

in a viral load study using cell culture as surrogate for infectivity (6). Thus, nucleic acid detection does not prove the presence of viable or infectious virus, as Cohen et al. demonstrated in a smallpox-vaccine study (7). We pooled and extensively prepared platelet products from multiple donors, which may have diluted out any residual virus before transfusion 1 week later. In conclusion, our study shows that a blood donation from a donor with detectable MPXV viral DNA did not appear to transmit the infection to a pooled-platelet recipient.

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Detection of Invasive *Anopheles stephensi* Mosquitoes through Molecular Surveillance, Ghana

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The invasive *Anopheles stephensi* mosquito has rapidly expanded in range in Africa over the past decade. Consistent with World Health Organization guidelines, routine entomologic surveillance of malaria vectors in Accra, Ghana, now includes morphologic and molecular surveillance of *An. stephensi* mosquitoes. We report detection of *An. stephensi* mosquitoes in Ghana.

Anopheles stephensi is an invasive mosquito species originating from parts of Southeast Asia and the Arabian Peninsula (1). Over the past decade, *An. stephensi* mosquitoes have been expanding in range and have now been documented in several countries in Africa (2). First detected in Djibouti, on the Horn of Africa, in 2012, this vector has been implicated in urban malaria outbreaks (3). They were also detected in Ethiopia in 2016 and 2018 (4,5). *An. stephensi* mosquitoes were subsequently detected in Sudan (2016), Somalia (2019), Nigeria (2020), and Kenya (2023) (2,3,5–7). This invasive vector poses a major threat to current malaria control and elimination efforts. The ability of *An. stephensi* mosquitoes to breed in artificial containers enables them to thrive in urban areas, setting them apart from other major



Figure. Routine entomologic surveillance sites, Accra, Ghana, January 2022–July 2022. Inset map shows location of Ghana in Africa.

malaria vectors (8). This species can also transmit both *Plasmodium falciparum* and *P. vivax* protozoa (1). Although malaria is widely a rural disease, transmission in urban areas may rise because of the establishment of *An. stephensi* mosquitoes, putting ≈126 million persons at risk of malaria (2,8). The World Health Organization issued an initiative in 2022 aimed at strengthening surveillance to help stop the

spread of *An. stephensi* mosquitoes in sub-Saharan Africa (2). Morphologic and molecular surveillance of *An. stephensi* mosquitoes were incorporated into routine entomologic surveillance of malaria vectors in the city of Accra, Ghana, after the World Health Organization initiative (2). This study outlines the entomologic surveillance that documents the identification of this invasive species in Ghana.

Table. Sequencing results of suspected *Anopheles stephensi* mosquito samples, Accra, Ghana

Sample	ITS2 contig	BLAST result†	GenBank accession no. of best match	% Identity match	Final species identification	GenBank accession no.
DN 035	283	<i>An. stephensi</i> voucher	MH650999.1	100	<i>An. stephensi</i>	OR711900
TP 002S	283	<i>An. stephensi</i> voucher	MH650999.1	100	<i>An. stephensi</i>	OR711899

*ITS2, internal transcribed spacer 2 region.

†BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>.

We conducted routine entomologic surveillance in 8 sites within the city of Accra, Ghana, during January 2022–July 2022 (Figure). We conducted larval sampling in all mosquito larval breeding habitats encountered in each of the sites. We recorded the total number of dips, larvae, and pupae, and we calculated the larval density as the ratio of the number of larvae collected per dip. We conducted larval sampling in the dry (February–March) and rainy (June–July) seasons of 2022. We transported larval samples to the insectary at the Department of Medical Microbiology, University of Ghana Medical School (Accra, Ghana), where we raised them into adults for morphologic and molecular species identification. We further identified members of the *An. gambiae* sensu lato complex and sibling species by using PCR. We performed PCR amplifications to detect *An. stephensi* mosquitoes by using primers targeting the internal transcribed spacer region on the basis of on previously described protocols by Singh et al. (9). After PCR, were subjected 2 mosquitoes to Sanger sequencing of the internal transcribed spacer 2 regions and analyzed them on the basis of comparisons to the National Center for Biotechnology Information database.

We identified a total of 1,169 mosquitoes obtained from the larval sampling by using morphologic keys and PCR methods for speciation. Out of that number, 551 (47.13%) were *An. gambiae* sensu stricto, 582 (49.79%) *An. coluzzii*, and 32 (2.74%) hybrids of both species. We identified 4 samples (0.34%) as *An. stephensi* by using a modified PCR-based method by Singh et al. (9) and sequencing (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/30/2/23-1638-App1.pdf>). Results from BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that the *An. stephensi* mosquito samples had 100% sequence similarity with *An. stephensi* voucher A268 5.8S ribosomal RNA gene and internal transcribed spacer 2 (GenBank accession no. MH650999.1) (Table).

We found *An. stephensi* mosquitoes in larval samples from urban areas of Accra, Ghana, specifically the suburbs of Tuba, Dansoman, and Nima. We found *An. stephensi* mosquitoes breeding in dugout wells within irrigated vegetable farms and roadside ditches (Appendix Figure), habitats that are distinct from the typical ones observed in Asia and East Africa. In addition, *An. stephensi* larvae were present alongside *An. gambiae* s.s. and *An. coluzzii* mosquitoes, even though *An. stephensi* larvae are usually present alongside *Aedes* mosquitoes.

The spread of *An. stephensi* mosquitoes in Africa is thought to have occurred through land borders, air travel, or seaports. However, we discovered the

mosquitoes at considerable distances from those points of entry, suggesting possible earlier introductions. Expanding surveillance efforts for *An. stephensi* mosquitoes is crucial to curbing the dissemination of this invasive species within Ghana, which could potentially elevate malaria prevalence in the city of Accra, traditionally considered a low malaria transmission zone within Ghana.

This report of the invasion of *An. stephensi* mosquitoes in Accra, Ghana, represents a major public health concern, given the heightened risk of urban malaria outbreaks. It is imperative to reinforce surveillance and response strategies in both rural and urban settings across Ghana, with specific attention directed toward *An. stephensi* mosquitoes, to mitigate the spread of this invasive species.

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***Streptobacillus moniliformis* and IgM and IgG Immune Response in Patient with Endocarditis¹**

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We describe a case of endocarditis caused by *Streptobacillus moniliformis* bacteria, a known cause of rat-bite fever, in a 32-year-old woman with pet rats in Germany. The patient had a strong serologic response, with high IgM and IgG titers. Serologic analysis is a promising tool to identify *S. moniliformis* bacterial infection.

Rat-bite fever (RBF) is a rare disease that typically manifests with fever, rash, and arthritis (1). Possible complications are abscess formation, endocarditis, and death if left untreated (1,2). *Streptobacillus moniliformis* bacteria is the main causative pathogen of RBF (3). Norway rats (*Rattus norvegicus*) are the natural host and usually carry *S. moniliformis* bacteria asymptotically in their nasopharynx (3,4). Transmission occurs typically by rat bite or scratch but also by nontraumatic indirect contact.

We describe a case of a 32-year-old woman who came to an emergency department in Germany in May 2022 with fever, fatigue, and migrating arthralgia in the large and small joints of all 4 extremities, without signs of joint swelling or rash. She had a short history of diarrhea, and her first set of blood cultures were negative. She was initially diagnosed with reactive arthritis and transferred to the rheumatology department. We initiated treatment with 20 mg prednisolone and etoricoxib. The patient had initial relief of symptoms and was discharged after 6 days in the hospital. A small papule on her right foot

¹Preliminary results from this study were presented at the 51st Congress of the German Rheumatological Society, August 30–September 2, 2023, Leipzig, Germany.

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Appendix

Study Sites

Sampling was conducted in 8 sites within the city of Accra, Ghana, as part of routine entomological surveillance from January 2022 to July 2022. These sites were categorized to represent different environments and socio-economic status; irrigated urban farming (IUF) sites (Tuba and Dzorwulu), lower socioeconomic (LS) sites (Nima and Chorkor), middle socioeconomic (MS) sites (Dansoman and Teshie) and high socioeconomic (HS) sites (East Legon and Cantonment). Tuba ($5^{\circ} 30' 47''\text{N}$ $0^{\circ} 23' 16''\text{W}$) and Dzorwulu ($5^{\circ}36'53''\text{N}$ $0^{\circ}12'03''\text{W}$) are sites where irrigated farming is practiced all year round leading to the creation of mosquito breeding sites. Socio-economic sites were classified based on their population, housing structures and the availability of proper drainage and sanitation systems. Low socioeconomic sites, Nima ($5^{\circ} 35' 0''\text{N}$, $0^{\circ} 12' 0''\text{W}$) and Chorkor ($5^{\circ}31'39''\text{N}$ $0^{\circ}13'55''\text{W}$) are densely populated slums with poor sanitation and inadequate drainage systems. Dansoman ($5^{\circ} 33' 0''\text{N}$, $0^{\circ} 16' 0''\text{W}$) and Teshie ($5^{\circ} 35' 0''\text{N}$, $0^{\circ} 6' 0''\text{W}$) are middle socioeconomic sites with more standard residential structures with well-designed drainage and sanitation systems but poorly managed. High socioeconomic sites, Cantonment ($5^{\circ} 35' 10''\text{N}$, $0^{\circ} 10' 35''\text{W}$) and East Legon ($5^{\circ}38'16.39''\text{N}$, $0^{\circ}9'40.33''\text{W}$) have proper housing structures with good sanitation and drainage systems. Accra is the capital city of Ghana and it is the most populous. Accra lies in the coastal savannah zone of Ghana, with an annual mean temperature of 26.5°C and an average annual precipitation of 787 mm. Figure in main text (<https://wwwnc.cdc.gov/EID/article/30/3/23-1638-F1.htm>) shows a map of the routine surveillance sites.

Anopheles Larval Densities in Different Habitat Types across Different Sites

Ten different habitat types were encountered during the larval sampling. The highest larval density during the dry and wet seasons was observed in drainage ditches from Chorkor (9.72 larvae/dip) and swamps in Teshie (20.3 larvae/dip) respectively. Drainage ditches were consistently productive across almost all the sites in both seasons. The most productive habitat type across all the sites was drainage ditches. However, habitat types such as footprints, swamps and tire tracks also recorded low to high larval densities in some of the sites (0.25 to 20.3 larvae/dip). In Tuba, Nima and Dansoman, where *An. stephensi* mosquitoes were found, and some of the more productive habitats were drainage ditches (1.45 to 8.39 larvae/dip) and tire tracks (0.77 to 14.96 larvae/dip) (Appendix Table 2). Appendix Figure 2 shows habitats where *An. stephensi* mosquitoes were found. *An. gambiae s.l.* larval density was significantly associated with season ($t = 4.14$, $p = 0.00$).

Appendix Table 1. *Anopheles* larvae species distribution across different sites

Site	Site Category	Species, no. (%)				Total
		<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrids	<i>An. stephensi</i>	
Tuba	IUF	197 (61)	116 (35.9)	8 (2.5)	2 (0.6)	323 (100)
Dzorwulu		5 (31.3)	11 (68.7)	0	0	16 (100)
Nima	LS	67 (33.5)	120 (60)	12 (6)	1 (0.5)	200 (100)
Chorkor		17 (29.3)	41 (70.7)	0	0	58 (100)
Dansoman	MS	7 (7.1)	84 (85.7)	6 (6.1)	1(1.1)	98 (100)
Teshie		166 (46.62)	186 (52.2)	3 (1.2)	0	355 (100)
East Legon	HS	77 (77.7)	19 (19.3)	3 (3)	0	99 (100)
Cantonment		15 (75)	5 (25)	0	0	20 (100)
Total		551 (47.13)	582 (49.79)	32 (2.74)	4 (0.34)	1169 (100)

Appendix Table 2. *Anopheles* larval density in the dry and rainy seasons*

Habitat type	Sites/Seasons															
	Tuba		Dzorwulu		Nima		Chorkor		Dansoman		Teshie		East Legon		Cantonments	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Man-made pond	5.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Car tire	0	0	0	0	0	0	0	0	0	0	2.3	0	0	0	0	0
Drainage ditch	6.08	0	1.68	0.59	8.39	2.25	9.72	4.35	1.83	1.45	6.7	5.78	1.14	0.9	2.33	1.43
Footprint	0	1.6	0	3.53	0	1.97	0	6.44	0	4.52	0	5.67	0	0	0	0
Furrow	3.18	6.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Natural pond	0	0	6.15	0	0	0	0	0	0	0	0	0	0	0	0	0
Puddle	0	4.16	0	0	0	0	0	0	0	4	0	4.06	0	0	0	0
Swamp	0	0	5.75	1.27	0	2.67	0	0	0	2.31	0	20.3	0	1	0	2.85
Tire track	12	14.96	0	0	0	2.69	0	3	0	3.16	0.77	8.95	0	1.52	0	1
Well	0.96	0	0	0	0	0	7.5	4	0	0	0	0	0	0.25	0	0

*Values in bold represent habitat types where *An. stephensi* larvae were found.



Appendix Figure. Habitats where *An. stephensi* larvae were found. A) Dug-out well (Tuba); B) drainage ditches (Dansoman); C) swamp (Nima).