Acta Tropica 139 (2014) 39–43

Contents lists available at ScienceDirect

Acta Tropica

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

First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa

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ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 20 June 2014
Accepted 29 June 2014
Available online 5 July 2014

Keywords:
Anopheles stephensi
Invasive species
Plasmodium falciparum malaria
Outbreak
Djibouti

ABSTRACT

Anopheles stephensi is an important vector of urban malaria in India and the Persian Gulf area. Its previously known geographical range includes southern Asia and the Arab Peninsula. For the first time, we report *A. stephensi* from the African continent, based on collections made in Djibouti, on the Horn of Africa, where this species' occurrence was linked to an unusual urban outbreak of *Plasmodium falciparum* malaria, with 1228 cases reported from February to May 2013, and a second, more severe epidemic that emerged in November 2013 and resulted in 2017 reported malaria cases between January and February 2014. *Anopheles stephensi* was initially identified using morphological identification keys, followed by sequencing of the Barcode cytochrome c-oxidase I (COI) gene and the rDNA second internal transcribed spacer (ITS2). Positive tests for *P. falciparum* circumsporozoite antigen in two of six female *A. stephensi* trapped in homes of malaria patients in March 2013 are evidence that autochthonous urban malaria transmission by *A. stephensi* has occurred. Concurrent with the second malaria outbreak, *P. falciparum*-positive *A. stephensi* females were detected in Djibouti City starting in November 2013. In sub-Saharan Africa, newly present *A. stephensi* may pose a significant future health threat because of this species' high susceptibility to *P. falciparum* infection and its tolerance of urban habitats. This may lead to increased malaria outbreaks in African cities. Rapid interruption of the urban malaria transmission cycle, based on integrated vector surveillance and control programs aimed at the complete eradication of *A. stephensi* from the African continent, is strongly recommended.

1. Introduction

The Republic of Djibouti is a small country (43°00′E, 11°30′N; 23,200 km^2 total area; 860,000 estimated population) located on the Horn of Africa (World Factbook, 2013; WHO, 2014). According to WHO data, *Plasmodium falciparum* malaria is exclusively transmitted by *Anopheles arabiensis* Patton and *Anopheles gambiae* Giles in Djibouti (WHO, 2014). Although the Horn of Africa is known to be highly susceptible to mosquito-borne infectious diseases, Djibouti was formerly thought to be a meso- to hypo-endemic country with unstable malaria transmission (Carteron et al., 1978; Fox et al., 1989, 1991). Low levels of *P. falciparum* malaria endemicity in 2007 were also predicted for this area after employing a global mapping approach based on 7953 *P. falciparum* parasite rate surveys (Hay et al., 2009). The last malaria epidemic occurred in Djibouti City from March to June 1999 (Rogier et al., 2005). Since then, malaria cases had dropped to an incidence rate of <1/1000 persons/year (Ollivier et al., 2011). Studies further revealed that Djibouti was entering a malaria pre-elimination phase because conditions for its eradication had been established (Noor et al., 2011; Khaireh et al., 2013).

From January to May 2013, Djibouti experienced an unusual urban outbreak of *P. falciparum* malaria, with 1228 reported cases, of which 1016 (82.7%) were from Djibouti City alone (United Nations, 2013). Beginning in late January 2013, approximately 100 imported cases occurred in Dikhil State among a nomad

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http://dx.doi.org/10.1016/j.actatropica.2014.06.016
0001-706X/

Fig. 1. Overview of vector monitoring locations established in Djibouti since 2008: ML 1, Ile du Héron; ML 2, Sheraton Hotel; ML 3, airport area 1; ML 4, airport area 2; ML 5, central market place; ML 6, animal quarantine station. Maps were accessed at www.google.de/maps.

population returning from Ethiopia. From late February 2013, malaria was increasingly detected in urban parts of the capital, Djibouti City. Cases there peaked in April, and the outbreak ended in late May 2013. Until late February 2013, it had been suggested that only imported cases were occurring. Within Djibouti City, Arhiba Quarter was identified as a previously unknown malaria hot spot, accounting for 62% of all urban cases reported (United Nations, 2013). No malaria cases were reported between July and August, and only a few cases (28–51 per month) were reported from September to November 2013 (Dr. Houssein Darar, Director, National Institute of Public Health, Djibouti; personal communication). Recent on-site investigations have shown that a second, more severe epidemic began in December 2013, with 2017 malaria cases reported for January and February 2014 alone (Dr. Houssein Darar, Director, National Institute of Public Health, Djibouti; personal communication).

In order to monitor malaria and other mosquito-borne diseases affecting military personnel stationed in Djibouti City as part of the international European Union Naval Force Somalia mission "Atalanta", continuous vector surveillance had been established there in 2008 (Faulde and Ahmed, 2010; Faulde et al., 2012). Between January 2010 and June 2012, surveillance sites yielded 20,431 mosquitoes, of which 99.9% were *Culex* sp., 0.09% were *Aedes aegypti* (Linnaeus), and only 0.01% were *Anopheles sergentii* (Theobald), indicating that urban malaria transmission risk was extremely low during this period (Faulde et al., 2012). The first evidence of non-native *Anopheles stephensi* Liston was reported in September 2012 at a surveillance site near the animal export and quarantine station, located approximately 14 km from Djibouti City and four km away from the Somalian border. Since early March 2013, *A. stephensi* had been detected in urban parts of Djibouti City. The aims of the present study were to: (1) confirm the preliminary identification of *A. stephensi*, (2) monitor any geographical extension of its range, and (3) gather further evidence as to whether the occurrence of this species is linked to the unusual ongoing urban malaria outbreak in Djibouti City.

2. Materials and methods

2.1. Vector surveillance and sampling locations

Since 2008, continuous vector surveillance has been conducted in the vicinity of Djibouti City using standard miniature CDC light traps (No. 1012, John W. Hook Co., Gainesville, FL, USA) and BG lure traps (BioGents AG, Regensburg, Germany). In Djibouti City itself, five monitoring locations (ML) were established: ML 1, Plateau du Héron (43°09′01″E; 11°37′17″N); ML 2, Sheraton Hotel (43°09′29″E; 11°36′09″N); ML 3, civilian airport area (43°08′54″E; 11°33′07″N); ML 4, military airport area (43°09′13″E; 11°33′11″N). ML 5 was situated in the much-frequented, populous central marketplace (43°08′96″E; 11°35′55″N) in order to detect anthroponotic transmission cycles of mosquito-borne diseases. A sixth monitoring location (ML 6) was installed at the Djibouti animal export and quarantine station (43°13′52″E; 11°28′38″N), located approximately 14 km from Djibouti City and 4 km from the Somalian border, close to national route N2. This last surveillance site is considered an excellent sentinel site for enhanced detection of zoonotic mosquito-borne disease agents. Fig. 1 shows the locations of ML 1-6.

During mid-March 2013, in an effort to detect autochthonous transmission, additional CDC light and BG lure traps were run for two days in two houses of malaria patients that did not have a history of travel in Arhiba Quarter (house 1, 43°08′15″E; 11°34′50″N; house 2, 43°04′51″E; 11°20′43″N; Fig. 1).

Using the WHO-recommended Frisbee disk method (Service, 1993), anopheline larval monitoring was conducted to investigate