Published in final edited form as:
Lancet Planet Health. 2023 December ; 7(12): e999–e1005. doi:10.1016/S2542-5196(23)00250-4.

Building the vector in: construction practices and the invasion and persistence of *Anopheles stephensi* in Jigjiga, Ethiopia

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Abstract

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Contributors

Data Collection: SY, AG, EAk, ODK, GG-O, AC-M, WB-M, PM-S, GMV-P, TC, and EW collected data for the study. GMV-P, TC, and EW did the data analysis. SY, AG, EAk, ODK, GG-O, AC-M, WB-M, PM-S, and GMV-P designed the study. SY, AG, EAk, EAb, ODK, GG-O, TC, JL, AL, UK, PM-S, GMV-P, and EW wrote the manuscript. GMV-P secured funding.

Declaration of interests

We declare no competing interests.

Anopheles stephensi is a major vector of malaria in Asia and the Arabian Peninsula, and its recent invasion into Africa poses a major threat to malaria control and elimination efforts on the continent. The mosquito is well adapted to urban environments, and its presence in Africa could potentially lead to an increase in malaria transmission in cities. Most of the knowledge about *An stephensi* ecology in Africa has been generated from studies conducted during the rainy season, when vectors are most abundant. Here, we provide evidence from the peak of the dry season in the city of Jigjiga in Ethiopia, and report *An stephensi* immature stages infesting predominantly in water reservoirs made to support construction operations (ie, in construction sites or associated with brick-manufacturing businesses). Political and economic changes in Ethiopia (particularly the Somali Region) have fuelled an unprecedented construction boom since 2018 that, in our opinion, has been instrumental in the establishment, persistence, and propagation of *An stephensi* via the year-round availability of perennial larval habitats associated with construction. We argue that larval source management during the dry season might provide a unique opportunity for focused control of *An stephensi* in Jigjiga and similar areas.

Introduction

Most African countries have been remarkably successful in reducing malaria burden since the year 2000, thanks to the scaling up of vector control tools (insecticide-treated nets and indoor residual spraying) and effective preventive and treatment drugs.[1] Increasing evidence suggests that rapid urbanisation of Africa's human population (driven primarily by rural to urban migration) is also contributing to a reduction in malaria burden.[2–5] Lower habitat suitability for *Anopheles* spp breeding and improvements in housing within African cities reduce human–mosquito contact and can lead to lower *Plasmodium* spp inoculation rates compared with rural settings.[2–5] Environmental management in the form of housing improvement has gained research interest due to its sustained effect on *Anopheles* spp mosquitoes and its positive effect on livelihoods.[6,7] WHO calls this approach "building the vector out", and it involves the adoption of practices that range from improved housing structures to retrofitting eave tubes and other approaches to limit mosquito entry indoors.[8] This approach is also seen as a novel aspect of malaria control in urban settings, given that most human population growth over the next century will be accounted for by the growing number of city dwellers.[9]

As most sub-Saharan countries continue their push towards malaria elimination with large-scale delivery of long-lasting insecticide nets and indoor residual spraying, increased insecticide resistance in *Anopheles* spp is becoming a serious challenge.[10] Currently, resistance to insecticides is reinforcing the need to consider novel approaches, such as attractive targeted sugar baits, spatial repellents, and housing improvement in the form of eave tubes and screening.[11,12] A new threat with the potential to derail public health gains in the reduction of malaria burden in Africa is the invasion and establishment of *Anopheles stephensi*, a malaria vector native to Asia and commonly found in cities throughout India, Iran, Pakistan, and the Arabian Peninsula.[13,14]

Since it was first detected in Africa in Djibouti in 2012,[15] *An stephensi* has spread to Ethiopia,[16] Somalia,[17] Sudan,[18] Kenya, Nigeria, and Ghana.[19] Niche modelling predicts suitable environmental conditions for *An stephensi* establishment throughout tropical African cities, putting an additional 126 million people potentially at risk of malaria.[20] While in its native range *An stephensi* has different forms, with the type form mainly found in urban environments and the mysorensis form being mostly rural,[21] reports from Africa have predominantly described the type form in urban areas.[22,23] Given *An stephensi*'s dependency on artificial containers as primary larval breeding habitats, rainfall alone was a poor predictor of *An stephensi*-driven malaria transmission.[24] In Djibouti, a 2000-times exponential increase in the number of malaria cases has been observed since the detection of *An stephensi*.[14,19] The contribution of *An stephensi* to increases in malaria transmission outside of Djibouti has started to be investigated, especially in light of the malaria outbreak (2018–2022). From 2018 to 2020 in Ethiopia, *Plasmodium vivax* was detected in wild-caught *An stephensi* from the cities of Dire Dawa (infection rate 0·5%) and Kebridehar (0·3%),[23] and *P vivax* (2·8%) and *P falciparum* (1·4%) infection was reported in *An stephensi* from Awash, in 2021.[25] Furthermore, experimental membrane feeding experiments showed that field-caught *An stephensi* from Ethiopia became considerably more infectious with local *P vivax* and *P falciparum* than *Anopheles arabiensis* (the primary malaria vector in Ethiopia), indicating that *An stephensi* is a highly competent vector for African *Plasmodium*.[25]

Given the entomological and epidemiological evidence gathered so far, WHO launched a new initiative to stop the further spread of *An stephensi* in Ethiopia that is based on a 5-pronged approach: (1) increasing collaboration, (2) strengthening surveillance, (3) improving information exchange, (4) developing guidance, and (5) prioritising research.[19] To execute an effective plan for *An stephensi* elimination, key sources of information about its biology and bionomics in its new habitats are needed, and vector control tools that are better suited for urban settings will need to be investigated.

Several studies (most from Ethiopia, cross-sectional, and conducted during the rainy season) have characterised *An stephensi* habitats and bionomics, with many knowledge gaps remaining.[22,23,25,26] The evidence gathered so far shows that in the rainy season, *An stephensi* larvae are found in a wide array of small and large artificial containers, ranging from large water cisterns to car tyres and buckets.[22,23,25] In addition to *Plasmodium* infection, such studies have characterised up to 48% human biting (14 of 29 mosquitoes) in Awash,[25] but low human biting (<1% human biting) in Dire Dawa and Kebridehar (where a high frequency of domestic animal feeding was also observed).[23] Such discrepancies might have originated, in part, due to the opportunistic collection of adult mosquitoes in or near animal shelters. Indeed, the finding of *P vivax* and *P falciparum* infected mosquitoes can only be explained by human biting. Furthermore, given its egg-laying behaviour (eggs that resist desiccation[27] and are laid in small containers), the fact that it bites humans not only at night when they are sleeping and that it is found in urban and peri-urban areas, *An stephensi* has more similarities with *Aedes aegypti* mosquitoes (which vector viruses such as dengue, chikungunya, and Zika) than with other *Anophelines*.[28] One of the many factors that remains to be studied is how *An stephensi* persists in Ethiopia and other African countries that have a prolonged dry season, as this period might offer unique opportunities for surveillance and control.

Here, we report the habitat use of *An stephensi* during the dry season in eastern Ethiopia. Although increased focus on characterising larval habitats in rainy periods can provide information of niche breadth for the species, our goal of focusing on the dry season was to explore possible windows for control in periods where the population size might be smallest.

Dry season *An stephensi* collections in Jigjiga, Ethiopia

During March 6 to March 14, 2023, mosquito surveys were conducted in Jigjiga city (capital of Somali Region with a population of approximately 800 000) during the dry season. *An stephensi* was first detected in Jigjiga in 2018[22] and has persisted in the city since then despite a harsh dry season (the rainless period of the year lasts for around 3 months). Molecular analysis of cytochrome oxidase subunit I (*COI*) and cytochrome B genes shows Jigjiga as one of the locations with highest diversity, suggesting it was likely an early introduction point of *An stephensi* into Ethiopia.[29] Jigjiga is of relevance because of its large population size, rapid urbanisation, and connection to other malaria-endemic regions and the port of Berbera in Somaliland.

We used methods developed for standard larval and pupal sampling of container-breeding mosquitoes[8] that included collecting all the larvae and pupae in small water-holding containers and using dippers and large fish nets to sample large water-holding habitats. Dipping and nets were used to exhaustively collect immature mosquitoes from the sides and centre of each habitat. All the larvae and pupae were reared to adulthood at Jigjiga University Entomology Laboratory. The emerged *Anopheles* spp adults were identified to species level with standard keys[30] and molecular means.[16] From a total of 60 potential larval sites with water that were sampled across the city, we identified a major habitat consistently positive for *An stephensi* larvae and pupae during the dry season: man-made pits related with construction operations (figure 1A). We term such habitats construction pits, as they were primarily built for the storage of water in construction sites or in small-scale brick-manufacturing businesses (figure 1B). *An stephensi* positivity in construction pits was 62·5% (presence-absence), which was significantly higher (by an order of magnitude) than the 5·9% positivity in water cisterns made of cement (Fisher's exact test; χ^2=0·0008; p<0·01) and the 0% positivity in 200 L plastic drums (χ^2=0·0001; p<0·01; figure 1B). All abandoned tyres sampled did not contain any water. Of note, from all the sites that we found positive for *An stephensi* larvae and pupae, 63·6% of them also had *Culiseta longiareolata* and *Aedes hirsutus* larvae in them, whereas only 18·2% and 9·1% sites positive for *An stephensi* were cohabited by *C longiareolata* or *Ae hirsutus* only, respectively (figure 1C). In construction pits, the frequency of positivity of the three species did not differ significantly (χ^2=0·740, p>0·05 for *An stephensi vs C longiareolata*; χ^2=1, p>0·05 for *An stephensi vs Ae hirsutus*; and χ^2=0·740, p>0·05 for *C longiareolata vs Ae hirsutus*). Although the drivers that enabled cohabitation of multiple species remain to be studied, we found that all positive containers for all species were open and exposed to direct sunlight, and also had a presence of algae, but not turbid water.

A subset of 20 adults emerging from the pupae collected in construction pits and visually identified with standard keys was molecularly confirmed to be *An stephensi* using an allele specific PCR and the sequencing of *ITS2* and *COI* loci.[16] While *ITS2* haplotypes were all identical for the *An stephensi* samples, three *COI* haplotypes were detected: Hap 1 (7 of 14 adult *An stephensi*), Hap 2 (6 of 14), and Hap 3 (1 of 14; using published haplotypes designations),[29] mostly consistent with previous studies. Notably, the presence of the *COI* Hap 1 (common to south Asia and detected in northern Ethiopia and Djibouti) supports the notion of Jigjiga's connectivity with regions outside of the continent with long-established *An stephensi* populations and as a likely entry point for *An stephensi* into the southern part of the country.

We incorporated the GPS coordinates from the construction pits sampled in March 2023 into Google Earth to visualise their location within Jigjiga (figure 1A). After mapping the construction pits to Google Earth, it was evident that their unique spectral signature (size, colour contrast, and presence of water) allowed the visual identification of other pits not sampled by our team (figure 2A, B). In Google Earth, we then used a satellite image taken in November, 2022 (4 months previous to sampling and the closest point in time to our collections) to digitise all construction pits visible in a swath of Jigjiga centred on the road connecting the city with Somaliland and ranging from a dense urban area (the city's downtown) at the city's edge (figure 2C). Within this urban to rural area of transition, we identified a total of 101 construction pits. In ArcMap 10.8 (ESRI, Redlands, CA), we implemented a kernel density estimation of pits per hectare within the swath and found that pits were concentrated to the centre of the swath (figure 2D). This area of Jigjiga was experiencing rapid construction and development at the time.

We acknowledge several limitations to our findings. We might have missed habitat types that, while common and infested by *An stephensi* in the rainy season,[22,23] were not found holding water in the dry season. Increasing the number of sampled locations might have provided an opportunity to detect such infested habitats. Although we provide information on the infestation of construction pits, we were unable to estimate the productivity of such habitats. For container-breeding mosquitoes (ie, *Ae aegypti*) pupal surveys are used to quantify productivity.[32] Unfortunately, *An stephensi* sampling has not relied on such measures and is restricted to the counting of larvae collected from dipping, which has challenges when comparing habitats of different sizes. As more evidence from studies similar to this emerge, formal guidelines for immature and adult *An stephensi* sampling (as the ones developed for *Ae aegypti*)[33] will provide an opportunity to better quantify productivity and the effect of vector control interventions.

Political and economic development and *An stephensi* invasion in Jigjiga

We suggest that an unprecedented urban development boom in Jigjiga has been crucial in favouring *An stephensi* establishment and rapid spread. Since its first rare detection in Jigjiga from 2018, *An stephensi* has been consistently and more commonly found in 2020 and now in 2023. Jigjiga increased its built-up area from 4·2% in 1985 to 5·2% in 2005 and then to 24·0% in 2015, primarily driven by a change in status from zonal capital to regional capital, which opened political and economic opportunities, leading to high rural to urban migration.[34] Since 2018, when transformative political reform of Ethiopia was enacted by the Ethiopian Government, Jigjiga has seen an even larger population and urban footprint increase. The recent declaration by the national Government of Ethiopia that 19·0%

of commodity imports for the country should enter via Berbera Port in Somaliland and be transported through Jigjiga to the rest of the country,[35] led to an increased interest in investment and even higher migration into the city.[35] Jigjiga's population grew from 125 876 inhabitants in 2007 to more than 700 000 in 2020.

Since the 2018 political reform in Ethiopia, different political groups began to accept Jigjiga's new regional status as a safe regional hub, opening the window of opportunity to increased investment and business development.[35] Diaspora Somalis started to make investments, purchase land, and construct homes leading to a construction boom and increases in the price of land.[35] New hotels, restaurants, and businesses are being built in preparation for the increased trade (and truck traffic) with Berbera Port and from Somaliland.[35] As the city continues its unprecedented expansion, it is also increasingly facing crucial water shortages (particularly during the dry season); the mean water accessibility of Jigjiga in 2016 was only 19·0%.[36] In response to these water shortages, communities build cement cisterns to store water for domestic use.[36] Similarly, for building construction or brick-manufacturing purposes, people in the town are accustomed to construct temporary construction pits lined with plastic sheets (figure 1). During the dry season, water for construction pits is generally purchased and delivered in truck cisterns, which source the water from underground wells located outside the city. We can see evidence of the unprecedented construction boom in Jigjiga using historical satellite imagery (figure 3). From the images, the striking expansion of construction pits in 2018 and the construction further along the periphery of the city can be seen. The sector went from 62–84 pits between 2016–18 to 232 in 2020 and 192 in 2021, showcasing the rapid urban expansion of Jigjiga during that time (figure 3).

Building *An stephensi* into Africa

In sub-Saharan Africa, residents of modern rural houses experience half the risk of malaria infection compared with those living in traditional rural houses.[37] Such findings have led to a package of interventions intended to build out malaria that focuses on practices, such as house screening in windows, doors and eaves, and sensitive building designs that lower the suitability of the indoor environment for *Anopheles* spp mosquitoes.[8,38] Improved housing and the increased number of construction sites in rural and peri-urban areas might be perceived as a sign of positive development with the potential to reduce malaria transmission. We argue that with *An stephensi*, construction sites might become a pivotal environment for species establishment and propagation within sub-Saharan cities.

As new evidence emerges, it is apparent that the presence of *An stephensi* in construction areas might not be a unique feature of Jigjiga City. Balkew and colleagues[22] described construction pits infested by *An stephensi* larvae during the rainy season across multiple cities of Ethiopia in the Afar, Dire Dawa, and Amhara regions. In Sudan, construction pits made of cement were also identified as productive *An stephensi* larval habitats in Port Sudan and Tokar City.[39] In India, it is widely recognised that many *An stephensi* breeding sites are built into the finished structures of offices, homes, and factories in urban areas.[40] Less widely recognised but also as important as *An stephensi* breeding sites are the transient structures created during and as part of the construction process.[41] Therefore, by capitalising on the temporary water reservoirs predominant in construction sites, *An stephensi* might be exploiting unique environmental conditions that could be favouring its establishment and spread in Africa. This association between urban development and *An stephensi* resembles the finding of cutaneous leishmaniasis outbreaks associated with urban growth and, more specifically, construction sites in Israeli settlements.[42,43]

Opportunities for *An stephensi* containment: larval source management of construction pits

The finding of discrete and easily identifiable *An stephensi* larval habitats in Jigjiga could provide a unique opportunity for immediate larval source management (LSM) and targeted control during the dry season, particularly with larviciding or biological control. A similar concept of dry season LSM has been proposed for *An gambiae* in semi-arid Kenya as an approach to maximise the effectiveness of larval control.[44] An extensive list of larvicides prequalified by WHO for vector control is available.[45] While *temephos* and *Bacillus thuringiensis* have shown important larviciding effects on *An Stephensi* from Ethiopia,[46] they require frequent reapplication, which can be challenging given the number of construction pits that need to be treated. Long-lasting larvicide formulations, that could be potential candidates for control in large water volumes are Spinosad 7·48% DT (Clarke Mosquito Control Products) and SumiLarv 2 MR (Sumitomo Chemical Company). Spinosad DT is a tablet for direct application used at a dose of 0·5 mg per L active ingredient (1 tablet per 200 L of water) for control of container-breeding mosquitoes with a minimum expected duration of optimum efficacy of 4–6 weeks under field conditions.[47] SumiLarv 2 MR is a 2 g plastic disc containing 2% (20 g active ingredient per kg ± 25% w/w) pyriproxyfen used at the dose of one disk in a water container with a volume of 40 L.[48] Long-lasting methoprene briquettes are commonly used for *Culex pipiens* control in catch basins in the USA and, if prequalified, would provide an additional long-lasting tool since there is a 6-month extended release formulation (Altosid 150-Day Briquets, Zoecon).[49]

Given the water source and use, long duration of construction pits, and constant availability of water, a biological control option that can be considered is the use of larvivorous fish.[50] Fish that feed on mosquito larvae have been widely used around the world in attempts to control malaria, other mosquito-borne diseases, and mosquito nuisance biting,[50] and could be used in this case as a textbook example.[51] Locally native larvivorous fish exist near Jigjiga.[50] Furthermore, LSM in Jigjiga could include both larviciding and larvivorous fish if larvicides with low toxicity (Spinosad, metoprene, or pyriproxyfen) are chosen. More importantly, our finding of high cohabitation between *An stephensi* with *Culiseta spp* and *Aedes spp* mosquitoes provides an opportunity for integrated LSM across vectors, which can lead to important co-benefits and a higher justification for the implementation of such programmes within Jigjiga and other cities. The emphasis for malaria is typically on the protection of people inside their home by deployment of insecticide-treated bed nets and indoor residual spraying. In the case of *An stephensi* in Jigjiga, vectors could be controlled outside the house by conducting LSM during the dry season (the period when mosquito populations are lower and primary larval sites are easier to identify) to reduce the risk of vector establishment and further transmission of malaria. More broadly, incorporating LSM within a comprehensive integrated vector management package[52] that includes adult control and targets other cohabiting species, such as *Aedes aegypti*, might provide an opportunity to tackle both the invasion of *An stephensi* and the growing threat of arbovirus transmission in African cities.

Conclusions

The spread of *An stephensi* in Africa might be facilitated in some Ethiopian cities by high urban migration and immigration and an unprecedented construction boom, which is generating novel larval habitats that the vector exploits during the dry season. We highlight that the spread of *An stephensi* is a planetary health problem that requires holistic consideration of the environmental, social, and political changes ongoing in rapidly growing African cities.

Acknowledgments

We thank the Jigjiga residents for allowing us to conduct this research. Emory Global Health Institute Rapid Response Grant funding was provided by the Emory Global Health Institute to conduct this research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Emory Global Health Institute or the official policy or position of the Centers for Disease Control and Prevention.

Data sharing

Files used for generating figure 2 and figure 3 are shared as supplementary Google Earth kml files. Please contact the corresponding author Gonzalo M Vazquez-Prokopec for data used in the study.

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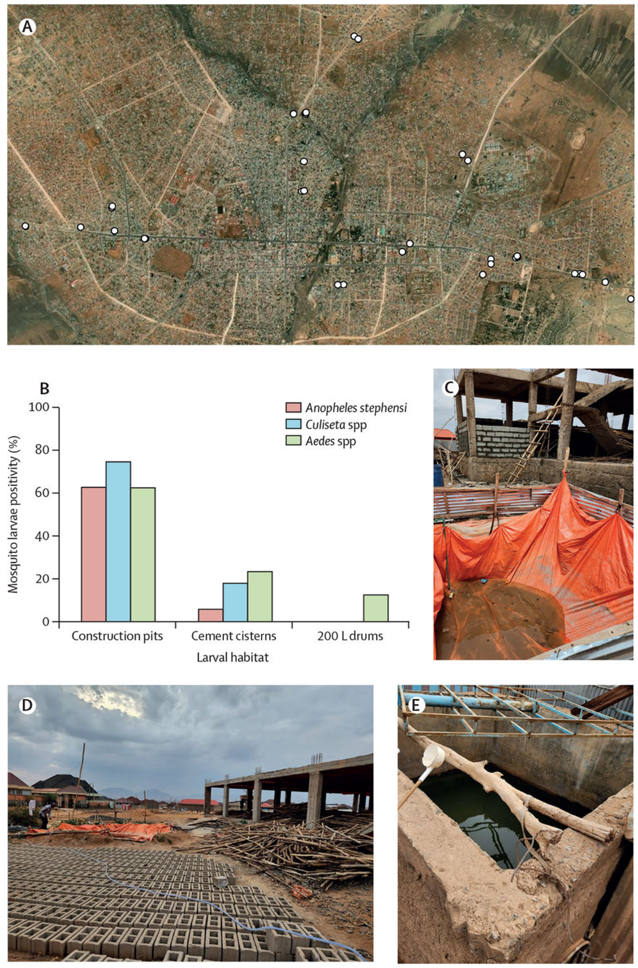

Figure 1: Results from field collections in Jigjiga during the dry season of 2023

(A) Distribution of *Anopheles stephensi* positive larval habitats in the city of Jigjiga, Somali Region, Ethiopia. Map scale 1:39840. (B) Positivity of *An stephensi* immature stages stratified by habitat type and by species or genus of mosquito found. Examples of sampled habitats, such as (C) construction pits associated with house construction, (D) a construction pit associated with brick manufacturing, and (E) a cement cistern. Image (A) sourced from ESRI World Imagery, MAXAR, and the GIS User Community.[31]

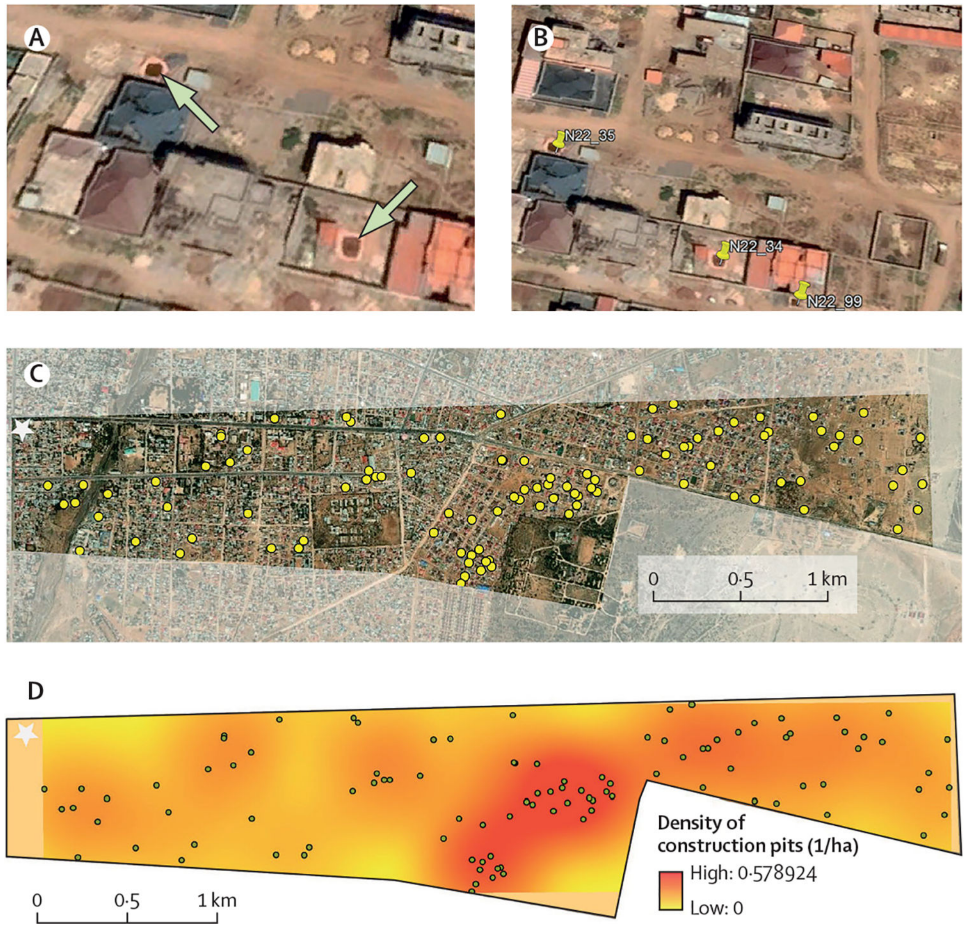

Figure 2: Construction pits can be mapped using high-resolution satellite imagery

(A) Construction pits identified as positive for *Anopheles stephensi* larvae during March, 2023 in Jigjiga, Ethiopia (indicated by the arrows). (B) Use of Google Earth Pro to digitise all visible construction pits (a total of three pits are identified with a pin). (C) Distribution of the 101 construction pits visually identified in November, 2022, 4 months before our sampling, within a rural-urban swath measuring 4·3 km^2 and centred on the highway connecting Jigjiga with Somaliland (one of the busiest corridors in the region). (D) Kernel density estimate of the density of construction pits per hectare (coloured surface) and location of all identified pits (dots) using a bandwidth of 500 m and a pixel size of 10 m. Stars in (C) and (D) indicate the location of Jigjiga's downtown. Images (A–C) were sourced from ESRI World Imagery, MAXAR, and the GIS User Community.[31]

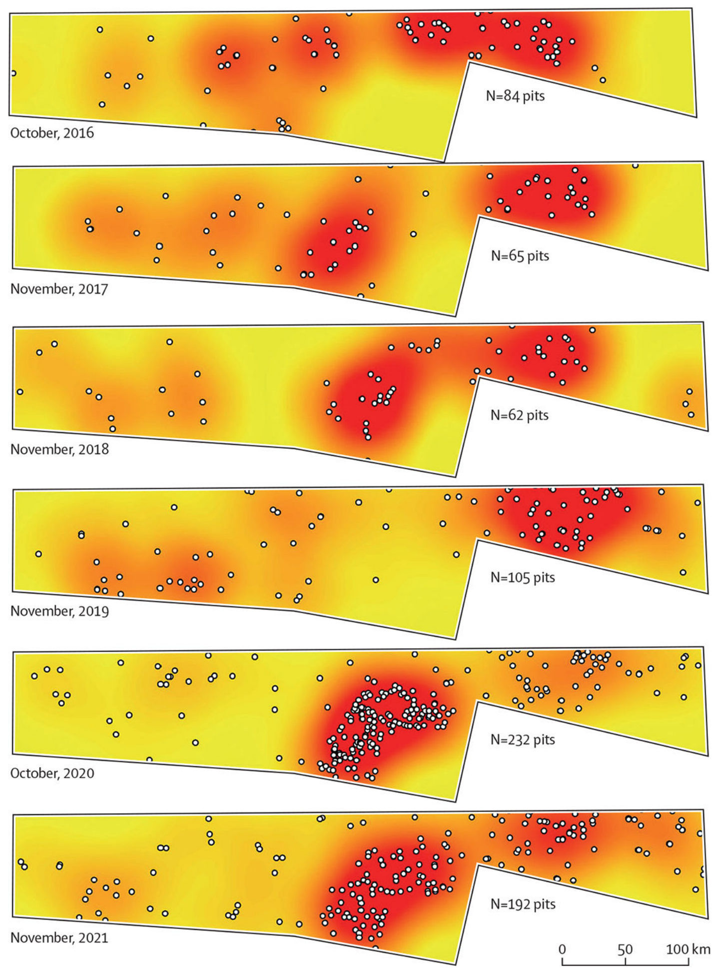

Figure 3: Historical sequence of the distribution of construction pits in an urban-rural swath of Jigjiga, Somali Region, Ethiopia

The white dots represent digitised construction pits, observed with high-resolution satellite imagery historically archived in Google Earth. The surface was generated using kernel density function in ArcMap 10.8. For each year we used October–November, as they were the months that had most complete information.