Infection, Genetics and Evolution 33 (2015) 189–196

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Molecular inference of sources and spreading patterns of *Plasmodium falciparum* malaria parasites in internally displaced persons settlements in Myanmar–China border area

Eugenia Lo [a], Guofa Zhou [a], Winny Oo [a], Ming-Chieh Lee [a], Elisabeth Baum [b], Philip L. Felgner [b], Zhaoqing Yang [c], Liwang Cui [d], Guiyun Yan [a,*]

[a] *Program in Public Health, University of California, Irvine, CA, USA*
[b] *Department of Medicine, Division of Infectious Diseases, University of California, Irvine, CA, USA*
[c] *Department of Pathogen Biology and Immunology, Kunming Medical University, Kunming, China*
[d] *Department of Entomology, Pennsylvania State University, University Park, PA, USA*

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form 29 April 2015
Accepted 2 May 2015
Available online 4 May 2015

Keywords:
Plasmodium falciparum
Malaria transmission
Border migration
Genetic bottleneck
Microsatellites
Genetic diversity

ABSTRACT

In Myanmar, civil unrest and establishment of internally displaced persons (IDP) settlement along the Myanmar–China border have impacted malaria transmission. The growing IDP populations raise deep concerns about health impact on local communities. Microsatellite markers were used to examine the source and spreading patterns of *Plasmodium falciparum* between IDP settlement and surrounding villages in Myanmar along the China border. Genotypic structure of *P. falciparum* was compared over the past three years from the same area and the demographic history was inferred to determine the source of recent infections. In addition, we examined if border migration is a factor of *P. falciparum* infections in China by determining gene flow patterns across borders. Compared to local community, the IDP samples showed a reduced and consistently lower genetic diversity over the past three years. A strong signature of genetic bottleneck was detected in the IDP samples. *P. falciparum* infections from the border regions in China were genetically similar to Myanmar and parasite gene flow was not constrained by geographical distance. Reduced genetic diversity of *P. falciparum* suggested intense malaria control within the IDP settlement. Human movement was a key factor to the spread of malaria both locally in Myanmar and across the international border.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Since the inception of the Roll Back Malaria Initiative by World Health Organization (WHO) in 1999, malaria control has been greatly intensified in endemic countries. In conjunction with better financial support and improved technology, malaria morbidity and mortality have been reduced tremendously worldwide (WHO, 2008, 2013). However, the reductions in malaria are not universal. While some countries such as Morocco and Syria did eliminate malaria in the past decade, many others such as Thailand and China are still experiencing a sustained low-level transmission (Coker et al., 2011). In Myanmar, the situation is particularly concerning because of the resurgence of the disease and lack of sufficient, consistent information on malaria transmission (Cui et al., 2012a; WHO, 2013). Myanmar has the highest malaria burden among other Southeast Asian countries with approximately 200,000 cases per year (Coker et al., 2011; WHO, 2013). The number of malaria cases and malaria-induced mortality in Myanmar has been consistently high for the past three decades (Cui et al., 2012b). Factors such as the emergence/spread of *Plasmodium falciparum* resistance to artemisinins and *P. vivax* resistance to chloroquine, inadequate epidemiological data to assess malaria situations, complex vectorial systems, and above all civil unrest make malaria control very difficult in Myanmar (Coker et al., 2011; Cui et al., 2012a,b; Delacollette et al., 2009; Cheeseman et al., 2012; Phyo et al., 2012; Li et al., 2013).

Myanmar has been engaged in the world's longest-running civil unrest. Commonly known as Burma, Myanmar succumbed to ethnic civil unrest and turmoil since its independence in 1948, and this conflict remains unresolved today (Socheat et al., 2003). Political instability and military conflicts have driven hundreds of thousands of citizens into relocation camps known as Internally

* Corresponding author at: Program in Public Health, University of California at Irvine, Irvine, CA 92697-4050, USA.
E-mail address: guiyuny@uci.edu (G. Yan).

http://dx.doi.org/10.1016/j.meegid.2015.05.002
1567-1348/© 2015 Elsevier B.V. All rights reserved.

Displacement Persons (IDP) settlement scattered throughout the country's borders, specifically the Myanmar–China and Myanmar–Thailand borders. Large-scale human movement has led to intensive transmission of malaria in the IDP settlement (Delacollette et al., 2009; Archavanitkul and Vajanasara, 2010; Kumar et al., 2012). In addition, the high proportion of ethnic minorities who live in remote countryside receives very little attention and healthcare resources from the central government (WHO, 2008). For example, in Kachin State, the remote border region of northeast Myanmar, the estimated malaria incidence and morbidity and mortality rates have been shown higher than other parts of Myanmar (WHO, 2008; Lee et al., 2006; Li et al., 2013). Regional seasonality coupled with population movement along country borders and countryside heavily impact malaria transmission in both Myanmar and China.

This study aimed to examine the sources and spreading patterns of *P. falciparum* between IDP settlement and surrounding villages in eastern Myanmar and western China along the international border. Moreover, we compared the parasite samples collected in the past three years to determine if there were changes in genotype structure over time within the IDP settlement and local community. In-depth knowledge and information on the extent of malaria spread are keys to target disease control efforts in high-risk areas. This is of particular relevance when most other parts of Southeast Asia are entering the malaria elimination phase.

2. Materials and methods

2.1. Blood sample collection

Nearly 300 samples that were diagnosed with *P. falciparum* infections were collected from clinics or hospitals located in two IDP settlements, Je Yang Hka (JYH) and Hpum Lum Yang (HLY), and four surrounding villages/towns including military base (CMH), Ja Htu Kawng (JHK), Laiza (LZCH), and Mai Sak Pa (MSP) in Myanmar along the international border of China, in addition to two town hospitals Tengchong (TC) and Yingjiang (YJ) in Yunnan, China (Fig. 1; Supplementary Table S1). Among the village localities, LZCH is a major regional hospital that represents a larger catchment area of nearly 100,000 people from surrounding smaller villages. By contrast, CMH, JHK, and MSP are local clinics that represent a smaller catchment area of approximately 3000 people. For the IDP settlement population size is variable; in 2012 the population size of HLY and JYH were about 1600 and 8600 respectively. Because of uneven sample size among localities, genetic variation was compared among three locality settings: regional hospital (LZCH), villages (CMH, JHK, and MSP), and IDP settlement (JYH and HLY). Samples from JYH and LZCH were collected in three consecutive years 2011, 2012, and 2013 for genetic composition comparison. All studied individuals showed fever or malaria-related symptoms at the time of sampling, and were detected with falciparum-infection by microscopic examination and PCR assays. For each individual, 30–50 μl of blood was blotted on Whatman 3MM filter papers. Filter papers were air-dried and stored in zip-sealed plastic bags with silica gel absorbent at room temperature until DNA extraction. Parasite DNA was extracted from dried blood spots by the Saponin/Chelex method (Bereczky et al., 2005).

2.2. Microsatellite genotyping

Thirteen single-copy microsatellites (SSRs) with tri- or tetranucleotide repeats, which map to 14 chromosomes are typed for *P. falciparum*. Alleles were PCR-amplified with the published oligonucleotide primers following the published protocol (Su et al., 1999; Anderson et al., 1999). After PCR amplification, products were pooled as follows: POLY2 + TAA124 + POLYα + TAA81, TAA42 + TAA87 + TAA109, PE87a + 9735 + PFPK2, TAA80 + PfG377 + TAA116 according to their sizes and fluorescent labels (Supplementary Table S2). The pooled products were separated on an ABI 3730 capillary sequencer and all allele sizes were determined and visualized in Peak Scanner.

2.3. Data analyses

2.3.1. Linkage disequilibrium genetic diversity, and clonal assessment

To examine whether the SSR loci represent an independent set of markers in the *P. falciparum* genome, genotyping linkage disequilibrium (LD) was tested by Fisher's exact test for each pair of loci with GenePop version 4.2, using the Markov chain method with 100 batches and 10,000 iterations per batch (Raymond and Rousset, 1995). Significance values were adjusted by sequential Bonferroni correction for multiple comparisons.

Genotypic diversity and clonal variation were calculated in GenoDive version 2.0b4 (Meirmans and Van Tienderen, 2004) and GenClone version 2.0 (Arnaud-Haond and Belkhir, 2007). We first calculated genetic distances using the method of Smouse and Peakall – a squared Euclidean distance based on the number of times a certain allele is found in the two individuals (Smouse and Peakall,1999). The minimal distance class was set as threshold to identify the follow: (i) the number of multilocus genotypes (G) where parasite samples with identical genotypes at all the examined microsatellite loci were identified as a single clone and thus the number of unique multilocus genotypes was referred to the number of unique clones; (ii) Simpson's diversity index (D), also referred as clonal diversity corrected for sample size (a measure of the proportion of unique genotypes/clones in a population) that ranges from zero (where two randomly chosen individuals in a population represent a single clone) to one (where individuals all represent different clones); and (iii) genotype evenness (E) that ranges from zero (where one or a few clones dominate in a population) to one (where all clones are of equal frequency in a population). In addition, the number of effective alleles and expected heterozygosity were estimated with GENALEX for each of the localities (Peakall and Smouse, 2006).

2.3.2. Population structure and isolation-by-distance analyses

A model-based Bayesian method implemented in STRUCTURE (version 2.3.4) was performed to examine partitioning of individuals to genetic clusters (Pritchard et al., 2000). All samples from the different years were included in a single analysis to allow comparison of clustering patterns among sites as well as among years. The number of clusters (K) was determined by simulating a range of K values from 1 (no genetic differentiation among all localities) to 8 (all localities were genetically differentiated from one another). The posterior probability of each value was then used to detect the modal value of ΔK, a quantity related to the second order rate of change with respect to K of the likelihood function. Posterior probability values were estimated using a Markov Chain Monte Carlo (MCMC) method, and a burn-in period of 500,000 iterations followed by 10^6 iterations of each chain were performed to ensure convergence of the MCMC. Each MCMC chain for each value of K was run eight times with the 'independent allele frequency' option that allows individuals with ancestries in more than one group to be assigned into one cluster. Individuals were assigned into K clusters according to membership coefficient values (Q) ranged from 0 (lowest affinity to a cluster) to 1 (highest affinity to a cluster). The partitioning of clusters was visualized with the program DISTRUCT (Rosenberg, 2004).

An F_{ST} analysis was conducted using θ, an F_{ST}-estimator in SPAGeDi version 1.2e (Hardy and Vekemans, 2002). F_{ST} values were tested for significance using 10,000 permutations. Genetic

Download English Version:

https://daneshyari.com/en/article/5908862

Download Persian Version:

https://daneshyari.com/article/5908862

Daneshyari.com