Risk of vector-borne diseases in relation to rubber plantations in Lao PDR

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Background:

Currently more than 80 % of the global population lives in regions with risk of at least one vector-borne disease, with more than 50 % of the global population at risk of at least

two vector-borne diseases [1]. An increasing number of studies are showing changes in the environment as a major driver of vector-borne disease incidence [2-7]. However environmental changes do not necessarily cause increase in vector-borne disease incidence. Changes in mosquito ecology and disease risk after ecological disruption is difficult to predict and even more difficult to relate to vector-borne disease incidence.

There is a complex balance dependent on the ecosystems affected, type of land-use change, disease-specific transmission dynamics, sociocultural changes and the susceptibility of human populations [8, 9].

Since the economic reforms in the late 1980s Lao PDR has become one of the fastest growing economies in Asia with Gross Domestic Product (GDP) in 2014 growing 7.5 %. As a member of the Association of South-East Asian Nations (ASEAN) Lao PDR is slowly becoming a land-linked country more than a land-locked country, with increased integration in the regional and global economy. The economic reforms in Lao PDR are changing the dynamics of the country in an unprecedented speed, presenting new challenges such as changes in disease dynamics. An example is the establishment of rubber plantations. Rubber cultivation is seen as a sustainable alternative for poor farmers in South-east Asia (SEA) as they live on land suitable for rubber plantations and these plantations commonly provide a relatively high income [10, 11]. Lao PDR has therefore seen a rapid increase in rubber plantation area, with 900 ha of mature plantations in 2010 increasing 163 fold to 147,000 ha in 2015 [12]. In the future this is likely to further expand to 342,400 ha [12]. Rubber tree cultivation is a new kind of mass farming not seen in Lao PDR before and the impact of these changes on the local ecosystems and disease dynamics remain poorly understood.

Currently there is a paucity of information on vector-borne disease risk in rubber plantations despite the fact malaria

epidemics have been linked to rubber plantations for over a century, as have outbreaks of dengue and the presence of lymphatic filariasis [13-18]. The rubber plantations are unnatural forests with generally higher humidity and lower temperatures than other non-tree crops. This is a habitat that some important malaria vector species like Anopheles dirus and dengue vector species like *Ae. albopictus* thrive in [16, 19-22]. The mosquitoes favour these unnatural forested areas as there are sufficient larval breeding sites, high humidity, stable temperatures and contact with people. In the next decade an estimated 4.5 to 6 million people will be working as seasonal tappers on rubber plantations in SEA. Seasonal workers hired to work on the plantations could create a different dynamic for vector-borne diseases. These temporary workers may not have immunity against the local diseases and are more likely to develop serious adverse effects. Additionally, these workers can spread diseases when they travel to new areas. Most worrying is the threat that they might spread a drug resistant strain, like ACT resistance, in an area by either returning home with a resistant strain or transporting the resistant strain to the place of work.

It is suggested that the presence of high numbers of different vector mosquitoes combined with the increase in seasonal rubber workers and their high exposure to the vector mosquitoes is creating a 'perfect storm' in and around rubber plantations for future disease outbreaks. With the ECOMORE study in Lao PDR we identified the risk of exposure to vector-borne diseases in the rubber plantations in the north of the country and how to mitigate these risks.

Rationale:

The area of land cultivated for rubber is expanding rapidly in Lao PDR. We anticipate that the changes in ecology from primary and secondary rainforest, to cleared land cultivated for rubber and the maturation of these rubber trees is likely to result in an altered risk from vector-borne diseases;

predominantly malaria and dengue. This study will examine the vector ecology in rubber plantations compared to village and forest habitats, identify risk factors for vector-borne diseases and make recommendations on how best to reduce the incidence of vector-borne disease for public health workers, governments, and those working in the rubber industries of Lao PDR and other countries in SEA. The goal of this study is to assess the potential risk of vector-borne diseases in rubber plantations in northern Lao PDR.

The study objectives are:

1. Determine the most efficient, reliable and ethically sound method for sampling human-biting mosquitoes in dengue-endemic areas.
2. Compare the adult mosquito diversity and abundance in the four major rural habitats: secondary forests, immature rubber plantations (<5 years), mature rubber plantation (>8 years) and rural villages situated close to rubber plantations. 3. Describe the behaviour of villagers and rubber workers in the study area that are likely to increase the risk of vector-borne diseases.
4. Investigate where mosquitoes are breeding in rural villages, immature and mature rubber plantations.

Key findings:

During 2016 the ECOMORE team in Lao PDR has been focused on analysing the final results, identifying the key findings and writing recommendations for the rubber industry and Lao government. Below we describe the key findings of our project for each objective.

1. Sampling outdoor-biting mosquitoes

Estimating the number of mosquito bites per person per day or night is a key metric used for quantifying the risk of infection with mosquito-borne pathogens. This human-biting rate is typically estimated using human landing catches (HLC)

with which mosquitoes are collected off exposed limbs. However this method potentially exposes individuals to infective mosquito bites. There has been no suitable alternative method identified for outdoor mosquito collections in SEA. Hence we tested a range of sampling techniques which do not expose participants to mosquito bites. In the first experiment we compared the human-baited double net trap (HDN), CDC light trap, BG Sentinel trap and Suna trap. The HDN trap method collected 11 to 44 times more mosquitoes than the other traps. In the succeeding experiment the trap collecting the highest number of mosquitoes, the HDN trap, was compared directly against HLC to determine whether it could be an alternative for outdoor mosquito sampling. The HDN collected similar numbers of *Anopheles* (Rate Ratio (RR) 1.2, 95 % confidence interval (CI) 0.6-2.2) and Culex (RR 1.3, 95 % CI 0.7-2.2) mosquitoes as HLC, but under-estimated the numbers of *Ae. albopictus* by half (RR 0.45, 95 % CI 0.27-0.77). It should be recognised that both HDNs and HLCs are only proxy estimates of exposure. It is likely that HDNs slightly under-estimates biting rates, whilst HLCs over-estimates biting rates. The HDN is a simple and cheap method to estimate the human-biting rate outdoors without exposing collectors to mosquito bites.

2. Adult mosquito diversity and abundance

In Lao PDR no study on the vector-borne dynamics in rubber plantations have been conducted, even though both malaria and dengue outbreaks occur regularly in South-east Asian rubber plantations. We therefore carried out a study using HDN traps to compare the abundance and diversity of adult mosquitoes in four rural habitats common in northern Lao PDR: the secondary forests, the immature rubber plantations, the mature rubber plantations and the local villages. A total of 24,927 female mosquitoes were collected during the nine months of adult mosquito surveys, including 61 species not documented in the country before. This study has shown that mosquito abundance was highest in the secondary forests, two to three times lower

in the immature rubber plantations, four times lower in the mature rubber plantations and five to seven times lower in the villages. High species diversity was found in all habitats (Simpson’s Indexes ranging from 0.82 to 0.86), including vectors of dengue, Japanese encephalitis, lymphatic filariasis and malaria. *Aedes albopictus* is the dominant mosquito species in the secondary forests and rubber plantations, indicating that these natural and man-made forests could play an important role in the establishment and spread of dengue and chikungunya in the region.

Furthermore, as this mosquito is an opportunistic feeder that can be a bridge vector between the sylvatic and human population [23, 24], there could be a substantial risk of emerging infectious diseases in rubber plantations. Overall there is a low risk of exposure to vectors of Japanese encephalitis, lymphatic filariasis and malaria in all habitats investigated. However risk of exposure to dengue vectors was considerable in both the natural and man-made forests.

3. Risky behaviours

In the next decade an estimated 4.5 to 6 million seasonal workers will be working in rubber plantations of South-east Asia. The rubber tappers work in the plantations during both the day and night, exposing them to different vectors compared to the local population. In this study we identified the presence of flavivirus and alphaviruses in the *Ae. albopictus* mosquitoes collected using the HDN trap. Furthermore we explored differences in human behaviour between rural villagers and rubber workers using rapid rural appraisals and surveys to identify risky behaviour. The human presence in the different habitats was related to the mosquito dynamics using four scenarios. In all natural and man-made forest habitats flavivirus present *Ae. albopictus* were identified. Furthermore the dengue basic reproductive number (R0) was between 2.8 and 42.0, which indicates that the disease can spread and established itself in these habitats. Risky behaviour for

dengue vector exposure compared to staying in the village was visiting the secondary forests during the day (36 times higher), working and living in the rubber plantations (16 times higher) and rubber worker activity (> 3 times higher). Furthermore when visiting the secondary forests during the day risk of exposure to Japanese encephalitis vectors was 1.38 times higher and exposure to malaria vectors 1.3 times higher compared to staying in the village. Although other studies in SEA have shown the increased risk of malaria vector exposure for rubber workers [25-29], we did not recognise rubber tapping as a risky behaviour for malaria vector exposure in our study sites. On the contrary working and living in the plantations decreased risk of malaria exposure by 1.6 times compared to staying in the village. The malaria R0 did indicate that once the malaria parasites are introduced to rubber plantations, malaria could establish itself and result in outbreaks with *Anopheles maculatus* s.l. the main vector in the rainy season (R0 16.6-64.0) and *Anopheles minimus* s.l. the main vector in the dry season (R0 31.3-44.3). This study highlights the importance of implementing mosquito control in the secondary forests and rubber plantations for the control of dengue disease and emphasizes the importance of including local human behaviour in the risk analysis.

4. Mosquito breeding sites

According to the adult mosquito survey, rubber plantations are important habitats for adult vectors of dengue, Japanese encephalitis, lymphatic filariasis and malaria. Vector control in Lao PDR has been dependent largely on the distribution of long lasting insecticide-treated nets, the application of temephos in village water containers and, to a lesser extent, the use of indoor residual spraying. However these methods are not sufficient to control outdoor vectors nor mosquitoes breeding in other waterbodies. Here we identified the major breeding sites of vector mosquitoes and their waterbody characteristics in rubber plantations and in nearby villages

for vector control purposes. During five months of survey 1,379 waterbodies were identified, of which 53 % contained immature mosquito stages. *Aedes* immature stages, primarily *Ae. albopictus*, were associated with small temporary waterbodies (< 5 L). Compared to puddles *Aedes* larvae were 29 times more likely to be found in cut bamboo, 17 times more likely to be found in tyres, 16 times more likely to be found in tree trunks, 9 times more likely to be found in leaf axils and 8 times more likely to be found in latex collection cups and water containers. *Culex* larvae, mainly *Cx. brevipalpis*, were 2.5 times more likely to be collected in tyres and 2.7 times more likely to be collected in other artificial waterbodies (ponds, ditches and rice fields) than in puddles. *Culex* larvae present waterbodies were 5 times more associated with the presence of other vertebrates than *Culex* larvae absent waterbodies. Anopheles larvae were equally associated with puddles, tree trunks, other natural waterbodies (pools and leaf puddles) and other artificial waterbodies. A high proportion of the emerged *Anopheles* larvae and pupae were *An. dirus* s.l. (27/38), a major malaria vector in SEA. Rubber plantations have more suitable breeding habitats for *Aedes, Culex* and *Anopheles* species than rural villages. Current focus on village larval control should therefore broaden to include rubber plantation areas. The findings suggest that in the rubber plantations latex collection cups should be turned upside down after emptying latex, rubbish such as unused tyres should be cleared, puddles should be filled up and tree trunks removed. In the villages, additional to the control methods suggested for rubber plantations, larval control should focus on filling the openings in cut bamboo, covering water containers [30] and the use of *Mesocyclops* in the large permanent water containers and ponds [31, 32]. Vector control intervention strategies should target these water bodies in a community-based manner every three to six months, focussing on education and participation of the population.

Recommendations for the Lao health officials and rubber

industry:

This study was conducted to share information and recommendations with the local Lao health officials and the rubber industry stakeholders. The most important recommendation from this study is the necessity to include rubber plantation habitats in vector control programmes, with vector control programmes currently focussed on the villages. In the rubber plantations the following larval control can be implemented. If latex tapping is not conducted for more than one week, all latex collection cups should be turned upside down to avoid *Aedes* and to a lesser degree *Culex* breeding. It is important to note that mosquitoes can also breed well in water that collects on top of the latex layer. After latex tapping is completed for the season, all latex collection cups should be collected in roofed sheds to diminish breeding sites. To further decrease *Aedes* and *Culex* mosquitoes the water containers and other waterbodies surrounding the rubber and village houses should be covered with a lid or netting, or treated with an insecticide such as temephos and the microbial larvicide *Bacillus thuringiensis israelensis* (*Bti*). Moreover all rubbish in plantations and villages should be properly disposed of in closed containers, including unused tyres. When using bamboo for construction, the open end of bamboo poles should be filled with gravel, cut at the joint or in the length to further decrease *Aedes* mosquitoes breeding. Tree trunks in the villages and rubber plantations should be removed as they are good breeding sites for *Aedes* and *Anopheles* mosquitoes. Mud roads in the plantation and villages contain road puddles where Anopheles mosquitoes breed. These puddles should therefore be levelled by filling the cavities with gravel. This needs to be done regularly during the rainy season when road use is intensive. Tarmac roads would reduce pooling more permanently, providing there was good drainage on either side of the road. To achieve sustainable larval control, the recommendations should be communicated and implemented during regular community-based mosquito source

reduction activities.

Rubber workers should be encouraged to live in villages instead of inside the plantations, as this decreases exposure to dengue (which is a bigger risk in the study area than Japanese encephalitis or malaria is). Additionally this study has shown the necessity of outdoor vector protection methods to complement the larval control in rubber plantations and for protection in secondary forests. There is a need to compare personal protection methods such as insecticide-treated clothing and insecticide emitters for their efficacy outdoors in field settings to identify the methods which are most protective against mosquito bites. More importantly, it is essential to understand the protection of these methods at the community level. When this information is available, the mandatory use of personal protection methods by plantation workers can be discussed. Until such information is available the local population should be encouraged to use methods that are known to decrease mosquito bites, including wearing thick long-sleeved clothing and applying topical repellents when visiting the natural and man-made forests, as exposure to vector mosquitoes is highest in these habitats. Moreover it should be safeguarded that all rubber worker families and villagers have access to long lasting insecticide-treated nets, as Japanese encephalitis and malaria vectors are seeking blood meals in the evening and at night when people (including the families of the rubber worker) are asleep.

Contributions to the field of medical entomology in Lao PDR:

To our knowledge no entomological studies have been published on the mosquito population in the north of the country with relatively few entomological studies from central and southern Lao PDR [30, 32-46]. The entomological studies that have been conducted in Lao PDR focus mostly on malaria vectors [34, 36, 38, 40-43, 45] with few studies on dengue and Japanese encephalitis vectors [30, 32, 33, 35, 39]. Luckily this emphasis is slowly shifting. A recent publication in Lao PDR

underlined the importance of non-malarial vector-borne diseases present in Lao PDR including dengue, scrub typhus and Japanese encephaltis virus infections [47]. Data on disease vectors in Lao PDR are still limited for many vector species with few descriptions of diseases transmitted by other vectors like fleas, mites, ticks and sand-flies [48-52]. The high diversity of mosquito species identified in this study, including a variety of vector species, have contributed to knowledge of the general mosquito fauna in Lao PDR and to knowledge of vector species dynamics and behaviour.

This study has been a first attempt at using both rapid rural appraisals and surveys in the identification risky behaviour for exposure to dengue, Japanese encephaltis and malaria vectors. These social scientific methods not only provided information on the human behaviour of the local population, it also gave the villagers a sense of ownership by involving them in the study from the start. The results emphasize the importance of including local human behaviour into the risk analysis, with risk to vector-borne diseases changing depending on the population behaviour.

In Lao PDR behavioural analysis have rarely been included in vector-borne disease studies, even though this is especially important in a country with 57 recognized ethnic groups.

From the beginning of this project both the Ministry of Health and Ministry of Forestry and Agriculture of Lao PDR have been actively involved. This has been formally achieved by the signing of a Memorandum of Understanding (MOU) at the beginning of the project between the National Agriculture and Forestry Research Institute (NAFRI) and Institut Pasteur du Laos. Practically this cooperation entailed the organization of regular stakeholder meetings at country, provincial, district and village level which were attended by experts from neighbouring countries, representatives of both ministries and rubber stakeholders. During the different stakeholders meetings NAFRI regularly complemented our presentations with

presentations on the rubber plantation dynamics from an agricultural perspective. Furthermore members of the NAFRI staff supported us during the collection of environmental data and visited us in the field to see the fieldwork in action. This study is the first study in Lao PDR to have integrated the Ministry of Health and Ministry of Forestry and Agriculture in one project to mitigate vector-borne disease risk. This approach has been novel for all people involved and has created awareness of the overlap between the goals of different ministries. The cooperation between the Ministry of Health and the Ministry of Agriculture from the beginning of this project has facilitated this study in achieving its study objectives and has improved the communication of our recommendations to the important stakeholders in Lao PDR and the region. The study has resulted in the contribution of one chapter on 'health risks for rubber workers' in an information book on rubber plantations published by the Ministry of Forestry and Agriculture. This book will be distributed to the rubber industry, provincial offices and district offices all over Lao PDR.

Publications:

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In review for publication 2016

Tangena J-AA, Thammavong P, Malaithong N, Inthavong T, Ouanesamon P, Brey PT, Lindsay SW: Density and diversity of mosquitoes (Diptera: Culicidae) in natural and man-made forest

habitats in rural Lao PDR

Tangena J-AA, Thammavong P, Chonephetsarath S, Brey PT, Lindsay SW: Surveillance of larval habitats in rubber plantations and villages: A baseline study in northern Lao PDR

Tangena J-AA, Thammavong P, Lindsay SW, Brey PT: Risk of vector mosquito exposure for rubber workers compared to villagers in northern Lao PDR