the implementation of the NMEP from 2010 to 2014, local malaria incidence has declined continuously, only remaining in the Yunnan Province and Tibet Autonomous Region in 2014. By the end of 2015, 75.6 % (1 636/2 163) of the malaria-endemic counties passed the sub-national elimination assessment. The main challenges are cases of border malaria and imported malaria from other countries. Sustainable support and investment from the government, the establishment of an effective surveillance and response system, and risk assessments for the potential reintroduction of malaria are priorities for the next stage of the elimination programme.

Conclusions: The NMEP in China has been successfully implemented thus far and the malaria map has shrunk dramatically. The priorities for malaria elimination are interventions to block transmission at border areas, management of imported malaria cases, preventing malaria reintroduction, capacity building, and sustainability of malaria surveillance and response.

Keywords: Malaria elimination, Incidence, Progress, Experiences, Challenges, China

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Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the six official working languages of the United Nations.

Background

Malaria remains one of the most serious public health issues in the world. According to the latest available data, about 3.2 billion people were at risk of contracting malaria in 2015, and an estimated 214 million new cases and 438 000 deaths associated with malaria were reported that same year [1]. Historically, malaria was extensively endemic in China and large-scale outbreaks occurred in the 1960s and 1970s, which had a serious impact on people's health and inhibited socio-economic development [2, 3]. After intensive efforts for many years, malaria has been effectively controlled in China through the implementation of effective strategies and measures, such as mass malaria control activities, integrated vector control and joint control strategies. The intensity of malaria epidemics and the number of endemic areas have decreased significantly, and falciparum malaria transmission was successfully interrupted in central China in early 1990s [2, 4, 5]. In 2010, the number of malaria cases nationwide was 7 855, a reduction of 45.8 % compared with the 14 491 cases reported in 2009. Over 95 % of the counties in China have a malaria incidence below 1/10 000 [6].

In 2008, the United Nations issued the Millennium Development Goals, one of which was to globally eradicate malaria. Many countries have made remarkable progress in malaria elimination and 109 countries were malaria free by 2010 [7]. To respond to this global action plan, the Chinese government developed a National Malaria Elimination Programme (NMEP) in 2010, and the Action Plan of China Malaria Elimination (2010–2020) (APCME) was officially endorsed in the same year by the Ministry of Health in conjunction with 12 other ministries including Finance, Education, Science and Technology, Entry-Exit Inspection and Quarantine, and so on. The general goal of the NMEP is to "eliminate local malaria transmission except in some of the Yunnan-Myanmar border areas by 2015 and realize malaria elimination across China by 2020" [8].

Since the launch of the elimination programme in 2010, along with socio-economic development, progress of urbanisation, and changes in the natural environment and malaria vectors, the incidence of locally-acquired malaria in China has declined sharply and malaria-endemic areas have also dramatically shrunk. There were 3 078 malaria cases reported in 2014, but only 56 were indigenous cases [9]. China is currently on the path towards malaria elimination and has developed a roadmap for nationwide elimination of the disease [10]. In order to understand the current status of the NMEP and ensure the realisation of the final goal of malaria elimination countrywide in 2020, the achievements and experiences of the past five years since the launch of the programme are reviewed in this paper. Challenges and priorities for the next steps are also identified.

Methods

Brief profile of China

China is located in East Asia and the Western Pacific Region. Covering approximately 9.6 million square kilometres, China has a population of 1.37 billion, with an average population density of 145 per square kilometre in 2014. China is divided into provinces, autonomous regions and municipalities (P/A/M) directly under the administration of the Central Government. Currently, the country is divided into 23 provinces, five autonomous regions, four municipalities and two special administrative regions [11].

Data collection

Data on the NMEP from 2010 to 2014 were collected and the progress towards the elimination of malaria in China was reviewed. Data were obtained from the web-based China Information System for Disease Control and Prevention (CISDCP) and the China Annual Report of Malaria Elimination. Data collected included the number of reported malaria cases sorted by indigenous cases and imported cases from other countries; strategies and interventions implemented, including number of blood examinations conducted, distribution of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), provision of training, administering of health education; and amount of financial investment. Data from Hong Kong, Macao and Taiwan were not included in this analysis.

Data analysis and mapping

Data were double entered into Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and then a descriptive analysis was conducted. The maps showing the geographical distribution of indigenous malaria cases were generated using ArcGIS software version 10.1 (Environmental Systems Research Institute. Inc., Redlands, CA, USA).

Results

Implementation of malaria elimination strategies and interventions

Since the launch of the NMEP, the Chinese government has been massively promoting the implementation of malaria elimination strategies and interventions (see Table 1). A special fund from central government finance was set up for malaria and the total amount given from 2011 to 2014 was 426 million CNY (US$ 66.6 million).

Table 1 Implementation of strategies and interventions for malaria elimination in China, 2010–2014

Year	Blood examination (Persons)	LLINs (Nets)	IRS (Persons covered)
2010	7 115 784	1 030 373	854 701 275
2011	9 189 270	1 840 792	1 043 963
2012	6 918 657	251 555	1 092 158
2013	5 554 960	58 874	447 639
2014	4 403 633	19 899	504 936
Total	33 182 304	3 201 493	857 789 971

A total of 33 182 304 blood tests were performed using both passive and active case detection for fever patients, with 22 277 malaria cases found from 2010 to 2014. By the end of 2014, the proportion of suspected malaria cases that received parasitological tests and the proportion of reported malaria cases based on parasitological confirmation reached 99.68 and 98.15 %, respectively. The proportion of cases reported within one day (24 h) after diagnosis, cases investigated within three days after reported, and foci investigation and action conducted within seven days after case reported reached 100, 98.95 and 100 %, respectively in 2014.

A total of 3 201 493 LLINs were distributed and 857 789 971 people were protected by IRS during the period from 2010 to 2014. Trainings that mainly targeted public health staff, clinicians and microscopists were carried out at different levels. About 130 000 technicians were trained in malaria microscopy during the period from 2010 to 2014. Health education campaigns for residents and students were carried out combined with the National Malaria Day (April 26th) every year across China. At present, an effective surveillance system that combines routine surveillance and sentinel surveillance has been established. The routine surveillance includes a case reporting system based on the web-based CISDCP and a web-based information system specifically designed for parasitic diseases. A total of 49 sentinel sites have been set up, which covered 25 P/A/M across China, to provide information and technical support for malaria elimination in China. The activities of the sentinel sites include active case detection, vector surveillance (species, population density and insecticide resistance) and anti-malarial drug efficacy. A laboratory network for malaria diagnosis has been developed [12] and 22 provincial-level malaria diagnosis reference laboratories in 24 malaria-endemic provinces were established by the end of 2014.

Indigenous malaria incidence has continuously been declining

Since the launch of the NMEP, indigenous malaria incidence has declined significantly. A total of 4 262 indigenous cases were reported in China in 2010, however, only 56 cases were reported in 2014, a reduction of 98.6 % (see Fig. 1a). There was only one county (Motuo County in the Tibet Autonomous Region) that had an incidence of indigenous malaria of more than 1/10 000 in 2014. Hainan Province was the most seriously affected malaria-endemic area, as it had the highest transmission of *Plasmodium falciparum* and *P. vivax* malaria in history [13], however, no locally-acquired falciparum malaria cases have been reported since 2010 and the number of vivax malaria cases has declined sharply to

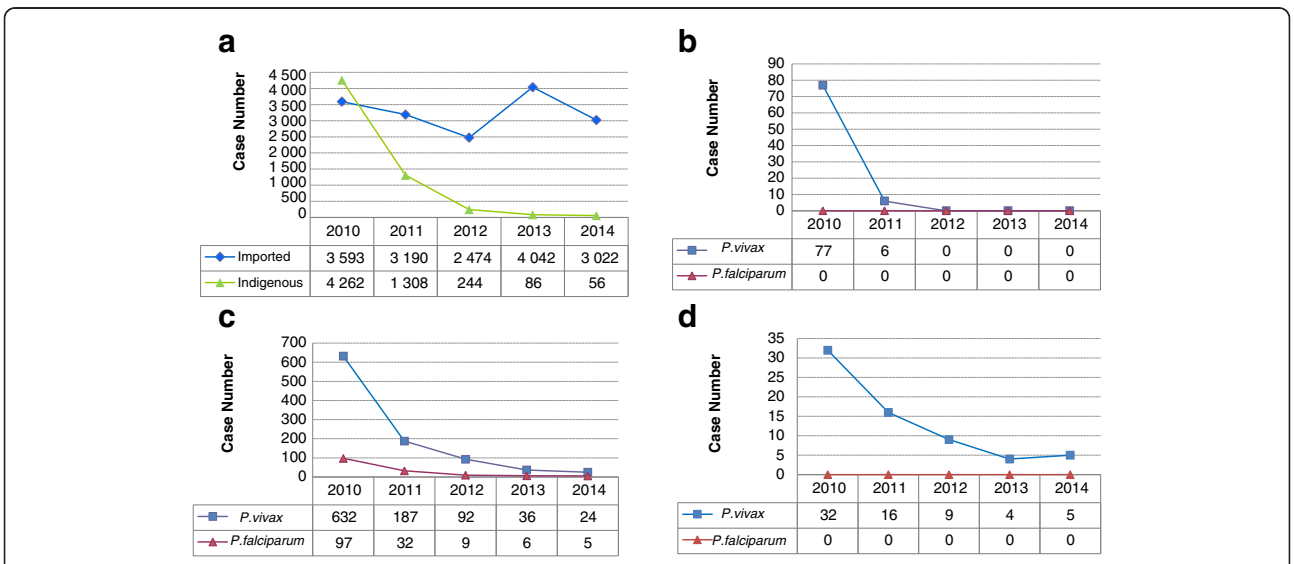

Fig. 1 The changing malaria situation in China, 2010–2014. **a** Number of malaria cases (indigenous versus imported) reported in China. **b** Number of indigenous malaria cases (*P. vivax* versus *P. falciparum*) reported in Hainan Province. **c** Number of indigenous malaria cases (*P. vivax* versus *P. falciparum*) reported in Yunnan Province. **d** Number of indigenous malaria cases (*P. vivax* versus *P. falciparum*) reported in Tibet

zero since 2012 (see Fig. 1b). The number of indigenous malaria cases in Yunnan Province has continuously been decreasing with only five falciparum cases and 24 vivax cases reported in 2014 (see Fig. 1c). The number of indigenous malaria cases in the Tibet region also declined steadily from 2010 to 2014 (see Fig. 1d).

Malaria-endemic areas have shrunk dramatically

In total, 2 194 counties in 24 provinces across China were identified as malaria-endemic counties and 762 counties reported local malaria cases when the elimination programme was first launched in 2010. The number of counties with local cases decreased to 155, 41, 12 and 10 by the end of 2011, 2012, 2013 and 2014, respectively. Only Yunnan Province and Tibet Autonomous Region reported locally-acquired malaria cases by the end of 2014, with the cases mainly distributed in nine counties along the China-Myanmar border and one county (Motuo County) in the Tibet Autonomous Region (see Fig. 2).

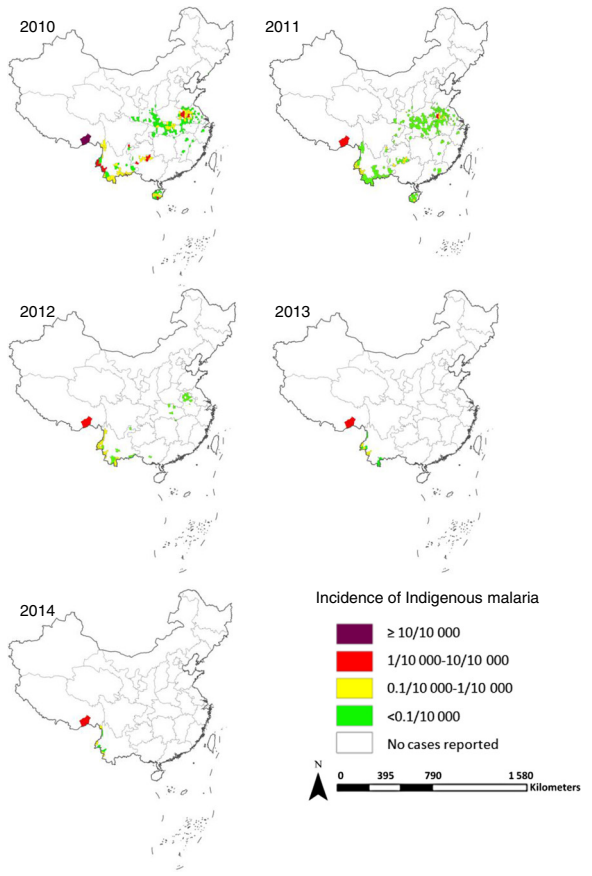

Fig. 2 Changes in the distribution of indigenous malaria cases in China between 2010 and 2014

Progress towards malaria elimination

To ensure progress towards malaria elimination, a sub-national elimination assessment was conducted starting from 2012. The assessment of each county was carried out by up usually prefecture-level authority, according to the Criteria for Control and Elimination of Malaria (GB26345-2010) and the protocols developed by the National Health and Family Planning Commission of China. By the end of 2015, 75.6 % (1 636/2 163) of malaria-endemic counties passed the assessment and were officially recognised as reaching the goal of malaria elimination. All other counties are expected to be assessed by the end of 2020, as according to the APCME (see Fig. 3).

Discussion

The Global Technical Strategy has proposed to eliminate malaria in 35 new countries by 2030 [14]. At the Ninth East Asia Summit, regional leaders agreed to the goal of an Asia Pacific free of malaria by 2030 and proposed a roadmap for malaria elimination [15]. Malaria elimination in China is one of the most important goals of global malaria elimination and is of great concern to the international community [16, 17]. Recent progress made by the NMEP has shown that remarkable strides can be made with adequate investment and the proper strategies.

Overall, the NMEP in China is proceeding as planned and the malaria map has successfully been shrunk to limited border areas. This success is driven by several factors. Firstly, it has benefited from the attention and strong political commitment of the Chinese government. The APCME proposed clear objectives, strategies, measures and schedules for malaria elimination at the national level from the beginning of the programme, and the Chinese government has continued to financially support malaria elimination strategies despite the Global Fund to Fight AIDS, Tuberculosis and Malaria stopping its funding for China in 2012 [18]. Secondly, regional collaboration, which is called China's joint malaria prevention and control mechanism, has largely helped the progress towards malaria elimination. The joint mechanism, which is under the guidance of the different levels of governments and health authorities, groups together regions adjacent to each other with similar natural and geographical conditions, consistent transmission intensities and epidemic factors. These regions then implement the same strategies through unified planning and conduct simultaneous actions, joint training, unified monitoring and evaluation, as well as hold regular meetings to exchange experiences [19]. In addition, cooperation between China and neighbouring countries including Myanmar, Laos and Vietnam has played an important role in improving control of malaria at cross-border areas and solving the cross-border challenge of imported malaria. Thirdly, the national web-based case reporting system combined

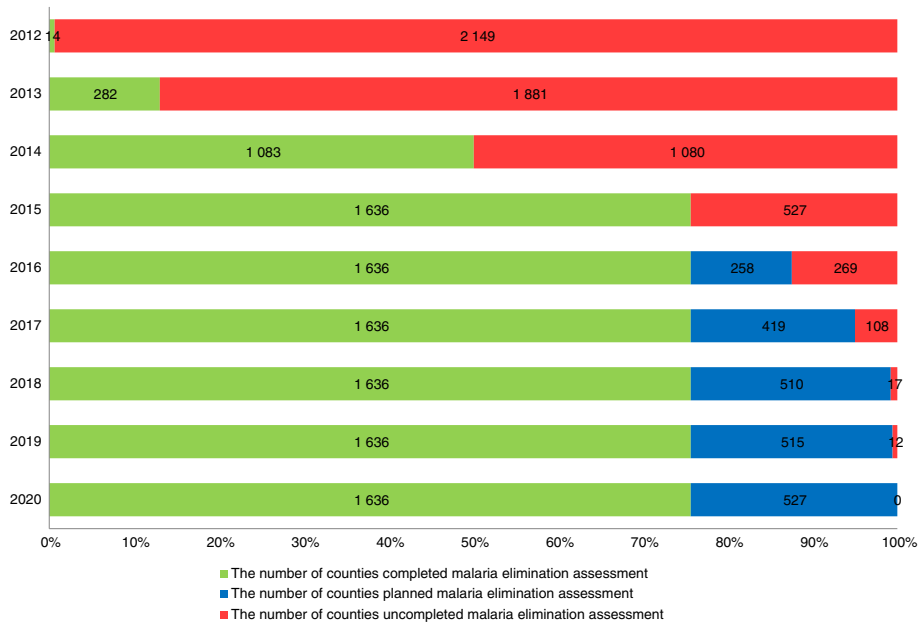

Fig. 3 Progress of the sub-national malaria elimination assessment in China

with a specific system for parasitic diseases provides a strong support for data collection. Fourthly, the strategy for surveillance and response called "1-3-7" has been developed and implemented in China since early 2012, and has played an important role in efficiently detecting, treating and responding to individual malaria cases and eliminating the source of infection promptly [20–23]. In addition, a lot of training has been conducted to strengthen capacity building. For example, a national competition measuring the skills of microscopists has been held every year since 2011, which has improved the competency of microscopists at different levels and provided strong technical support for malaria elimination [24, 25].

Challenges to achieve the final goal of a malaria free China by 2020 are mainly related to cross-border and imported malaria. There are 25 counties along the 4060-km borderline of Yunnan Province with Myanmar, Laos and Vietnam. The border areas belong to a mixed endemic area with transmission of *P. falciparum* and *P. vivax* malaria throughout the year. The natural environment in these areas is complex and a variety of malaria vectors, such as *Anopheles dirus* and *A. minimus*, usually co-exist in one setting and have a high vector capacity for transmitting malaria [26–28]. Furthermore, there is a large cross-border mobile population, as there is no natural barrier in China-Myanmar border areas and this makes management of imported malaria a great challenge [29, 30]. The other border area is Motuo County in Tibet Autonomous Region, which is one of the poorest areas in China and borders with India. The population of Motuo is only 10 000, but the county's ability to carry out a malaria elimination programme is limited due to the poor quality transportation and health system. Furthermore, because basic information including malaria epidemiology, biology of local parasites and vectors still remains poorly understood in Motuo, there are currently no effective strategies and measures for malaria elimination in this county [9, 31].

Another challenge is the increase of imported malaria cases from other countries, especially from Africa. Local malaria has been effectively controlled in most areas of China, however, with the increase of China's cross-border trade and foreign aid projects, imported cases have increased rapidly in recent years [9, 32, 33]. An outbreak with more than 874 imported cases even occurred in Shanglin County from May to August 2013 [34]. Almost all of the P/A/M (except for Inner Mongolia) in China (31/32) has reported imported malaria cases in recent years [9, 35]. Without sufficient awareness and experience of handling imported malaria cases, most clinicians are facing new challenges in diagnosis and treatment of this type of malaria. Severe cases and even death have occurred in China every year. Surveys showed that in some areas, the proportion of patients diagnosed within 24 h of onset was only 13.3 % and the proportion of patients diagnosed at township-level hospitals was only 4.3 %. Misdiagnosis and missed diagnosis also occurred in some provincial/city hospitals [36], which undoubtedly brings the potential risk of reintroducing malaria into areas where transmission has been eliminated, but vectors still remain.

When planning for the next stage towards the elimination of malaria in China, the first point to emphasise is governmental support. Governments' commitment at all levels should further strengthen and ensure substantial and consistent funding. The funding mechanisms of the Central Government need to be changed to address the new malaria situation. For example, funding coverage needs to be extended from historically malaria-endemic areas to 31 P/A/M, as imported cases are being reported in almost every part of China. In addition, the funding priority should shift to controlling malaria at border areas and the management of imported malaria. Secondly, new operational strategies need to be developed to achieve and maintain malaria elimination [37]. For example, network methods can be used to reach hard-to-reach populations such as migrant labourers and to improve the management of imported malaria [38]. New mechanisms for intersectorial cooperation and information exchange need to be further explored in order to deal with imported malaria more efficiently, such as through international cooperation at cross-border areas in Yunnan Province and neighbouring countries. This should include such factors as strengthening the village-level capacity for malaria diagnosis and treatment and county-level surveillance and response ability, and discovering malaria cases and managing them among the cross-border mobile population, thus reducing malaria on the Myanmar side. Other international collaborations such as through the Asia Pacific Malaria Elimination Network [39], the Asia Pacific Leaders Malaria Alliance [40], Chinese aid programmes for African countries and China's Belt and Road Initiative will also be critical for the control of imported malaria; such collaborations will also contribute to malaria elimination in other countries by introducing China's experiences, lessons and expertise in malaria control [41, 42]. In addition, operational researches need to be carried out for malaria elimination such as diagnostic methods for cases of malaria with low parasitemia, molecular techniques to distinguish local and imported cases, effective methods for monitoring drug resistance and susceptibility of local malaria vectors to imported cases [43]. Thirdly, an effective and sustainable malaria surveillance and response system tailored to local settings needs to be further developed [44]. Although no local malaria cases have been reported in most parts of China by 2014, malaria vectors still exist and the capacity of malaria control is still limited in some regions. This means that constant vigilance for imported cases will be important for the regions that are in the phase of preventing the reintroduction of malaria, especially in the cross-border region where importation risk is relatively high [45]. Fourthly, assessments for transmission risks need to be urgently conducted in order to develop targeted strategies and measures for surveillance and response in the regions that have passed the sub-national elimination assessment [46].

Conclusion

The NMEP in China is successfully being implemented and the malaria map has been shrunk dramatically. The next five years are critical for achieving the goal of a completely malaria-free China by 2020. The priorities are malaria elimination at border areas, management of imported malaria, preventing malaria reintroduction, capacity building, and sustainability of malaria surveillance and response.

Additional file

Additional file 1: Multilingual abstract in the six official working languages of the United Nations. (PDF 210 kb)

Abbreviations

APCME: action plan of China malaria elimination; CISDCP: China information system for disease control and prevention; iRS: indoor residual spraying; LLIN: long-lasting insecticide-treated net; NMEP: National Malaria Elimination Programme; P/A/M: provinces autonomous regions and municipalities.

Authors' contributions

TH, JC and ZF conceived and planned the study. TH and YL conducted the analysis and wrote the paper. SZ (Shaosen Zhang) collected and managed the data and drew the maps. ZX, SZ (Shuisen Zhou) and JY contributed to the data interpretation and coordination. All authors discussed the results and contributed to the revision of the final paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the paper.

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RESEARCH ARTICLE Open Access

Mapping transmission foci to eliminate malaria in the People's Republic of China, 2010–2015: a retrospective analysis

Jun Feng, Hong Tu, Li Zhang, Shaosen Zhang, Shan Jiang, Zhigui Xia and Shuisen Zhou*

Abstract

Background: China has initiated the National Malaria Elimination Action Plan, which aims to eliminate malaria by 2020. However, the transmission of malaria occurs sporadically or in distinct foci, which greatly hampers progress toward elimination in China and other countries. The object of this study was to foci categorization and evaluates whether the response met the requirements issued by the nation or WHO.

Methods: Residual transmissions were investigated and located with fine spatial resolution mapping from parasitological confirmed malaria cases by use of routine national surveillance data. The "1–3-7" timeframes were monitored for each focus between 2012 and 2015. Each focus was identified, and the application of appropriate measures was evaluated.

Results: A total of 5996 indigenous cases were recorded between 2010 and 2015; during this period, the number of cases declined by 99.1% (2010, $n = 4262$; 2015, $n = 39$). Most indigenous cases (92.5%) were reported in Anhui ($n = 2326$), Yunnan ($n = 1373$), Henan ($n = 930$), Hubei ($n = 459$), and Guizhou ($n = 458$). The temporal distribution showed that the indigenous malaria cases were clustered during the period of May to August. A total of 320 foci were carefully investigated and analyzed: 24 were active foci; 72, residual non-active foci; and 224 cleared-up foci. For the foci response evaluation, all the active foci were investigated within 7 days, while 80.2% of the residual non-active foci were responded within 7 days. In addition, reactive case detection (RACD) was carried out with 92.9% of the active foci and vector investigation carried out with 75%. For residual non-active foci, RACD was carried out with 83.2% and vector investigation with 78.2% of the foci.

Conclusions: This study used nationwide data to categorize foci in China and evaluate the response of these areas during the control and elimination phases. Our approach stratifies future control responses by identifying those locations where the elimination of endemic transmission is needed, such as in the counties at the China–Myanmar border and in Tibet. In addition, this study will help local CDC staff to reassess their needs and responses against different types of foci during the elimination and post-elimination phases.

Keywords: Malaria elimination, Foci, China

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Background

China initiated the National Malaria Elimination Action Plan (NMEAP) in 2010, which aimed to eliminate indigenous malaria in non-border areas by the end of 2015, and eliminate it nationwide by the end of 2020 [1]. All the counties in China were classified into 4 types, and each type has its own strategy and interventions [2] (Table 1).

To achieve the national goal of malaria elimination, China has made great strides in controlling indigenous malaria over the past several decades [3]. After implementing an integrated control strategy and interventions, along with socio-economic development, urbanization progress, and changes in the natural environment and malaria vectors, the incidence of indigenous malaria in China has sharply declined, and the malaria-endemic areas have dramatically shrunk [4]. Since 2010, transmissions mainly occurred in the counties along the China–Myanmar border and in Motuo County of the Tibetan Autonomous Region of China.

Malaria tends to persist in "foci" or localized areas of self-sustaining transmission, due to spatially heterogeneous influences, such as social and ecological factors that include *Anopheline* mosquito density, agricultural practices, human behavior, wealth, education, access to and utilization of health care, and urbanization [5]. In the elimination phase, cases occur sporadically or in distinct foci. In our investigation, the foci were classified into 3 types (active, residual non-active and cleared-up) according to the guidelines issued by the World Health Organization (WHO) [6]. The elimination of residual foci is a dynamic process, taking place mainly during the late stage of malaria elimination.

Monitoring the status of foci, with the precise identification of their functional status, is the cornerstone for success in interrupting malaria transmission and preventing the re-introduction of malaria where potential foci (foci with imported cases but without proof of local transmission) may be present. This step was important for China because there were many areas where transmission was sharply reduced but the vectors still exist. To interrupt malaria transmission, a two-pronged approach is required: disease management (alleviation of symptoms and prevention of biting by mosquitoes) and disease prevention through vector control.

The object of this study is to categorize foci by using country-wide data collected under operational conditions during the important period from the control phase to the elimination phase in China, and to evaluate whether the responses meet the requirements set out by national or WHO guidelines. Many factors would be challenges in the performance of the case investigation, foci investigation and response. The findings are likely to reflect the real situation and would help local CDC staff to reassess their needs and responses against different types of foci during the elimination and post-elimination phases.

Methods

Study design

A retrospective study was conducted to explore malaria-endemic characteristics from January 1, 2010 to December 31, 2015 at the Chinese Center for Disease Control and Prevention (CDC) [7–12]. All individual cases from the Infectious Diseases Information Reporting Management System (IDIRMS, http://chinacdc.cn) were carefully reviewed and analyzed [13]. The IDIRMS, which was set up in 2004 after the SARS outbreak, is a standardized platform that provides health care systems nationwide the ability to detect, analyze, prevent, and respond to any communicable disease in the country. The data for the study were selected by use of the reporting data and reporting area, but the data from Hong Kong, Macao, and Taiwan were excluded from these statistics. The IDIRMS parameters consisted of the geographical distribution, species composition, gender, and age distribution of the cases. Both clinically diagnosed cases and laboratory-confirmed cases were included in this analysis (Table 2).

Another reporting system, the Parasitic Diseases Information Reporting Management System (PDIRMS, http://chinacdc.cn), was set up in 2012 to report on 3

Table 1 Classification of county types and strategy implemented according to NMEAP

Type	Classification	Strategy
I	Local infections are detected in 3 consecutive years, and the incident rate is equal to or greater than 1 in 10,000.	The integrated interventions of case management and vector control will be scaled up to reduce disease incidence because annual parasite incidence (API) < 1 compared to 2010.
II	Local infections are detected in 3 consecutive years, and the incident rate is lower than 1 in 10,000 in at least one of those 3 years.	Response to any possible malaria cases and active foci is the main strategy to interrupt local transmissions.
III	No local infections reported for 3 years.	Enhance monitoring and surveillance of imported cases to prevent secondary transmission.
IV	Areas without malaria epidemic.	Sensitively detect and promptly respond to imported cases.

Table 2 Definitions used in this study

Type of malaria	Description
Clinically diagnosed case	An individual with malaria-related symptoms (fever [axillary temperature ≥ 37.5 °C], chills, severe malaise, headache, or vomiting) at the time of examination.
Laboratory-diagnosed case	A clinical case confirmed by microscopy, polymerase chain reaction, or rapid diagnostic tests in the laboratory.
Indigenous case	A case contracted locally with no evidence of importation and no direct link to transmission from an imported case. In this study, an indigenous case refers to malaria acquired by mosquito transmission in China.
Imported case	A malaria case or infection in which the infection was acquired outside the area in which it was diagnosed. Here, it refers to the patient who acquired the illness from a known malaria-prevalent region outside China.
Induced case	A case in which the origin of the illness can be traced to a blood transfusion or other form of parenteral inoculation of the parasite but not to transmission by a natural mosquito-borne inoculation.
Introduced case	A case contracted locally, with strong epidemiological evidence linking it directly to a known imported case (first-generation local transmission).
Recrudescent case	Recurrence of asexual parasitemia of the same genotype(s) that caused the original illness, due to incomplete clearance of asexual parasites after antimalarial treatment.
Death from malaria	Patient with signs and symptoms of complicated malaria, with confirmed diagnosis of *P. falciparum* (or *P. vivax*) or associated infection.
Focus	A defined and circumscribed area situated in a currently or formerly malarious area that contains the epidemiological and ecological factors necessary for malaria transmission.
Reactive case detection	A process that involves an active response after the identification of a local or imported case in a receptive area where the transmission intensity is low or assumed to be interrupted.

diseases: malaria, schistosomiasis, and echinococcosis. Malaria was monitored and reported on within the time frame indicators using the "1–3-7" strategy. The "1–3-7" strategy, which refers to the reporting of malaria cases within 1 day, case confirmation and investigation within 3 days, and foci investigation and response to prevent further transmission within 7 days, was launched in 2012 to guide and monitor the elimination process nationwide [14]. Each case was investigated by staff at the county CDC to classify it as indigenous or imported; the definitions appear in Table 2 [15]. The PDIRMS contains the date of diagnosis, date of reporting, date of the case investigation, case classification (indigenous, imported, or other [induced, introduced, or recrudesce]), focus investigation, and foci response, such as reactive case detection (RACD) and indoor residual spraying (IRS). Both the website for the IDIRMS and the PDIRMS are private. Malaria department in NIPD was responsible for national malaria data for these two systems and have permission to access them.

Data extraction for foci identification and response evaluation

In this study, a natural village is considered the smallest unit of focus. "Active focus" refers to a focus with ongoing transmission; "residual non-active focus" refers to a transmission that was interrupted recently (1–3 years ago). A "cleared-up focus" is defined as a focus with no local transmission for more than 3 years.

For foci identification, the data of the indigenous cases from 2012 to 2015 in the IDIRMS were carefully reviewed and analyzed. Because it was not possible to distinguish the precise foci location for domestically mobile cases within the country (these cases are also defined as indigenous cases), only the foci with exact information were selected for categorization. If 2 or more cases were reported at 1 focus, they were considered and recorded as only one focus because the aim of this study is to classify foci and evaluate the response strategy. For the foci response evaluation, only the indigenous cases from the IDIRMS that matched the data in the PDIRMS were selected and analyzed. The population data for every county in China from 2010 to 2015 were obtained from the National Bureau of Statistics of China (http://data.stats.gov.cn/). All malaria cases reported were geo-coded and matched to the county-level layers of polygon and point using the software ArcGIS 10.1(Environmental Systems Research Institute, Inc., Redlands, CA).

Results

Indigenous malaria in China, 2010–2015

From 2010 to 2015, 5996 indigenous cases were recorded by the IDIRMS. During this period, the reported indigenous cases sharply declined by 99.1% (2010, n = 4262; 2015, n = 40). Since data of indigenous malaria cases were not available for 2010–2011, the epidemiological data for *Plasmodium* species from 2012 to 2015 were collected. These data were analyzed, and the results indicated that most of the indigenous cases (63.3%) were

attributed to *P. vivax*. Like *P. vivax*, the number of local *P. falciparum* cases declined from 97 in 2010 to 1 in 2015 (this strain only occurred in Yunnan Province).

Transmission largely decreased during the study period; indigenous cases occurred in 303 counties in 18 provinces in 2010, while in 2015, transmissions occurred in only 9 counties in 4 provinces (Table 3).

In this study, indigenous cases occurred in 93.6% of the Type I and Type II counties. From 2010 to 2015, transmissions in Type I and Type II counties significantly decreased, particularly for Type II counties, while in 2015 only 1 Type II county in Dandong city, Liaoning Province, reported indigenous cases ($n = 2$; Fig. 1).

Most indigenous cases (92.5%) were reported in Anhui ($n = 2326$, 38.8%), Yunnan ($n = 1373$, 22.9%), Henan ($n = 930$, 15.5%), Hubei ($n = 459$, 7.7%), and Guizhou ($n = 458$, 7.6%). However, in Liaoning and Hainan, where local transmission was blocked for 4 years, officials reported 2 local *P. vivax* and 6 local *P. malariae* cases in 2015 (Fig. 2).

Temporal distribution of indigenous malaria

The temporal distribution showed that indigenous malaria clustered between May and August. The highest number of transmissions was reported in June 2012, with 63 indigenous cases. The indigenous cases have sharply decreased since 2013: for example, only 12 indigenous cases were reported between May and August 2015, a 92.7% reduction compared with the same months in 2012 (Fig. 3). The residual auto correlation function (ACF) and residual partial correlation function (PACF) for the indigenous model also proved that the summer season (May–August) was the time frame with the most reported cases (Fig. 4).

Foci identification and classification

A total of 426 indigenous cases that were reported between 2012 and 2015 were carefully investigated and analyzed for foci identification and classification. Despite some unclear foci investigations ($n = 60$) and 2 or more cases reported in 1 focus ($n = 46$), 320 foci were finally obtained and used for foci identification (Fig. 5).

The 320 foci were investigated and classified as 24 active foci, 72 residual non-active foci, and 224 cleared-up foci. The 24 active foci were distributed in 6 counties of Yunnan, 1 county of Hainan, 1 county of Liaoning, and 2 counties of Tibet (Table 4). The 72 residual non-active foci were distributed across 15 counties of Yunnan, 2 counties of Tibet, and 2 counties of Anhui.

Foci response evaluation

For the foci response evaluation, 43.9% ($n = 182$) of the 426 indigenous cases selected from the IDIRMS were matched in the PDIRMS (Fig. 5). Further analysis found 32 of them were classified as 2 or more cases reported in 1 focus; therefore, 150 foci were evaluated for foci response.

In 2015, 28 indigenous cases were reported and foci response was carried out. All the active foci were investigated within 7 days; 92.9% ($n = 26$) of foci carried out RACD, and 75% ($n = 21$) carried out vector investigations (Table 5). Two positive patients were screened by RACD and found to have *P. vivax* (Yingjiang County) and *P. malariae* (Sanya County). A total of 101 residual non-active foci were evaluated, and most of them ($n = 81$, 80.2%) responded within 7 days (Table 5). In addition, 83.2% ($n = 84$) of the foci carried out RACD, and 78.2% ($n = 79$) carried out vector investigations. Four people were diagnosed as malaria-positive by RACD, and 83.2% ($n = 84$) of the foci carried out IRS. For the cleared-up foci, 21 foci were matched in both systems: only 1 focus investigated and responded within 7 days, while 16 carried out RACD and 19 conducted vector investigations (Table 5).

Discussion

During the elimination phase, population-level measures become inefficient and inadequate, so as countries

Table 3 Indigenous cases in China, 2010–2015

Year	Total cases	Indigenous (%)	Local *P. v* (%)	Local *P. f* (%)	No. of counties with transmissions	No. of Type I[a] counties with transmissions	No. of Type II[b] counties with transmissions
2010	7855	4262 (54.3)	NA[c]	97 (2.3)	303	71	203
2011	4498	1308 (29.1)	NA[c]	32 (2.4)	155	60	92
2012	2718	244 (9.0)	228 (93.4)	16 (6.6)	41	30	9
2013	4128	86 (2.1)	77 (89.5)	9 (10.5)	12	9	3
2014	3078	56 (1.8)	45 (89.3)	6 (10.7)	10	9	1
2015	3116	39 (1.2)	38 (97.5)	1 (2.5)	9	8	1
Total	25,393	5995 (23.5)	393	32	530	187	309

[a]Type I counties refer to areas with local infections that are detected in 3 consecutive years with an incidence rate equal to or greater than 1 in 10,000
[b]Type II counties refer to areas with local infections that are detected in 3 consecutive years with an incidence rate lower than 1 in 10,000 in at least 1 of those 3 years
[c]NA indicates that data were not available in the annual reporting system

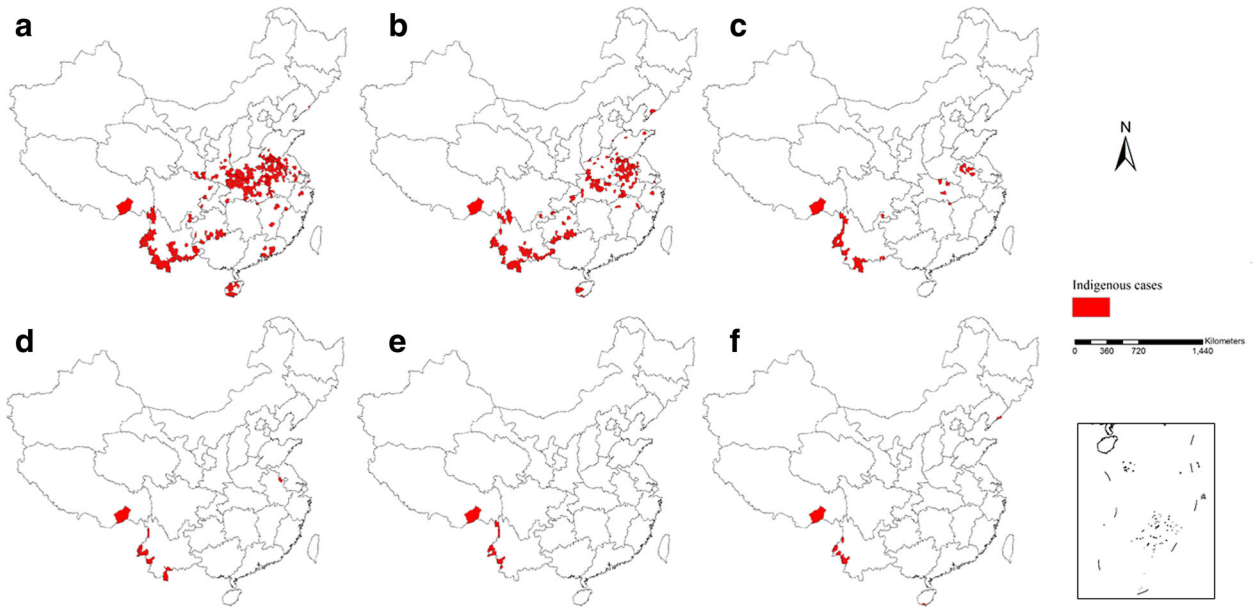

Fig. 1 Indigenous malaria in China, 2010–2015. **a** 2010, **b** 2011, **c** 2012, **d** 2013, **e** 2014, and **f** 2015. The red zones represent the areas where indigenous cases occur (county level)

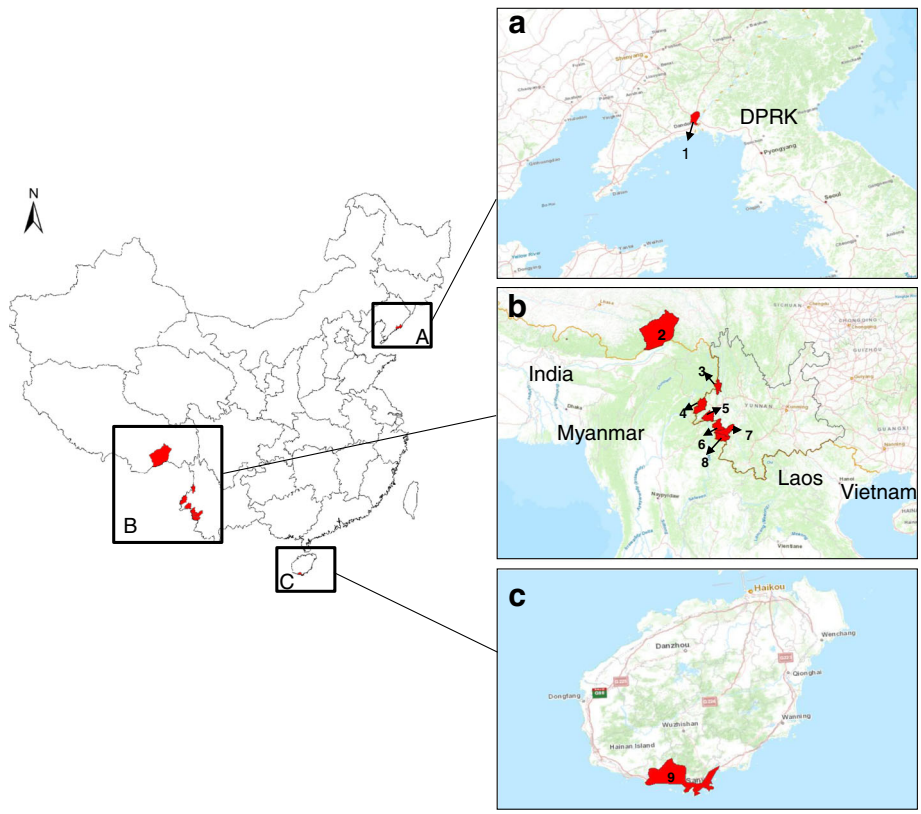

Fig. 2 The foci at county-level and its neighboring countries reported in 2015. **a** 1 represents Donggang County in Liaoning province neighboring with Democratic People's Republic of Korea (DRPK), **b** 2 represents Motuo County in Tibet, 3, 4, 5, 6, 7, 8 represent counties of Lushui, Yingjiang, Mangshi, Zhengkang, Gengma, Cangyuan in Yunnan province, respectively, where neighboring with Myanmar, **c** 9 represents Sanya County in Hainan province

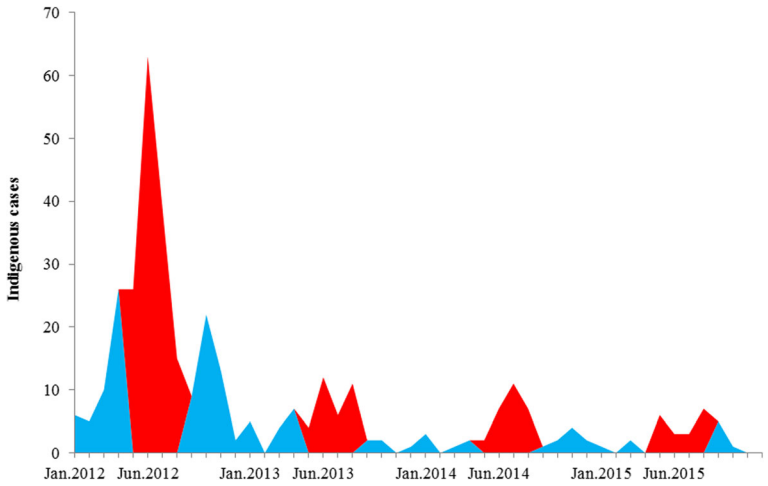

Fig. 3 Temporal distribution of indigenous malaria, 2012–2015. Red zones represent the period from May to August, and blue zones represent the period from September to April (the next year)

approach the goal of eliminating malaria, individual-based estimates of transmission must identify foci where resources should be targeted because transmission remains high [16]. Aggregate ratios of indigenous-to-imported cases in time (or in space) alone, could obscure localized transmission if, for example, most cases failed to transmit but some pockets of transmission remain.

The border areas are a great challenge for malaria elimination in China because they harbor a mixed endemic region with both *P. falciparum* and *P. vivax* malaria transmission [17]. The natural environment in these areas is complex and a variety of malaria vectors, such as *Anopheles dirus* and *An. minimus*, usually co-exist in a single setting and have a high vector capacity for transmitting malaria [18]. In addition, there is a large mobile cross-border population since there is no natural barrier in this region, which makes management of imported malaria a significant challenge [19–21]. Poor transportation also makes it difficult to conduct epidemiological studies and blood smear verification within 3 days [22]. In this study, the healthcare workers' response in these foci was inefficient, although all the cases at the foci were treated within 7 days. First, not all the active foci carried out RACD and IRS, which are required by the national strategy. Second, only three-quarters of the active foci conducted a vector investigation; without favorable entomological information, 2 potential risks remain.

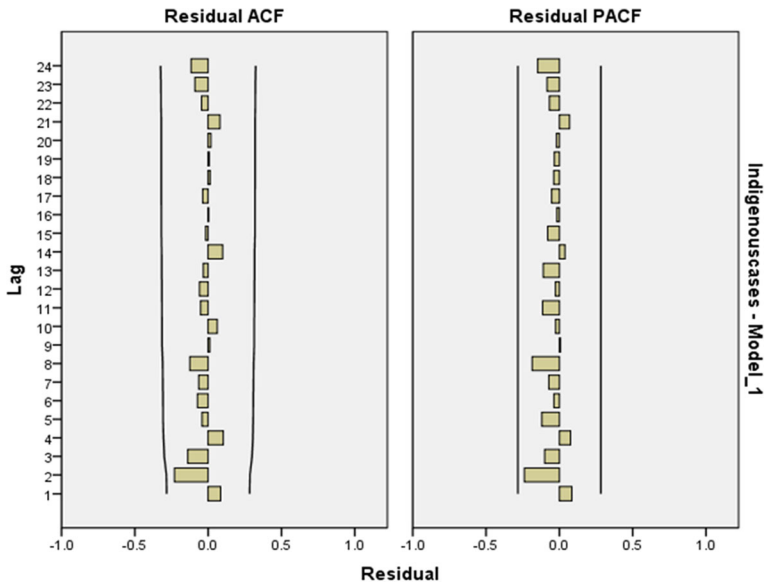

Fig. 4 Residual ACF and residual PACF of indigenous malaria, 2012–2015

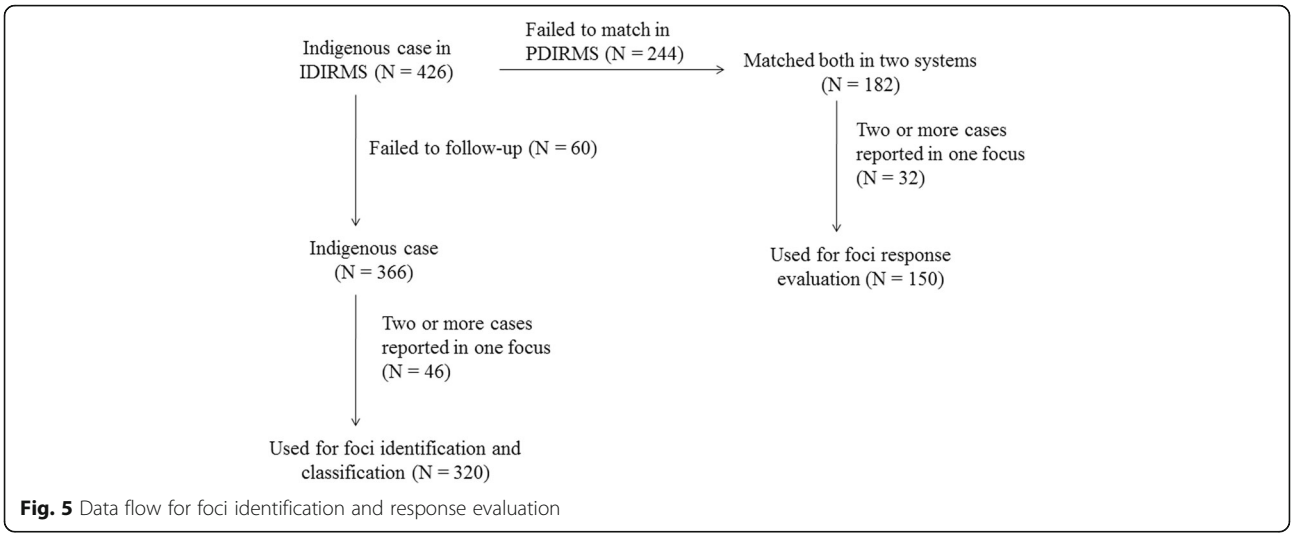

Fig. 5 Data flow for foci identification and response evaluation

Table 4 Active foci in China (the foci that were investigated in each natural village[a])

Foci No.[b]	Province	County	Township	Village	Case
1	Yunnan	Yingjiang	Nabang	Jiedao	2
2				Nabang	1
3				Lishu	1
4				Daonong	1
5				Qiaotou	1
6			Kachang	Caobei	1
7		Gengma	Mengding	Hanhong	1
8				Qiushan	1
9		Mangshi	Xuangan	Qincaitang	1
10			Santaishan	Yunqian	1
11		Zhengkang	Mengdui	Yakou	1
12		Lushui	Pianma	Pianma	1
13		Canyuan	Banlao	Banlao	1
14	Tibet	Motuo	Beibeng	Gelin	1
15				Jiangxin	1
16				Beibeng	1
17			Motuo	Yadong	1
18				Bodong	1
19			Dexing	Dexing	1
20		Linzhi	Bayi	Yingbin	1
21	Hainan	Sanya	Fenghuang	Lixin	4
22				Baolong	2
23				Nandao	1
24	Liaoning	Donggang	Jiangzi	Zhangdao	2

[a]A natural village has approximately 50 households with a population of 200 to 250 people and is the lowest administrative level in China. In China, a natural village with a reported malaria case is considered a focus
[b]If 2 or more cases were reported at 1 focus, only 1 focus was recorded. In this study, more than 1 case was reported in Jiedao, Lixin, Baolong, and Zhangdao; therefore, those 4 sites were considered as 4 foci

One is that the imported *P. vivax* can be re-introduced under conducive ecological conditions (transmission season) if the area harbors an appropriate vector, although there is little evidence of re-introduction of imported malaria in China [23]. Another is the increasing importation of *P. falciparum* to China due to a lack of knowledge about the efficient vector capacity to sustain imported *P. falciparum* for the re-establishment of transmission. A foci investigation consists of an assessment of potential *Anopheles* breeding sites, the collection of adult mosquitoes to identify the species responsible for transmission, and the assessment of the vector's susceptibility to insecticides; these steps are absent in routine surveillance work, but they are required by national guidelines. In addition, 2 malaria-positive patients who were screened by RACD revealed an insensitivity for passive case detection, this insensitivity is a particularly difficult issue for some regions where transmission was absent for several years, such as Hainan, where there was no transmission in 2011. However, in 2015, 7 indigenous cases were reported in this province [24]. This resurgence shows the significance of surveillance systems during the elimination stage, and especially in the post-elimination stage, to monitor for the potential re-introduction of malaria into these areas.

The active foci response required a strategy that combined RACD with vector control to clear the foci [25]. Given proof that asymptomatic people are present at the China–Myanmar border [26], a more sensitive technology is required to screen local residents and frequently mobile populations, including temporary workers and illegal immigrants, who may not routinely use established health services. In China, RACD was performed in the households where a case was identified and neighboring households within a 300-m radius if the focus was considered large (an entire village) [27]. The RDT was

Table 5 Evaluation of foci response for the 3 types of foci in China

Response	Foci classification		
	Active[a]	Residual non-active[b]	Cleared-up
Total cases	28	101	21
Population at risk[c]	6452	46,809	10,006
No. of foci responding within 7 days (%)	28 (100)	81 (80.2)	1 (4.8)
No. of foci that carried out RACD (%)	26 (92.9)	84 (83.2)	16 (76.2)
Screened population	1447	2985	712
No. of malaria-positive patients	2	4	0
No. of foci that carried out entomological investigations (%)	21 (75)	79 (78.2)	19 (90.5)
No. of foci that carried out IRS	26 (92.9)	84 (83.2)	18 (85.7)

[a]All the indigenous cases reported were considered active foci. In 2015, 28 indigenous cases were reported, and foci response was carried out for all of them
[b]A total of 101 cases reported from 2013 to 2014 were categorized as residual non-active foci
[c]The population at risk represents the recorded data for each type of foci

adopted in the field and all blood samples were taken and sent to the provincial CDC or national CDC for PCR verification. Currently, the CDC staff at the China–Myanmar border use a high-throughput, low-cost, and highly sensitive screening method based on 18S ribosomal RNA to detect asymptomatic sub-patient infections. In 96-well plates, the samples are quantified by the amount of ligated probes that bind continuously to the 18S rRNA of the genus *Plasmodium*; this method may offer an alternative for sensitive, large-scale molecular screening that can be used for RACD [28].

All the measures implemented in these foci, e.g., RACD, IRS, targeted mass drug administration (tMDA), and health education, should follow the national guidelines because they significantly control the foci in the next year. Malaria elimination programs follow-up malaria cases reported by health facilities in order to carry out case investigations that will determine the origin of the infection, whether it has been imported or is due to local malaria transmission. All the active foci should carry out RACD and IRS to reduce the reservoir of asymptomatic and low-density infections, and prevent mosquitoes from biting to block transmission. Targeted interventions such as tMDA may be considered for patients, tMDA was performed on a smaller scale, used for close contacts, households, and villages of index cases.

The residual non-active foci in China cover 19 counties in 3 provinces. Not all the foci investigated and/or responded within 7 days because of poor transportation in Yunnan and Tibet, which caused delays for the CDC staff. To solve this problem, the government has improved road infrastructure; thus, the CDC staff could arrive at the foci in a timely manner. For remote areas, provincial CDC staff designated a central location at which they carry out to carry out case diagnosis, investigation, and treatment. For example, Yunnan Institute of Parasitic Diseases has set up a work station in Nabang village (one village of Yingjiang County, neighboring Myanmar) to conduct case diagnosis by use of PCR technology during the transmission season.

Despite a lack of indigenous cases occurring at these foci, there is still the possibility of relapse for *P. vivax* and *P. ovale*. A relapse would require that the local CDC staff emphasize the quality of treatment when visiting each house at the time of radical treatment. An attempt should be made to contact people who were absent during the healthcare visit. Although it is hard to carry out in the primary health care sectors, especially with minority populations who were present at the China–Myanmar border and in Tibet, the use of glucose-6-phosphate dehydrogenase deficiency assays should also be considered. In China, people who relapsed with *P. vivax* and *P. ovale* should undergo directly observed therapy, taking primaquine for radical treatment [29]. After 1 or 2 years without evidence of transmission, the 72 residual non-active foci may be re-categorized as cleared-up foci.

In addition, we have also summarized the similarities and differences between the frameworks of the WHO and China. While both frameworks could guide classification of foci, WHO's definition and identification of foci mainly classifies foci by tracking them over the last 3 years, and China investigates the response of foci to any current cases, including imported cases. A natural village with any reported cases was assumed to be a focus and was classified based on the *Plasmodium* species, season, and vectors. Once an imported case was reported, it was checked by microscopy or PCR in a reference lab to determine the *Plasmodium* species, and then the case was investigated to determine whether it occurred during transmission season. For example, if imported *P. vivax* was reported during transmission season, the CDC staff would carry out a response appropriate for the type of focus, including RACD and IRS, combined with tMDA if it was an active focus. Unlike the WHO classification, the purpose of foci classification

in China is to allow the CDC staff to determine the appropriate response so that each patient can be identified early and obtain appropriate treatment.

Limitations

Firstly, not all indigenous cases were well recorded for the exact epidemiological information, especially for domestically mobile (within the country) cases; in this study we missed 60 indigenous cases information. Secondly, the 2 web-based reporting systems were set up in different years, which led to a mismatch in the cases posted in these systems. This issue is also a problem with the PDIRMS, which was established in 2012. More time should be invested in integrating the IDIRMS data with the PDIRMS data and in training local CDC staff. Thirdly, we did not know the exact reasons for the delay in foci investigation and response though these are relatively minimal, they still need specific correction. Fourthly, because the ArcGis software used for foci mapping at village level is not presently available, and because the presentation of all foci in one map would give a low-resolution picture due to the number of foci (more than 200 foci in 2012 and dozens between 2013 and 2015), in this report we provided the indigenous cases at county level from 2010 to 2015.

Conclusion

This study used nationwide data to categorize foci in China and evaluate the response of these areas during the control and elimination phases. Our approach stratifies future control responses by identifying those locations where the elimination of endemic transmission is needed, such as in the counties at the China–Myanmar border and in Tibet. In addition, this study will help local CDC staff to reassess their needs and responses against different types of foci during the elimination and post-elimination phases.

Abbreviations

ACF: Residual auto correlation function; CDC: Center for Disease Control and Prevention; IDIRMS: Infectious Diseases Information Reporting Management System; IRS: Indoor residual spraying; LLTN: Long-lasting insecticidal nets; NMEAP: National Malaria Elimination Action Plan; PACF: Residual partial correlation function; PCR: Polymerase chain reaction; PDIRMS: Parasitic Diseases Information Reporting Management System; RACD: Reactive case detection; tMDA: targeted mass drug administration; WHO: World Health Organization

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Availability of data and materials

All individual cases in this study could be traced through the Infectious Diseases Information Reporting Management System (IDIRMS, http://chinacdc.cn) or the Parasitic Diseases Information Reporting Management System (PDIRMS, http://chinacdc.cn).

Authors' contributions

JF and HT conceived the study and drafted the manuscript. LZ, SSZ (Shaosen Zhang), and ZGX analyzed the data and provided suggestions for improving the quality of the data. HT undertook statistical analysis and model construction. JS carried out the ArcGIS software mapping and data exportation. SSZ (Shuisen Zhou) initiated the study and made major contributions to drafting the manuscript. All authors contributed to the writing of the manuscript and approved the submitted version of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The data of this study was collected from web-source, so the ethics and participatory consent was not required, and this was approved by National Institute of Parasitic Diseases, Chinese Center for Diseases Control and Prevention.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Chapter 10

Malaria Elimination in the People's Republic of China: Current Progress, Challenges, and Prospects

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Additional information is available at the end of the chapter

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Abstract

In China, the malaria elimination program was launched in 2010 with the objective to eliminate this disease by 2020. Large-scale malaria control and elimination actions have been conducted with significant success since inception of the nationwide program. The incidence of locally acquired malaria has declined sharply along with the concomitant decrease of malaria-endemic areas from 762 counties reporting malaria in 2010 to just two counties adjacent to border areas (Yunnan, China-Myanmar and Tibet, China-India) in 2016. In total, 1723 counties (79%) and 134 prefectures (52%) had completed the malaria elimination internal assessment by the end of 2016. The year 2017 was the first year without report of indigenous malaria cases throughout the country. Hence, this chapter is meant to share the lessons learned from malaria elimination in China benefiting countries on the way to malaria elimination.

Keywords: malaria elimination, China, surveillance and responses, 1-3-7 model

1. Introduction

Although significant progress on malaria control and elimination has been made worldwide, malaria remains a major public health threat to human beings. According to the World Malaria Report published by the World Health Organization (WHO), a total of 216 million malaria cases were reported worldwide with 445,000 deaths in 2016 [1]. These malaria cases were mainly reported from Africa (90%) and Southeast Asia (7%). With the available intervention tools, several countries have been certified to be malaria-free, and others are in the

Anopheles species/ taxa	Sibling species prevalent in the People's Republic of China	Species identification tools	Breeding habitats	Feeding behavior (peak biting activity)	Resting behavior	Insecticide susceptibility status	Distribution range
Anopheles sinensis	—	Morphological characters	Rice field, canal, ditch, pond	Zoophily (first option) Anthropophily (second option)	Exophily	Resistance to organochlorine, dichloro-diphenyl-trichloroethane (DDT), and deltamethrin in some provinces	Latitude below 33°N
Anopheles minimus s.l.	*An. minimus*, *An. harrisoni*	Morphological characters; PCR	Heliophobic stream, canal, ditch, rice field	Zoophily/ anthropophily (reported only in Hainan Province)	Endophily	Sensitive to all insecticides used currently	Latitude below 32.5°N
Anopheles lesteri	—	Morphological characters; PCR	Heliophobic, canal, ditch, rice field, filter well	Anthropophily	Endophily	Sensitive to all insecticides used currently	Latitude 22°N~33°N
Anopheles dirus s.l.	*An. dirus*, *An. baimaii*	Morphological characters; PCR	Heliophobic, stream in forest, pit with water, footprint of cattle	Anthropophily	Exophily	Sensitive to all insecticides used currently	Latitude below 23°N

Table 1. Bionomical characteristics of malaria vectors in the People's Republic of China (reference [14]).

process of elimination in the foreseeable future [1]. Among others, within the broad objective of worldwide malaria elimination [2], 21 countries have the potential to eliminate malaria by 2020; these are marked as E-2020 countries by WHO [1]. Although some of E-2020 countries were moving forward to achieve elimination goals, 11[1] have reported an increase of indigenous malaria cases since 2015, and 5 countries[2] reported >100 cases in 2016 compared to 2015. World malaria elimination is currently at crossroads [3].

Among the E-2020 countries, China has made a significant progress on malaria elimination. Both the malaria-endemic territories and indigenous cases have decreased dramatically [4–6]. Furthermore, no indigenous cases were reported in China in 2017. Along with the decrease of indigenous malaria cases, the distribution of *Plasmodium* species associated to the reported malaria cases had changed as well. Only 2 *Plasmodium* species, i.e., *Plasmodium falciparum* and *P. vivax*, were present prior to the elimination program, but now all 4 human malaria parasites are encountered in China (i.e., *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*), as well as the simian species *P. knowlesi* [1, 7–12]. The predominant *Anopheles* vectors had also changed over the same period due to environmental changes and anti-malaria interventions. Prior to malaria elimination, 4 main species/complexes of vectors were recorded, i.e., *Anopheles lesteri*, *An. dirus s.l.*, *An. minimus s.l.*, and *An. sinensis* (**Table 1**). Currently, after 7 years of malaria elimination efforts, only 2 species/complexes are recorded to be prevalent, i.e., *An. minimus s.l.* (mainly *An. minimus* and *An. harrisoni*) and *An. sinensis.* Moreover, the geographic distribution range of *An. sinensis* has expanded and the proportion increased too [13–16]. Considering the progress of malaria elimination in China and the challenges still to be met, useful information has been generated which could be shared with the communities working on malaria elimination. This chapter is thus aiming at detailing the strategy of the Chinese national malaria elimination program, current progress, and lessons learned in defeating malaria.

2. Malaria elimination strategy in China

The national malaria elimination strategy was developed based on the malaria control situation and response to the Global Eradication of Malaria Initiative proposed by the United Nation Millennium Development Goals (MDGs) in September 2000 [5, 17, 18]. The overall strategy comprised specific objectives and key measures taking into account the different epidemiological contexts and diversity of malaria transmission models all over the country. The overall goals of malaria elimination in China were set to achieve zero indigenous cases in the country by 2015, leaving apart the border areas of Yunnan Province with Myanmar and Tibet Autonomous Region with India to achieve complete elimination in the country by 2020.

2.1. Intermediate objectives

The strategy and key measures for malaria elimination in China were developed in line with the WHO guidelines [19]. However, counties had variable endemicity based on which a classification

[1]*Botswana, Cabo Verde, South Africa, Swaziland, Costa Rica, Ecuador, El Salvador, Mexico, Saudi Arabia, Timor-Leste, Malaysia.*
[2]*Botswana, South Africa, Swaziland, Ecuador, Saudi Arabia.*

was established according to the different types of area and intensity of malaria transmission. According to the magnitude of transmission and incidence, all counties were classified into 4 types, including Type I, local transmission and incidence ≥1/10,000 over the past 3 years; Type II, local transmission and incidence <1/10,000 over the past 3 years; Type III, no indigenous cases reported over the past 3 years but still with risk of transmission; and Type IV, malaria-free [20]. The classification of malaria-endemic areas is presented in **Figure 1** [17, 20].

	Classification	No of counties	Target population
Type I	Local infections are detected in the past three consecutive years and the incidence rate equals or higher than 1/10,000	75	30,965,000
Type II	Local infections are detected in the past three consecutive years, and the incidence rate is lower than 1/10,000 at least in one of those 3 years	684	440,792,000
Type III	No local infections reported in the past three consecutive years but risk of transmission exists	1,430	620,281,000
Type IV	Malaria-free area	669	180,882,000

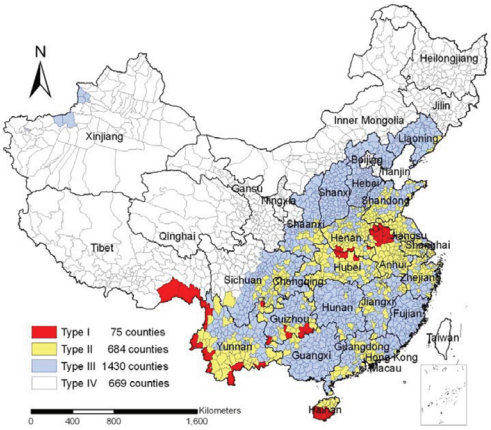

Figure 1. Stratification of malaria-endemic areas for data based on 2010 (references [5, 17]).

Several intermediate progress objectives were also set in a graduated way depending on the type of area. By 2015, (i) all Type III counties should achieve the objective of malaria elimination; (ii) at the same time, Types I and II counties, except Yunnan border counties, are expected to report zero indigenous malaria cases; (iii) the incidence of indigenous malaria cases in Type I counties located in the Yunnan border areas should be reduced to <1/10,000; (iv) it is expected that by 2017, no indigenous cases should be reported in the whole country; (v) by the year 2018, all Types I and II counties, except Yunnan border counties, must have fully achieved malaria elimination; and (vi) malaria elimination should be achieved all over the country by the year 2020.

2.2. Implementation requirements for malaria elimination

In order to achieve malaria elimination, specific requirements were assigned depending upon the type of county. The Type I counties should strengthen the management of infectious source and implement vector control measures to reduce the incidence of malaria. The Type II counties should eliminate the infectious source of malaria to interrupt local malaria transmission. The Type III counties should enhance the monitoring and disposition of the imported cases to prevent the secondary transmission. The Type IV counties should deal appropriately with imported cases. These requirements were also made flexible enough to be adjusted according to the control process and changes in the dynamics of the disease. These requirements were based on specific and standardized key measures [17].

2.2.1. Strengthening control and management of infectious sources

- **Timely malaria case detection**. Both public and private clinics and health facilities at all levels should take blood samples from patients including clinical diagnosed malaria cases, suspected malaria cases and febrile patients without confirmed origin of infection. Blood smear for microscopic tests or auxiliary detection using Rapid Diagnosis Test (RDT) kits should be carried out with blood samples. For RDT-positive samples, blood smears must be collected and kept for verification.
- **Timely surveillance and response to all malaria cases** [20, 21]
  - **Strengthening malaria case reporting.** Public or private clinics and health facilities at all levels have the responsibility to report malaria cases within 24 hours (**1 day**) after diagnosis. This is a requirement from the law on the Prevention and Control of Infectious Diseases in the People's Republic of China (http://www.gov.cn/banshi/2005-08/01/content_19107.htm).
  - **Case verification.** Centers for Disease Control and Prevention (CDCs) at the county level are made responsible for the verification of individual cases reported through the information system and for laboratory test assessment (RDT and microscope). Case investigation and verification are required to be accomplished within **3 days** after reporting.
  - **Management of malaria foci.** CDCs at the county level are responsible for investigation on identified malaria foci and further disposal within **7 days** after index case reporting. A reactive case detection must be carried out by collecting blood samples from inhabitants around the index case (fever displayed over the last 2 weeks). Collected blood samples must be analyzed by microscope or RDT. Meanwhile, vector control measures (i.e., indoor residual spraying (IRS)) must be implemented, and information materials for public awareness and health education must be provided to all families in different foci.
- **Treatment**
  - Full dose and **whole medication**[3] should be given to treat malaria patients according to the national guidelines. Public or private clinics and health facilities at all levels should treat all the detected malaria patients according to the national guidelines for anti-malarial drug use, issued by the Ministry of Health. All patients must be followed up across the whole course of treatment.
  - **Anti-relapse treatment.** In non-transmission malaria season, patients diagnosed with *vivax* malaria during the previous year must be given anti-relapse treatment.

2.2.2. Strengthening vector control

- **Anti-mosquito interventions.** During the malaria transmission season, it is encouraged to transform and improve the environment so as to reduce the number of breeding sites and

[3]Whole medication refers to the 8-day treatment for vivax malaria with primaquine (total dose 180 mg over 8 days) and chloroquine (total dose 1200 mg over 3 days) as first-line drugs. Artemisinin combination therapy (ACT) was used as first-line treatment to treat uncomplicated falciparum malaria.

decrease the density of mosquitoes in combination with the patriotic health movement[4] and new rural village development [22]. Measures of IRS and insecticide-treated net (ITN) are required to be applied in malaria foci.

- **Strengthening personal protection**. To prevent or minimize mosquito bites during the malaria transmission season, personal protective measures must be implemented such as repellents, mosquito-repellent incense, mosquito nets, wire mesh screening of doors and windows, *etc*.

2.2.3. Strengthening health education

- **Strengthening people awareness.** Mass media such as newspapers, radio, TV, and internet posts should cover "World Malaria Day and National Malaria Day" activities and implement various ways to widely broadcast knowledge on malaria and the malaria elimination policy to improve public awareness and motivate community participation in malaria control and elimination.
- **Strengthening health education for primary and junior high school students.** Education department should deploy and arrange the primary and junior high school health education. CDCs should strengthen the technical support to health education on malaria in these schools. Primary and junior high schools in Types I and II counties should keep malaria and malaria control awareness as a recurrent topic in regular health education courses or theme activities. They should encourage pupils to pass such awareness to their family members by the way of "child educate adult."
- **Strengthening health education at the community level.** In Types I and II counties, the local CDCs should organize and support malaria public awareness by the way of advertisements and regularly update posted news in hospital waiting rooms, community health service centers, rural hospitals, village clinics, and large construction sites. They should also develop awareness material in minority nations' language.

2.2.4. Strengthening malaria control in mobile/migrant populations

- **Strengthening the management of malaria among travelers**. The Department of Health and Exit & Entry Administration should regularly release public information about the world malaria situation and related information inside the country. Tourism departments should release such information to tourists too. The Department of Tourism and Commerce has the obligation to aid the Department of Health in providing information to people traveling to malaria-endemic areas, as well as track information on malaria patients. Mechanism for shared information should be established among different sectors. This cross sector mechanism is responsible for increasing the anti-malaria public awareness, for providing consultation services to cross-border populations, for screening individuals with fever who have been traveling to malaria-endemic areas, and for reporting the detected malaria cases.

[4]The patriotic health movement is referring to a community-based health movement focusing on cleaning, pest control, environment reforming, and other health-related activities, with the aim of preventing infectious diseases.

- **Strengthening malaria control and prevention among expatriates**. China is involved in many large construction projects in malaria epidemic areas in different countries, e.g., Ethiopia and Zambia in Africa, Myanmar and Indonesia in Southeast Asia. Some of the projects like road, railway, or dam constructions involve high malaria risk exposure. CDCs provide appropriate information and training to the personnel employed in these projects before expatriates' deployment. The management and registration of malaria cases among expatriates are conducted locally in coordination with health agencies and CDCs. Public security departments provide assistance to investigate malaria cases among expatriates as requested by the Department of Health.

2.2.5. Improving malaria surveillance

- **Strengthening capacity building for malaria diagnosis.** The National Institute of Parasitic Diseases, China CDC is responsible for managing national malaria diagnosis reference laboratory. Its mandate is to provide a final laboratory confirmation and identify the source of any malaria case. Reference laboratories at all levels should regularly assess the skills of test and conduct quality control to ensure operation of the laboratory network.
- **Malaria surveillance in the post-elimination stage.** The status of malaria-free area must be maintained in counties having achieved malaria elimination, as well as in previously non-endemic provinces. This must be done through regular training of the medical personnel in malaria diagnosis and treatment. Prevention of re-establishment of malaria transmission is also required and must be conducted by intensifying the monitoring of imported cases.

3. Progress on malaria elimination in China

3.1. Status after seven years of implementation of malaria elimination program

A total of 28,886 malaria cases were reported in Mainland China (excluding Hong Kong, Macau, and Taiwan) from 2010 to 2016 (**Table 2**) [9, 10, 23–26]. During this period, indigenous cases, as well as the number of areas with local transmission, were substantially reduced. There were 40 indigenous cases reported from 10 counties in 2015, a decrease of 99.06% from 2010. By 2015, all Type III counties had achieved malaria elimination goals (no occurrence of indigenous cases for at least 3 consecutive years). Except for border counties in Yunnan, 96.43% (54/56) of the Type I counties reported no indigenous case over the same period. The malaria incidence in 19 Type I counties in Yunnan border area was lower than 10/100,000. All positive cases were reported through the China Information System for Disease Control and Prevention (CISDCP). The increase in incidence and number of detected cases after 6 years of implementation of the malaria elimination program was linked to the large number of imported cases, while the number of indigenous cases was reduced by 99.93% between 2010 and 2016 (**Table 2**) [8, 27–30]. The number of provinces with imported cases increased from 22 in 2010 to 30 (all the provinces in Mainland China except Tibet) in 2015. A total of 3318 imported cases were reported in 2016 (**Table 2**). The top 5 countries of origin of the imported cases were Myanmar (15.9%), Angola (12.5%), Nigeria (7.7%), Equatorial Guinea (7.5%),

Year	Total reported cases	Local cases					Imported cases							No. of death cases
		Clinical diagnosis	*P. vivax*	*P. falciparum*	Mix	Subtotal	Clinical diagnosis	*P. vivax*	*P. falciparum*	*P. malariae*	*P. ovale*	Mix	Subtotal	
2010	7855	0	4165	97	0	4262	NA*	NA	1161	NA	NA	NA	3593	19
2011	4498	364	885	56	3	1308	372	1253	1468	62**	0	35	3190	33
2012	2718	32	228	16	5	281	35	900	1403	60**	0	39	2437	15
2013	4128	6	77	9	0	92	29	859	2899	51	133	65	4036	23
2014	3078	5	45	6	0	56	20	798	1876	52	232	44	3022	25
2015	3288	1	38	1	0	40	22	840	1991	76	272	47	3248	20
2016	3321	0	3	0	0	3	15	709	2158	64	311	61	3318	15

°The number of malaria cases reported in 2017 will be published by the end of 2018.

*NA indicates that data were not available in the annual reporting system.

**Before 2013, the data recorded in the annual reporting system did not separate *P. malariae* and *P. ovale*.

Table 2. Malaria-attributable morbidity in the People's Republic of China during 2010–2016°.

and Cameroon (7.1%). In response to the increasing risk from imported cases, joint coordination and transfer of information were established among different agencies, in particular between China CDC and port quarantines. The latter are responsible for frontline screening and detection providing timely reports of positive cases. Clinics and hospitals are in charge of case treatment, while CDCs must follow up all the reported cases and carry out the individual case investigation. A successful example of such organization is given by the Shanglin County, Guangxi [31], for reporting 1,052 imported malaria cases in 2013, all of which were successfully treated and no death cases occurred. Furthermore, although *Anopheles* mosquitoes were present [14], no secondary transmission occurred.

Along the border between China and countries of the Greater Mekong Subregion (GMS) (Myanmar, Lao PDR, and Vietnam), 3 frontline barriers were established jointly by CDCs and port quarantines [32]. These 3 barriers consisted of (i) a strengthened health system in all 25 border counties with a capacity to immediate and comprehensive response to each malaria case (first line), (ii) establishment of 68 malaria service points at the border to provide consultation and screening to the migrant/mobile population (MMP) (second line), and (iii) a coordination process for response to malaria along the international borders between China, Myanmar, Lao PDR, and Vietnam covering 42 border counties (20 in Yunnan and 22 in the 3 other countries).

The main risks clearly identified after implementation of the national malaria elimination program were re-introduction through imported cases and the associated secondary transmission by local malaria vectors. Sustainable vector control is therefore essential. With the support from the national malaria surveillance system and national malaria diagnosis reference laboratory network, all the confirmed malaria cases were examined, including a total number of 2,215 foci investigated within 7 days after case detection and verification (**Table 3**). Long-lasting insecticidal net (LLIN) or ITN was delivered to the communities with high malaria incidence and presence of highly efficient malaria vectors, such as *Anopheles dirus* s.l. or *Anopheles minimus* s.l. [14]. IRS was carried out in active malaria foci (the definition of active foci is given in Ref. [19, 33]). In 2015, a total number of 29,611 LLIN/ITN were delivered, and 1,697,188 persons were protected by IRS in response to malaria foci (**Table 4**). Another key element in the protection of people against imported malaria was the training and education of the personnel. Annual joint health training workshops were carried out by the Departments of Health, Education, and Inspection and Quarantine on the National Malaria Day (April 26th) since 2008. Altogether, 74.9 million educational documents were delivered during workshops from 2010 to 2015 (**Table 5**). To these, one must add all posters and flyers delivered through port quarantines. Capacity building for health professional personnel corresponded to 464,500 working days in CDCs; 848,764 working days for clinical doctors; and 186,368 working days for microscopists during 2010–2015 (**Table 5**). With respect to port quarantines, 16,141 working days of training were accomplished with a malaria awareness rate of 100%.

A key issue in successful implementation of a program relies on the governmental commitment and support for sustained allocation of resources. The government at all levels has adopted malaria elimination as a component of the socioeconomic strategy. A national action plan for malaria elimination was issued jointly by 13 ministries in 2010 with clear goals and strategy, followed by a sustainable budget plan to ensure the financial support for malaria elimination. As a

Province	Cases reported within 24 h			Case investigation within 3 days		Number of foci investigated and disposed within 7 days
	Total reported cases	Reported cases within 24 h	Proportion of reported cases	Investigated cases within 3 days	Proportion of investigated cases	
Beijing	89	89	100%	89	100%	0
Tianjin	17	17	100%	17	100%	0
Hebei	44	44	100%	44	100%	21
Shanxi	12	12	100%	12	100%	15
Inner Mongolia	6	6	100%	1	16.67%	0
Liaoning	65	65	100%	65	100%	62
Jilin	21	21	100%	21	100%	0
Heilongjiang	8	8	100%	8	100%	0
Shanghai	42	42	100%	42	100%	29
Jiangsu	408	408	100%	408	100%	408
Zhejiang	195	195	100%	195	100%	160
Anhui	129	129	100%	129	100%	117
Fujian	94	94	100%	94	100%	12
Jiangxi	52	52	100%	52	100%	43
Shandong	219	219	100%	217	99.09%	199
Henan	185	185	100%	184	99.46%	180
Hubei	125	125	100%	122	97.60%	12
Hunan	129	129	100%	128	99.22%	46
Guangdong	155	155	100%	144	92.90%	1
Guangxi	236	236	100%	236	100%	33
Hainan	14	14	100%	14	100%	12
Chongqing	33	33	100%	31	93.94%	26
Sichuan	294	294	100%	292	99.32%	272
Guizhou	17	17	100%	17	100%	0
Yunnan	622	622	100%	618	99.36%	481
Tibet	8	8	100%	8	100%	0
Shaanxi	81	81	100%	81	100%	80
Gansu	22	22	100%	21	95.45%	6
Qinghai	1	1	100%	0	0	0
Ningxia	6	6	100%	6	100%	0
Xinjiang	4	4	100%	4	100%	0
Total	**3333**	**3333**	**100%**	**3300**	**99.01%**	**2215**

*1-3-7 model is referring to case reported within **1 day** (24 hours), case verification and investigation within **3 days**, and foci investigation and disposal within **7 days**. This is summarized as work model for malaria surveillance and response for malaria elimination program [20, 21].

Table 3. Progress indicators of 1-3-7* model in 2015.

http://dx.doi.org/10.5772/intechopen.77282

Province	Number of delivered LLIN/ITN	Number of people protected by IRS/house*
Beijing	0	0
Tianjin	0	0
Hebei	6	353
Shanxi	0	188
Inner Mongolia	0	0
Liaoning	0	1120
Jilin	0	0
Heilongjiang	0	0
Shanghai	0	301
Jiangsu	0	7299
Zhejiang	0	1850
Anhui	207	1094
Fujian	180	535
Jiangxi	0	11,142
Shandong	0	336
Henan	2096	17,814
Hubei	79	918
Hunan	4	408
Guangdong	1552	1,327,650
Guangxi	10	1961
Hainan	6910	20,106
Chongqing	2	45,280
Sichuan	7	11,248
Guizhou	7418	12,771
Yunnan	628	229,535
Tibet	10,000	1537
Shaanxi	512	3546
Gansu	0	196
Qinghai	0	0
Ningxia	0	0
Xinjiang	0	0
Total	**29,611**	**1,697,188**

*Vector control measures mainly implemented in malaria foci for targeting population at risk.

Table 4. Progress indicators of vector control measures in 2015.

key player in malaria elimination in the central government, the National Health Commission (NHC, previously known as the Ministry of Health before 2011 and National Health and Family Planning Commission during 2011–2018) has established a multidisciplinary technical committee

Province	Number of trained people (person/time)			Number of delivered health education materials
	Malaria health workers	Clinical doctors	Microscopists	
Beijing	208	0	176	185,000
Tianjin	240	300	300	25,700
Hebei	41,499	95,926	10,652	3,064,121
Shanxi	596	0	871	57,000
Inner Mongolia	204	408	204	3200
Liaoning	7554	88,609	5835	143,600
Jilin	226	0	226	4000
Heilongjiang	0	0	0	0
Shanghai	21,516	48,584	9377	1,246,268
Jiangsu	26,415	23,963	16,468	7,365,562
Zhejiang	11,447	19,304	8749	1,676,164
Anhui	59,229	42,417	18,671	14,323,973
Fujian	7653	8777	3095	556,489
Jiangxi	12,696	24,829	9272	1,621,293
Shandong	34,624	56,382	15,494	7,040,504
Henan	62,005	183,085	19,486	7,968,270
Hubei	27,199	38,291	10,062	4,078,954
Hunan	22,018	46,761	11,666	4,722,609
Guangdong	737	375	1008	429,401
Guangxi	23,682	57,461	10,914	4,505,439
Hainan	10,838	5288	2607	2,058,430
Chongqing	1027	0	235	0
Sichuan	1900	0	825	372,200
Guizhou	21,643	28,120	8827	3,342,021
Yunnan	28,962	35,103	7479	4,200,909
Tibet	—	—	—	—
Shaanxi	21,951	34,313	10,970	3,615,648
Gansu	12,851	9136	2178	1,631,944
Qinghai	—	—	—	—
Ningxia	469	0	144	0
Xinjiang	5111	1332	577	670,755
Total	464,500	848,764	186,368	74,909,454

Note: "-" denotes data not available.

Table 5. Progress indicators of health education and capacity building during 2010–2015.

comprising malaria experts, i.e., epidemiologists, entomologists, clinical doctors, parasitologists, program managers, *etc*. With the support of this committee, NHC has issued a series of guidelines and standards adapting and updating the WHO guidelines [2, 19, 33, 34], such as technical guidelines for malaria elimination, malaria treatment and anti-malarial drug use, standards for malaria control and elimination, and malaria diagnosis, to cite a few [18].

3.2. Successful implementation

A working model, named 1-3-7, for malaria surveillance and response was implemented as a national malaria program. The definition of "1-3-7" is as follow:

- "1," within 1 day (24 hours): all malaria cases must be reported to the Chinese Information System for Disease Control and Prevention (CISDCP), an internet-based reporting system. The case information will be notified through a four-level system "county→ prefecture→ province→ national." The response at different levels is implemented according to national guidelines. Malaria is classified as a category B notifiable infectious disease, and case reporting through CISDCP has been implemented since 2004 [35]. All private and public registered clinics and hospitals must report malaria cases through CISDCP after diagnosis. CDCs are the key operators of CISDCP (**Figure 2**). This ensures that malaria case information is timely transmitted from bottom to top.

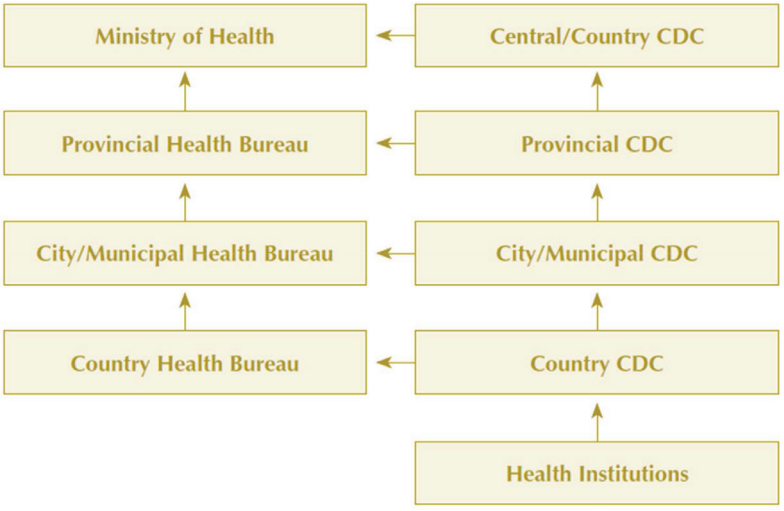

Figure 2. Vertical reporting structure of the China information system for disease control and prevention (CISDCP) (CDC, Centers for Disease Control and Prevention).

- "3," within 3 days: all the reported malaria cases should be confirmed and visited by CDCs' staffs at the county level to verify the infectious origin of the cases (whether imported or locally acquired). Meanwhile, blood samples of patients are taken and sent to the reference laboratory for further verification.

- "7," within 7 days: the outbreak focus should be investigated, and vector control and health promotion measures must be implemented. Vector control measures need to be conducted in active foci only, which are considered to have potential risk of onward transmission. The scope of investigation is the household of the reported patient and neighboring households. However, it can be expanded, if necessary.

Following the implementation of this 1-3-7 model, local malaria transmission was interrupted effectively in most parts of China, which accelerated the malaria elimination process [20, 21, 36]. Based on this success, the 1-3-7 model was recommended by WHO as an example for malaria surveillance model at elimination stage, in "Strategy for malaria elimination in the Greater Mekong Subregion: 2015-2030" and "Malaria surveillance, monitoring & evaluation: a reference manual" [37, 38].

However, the 1-3-7 strategy is not sufficient to successfully achieve malaria elimination. Other aspects must be considered. **The first aspect** to consider is the establishment of a network of malaria surveillance and diagnosis reference laboratories. After the launch of the malaria elimination program in 2010, and following the suggestions from the WHO guidelines [2, 19, 33, 34], a network for malaria diagnosis reference labs was gradually established [39]. By 2015, 23 provinces were enrolled into the National Reference Laboratory Network (NRLN). Laboratories at all levels worked together to ensure the quality of malaria diagnosis all over the country in a bottom-up approach (**Figure 3**) [39]. **Another key aspect** to consider is the involvement of communities. The community level is essential for a successful implementation. In addition to cross sector coordination, information was shared with different subnational divisions and in particular the community level. This is especially important when managing malaria cases among

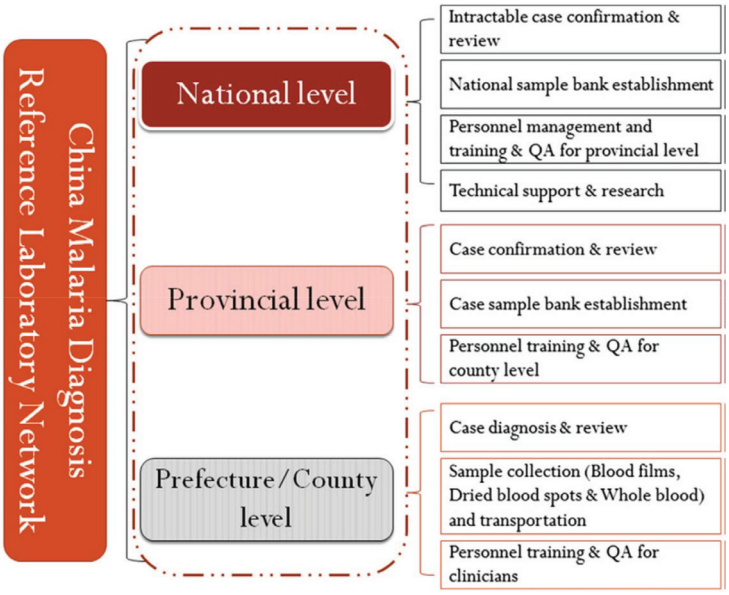

Figure 3. Structure of the National Reference Laboratory Network (QA, quality assurance).

mobile/migrant populations. Five provinces in Central China, i.e., Jiangsu, Shandong, Henan, Anhui, and Hubei, and 3 provinces in Southern China, i.e., Guangdong, Guangxi, and Hainan, coordinated their actions at all levels (**Figure 4**). This joint coordination efficiently contributed to control malaria outbreaks and reduce malaria incidence [40, 41]. **The last aspect** to be considered is international collaboration. Malaria elimination cannot be achieved through the efforts of few countries only. It must be a general and coordinated international effort. In this respect, China has received support from international agencies such as WHO and Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) to conduct this international collaboration [42]. At the same time, China developed international collaborations with African and GMS countries to implement a coordinated strategy for controlling and eliminating malaria [43, 44], and Chinese

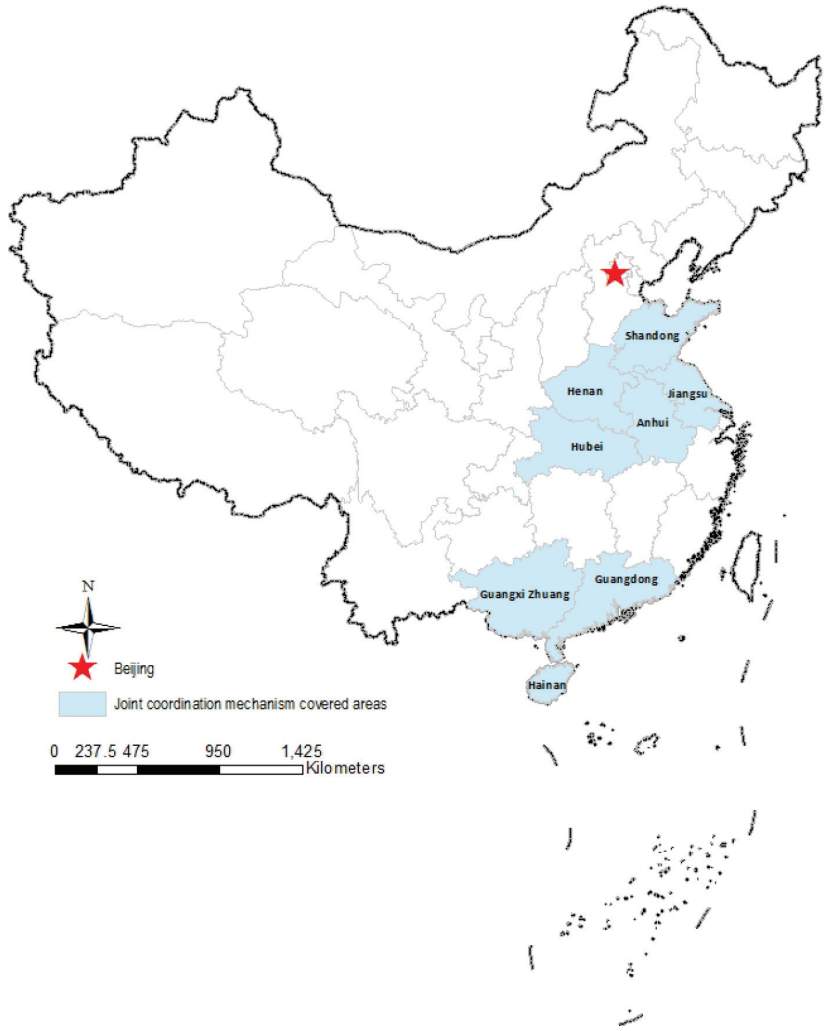

Figure 4. Cross province coordination mechanism for malaria control based on national mechanisms established since the 1950s–1970s. At that time, few population movements were occurring in the Yunnan Province.

students are being trained in Europe on molecular approaches applied on *Anopheles* mosquitoes [13–15]. These international collaborations on malaria and vector control toward elimination provided strong support to reduce malaria incidence in China and will be the basis for sustaining malaria elimination efforts.

4. Gaps and challenges

Although the malaria elimination program has made significant progress in China, there are still challenges. The main challenge is to accomplish malaria elimination along border areas. China is sharing 4060 km long international borders with GMS countries. These borders are devoid of natural barriers and porous, permitting population movement and facilitating cross border malaria transmission. The China-Myanmar border is particularly difficult to access for healthcare services due to ongoing armed conflict on the Myanmar side of the border resulting in proliferation of disease vectors and uninterrupted disease transmission [13, 15, 32, 45–48]. A related challenge is the prevention of re-introduction and re-establishment of malaria considered as a real threat to malaria elimination [2, 33, 34, 37]. Surveillance is highly recommended as a key intervention in the post-malaria elimination stage. However, decisive and rapid response to imported malaria is vital to prevent re-introduction and sustain malaria elimination [11, 27, 29, 49–53]. A shift from community to hospitals at the county and higher level for primary diagnosis was also recorded [8, 27], indicative of a more active role of the main city airports as ports of entry. In addition, owing to the sharp decrease of indigenous cases, it becomes difficult to maintain the capacity of intervention and proper training at the local level. Novel and innovative capacity building and training modules must thus be developed for both clinicians and CDC staffs.

5. Perspectives

The main objective in China with respect to malaria will be focused on how to sustain malaria elimination and prevent transmission re-establishment in accordance with the WHO's newly updated guidelines [33]. This requires a specific and sustainable investment even at post-elimination stage. Malaria is on the list of the "Health China 2030" blueprint issued by the State Council of China in 2016. This will ensure the required sustainable investment. Following this blueprint, a series of technical guidelines for malaria surveillance and response at post-elimination stage, as well as protocols for preventing malaria transmission re-establishment, are under development for short release. Meanwhile, mechanisms for maintaining anti-malaria capacity in health system are implemented. A National Technical Competition for Parasitic Disease Diagnosis and Test is organized annually for health workers from clinical agencies and CDCs [54]. This competition is an efficient way to maintain awareness and efficiency in malaria detection within the health system and prevent erosion of capacity along with malaria elimination. However, malaria elimination is primarily an international endeavor. Broad engagement and sustained investments are needed with support from multiple international partners [55–57]. In 2013, Chinese President Xi Jinping proposed the "One Belt and One Road Initiatives" to the world for international cooperation and development. Cooperation in health is one of the key components as it relates to the mutual benefits. China has already been actively involved

in global health governance [58], but a higher level of involvement is now expected, and the Chinese experience in malaria elimination will definitely be put at use within the "One Belt and One Road Initiative" [59]. Several platforms are currently under development, such as the Malaria Elimination Network in Lancang-Mekong Region (MENLMR) and the China-Africa Cooperation Program. Both GMS and sub-Saharan Africa are strongly affected by malaria, including drug resistance [60–62], high disease burden [1], and low level of resources [1, 3, 34]. They are thus primary targets for focused interventions enabling malaria elimination. Furthermore, these countries are experiencing innumerable challenges to achieve their planned malaria elimination program and in dire need of international support to bridge the funding gap [1–3, 37]. Although China has applied a successful model and did significant progress on malaria elimination, the Chinese model and experience cannot directly be implemented in these countries. Evaluation and field tests are needed as preliminary steps for operational feasibility. Pilot areas have been identified, and demonstrative projects have been therefore launched jointly by China and the targeted countries to assess the level of feasibility. These preliminary projects will provide evidence-based suggestions to develop a suitable strategy and model for each country to realize the ultimate goal of malaria elimination.

6. Conclusions

China has made substantial progress on malaria elimination and is on the way to achieve the elimination goal on time by 2020. The lessons drawn based on experiences in China will make a good reference for the countries aiming at malaria elimination. Challenges identified in the malaria elimination process in China might help other countries formulating appropriate strategies in time and place. International collaboration is strongly advocated to achieve the global issue to eliminate the most important infectious disease of the current times.

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Conflict of interest

The authors declare jointly that there is no conflict of interest.

Acronyms

ACT	Artemisinin combination therapy
CDC	Centers for Disease Control and Prevention

CISDCP	China Information System for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
E-2020	In 2016, the WHO identified 21 countries with the potential to eliminate malaria by the year 2020. These countries were known as "E-2020 countries
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GMS	Greater Mekong Subregion
IRS	Indoor residual spraying
ITN	Insecticide-treated net
LLIN	Long-lasting insecticidal net
MENLMR	Malaria Elimination Network in Lancang-Mekong Region
MDGs	UN Millennium Development Goals
MMP	Mobile and migrant population
NHC	National Health Commission
NRLN	National Reference Laboratory Network
QA	Quality assurance
RDT	Rapid diagnosis test
TDR	Special Programme for Research and Training in Tropical Diseases
WHO	World Health Organization

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Conclusion

Results in this chapter show that the National Malaria Elimination Program was successfully implemented and the distribution of malaria endemic areas has drastically shrunk. Both locations of residual foci and origin of malaria cases indicated that the main challenges of malaria elimination in China are now the management of imported malaria cases and the prevention of malaria reintroduction at international border areas, especially the China-Myanmar border.

The lessons drawn, based on experiences of malaria elimination in China, will make a good reference for the countries aiming at malaria elimination. Challenges identified in the malaria elimination process in China might help other countries formulating appropriate strategies in time and place. International collaboration is strongly advocated to achieve global and regional malaria elimination goals. Malaria is on the list of the "Health China 2030" blueprint issued by the State Council of China in 2016. This will ensure the required sustainable investment. Following this blueprint, a series of technical guidelines for malaria surveillance and response at post-elimination stage, as well as protocols for preventing malaria transmission re-establishment, are under development for short release. Meanwhile, mechanisms for maintaining anti-malaria capacity in the health system are implemented. A National Technical Competition for Parasitic Disease Diagnosis and Test is organized annually for health workers from clinical agencies and China CDCs. This competition is an efficient way to maintain awareness and efficiency in malaria detection within the health system and

prevent erosion of capacity along with malaria elimination. However, malaria elimination is primarily an international endeavor. Broad engagement and sustained investments are needed with support from multiple international partners. In 2013, the "One Belt and One Road Initiatives" was proposed to the world for international cooperation and development. Cooperation in health is one of the key components as it relates to mutual benefits. China has already been actively involved in global health governance, but a higher level of involvement is now expected, and the Chinese experience in malaria elimination will definitely be put at use within the "One Belt and One Road Initiative". Both GMS (Great Mekong Subregion) and sub-Saharan Africa are strongly affected by malaria, including drug resistance, high disease burden, and low level of resources. They are thus primary targets for focused interventions enabling malaria elimination. Furthermore, these countries are experiencing innumerable challenges to achieve their planned malaria elimination program and in dire need of international support to bridge the funding gap. Although China has applied a successful model and did significant progress on malaria elimination, the Chinese model and experience cannot directly be copied into these countries. Evaluation and field tests are needed as preliminary steps for operational feasibility. Pilot areas have been identified to implement the demonstrative projects for assessing the level of feasibility jointly supported by China and the targeted countries. These preliminary projects will provide evidence-based suggestions to develop a suitable strategy and model for each country to realize the ultimate goal of malaria elimination.

Chapter 5 Conclusion and perspectives

Conclusion

The work presented in the previous chapters summarized the spatio-temporal evolution of malaria control and elimination in China, with special focus on malaria vectors and cross-border transmission surveillance and response. China has made a substantial progress since 2010, the year of which launch of the National Malaria Elimination Program; and then achieved zero local indigenous malaria case report in 2017 (105, 175, 176). The residual malaria foci have been identified and eliminated (175, 178, 179). However, the findings of this study show that all former non-endemic areas are now reporting imported malaria cases in China, while the largest proportion of imported cases is still reported in former endemic areas. The demographic characteristics of imported malaria depend upon the country of expatriation, species of parasites, occupation and place of origin of workers. Health facilities in former endemic areas outperformed those in former non-endemic areas, most likely due to a better practice and experience of the medical staff in the former endemic regions. Information, treatment and surveillance must be provided to expatriates while capacity building and continuous training must be implemented at all level of health facility in China.

The former predominant malaria vectors were well controlled in most of the country (129), in particular for *Anopheles lesteri* (synonym: *An. anthropophagus*) and *Anopheles dirus* s.l. (two main malaria vector species occur in China, *An. dirus* and *An.*

baimaii), which nearly disappeared after several years of malaria control effort. *Anopheles sinensis*, which was previously reported to be less efficient for malaria transmission, is becoming the predominant species in Southwestern China. Besides, the field sampling results indicated the existence of high efficient malaria vectors, e.g. *Anopheles harrisoni* at the China-Myanmar border. In addition, elevated human-biting rates, high adult and larval densities, and important parous rates were found in both *An. sinensis* and *An. harrisoni* , which reveal a very high receptivity and risk of malaria re-introduction along the China–Myanmar border.

The malaria elimination assessment at county level and sub-national level is being carried out as planned (175, 176). The whole country is on the right track to achieve malaria elimination by 2020. The progress is attributed to the well-allocated health systems, effective strategy and efficient implementation, as well as the strong political commitment and funding support from the government (180, 181). However, the following aspects are identified as the challenges to the maintenance of the sustainability of malaria elimination in China: 1) the increasing risk of malaria re-introduction from imported malaria together with international mobile and migrant populations (MMP); 2) the decreasing knowledge on malaria in health facilities and delayed response to imported cases; 3) the presence of malaria vectors and possible changes in their bionomics due to environment alteration (16, 148, 149, 175, 182-185). In addition, more entomologists need to be trained and the knowledge on malaria vectors must be maintained and even improved in particular by introducing the use of molecular techniques, as malaria elimination relies also on effort carried on appropriate

vector control. Another effort should be directed towards the development of modeling and simulation of different scenarios matching conditions in different areas in order to prepare actions to be taken in case of reintroduction. Continuous studies and efforts addressed to these aspects need to be done, as well as successive investment in the future.

Perspectives

Limitation and future work

The knowledge gaps in malaria vectors in China have been identified during this work. However, due to the limited time and resources and the complexity of the factors influencing malaria in China, only some gaps were investigated in this study. Although the bionomics of predominant malaria vectors as *An. harrisoni* and *An. sinensis* have been preliminary investigated (129, 148, 155, 186), there are still some fields, as below, needing further investigation.

1. The specific bionomics of *Anopheles* species within complex and group, e.g. *An. minimus* complex, *An. maculatus* group, as well as their role in malaria transmission, specifically at the China-Myanmar border areas.

2. The updated geographical distribution of primary malaria vectors after their identification at the species level using molecular assays (e.g. *An. sinensis*, *An. minimus*, *An. harrisoni*, *An. dirus*, *An. baimaii*, etc.) in China, with respect to Land Use and Land Cover (LULC) and climate change.

3. Upgrade the entomological surveillance tools with Remote Sensing (RS), Geographic Information System (GIS) and mathematical modeling for more precise spatio-temporal information (e.g. Maxent model).

4. Develop modeling and simulation at various scales (local, national and regional) to prepare scenarios of intervention.

Call for joint efforts

Imported malaria caused by Chinese citizens returning from malaria endemic countries and migrant workers has been identified as one of the key challenges to malaria elimination in China (17, 175, 187). Moreover, malaria control along the international borders and hard-to-reach population as mobile/migrant population (MMP) have been considered as the bottleneck of malaria elimination at both regional and global levels (182, 188-191). Facing these challenges and solving the problems will require joint efforts with other countries. There is now an urgent need to combine efforts with those of the neighboring countries for setting up an international taskforce and a regional coordination network. Some approaches in areas along the China-Myanmar and China-Lao PDR borders could be considered as pilot zones (192). Since 2005, a joint mechanism to carry out disease surveillance and implement responses at the borders has already been established with the agreement from the Ministry of Health of three countries, China, Myanmar and Lao PDR (193). Within this joint mechanism,

there are platforms for regular information sharing on infectious disease outbreak (focus on malaria, dengue fever, plague, HIV/AIDS, etc.), joint outreach team for case detection and treatment, collaborative workshops for capacity buildings. After several years of efforts implementing this process, malaria morbidity and local transmission as well as dengue outbreaks have been reduced (194-198). Malaria control in cross-border areas and hard-to-reach populations are major challenges in the Great Mekong Subregion (GMS) (109, 110). The bilateral, and even better, a multi-lateral collaboration among the neighboring countries for implementing joint and coordinated malaria control programs along the international borders, and more efficiently controlling MMPs, is highly recommended for increasing the control efforts and for envisaging the elimination of this disease in the region.

Opportunity

Following rapid economic growth, China has now the second-largest economy in the world, and is transiting from aid recipient to aid provider, particularly in global health and global health governance (199). As an example of an emergence response to the Ebola outbreak in West Africa in 2014, China played an active role in the front-line with financial, technical and personnel support (199-201). WHO and aid receiving countries have applauded these actions, and then a MoU was signed between the Chinese government and WHO for further collaboration on Global health governance. This agreement will give China more responsibilities for participating in the world

campaign of infectious diseases control and elimination, i.e. World malaria elimination campaign. Moreover, in October 2013, the Chinese President Xi Jinping raised the initiative of jointly building the Silk Road Economic Belt and the 21st-Century Maritime Silk Road (hereinafter referred to as Belt and Road Initiative, BRI) (Figure 9). Health Cooperation along One Belt and One Road has been identified as key part of People-to-people bond (22, 199, 202). Hence, to answer the call for establishing a community with a shared future for mankind, particularly a Health Silk Road, series of aid projects and research grants have or will be released to support the health cooperation among China and the countries along One Belt and One Road (22, 199).

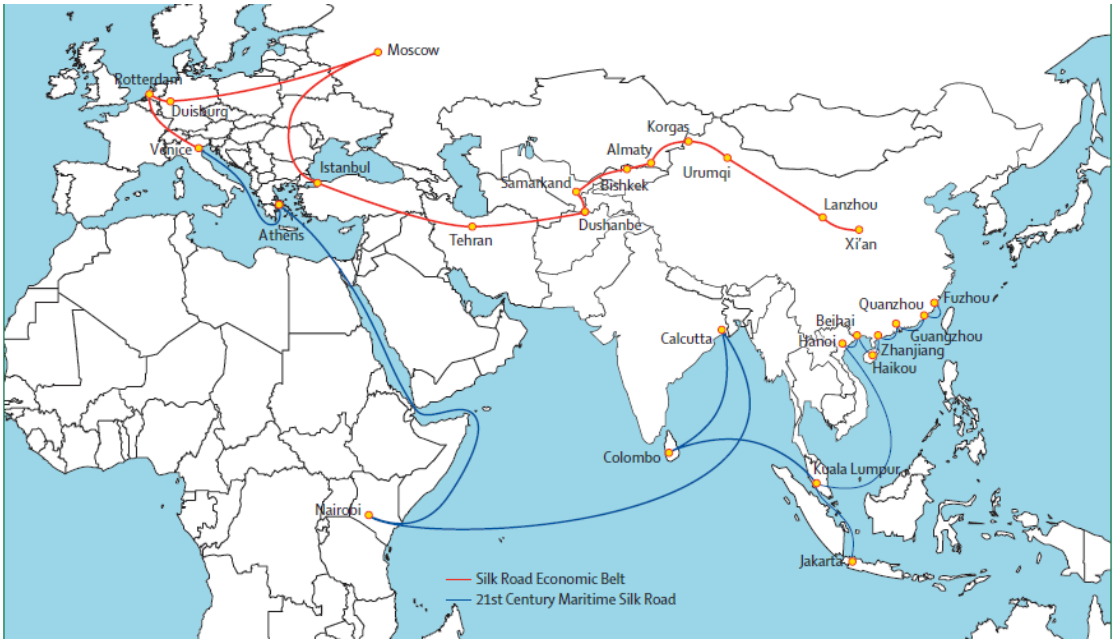

Figure 9 Map of One belt and One Road (199)

In 2018, a key research project on infectious disease control and prevention was launched with the support from the Ministry of Science and Technology (MOST) of China. This project was designed for a three-year duration (2018-2020) and is entitled "Study on epidemiological characters of key infectious diseases and early warning

techniques along One Belt and One Road". Malaria is one of the key infectious diseases in the countries along One Belt and One Road, particularly in Southeast Asia. One of the sub-projects was designated to focus on malaria with specific topics investigated in this PhD thesis (**Figure 10**). This project was carried out under the collaboration among National Institute of Parasitic Diseases-China CDC (NIPD), IRD and CIRAD in Montpellier, France and institutions from Southeast Asia, such as National Center for Parasitology, Entomology and Malaria Control (CNM) Cambodia, Kasetsart University (KU) Thailand.

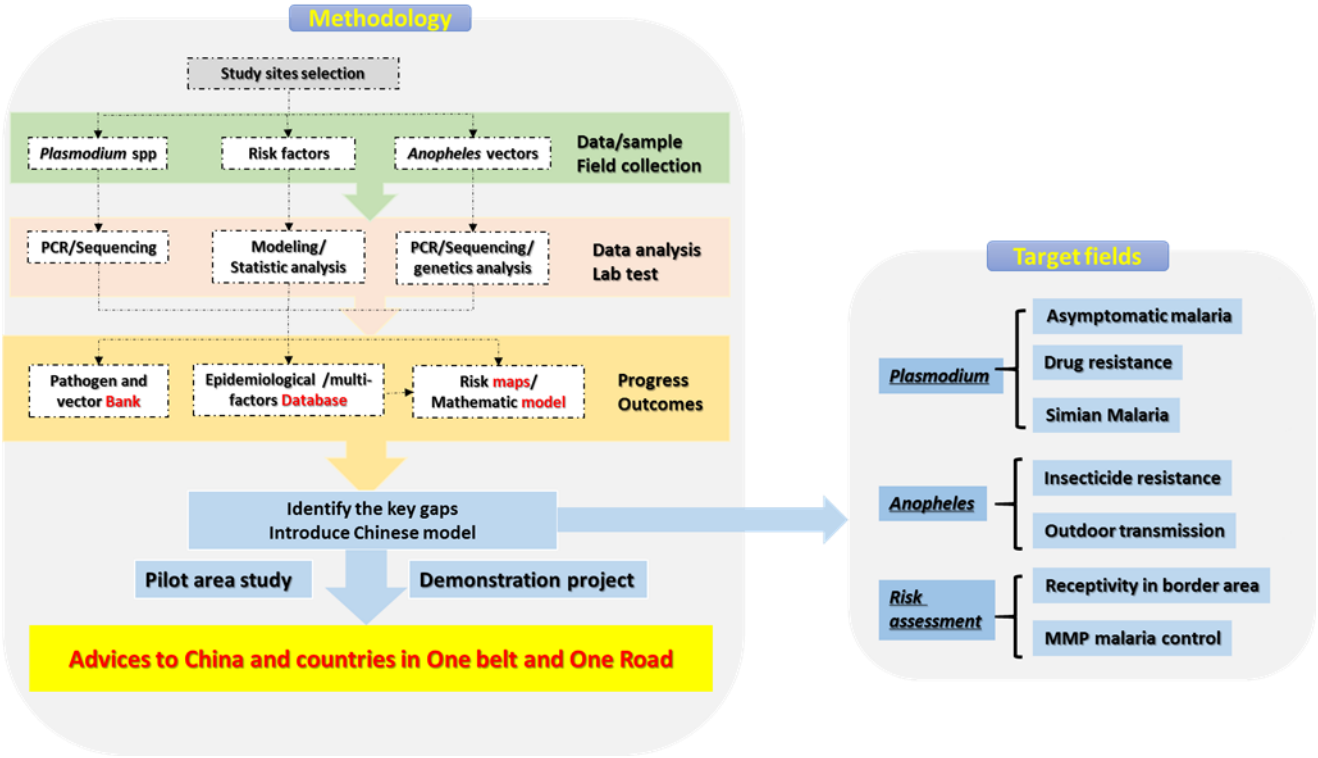

Figure 10. Proposed study design of BRI project on malaria

Chapitre 5 Conclusion et perspective

Conclusion

Les travaux présentés dans les chapitres précédents résument l'évolution spatio-temporelle de la lutte antipaludique et de son élimination en Chine, en mettant l'accent sur les vecteurs du paludisme, ainsi que sur la surveillance et le contrôle de la transmission transfrontalière. La Chine a réalisé des progrès substantiels depuis 2010, année du lancement du programme national d'élimination du paludisme, qui a permis d'atteindre zéro cas de paludisme autochtone en 2017 (105, 175, 176). Les foyers résiduels de paludisme ont été identifiés et éliminés (175, 178, 179). Cependant, les résultats de cette étude montrent que toutes les anciennes provinces non-endémiques signalent maintenant des cas importés de paludisme en Chine. Cependant, la plus grande proportion de cas importés est toujours signalée dans les anciennes zones d'endémie. Les caractéristiques démographiques du paludisme importé dépendent du pays d'expatriation, des espèces de parasites, de la profession et du lieu d'origine des travailleurs. Les établissements de santé situés dans les anciennes zones d'endémie étaient plus performants que ceux d'anciennes régions non-endémiques, sans doute grâce aux meilleures pratiques et à l'expérience du personnel médical des anciennes régions d'endémie. Des informations, des traitements et une surveillance doivent être fournis aux expatriés, tandis que le renforcement des capacités et la formation continue doivent être mis en œuvre à tous les types d'établissements de santé en Chine.

Les moustiques vecteurs du paludisme qui étaient historiquement prédominants ont été efficacement contrôlés dans la plus grande partie du pays (129), en particulier *Anopheles lesteri* (synonyme: *An. anthropophagus*) et *Anopheles dirus* s.l. (deux espèces vectrices majeures du paludisme existent en Chine, *An. dirus* et *An. baimaii*), qui ont presque disparu après plusieurs années de lutte antipaludique. *Anopheles sinensis*, qui avait déjà été signalé comme étant moins efficace dans la transmission du paludisme, est en train de devenir l'espèce prédominante dans le sud-ouest de la Chine. En outre, les résultats de l'échantillonnage sur le terrain ont révélé l'existence de vecteurs du paludisme efficaces comme par exemple *Anopheles harrisoni* à la frontière Sino-Birmane. De plus, des taux élevés de piqûres sur les humains, des densités élevées d'adultes et de larves, et des taux importants de femelles pares ont été trouvés chez deux espèces, à la fois chez *An. sinensis* et *An. harrisoni*, ce qui révèle une très grande réceptivité et un risque élevé de réintroduction du paludisme le long de la frontière Sino-Birmane.

L'évaluation de l'élimination du paludisme aux niveaux des comtés et à un niveau sous-national se déroule comme prévu (175, 176). L'ensemble du pays est en bonne voie de parvenir à éliminer le paludisme d'ici 2020. Ces progrès sont attribuables à des systèmes de santé bien répartis, une stratégie de mise en œuvre efficiente, au ferme engagement politique et au soutien financier du gouvernement (180, 181). Cependant, les aspects suivants sont identifiés comme des défis certains pour la pérennisation de l'élimination du paludisme en Chine: 1) le risque croissant de réintroduction du paludisme à partir de cas de paludisme importé par des populations à mobilité

internationale et des populations migrantes; 2) la diminution des connaissances sur le paludisme dans les établissements de santé et le retard de la réponse aux cas importés; 3) la présence de vecteurs du paludisme et les modifications possibles de leur biologie dues à une altération de l'environnement (16, 148, 149, 175, 182-185). De plus, il faut former davantage d'entomologistes et maintenir, voire améliorer, les connaissances sur les vecteurs du paludisme, notamment en introduisant l'utilisation de techniques moléculaires, car l'élimination du paludisme dépend pour beaucoup des efforts déployés pour lutter efficacement contre les vecteurs. Des efforts devraient également être consacrés au développement de la modélisation et de la simulation de différents scénarios correspondant aux conditions des diverses régions afin de prévoir les actions à déployer en cas de réintroduction. Etudes et efforts continus doivent être poursuivis sur ces aspects, ainsi qu'un niveau d'investissement soutenu.

Perspective

Limitation et futures orientations

Des lacunes dans les connaissances sur les vecteurs du paludisme en Chine ont été identifiées au cours de ces travaux. Cependant, en raison du temps et de ressources limitées et de la complexité des facteurs influant sur le paludisme en Chine, cette étude n'a porté que sur quelques-unes d'entre elles. Bien que la bionomique des vecteurs prédominants du paludisme comme *An. harrisoni* et *An. sinensis* ait fait l'objet d'une enquête préliminaire (129, 148, 155, 186), certains domaines doivent encore être investigués.

1. La bionomique spécifique des espèces d'*Anopheles* au sein d'un complexe et d'un groupe, comme respectivement par exemple le complexe *An. minimus* ou le groupe *An. maculatus*, ainsi que leur rôle dans la transmission du paludisme, en particulier dans les zones frontalières Sino-Birmanes.

2. La distribution géographique actualisée des vecteurs majeurs du paludisme après l'identification de chaque espèce à l'aide de tests moléculaires spécifiques (par exemple : *An. sinensis*, *An. minimus*, *An. harrisoni*, *An. dirus*, *An. baimaii*, *etc*.) en Chine, en lien avec l'analyse de l'utilisation et de la couverture des sols (LULC), ainsi que le changement climatique.

3. L'amélioration des outils de surveillance entomologique par la télédétection (RS), le système d'information géographique (SIG) et la modélisation mathématique pour obtenir des informations spatio-temporelles plus précises (par exemple, le modèle Maxent).

4. Le développement de la modélisation et de la simulation à différentes échelles (locale, nationale et régionale) afin de préparer des scénarios d'intervention.

Appel à des efforts communs

Le paludisme importé causé par des citoyens chinois revenant de pays d'endémie palustre et des travailleurs étrangers migrants en Chine a été identifié comme l'un des principaux défis de l'élimination du paludisme en Chine (17, 175, 187). En outre, les difficultés à conduire la lutte antipaludique le long des frontières internationales et à atteindre certaines populations comme celles à mobilité internationale et migrantes

(PMM) sont considérées comme le principal obstacle à l'élimination du paludisme aux niveaux régional et mondial (182, 188-191). Relever ces défis et résoudre ces problèmes nécessitera des efforts partagés avec les autres pays. Il est maintenant urgent de combiner les efforts avec ceux des pays voisins pour mettre en place un groupe de travail international et un réseau de coordination régionale. Certaines régions situées le long des frontières Sino-Birmane et Sino-Laotienne pourraient être considérées comme des zones pilotes (192). Depuis 2005, un mécanisme commun de surveillance des maladies et de mise en œuvre des méthodes de contrôle aux frontières, a déjà été mis en place avec l'accord des ministères de la Santé de trois pays : la Chine, le Myanmar et la République Populaire du Laos (193). Au sein de ce mécanisme commun, il existe à la fois des plateformes pour le partage régulier d'informations sur les épidémies de maladies infectieuses (ciblé sur le paludisme, la dengue, la peste, le VIH / SIDA, etc.), mais aussi une équipe de sensibilisation commune pour la détection et le traitement des cas et des ateliers collaboratifs pour le renforcement des capacités. Après plusieurs années d'efforts pour mettre en œuvre ce processus, la morbidité due au paludisme et sa transmission locale, ainsi que les épidémies de dengue, ont été réduites (194-198). La lutte antipaludique dans les zones transfrontalières et chez les populations difficiles à atteindre (PMM) constituent des défis majeurs dans la sous-région du Grand Mékong (GMS) (109, 110). La collaboration bilatérale, voire mieux, une collaboration multilatérale entre les pays voisins pour la mise en œuvre de programmes de contrôle du paludisme communs et coordonnés le long des frontières internationales et pour un contrôle plus efficace des PMM, est fortement recommandée pour renforcer les efforts

de contrôle et envisager l'élimination de ce fléau sanitaire dans la région.

Opportunité

Après une croissance économique rapide, la Chine est désormais la deuxième économie du monde et passe de bénéficiaire de l'aide à fournisseur de l'aide mondiale et tente de d'assister d'autres pays, en particulier dans les domaines de la santé mondiale et de la gouvernance mondiale de la santé (199). En tant qu'exemple d'une réponse à l'émergence de l'épidémie d'Ebola en Afrique de l'Ouest en 2014, la Chine a joué un rôle actif en première ligne avec un soutien financier, technique et en personnel (199-201). Un protocole d'accord a été signé entre le gouvernement Chinois et l'OMS pour une collaboration accrue sur la gouvernance mondiale en matière de santé. Cet accord donnera à la Chine davantage de responsabilités pour sa participation à la campagne mondiale de contrôle et d'élimination des maladies infectieuses, c'est-à-dire la campagne mondiale pour l'élimination du paludisme. De plus, en Octobre 2013, le président Chinois Xi Jinping a évoqué l'initiative de construction conjointe de la ceinture économique des nouvelles routes de la soie au XXIe siècle (dénommée One Belt & One Road) (Figure 9). La coopération sanitaire dans le cadre de ces nouvelles routes de la soie a été identifiée comme un élément majeur de collaboration internationale (22, 199, 202). Par conséquent, pour répondre à l'appel en faveur de la création d'actions d'intérêt global, en particulier une route de la soie pour la santé, une série de projets d'aide et de subventions de recherche ont été ou seront débloqués pour soutenir la coopération en matière de santé entre la Chine et les pays situés sur cette

nouvelle route de la soie (22, 199).

En 2018, un projet de recherche clé sur le contrôle et la prévention des maladies infectieuses a été lancé avec le soutien du ministère Chinois de la Science et de la Technologie (MOST). Ce projet, conçu pour une durée de trois ans (2018-2020), s'intitule « Étude sur les caractéristiques épidémiologiques des principales maladies infectieuses et techniques d'alerte rapide le long du tracé de la nouvelle route de la soie ». Le paludisme est l'une des principales maladies infectieuses dans les pays situés le long de cette route de la soie, en particulier en Asie du Sud-Est. L'un des sous-projets a été désigné pour se focaliser sur le paludisme avec des thèmes spécifiques abordés dans cette thèse (Figure 10). Ce projet est réalisé en collaboration avec l'Institut national des maladies parasitaires - CDC de Chine (NIPD), l'IRD et le CIRAD à Montpellier, en France, et des institutions de l'Asie du Sud-Est, telles que le Centre National de Parasitologie, d'entomologie et de lutte antipaludique (CNM) du Cambodge ou l'Université Kasetsart (KU) en Thaïlande.

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Summary

After several decades of effort, malaria prevalence decreased and China is currently approaching elimination throughout the country. The distribution of malaria vectors was reduced but former secondary species such as *Anopheles sinensis* are becoming predominant. Although China achieved zero indigenous case report in 2017, imported malaria cases are occurring throughout the country. Imported malaria cases and the presence of efficient malaria vectors in the country increase the risk of re-introduction of the disease. Former non-endemic provinces in China are now impacted by imported malaria but remain less efficient in detecting and managing cases. Profiles of imported malaria patients were analyzed and correspond mostly to overseas expatriate workers. International collaboration focusing on cross-border areas and mobile population should be intensified as well as surveillance. The lessons learned from malaria elimination in China could serve as a reference to other countries engaged in this process.

Keywords: Malaria elimination, China, Cross-border areas, Malaria transmission

Résumé

Après plusieurs décennies d'effort, la prévalence du paludisme a diminué et la Chine est sur le point d'éliminer cette maladie du pays. La distribution géographique des vecteurs du paludisme a été réduite, mais des vecteurs secondaires, tel que *Anopheles sinensis* deviennent prédominants. Bien que la Chine n'ait enregistré aucun cas de paludisme autochtone en 2017, des cas de paludisme importé sont répertoriés dans tout le pays. Ces cas importés et la présence de vecteurs efficaces dans le pays augmentent le risque de réintroduction de cette maladie. Les provinces chinoises où le paludisme était non-endémique sont maintenant impactées par des cas importés et sont moins efficaces dans la détection et la gestion des cas que les provinces où le paludisme était endémique. Les profils des patients à paludisme d'importation ont été analysés et correspondent principalement à des travailleurs chinois expatriés. La collaboration internationale, axée sur les zones transfrontalières et sur les populations mobiles entre pays, devrait être intensifiée, de même que la surveillance. Les enseignements tirés de l'élimination du paludisme en Chine devraient être utilisés au profit d'autres pays engagés dans ce processus.

Mots clés : Élimination du paludisme, Chine, Régions transfrontalières, Transmission du paludisme

摘要

经过几十年的努力，中国的疟疾发病率急剧下降，在全国范围内走向消除。主要的传疟媒介在全国范围内的分布减少，但中华按蚊成为全国分布广泛的主要媒介。尽管中国在 2017 年实现本地感染病例零报告，但输入性疟疾病例在全国均有报告。大量输入性疟疾病例和仍然存在的高效传疟媒介造成较高的输入继发传播风险。当前疟疾非流行省份也有输入性疟疾病例报告，但医疗机构在病例发现和病例管理方面仍存在不足。输入性疟疾病例的数据分析显示，海外务工人员为主要感染群体。因此，应加强针对边境地区和跨境流动人口为重点的国际合作及疟疾监测。中国消除疟疾的经验教训可为全球其他国家消除疟疾提供借鉴。

关键词：消除疟疾，中国，边境地区，疟疾传播