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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

Five years following first detection of *Anopheles stephensi* (Diptera: Culicidae) in Djibouti, Horn of Africa: populations established—malaria emerging

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Abstract

The Asian malaria mosquito, *Anopheles stephensi*, is a well-known and important vector of *Plasmodium falciparum* and *P. vivax*. Until 2013, its geographical distribution was confined to central and southern Asia including the Arabian Peninsula. In the Horn of Africa (HoA) Region, *An. stephensi* was first recorded from Djibouti in 2012, when it was linked geographically and temporally with an unusual outbreak of urban *P. falciparum* malaria. In 2016, *An. stephensi* was detected in the neighbouring Somali Region of Ethiopia. In order to determine whether *An. stephensi* populations have become established in Djibouti and contributed to the unusual rise in local malaria cases there, we carried out continuous vector surveillance from January 2013 to December 2017, investigated seasonal changes in *An. stephensi* population densities and bionomics, analysed available literature describing malaria in Djibouti since 2013, and investigated whether *An. stephensi* may have contributed to local malaria transmission by detecting circumsporozoite antigen of *P. falciparum* and *P. vivax* in female anophelines. From 2013 to 2016, seasonal activity of *An. stephensi* in urban Djibouti City primarily occurred during the colder, wetter season between September and May, with either no or rare trap catches from June to August. Unlike past years, this species was detected year-round, including the extremely hot summer months of June to August 2017. This change in seasonal occurrence may indicate that *An. stephensi* populations are adapting to their new environment in sub-Saharan Africa, facilitating their spread within Djibouti City. Among the 96 female *An. stephensi* investigated for malaria infectivity, three (3.1%) were positive for *P. falciparum* circumsporozoite antigen, including one *P. falciparum*/*P. vivax* VK 210 double infection. Subsequent to the unusual resurgence of local malaria in 2013, with 1684 confirmed cased reported for that year, malaria case numbers increased continuously, peaking at 14,810 in 2017. Prior to 2016, only *P. falciparum* malaria cases had been reported, but in 2016, autochthonously acquired *P. vivax* malaria cases occurred for the first time at a rate of 16.7% among all malaria cases recorded that year. This number increased to 36.7% in 2017. Our data indicate that the dynamics of malaria species in Djibouti is currently changing rapidly, and that *An. stephensi* can be involved in the transmission of both *P. falciparum* and *P. vivax*, simultaneously. Considering the extremely high potential impact of urban malaria on public health, the timely deployment of optimal multinational vector surveillance and control programs against *An. stephensi* is strongly recommended, not only for the HoA Region, but for the entire African continent.

Keywords *Anopheles stephensi* · Invasive species · Malaria · Outbreak · *Plasmodium falciparum* · *Plasmodium vivax*

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Introduction

To date, the World Health Organization (WHO) has identified 11 major global vector-borne human diseases causing 700,000 deaths annually, or approximately 17% of the estimated total burden of infectious diseases worldwide (WHO 2017a). Among mosquito-borne diseases, 60% are attributable to malaria, which together account for almost 96% of deaths (WHO 2017a). With an estimated 447,860 fatal cases annually, malaria is still the deadliest mosquito-borne disease on earth (WHO 2017a). Especially in sub-Saharan Africa, endemic anthropophilic and endophagic anopheline mosquitoes are currently the greatest vector-borne disease threat (WHO 2014a).

Despite the promising results of the WHO Roll Back Malaria Programme (WHO 2017a), multiple factors, including climatic and environmental changes, migrations of human malaria reservoirs, and/or the global spread of invasive anopheline vector species, are likely to negatively influence future malaria elimination efforts. One recent worrying event was the introduction of the primary Asian malaria vector *Anopheles stephensi* (Liston) into Africa, where this species was reported in Djibouti, near the Somalian border, for the first time in 2012, followed by its appearance in Djibouti City in early 2013 (Faulde et al. 2014) and in the nearby Somali Region of Ethiopia in 2016 (Carter et al. 2018).

Located at a latitude of 43° 00′ E and a longitude of 11° 30′ N in the Gulf of Aden Region on the Horn of Africa (HoA), Djibouti is a small, arid country with a rugged, volcanic topography, hypersaline soils, and an estimated population of 865,000 (World Factbook 2018). More than 70% of the country's people live in Djibouti City, which is densely urbanised. Historically, increases in the number of malaria cases in Djibouti City were observed during the 1970s and 1980s due to increased human settlement and expanded irrigation (Rodier et al. 1995). *Anopheles arabiensis* (Patton) had previously been identified as the primary malaria vector, and almost all cases were due to *Plasmodium falciparum*, with only sporadic cases of *Plasmodium vivax* (de Pécoulas et al. 1998), although a 2002 study revealed considerable human reservoir activity of *P. vivax*, with a mean seroprevalence rate of 17.5% (Khaireh et al. 2012). Since most African populations do not express the Duffy blood group antigen, as yet the only known *P. vivax* parasite receptor, the low clinical incidence rate in sub-Saharan Africa resulted in the perception that this type of malaria occurs there only sporadically or is completely absent (Howes et al. 2015). However, a recent study provided conclusive evidence of *P. vivax* transmission in this region, with a focus in Ethiopia (Howes et al. 2015).

Malaria in Djibouti has been categorised as meso- to hypoendemic, with unstable transmission. The country's last malaria epidemic occurred between March and June 1999, following an intense rainy season that caused severe floods (Rogier et al. 2005). Thereafter, the malaria incidence rate dropped to < 1/1000 persons/year (Ollivier et al. 2011). At that time, 100% of all registered malaria cases were caused by *P. falciparum* and vectored by *An. arabiensis* (WHO 2012), and favourable conditions for malaria eradication had been established for Djibouti, with the goal of entering the malaria pre-elimination phase (Khaireh et al. 2013). Continuous vector surveillance, carried out since 2008 primarily at military sites and on embassy-owned properties in Djibouti City, revealed almost no anopheline vector activity year-round (Faulde and Ahmed 2010).

In September 2012, *An. stephensi*, an important and effective vector of urban malaria in India and the Persian Gulf area, was recorded for the first time on the African continent (Faulde et al. 2014). Following its initial detection at the Djibouti animal export and quarantine station, located approximately 14 km from Djibouti City and 4 km from the Somalian border, *An. stephensi* specimens were trapped in February 2013 in areas of Djibouti City (Faulde et al. 2014). Beginning in late February 2013, autochthonous *P. falciparum* malaria cases were reported in increasing frequency from urban quarters of Djibouti City. Cases there peaked in April, and the outbreak ended in late May 2013 (United Nations 2013). Mosquito surveillance carried out at that time revealed that (a) *An. stephensi* had expanded its range within the urban areas of Djibouti City between 2013 and early 2014, and (b) *P. falciparum* circumsporozoite antigen was present in five of 36 *An. stephensi* females which were trapped near the living places of malaria patients (Faulde et al. 2014). These data strongly suggested that *An. stephensi* had become involved in malaria transmission in Djibouti.

The current malaria situation in Djibouti remains unclear, and published data describing the evolution and characteristics of malaria there are very sparse for the period after 2013. The purposes of this study are to (a) determine whether *An. stephensi* is still present and has further spread in Djibouti; (b) investigate seasonal changes in *An. stephensi* population densities and bionomics; (c) identify which indigenous anopheline species other than *An. stephensi* are abundant at surveillance locations and possibly contribute to malaria transmission; (d) analyse available literature describing malaria in Djibouti since 2013; and (e) determine whether *An. stephensi* may have contributed to local malaria transmission by detecting circumsporozoite antigen of *P. falciparum* and *P. vivax* in female anophelines.

Materials and methods

Mosquito surveillance and sampling locations

Mosquito vector surveillance was carried out continuously for 3 days each week from January 2012 to December 2017 at defined urban locations in Djibouti City employing standard miniature CDC light traps (model 1012; John W. Hook Co., Gainesville, FL, USA) and BG sentinel traps (BioGents AG, Regensburg, Germany) baited with BG-Lure® mosquito attractant. Trap catches were harvested following each 3-day trap run. In Djibouti City, six surveillance locations (SL) at military compounds and at the German Embassy were established as follows: SL 1, Plateau du Héron (43° 09′ 01″ E; 11° 37′ 17″ N); SL 2, vicinity of the Sheraton Hotel (43° 09′ 29″ E; 11° 36′ 09″ N); SL 3, military airport area I (43° 08′ 54″ E; 11° 33′ 07″ N); SL 4, military/civil airport area II (43° 09′ 13″ E; 11° 33′ 11″ N); SL 5, military/civil airport area III (43° 09′ 01″ E; 11° 33′ 23″ N); SL 6, German Embassy (43° 09′ 51″ E; 11° 33′ 53″ N) (Fig. 1).

Identification of anopheline mosquito species

Morphological identification of mosquitoes was carried out using dissecting microscopes at × 10–100 magnification as well as mosquito identification keys for the HoA Region (Gillies and Coetzee 1987; Das et al. 1990; Glick 1992), including the Walter Reed Biosystematics Unit's (WRBU 2012) online database. Final confirmation of *An. stephensi* was achieved by comparing new catches with voucher specimens trapped in 2013 (Faulde et al. 2014).

Sample preparation and detection of *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins (CSPs)

Freshly trapped female anopheline mosquitoes, labelled with trap date and location, were stored dry in a refrigerator at − 20 °C, subsequently transferred individually to 70% aqueous ethanol in Eppendorf screw vials until further processing and identified morphologically to species prior to malaria CSP testing. The VectorTest® Malaria Sporozoite Antigen Panel Assay, a dipstick ELISA (Vector Test Systems, Inc., Thousand Oaks, CA, USA), was used to detect the specific CSP of *P. falciparum* as well as the *P. vivax* polymorphs VK 210 and VK 247 (Bangs et al. 2002). All female anopheline mosquitoes trapped were investigated individually.

Literature search

Electronic databases, including PubMed, Web of Science, Scopus, the Literature Retrieval System of the US Armed Forces Pest Management Board, Google Scholar, and MEDLINE were searched from 2011 to 2018 using the terms "Djibouti & malaria", "Djibouti & paludisme", and "military & Djibouti & malaria".

Additionally, WHO World Malaria Reports 2011 to 2017 were analysed for Djibouti country malaria reports and profiles (WHO 2012, 2013, 2014b, 2015, 2016, 2017b). Annual malaria data listed in the "presumed and confirmed" columns were extracted. The Medical Surveillance Monthly Reports of the US Armed Forces Health Surveillance Center/Branch, published between 2011 and 2017, were analysed for reports on malaria in US Armed Forces autochthonously acquired in Djibouti (AFHSC 2012, 2013, 2014; AFHSB 2015, 2016, 2017, 2018). In the HoA Region, US Forces are primarily stationed at Camp Lemonnier, Djibouti City, close to Ambouli International Airport. Malaria data reported for 2017 are exclusively from the Department of Health Information, Ministry of Health, Republic of Djibouti.

Statistical analyses

Values were reported as mean ± standard deviation (SD). Differences in annual trap indices for *An. stephensi* were computed and compared by a two-sided *t* test at the fifth percentile of significance using the SPSS 8.0 program (SPSS Software GmbH, Munich, Germany).

Results

Between 2013 and 2017, *An. stephensi* was the only anopheline species detected at sentinel sites SL 1 to 6. *Anopheles stephensi* was detected regularly, but at very low densities, between the years 2013 and 2015, but its population density increased starting in 2016 (Fig. 2a). While 30, 11, and 8 specimens were trapped during 2013, 2014, and 2015, constituting mean species trap indices (male and female *An. stephensi* mosquitoes per trap night) of 0.14 ± 0.078, 0.13 ± 0.097, and 0.06 ± 0.027, respectively (Fig. 2b), 46 specimens (trap index 0.47 ± 0.22) were detected in 2016, and 185 (trap index 0.57 ± 0.41) in 2017. Mean trap indices were very low and not significantly different between the years 2013 and 2014 ($p = 0.841$), and 2014 and 2015 ($p = 0.145$), but were significantly different between 2013 and 2015 ($p = 0.0019$). However, a definite increase in *An. stephensi* population density was observed in 2016, and this increase was significant when compared with 2015 ($p < 0.0001$), as was the difference between 2015 and 2017 ($p < 0.0001$), but not between 2016 and 2017 ($p = 0.091$) (Fig. 2a and b). The mean annual female:male ratio of *An. stephensi* was 2.75 in 2013, 1.75 in 2014, 0.33 in 2015, 1.42 in 2016, and 0.52 in 2017. Our data show that *An. stephensi* population, after their establishment in urban areas of Djibouti City and first detection in 2013, has become endemic in the HoA Region at a continually rising population density since 2016.

The annual seasonal activity of *An. stephensi* in urban Djibouti City is depicted in Fig. 3a. While anopheline mosquitoes were detected between 2013 and 2016 primarily during the colder, wetter seasons between September and May, with no or rare trap catches in between, *An. stephensi* could be detected year-round by 2017 (Fig. 3b). Our results reveal a changing seasonal and annual occurrence of *An. stephensi* at

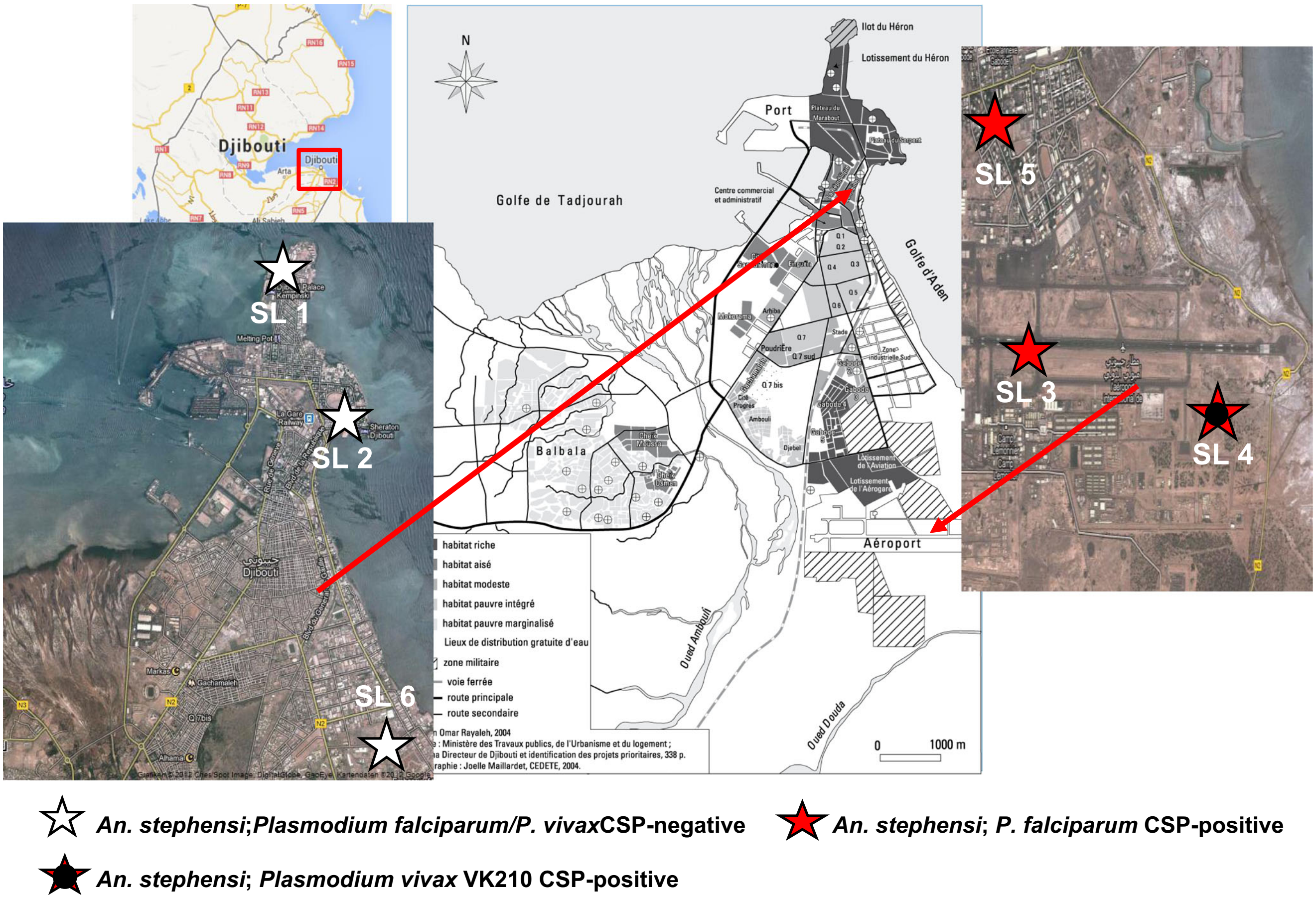

Fig. 1 Overview of vector surveillance locations established in Djibouti since 2012: SL 1, Plateau du Héron; SL 2, vicinity of Sheraton Hotel; SL 3, airport area I (military); SL 4, airport area II (military/civil); SL 5, airport area III (military/civil); SL 6, German Embassy. Maps were accessed at www.google.de/maps and https://goo.gl/images/NNFztM. (White star) *An. stephensi*; *Plasmodium falciparum*/*P. vivax* CSP-negative. (Red star) *An. stephensi*; *P. falciparum* CSP-positive. (Black star with red accent) *An. stephensi*; *Plasmodium vivax* VK210 CSP-positive

the different SLs, and an increasing population density at certain surveillance locations, especially in the vicinity of SL 2.

Malaria data from Djibouti, as gleaned from the limited literature, indicate meso- to hypo-endemic *P. falciparum* malaria associated with a continuous decrease of autochthonously acquired cases until 2012, when only 27 cases were reported and Djibouti was thought to have entered the malaria pre-elimination phase (Fig. 4). However, concurrent with the 2013 introduction and discovery of *An. stephensi* in Djibouti City, malaria resurged alongside a changing ecology of

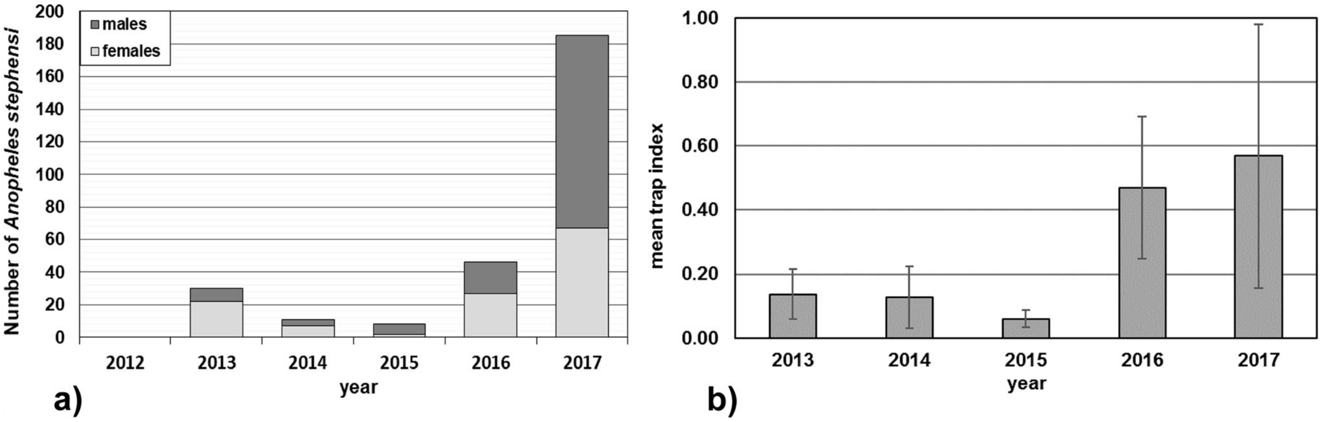

Fig. 2 **a** Occurrence of *Anopheles stephensi* in Djibouti City as documented by annual trap catch numbers, 2012–2017. **b** Mean annual trap indices of *Anopheles stephensi* (Σ♀ + ♂/trapnight) at the vector surveillance sites in Djibouti City, 2013–2017

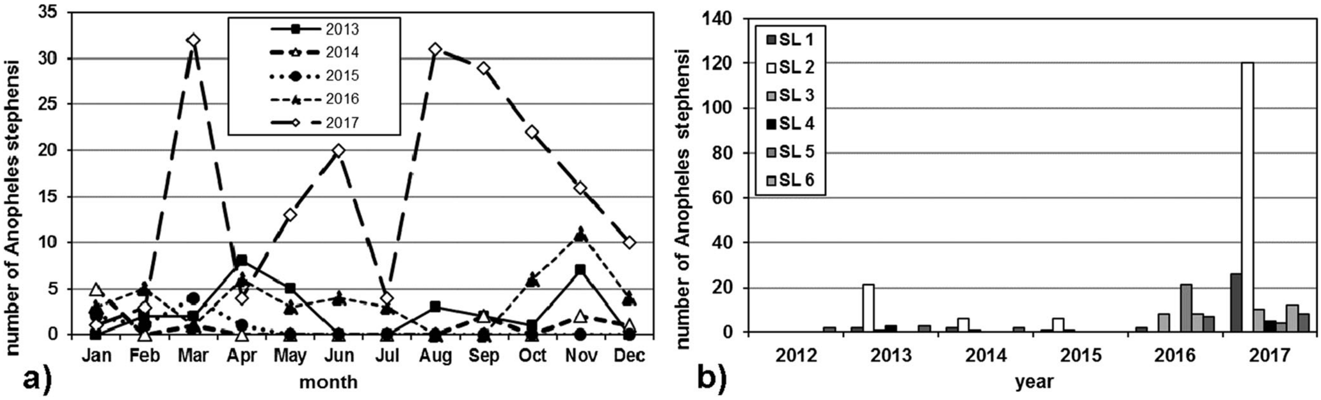

Fig. 3 **a** Seasonal activity of *Anopheles stephensi* (Σ♀ + ♂) measured by standardised vector surveillance in Djibouti City, 2013–2017. **b** Spatial distribution of *Anopheles stephensi* at vector surveillance sites SL 1–6, Djibouti City, 2012–2017

autochthonous transmission, and the disease is now prevalent in densely populated urban environments. Annual malaria cases reported by WHO and the Ministry of Health, Republic of Djibouti, rose continuously from 1684 in 2013 to 9439 in 2014, 9557 in 2015, 13,804 in 2016, and 14,810 in 2017. Together, with a change in autochthonously acquired malaria case numbers, a change in the distribution of malaria parasites has recently been observed. Until 2015, only *P. falciparum* malaria was reported to occur in Djibouti City. However, in 2016, *P. vivax* malaria transmission was reported for the first time, and at a remarkably high rate, constituting 16.7% (2041 cases) of all malaria cases, and increasing rapidly to 36.7% (5441 cases) in 2017. According to published reports, autochthonously acquired malaria cases in deployed US military personnel rose from zero during the years 2010 to 2014 to three each year between 2015 and 2017 (AFHSB 2016, 2017, 2018). *Plasmodium vivax* malaria cases have been diagnosed since 2016, with 2 cases that year, and two more cases in 2017.

Of the 96 female *An. stephensi* trapped at SL 1 to SL 6 between 2015 and 2017, three (3.1%) were positive for malaria CSP. Among the positives, one female trapped in February 2017 at SL 3 was *P. falciparum* CSP-positive, a second caught in March 2017 at SL 5 was also *P. falciparum* CSP-positive, and a third collected in December 2017 at SL 4 was doubly infected with *P. falciparum* and *P. vivax* VK 210 sporozoites. All three CSP-positive *An. stephensi* specimens were trapped in the vicinity of Ambouli Airport.

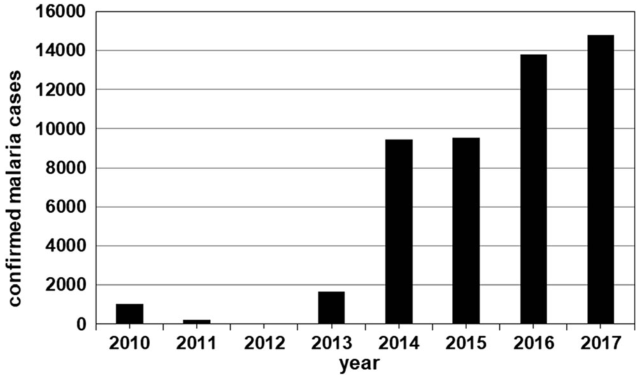

Fig. 4 Annual confirmed malaria cases in Djibouti, according to country reports provided by WHO (published data) and the Ministry of Health, Republic of Djibouti (unpublished data), 2010–2017

Discussion

Mosquito surveillance has been carried out in Djibouti City since August 2008, primarily at sites occupied by deployed German Armed Forces (Faulde and Ahmed 2010; Faulde et al. 2012). Before January 2013, no anopheline mosquitoes were detected, with the exception of two female specimens of *An. sergentii* found in 2009 at an airport location corresponding to SL 3 of our study. *Anopheles arabiensis*, the chief local malaria vector, which favours ecological niches such as wadi-like drying streams and rivers, open puddles, and artificial irrigation channels (Dida et al. 2018), was not detected at any of the surveillance sites in this study.

Within the greater Djibouti City area, suitable conditions for endemic anopheline species like *An. arabiensis* have been identified in the past only along the Ambouli River and its delta area in the City's periurban and rural outskirts, but not in the urban quarters where this study's SLs were situated (Ollivier et al. 2011). Ecological conditions in the vicinity of the Ambouli River include vegetation that may positively influence selection of mosquito oviposition sites and strengthen larval fitness during development (Asmare et al. 2017). Consequently, autochthonous malaria transmission and malaria foci were primarily confined to this area (Ollivier et al. 2011). Urban malaria transmission, especially in the privileged quarters chiefly addressed in this study, was not recorded until the appearance of *An. stephensi* in February 2013 (United Nations 2013; Faulde et al. 2014). Unlike *An. arabiensis*, *An. stephensi* typically breeds in a wide variety of artificial containers, such as domestic wells with cemented walls (Faulde et al. 2014; Carter et al. 2018).

Anopheles stephensi is known as an important synanthropic vector of urban malaria in India and the Persian Gulf area and transmits both *P. vivax* and urban *P. falciparum* in different parts of India (Wilson et al. 2015). When compared with *An. gambiae*, one of three principal African vectors of *P. falciparum*, *An. stephensi* shows similar high infection prevalence and susceptibility rates (Hume et al. 2007). Nevertheless, species-specific susceptibility has been shown to be variable among both species, depending on the geographical origin of both the parasite and the mosquito (Hume et al. 2007). The first infectious, *P. falciparum* CSP-positive *An. stephensi* were detected during a survey carried out in Djibouti between 2013 and 2014, demonstrating autochthonous transmission (Faulde et al. 2014). New data originating from our study confirm the involvement of *An. stephensi* in local urban *P. falciparum* transmission. Additionally, it should be borne in mind that infected *An. stephensi* introduced and released by non-disinsected aircraft may contribute to local malaria transmission.

In a malaria-endemic region of western India, high infection rates for *P. vivax* were detected in wild-caught *An. stephensi* (Nina et al. 2017). Djiboutian CSP-ELISA and epidemiological data from this study provide strong evidence that in Djibouti City, *An. stephensi* transmits both *P. falciparum* and urban *P. vivax* malaria simultaneously. The extent to which vector-parasite adaption processes and ecological and/or climatic changes enhance malaria transmission rates remains to be investigated. But the high vector potential of *An. stephensi* in urban malaria outbreaks, as experienced in India, led to increased prevention measures and eradication efforts following reintroduction of this species in Sri Lanka (Dharmasiri et al. 2017).

In 2016, *P. vivax* cases were reported for the first time from Djibouti, at a remarkably high infection rate of 16.7% of all malaria cases reported by WHO (2017b) and the Djiboutian Ministry of Health (unpublished data). Although a study revealed a *P. vivax* seroprevalence rate of 17.5% within the Djiboutian population as early as 2002 (Khaireh et al. 2012), thus indicating that human *P. vivax* malaria reservoirs had long been present, *P. vivax* transmission by the presumed chief malaria vector, *An. arabiensis*, obviously did not play a significant role. Nevertheless, *An. arabiensis*, the recently documented primary vector of *P. falciparum* in the lowlands of neighbouring Ethiopia (Daygena et al. 2017), has also been shown to be susceptible to *P. vivax*, especially in the highlands of central Ethiopia (Abduselam et al. 2016). In the past, malaria in sub-Saharan Africa has been attributed almost exclusively to *P. falciparum* (Howes et al. 2015). The argument for this view was that the Duffy blood group antigen, the only known receptor for *P. vivax* infection, is not present in most of the sub-Saharan human population. However, new data provide strong evidence that *P. vivax* transmission regularly occurs in Djibouti and neighbouring Ethiopia and Eritrea, all sub-Saharan states (Howes et al. 2015). There is increasing evidence that *An. stephensi*-associated *P. vivax* transmission has occurred in the Djiboutian lowlands since 2016, especially following the detection of a *P. falciparum*/*P. vivax* double-infected female mosquito trapped in Djibouti City in 2017. This abrupt rise in malaria cases and the sudden change in the composition of malaria species has been confirmed by data from US military personnel stationed at Camp Lemonnier, Djibouti City (AFHSB 2016, 2017, 2018).

Although grouped into two different, phylogenetically close clades according to their COI sequence bootstrap values, both the recently detected Ethiopian and the Djiboutian *An. stephensi* strains obviously originated from Pakistani strains (Carter et al. 2018). To date, it remains unclear whether these slightly different strains of *An. stephensi* had entered the HoA Region via a single route or whether they were introduced on different occasions. Because susceptibility to different *P. falciparum* and *P. vivax* strains can vary markedly among different *An. stephensi* strains (Hume et al. 2007), further research on transmission efficacy in the HoA Region is needed, with the goal of understanding this species' vector potential for pathogenic *Plasmodium* species and strains pathogenic to humans in Africa.

Following the December 2016 reappearance of *An. stephensi* in Sri Lanka, a country that had been certified as malaria-free, vigorous surveillance and eradication campaigns against this species, including measures designed to prevent malaria reintroduction, were implemented immediately in the affected areas (Dharmasiri et al. 2017). Considering the extremely high potential public health effects of endemized, rapidly spreading, urban *An. stephensi* populations capable of transmitting *P. falciparum* and *P. vivax*, the timely deployment of optimal multinational vector surveillance and control programmes is strongly recommended for both the HoA Region and the entire African continent.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Abduselam N, Zeynudin A, Berens-Riha N, Seyoum D, Pritsch M, Tibebu H, Eba K, Hoelscher M, Wieser A, Yewhalaw D (2016) Similar trends of susceptibility in *Anopheles arabiensis* and *Anopheles pharoensis* to *Plasmodium vivax* infection in Ethiopia. Parasit Vectors 9:552

Armed Forces Health Surveillance Branch (AFHSB) (2015) Update: malaria, U.S. Armed Forces, 2014. MSMR. 22(1):2–6

Armed Forces Health Surveillance Branch (AFHSB) (2016) Update: malaria, U.S. Armed Forces, 2015. MSMR. 23(1):2–6

Armed Forces Health Surveillance Branch (AFHSB) (2017) Update: malaria, U.S. Armed Forces, 2016. MSMR 24(1):2–7

Armed Forces Health Surveillance Branch (AFHSB) (2018) Update: malaria, U.S. Armed Forces, 2017. MSMR. 25(2):2–7

Armed Forces Health Surveillance Center (AFHSC) (2012) Update: malaria, U.S. Armed Forces, 2011. MSMR. 19(1):2–6

Armed Forces Health Surveillance Center (AFHSC) (2013) Update: malaria, U.S. Armed Forces, 2012. MSMR. 20(1):2–5

Armed Forces Health Surveillance Center (AFHSC) (2014) Update: malaria, U.S. Armed Forces, 2013. MSMR. 21(1):2–7

Asmare Y, Hill SR, Hopkins RJ, Tekie H, Ignell R (2017) The role of grass volatiles on oviposition site selection by *Anopheles arabiensis* and *Anopheles coluzzii*. Malar J 16:65

Bangs M, Rusmiarto S, Gionar YR, Chan AST, Dave K, Ryan JR (2002) Evaluation of a dipstick malaria sporozoite panel assay for detection of naturally infected mosquitoes. J Med Entomol 39:324–330

Carter TE, Yared S, Gebresilassie A, Bonnell V, Damodaran L, Lopez K, Ibrahim M, Mohammed S, Janies D (2018) First detection of *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) in Ethiopia using molecular and morphological approaches. Acta Trop 188:180–186. https://doi.org/10.1016/j.actatropica.2018.09.001. [Epub ahead of print]

Das BP, Rajagopal R, Akiyama J (1990) Pictorial key to the species of Indian anopheline mosquitoes. Zoology 2:131–162

Daygena TY, Massebo F, Lindtjørn B (2017) Variation in species composition and infection rates of *Anopheles* mosquitoes at different altitudinal transects, and the risk of malaria in the highland of Dirashe Woreda, South Ethiopia. Parasit Vectors 10:343

de Pécoulas PE, Tahar R, Quatas T, Mazabraud A, Basco LK (1998) Sequence variations in the *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase gene and their relationship with pyrimethamine resistance. Mol Biochem Parasitol 92:265–273

Dharmasiri AGG, Perera AY, Harishchandra J, Herath H, Aravindan K, Jayasooriya HTR, Ranawaka GR, Hewavitharane M (2017) First record of *Anopheles stephensi* in Sri Lanka: a potential challenge for prevention of malaria reintroduction. Malar J 16:326

Dida GO, Anyona DN, Abuom PO, Akoko D, Adoka AO, Matano A-S, Owuor PO, Ouma C (2018) Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. Infect Dis Poverty 7(1):2

Faulde MK, Ahmed AA (2010) Haematophageous vector monitoring in Djibouti City from 2008 to 2009: first records of *Culex pipiens* ssp. *torridus* (IGLISCH), and *Anopheles sergentii* (Theobald). J Egypt Soc Parasitol 40:281–294

Faulde MK, Spiesberger M, Abbas B (2012) Sentinel-site-enhanced near-real time surveillance documenting West Nile virus circulation in two *Culex* mosquito species indicating different transmission characteristics, Djibouti City, Djibouti. J Egypt Soc Parasitol 42:461–474

Faulde MK, Rueda LM, Khaireh BA (2014) First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. Acta Trop 139:39–43

Gillies MT, Coetzee M (1987) A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). South African Institute for Medical Research, Johannesburg, South Africa, pp 1–147

Glick JI (1992) Illustrated key to the female *Anopheles* of southwestern Asia and Egypt (Diptera: Culicidae). Mosq Syst 24:125–153

Howes RE, Reiner RC Jr, Battle KE, Longbottom J, Mappin B, Ordanovich D, Tatem AJ, Drakeley C, Gething PW, Zimmermann PA, Smith DL, Hay SI (2015) *Plasmodium vivax* transmission in Africa. PLoS Negl Trop Dis 9(11):e0004222

Hume JCC, Tunnicliff M, Ranford-Cartwright LC, Day KP (2007) Susceptibility of *Anopheles gambiae* and *Anopheles stephensi* to tropical isolates of *Plasmodium falciparum*. Malar J 6:139

Khaireh BA, Briolant S, Pascual A, Mokrane M, Machault V, Travaillé C, Khaireh MA, Farah IH, Ali HM, Abdi A-IA, Ayeh SN, Darar HY, Ollivier L, Waiss MK, Bogreau H, Rogier C, Pradines B (2012) *Plasmodium vivax* and *Plasmodium falciparum* infections in the Republic of Djibouti: evaluation of their prevalence and potential determinants. Malar J 11:e395

Khaireh BA, Assefa A, Guessod HH, Basco LK, Khaireh MA, Pascual A, Briolant S, Bouh SM, Farah IH, Ali HM, Abdi A-IA, Aden MO, Abdillahi Z, Ayeh SN, Darar HY, Koeck J-L, Rogier C, Pradines B, Bogreau H (2013) Population genetics analysis during elimination process of *Plasmodium falciparum* in Djibouti. Malar J 12:201

Nina PB, Mohanty AK, Ballav S, Vernekar S, Bhinge S, D'souza M, Walke J, Manoharan SK, Mascarenhas A, Gomes E, Chery L, Valecha N, Kumar A, Rathod PK (2017) Susceptibility of wild and colonized *Anopheles stephensi* to *Plasmodium vivax* infection. Malar J 16:284

Ollivier L, Nevin RL, Darar HY, Bougere J, Saleh M, Gidenne S, Maslin J, Anders D, Decam C, Todesco A, Khaireh BA, Ahmed AA (2011) Malaria in the Republic of Djibouti, 1998-2009. Am J Trop Med Hyg 85:554–559

Rodier GR, Parra JP, Kamil M, Chakib SO, Cope SE (1995) Recurrence and emergence of infectious diseases in Djibouti city. Bull World Health Organ 73:755–759

Rogier C, Pradines B, Bogreau H, Koeck J-L, Kamil M-A, Mercereau-Puijalon O (2005) Malaria epidemic and drug resistance, Djibouti. Emerg Infect Dis 11:317–321

United Nations (2013) Djibouti: Appel global – Revue à mi-parcours 2013. [accessed 2018 July 02]. Available from https://www.humanitarianresponse.info/fr/programme-cycle/space/document/revue-%C3%A0-mi-parcours-de-lappel-global-pour-djibouti-2013

Walter Reed Biosystematics Units (WRBU) (2012) Vector identification resources. [accessed 2012 Oct 25]. Available from http://www.wrbu.org/keys/CP_AN_A/Anopheles_W_Palearc_CENTCOM_A.html

WHO (2012) World malaria report 2011. Available from http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_full_report.pdf. Accessed 21 Aug 2018

WHO (2013) World malaria report 2012. Available from http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/. Accessed 21 Aug 2018

WHO (2014a) A global brief on vector-borne diseases Available from http://apps.who.int/iris/bitstream/10665/111008/1/WHO_DCO_WHD_2014.1_eng.pdf. Accessed 07 Aug 2018

WHO (2014b) World malaria report 2013. Available from http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/. Accessed 21 Aug 2018

WHO (2015) World malaria report 2014. Available from http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/. Accessed 21 Aug 2018

WHO (2016) World malaria report 2015. Available from http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. Accessed 21 Aug 2018

WHO (2017a) Global vector control response 2017–2030. Available from http://www.who.int/malaria/areas/vector_control/Draft-WHO-GVCR-2017-2030.pdf. Accessed 07 Aug 2018

WHO (2017b) World malaria report 2016. Available from http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/. Accessed 21 Aug 2018

Wilson ML, Krogstad DJ, Arinaitwe E, Arevalo-Herrera M, Chery L, Ferreira MU, Ndiaye D, Mathanga DP, Eapen A (2015) Urban malaria: understanding its epidemiology, ecology, and transmission across seven diverse ICEMR network sites. Am. J. Trop. Med. Hyg. 93(Suppl 3):110–123

World Factbook (2018) Djibouti. World Factbook, CIA, USA, June 07, 2018. [accessed 2018 June 22]. Available from https://www.cia.gov/library/publications/the-world-factbook/geos/dj.html