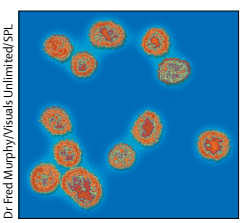
6 Costa-Pereira C, Campi-Azevedo AC, Coelho-Dos-Reis JG, et al. Multi-parameter approach to evaluate the timing of memory status after 17DD-YF primary vaccination. *PLoS Negl Trop Dis*. 2018; **12:** e0006462.
7 Vasconcelos PFC. Single shot of 17D vaccine may not confer life-long protection against yellow fever. *Mem Inst Oswaldo Cruz*. 2018; **113:** 135–37.
8 Collaborative group for studies on yellow fever vaccines. Duration of immunity in recipients of two doses of 17DD yellow fever vaccine. *Vaccine* 2019; **37:** 5129–35.
9 Roy Chowdhury P, Meier C, Laraway H, et al. Immunogenicity of yellow fever vaccine coadministered with MenAfriVac in healthy infants in Ghana and Mali. *Clin Infect Dis* 2015; **61** (suppl 5)**:** S586–93.
10 Monath TP, Vasconcelos PFC. Yellow fever. *J Clin Virol* 2015 **64:** 160–73.

The chameleonic genetics of Lassa virus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In *The Lancet Infectious Diseases*, Michael R Wiley and colleagues[1] report 23 near-complete genome sequences of Lassa virus from Liberia.[1] Lassa virus is a member of the Mammarenavirus genus and is the causative agent of Lassa fever, a febrile disease that can be associated with high morbidity and mortality. Lassa virus is maintained in nature as a chronic infection of its natural reservoir *Mastomys natalensis*, a rodent widely spread in sub-Saharan Africa. Lassa virus infection in humans can occur by inhalation of contaminated dust, contact of abraded skin with contaminated material, or ingestion of contaminated food. Lassa virus endemic areas cover large parts of the Mano River Union region (Guinea, Liberia, and Sierra Leone), as well as Nigeria, Togo, Benin, and some regions in Ghana. The alarming increase in Lassa fever cases in recent years, together with the lack of licensed vaccines and specific therapies to treat Lassa fever led WHO to include Lassa virus in the list of top priority pathogens for which vaccines and therapeutics are urgently needed.

The genetic diversity of Lassa virus poses challenges for the development of vaccines and therapeutics that are effective against all circulating Lassa virus lineages, as well as for the implementation of diagnostic tests. Despite a long history of Lassa fever in Liberia, information about the genetic diversity of Lassa virus strains circulating in this country is scarce—a knowledge gap that has been greatly narrowed by the work of Wiley and colleagues.[1]

The newly determined Liberian Lassa virus sequences were genetically very diverse, but most of them belonged to Lassa virus lineage IV, the prevalent lineage causing Lassa fever in the neighbouring Sierra Leone and, possibly, Guinea.[1–3] Liberian Lassa virus lineage IV genomes grouped into two clades: clade IV.A was mainly detected in Lassa fever cases in the central part of Liberia, whereas clade IV.B was associated with Lassa fever cases in the western part of Liberia, closer to Sierra Leone, where this clade is also dominant.[1] Phylogenetic analyses estimated that Lassa virus was introduced in the Mano River Union region 300–350 years ago, and that from Liberia the virus spread to Sierra Leone and Guinea.[1]

The findings by Wiley and colleagues[1] are consistent with previous observations on Lassa virus sampled in other countries, including studies done during the 2018 spike in Lassa fever cases in Nigeria,[4,5] indicating that Lassa virus sequences cluster more closely by geography than by isolation date, and that most cases result from spillovers from the natural reservoir. As with the geographical clustering of Lassa virus strains in Nigeria,[5] rivers might have contributed to the spatial structuring of rodent populations, and consequently of Lassa virus, in Liberia. Nonetheless, human movements exacerbated by armed conflicts in Liberia might also have played an important role in the spatial distribution of Lassa virus diversity, by facilitating incidental transport of rodents over geographical barriers. Lassa virus is thought to originate from Nigeria,[2] and the results by Wiley and colleagues[1] suggest Liberia as the Lassa virus entry point to the Mano River Union region. Therefore, a better understanding of the westward spread pattern of Lassa virus would be facilitated by examining Lassa virus diversity in countries between Nigeria and Liberia (ie, Benin, Togo, Ghana, and Côte d'Ivoire).

A limitation of the study by Wiley and colleagues[1] is that the majority of Lassa virus sequences examined were obtained from Lassa fever cases, whereas no Lassa virus genome sequences were obtained from the natural rodent reservoir in Liberia. An indication that more is to be uncovered on Lassa virus diversity in these regions is the identification of viruses with either small or large segments belonging to previously unrecognised Lassa virus lineages.[1,6]

The results reported by Wiley and colleagues[1] have important implications for Lassa virus diagnostic tests

and development of vaccines and therapeutics. The high degree of genetic diversity observed in Liberian Lassa virus strains included the target sites of most published assays to detect the presence of Lassa virus RNA, underscoring the need to match deployed diagnostic capabilities with available knowledge about the genetic diversity of locally circulating strains. Likewise, this novel information on Lassa virus genetic diversity should be taken into consideration for vaccine design and development of antiviral drugs. Lassa virus strains with novel unique genetic signatures should be incorporated into challenge studies to assess the protective efficacy of Lassa virus vaccine candidates, and into assays that test their susceptibility to antiviral drug candidates identified in screening campaigns.

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1 Wiley MR, Fakoli L, Letizia AG, et al. Genomic characterisation of Lassa virus circulating in Liberia. *Lancet Infect Dis* 2019; published online Oct 3. https://doi.org/10.1016/S1473-3099(19)30486-4.
2 Andersen KG, Shapiro BJ, Matranga CB, et al. Clinical sequencing uncovers origins and evolution of Lassa virus. *Cell* 2015; **162:** 738–50.
3 Olayemi A, Cadar D, Magassouba N, et al. New hosts of the Lassa virus. *Sci Rep* 2016; **6:** 25280.
4 Kafetzopoulou LE, Pullan ST, Lemey P, et al. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. *Science 2019;* **363:** *74–77.*
5 Siddle KJ, Eromon P, Barnes KG, et al. Genomic analysis of Lassa virus during an increase in cases in Nigeria in 2018. *N Engl J Med* 2018; **379:** 1745–53.
6 Olschläger S, Lelke M, Emmerich P, et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5′ region of S RNA. *J Clin Microbiol* 2010; **48:** 2009–13.

Strengthening diagnosis is key to eliminating malaria in India

The Indian Government has set the goal of eliminating malaria by 2030 in a systematic and progressive way. Although the commitment to a malaria-free India has met with considerable progress, evidenced by the 24% decline in malaria in 2018,[1] new diagnostic challenges are emerging. Early and accurate diagnosis is crucial to attaining the target of a malaria-free India, but malaria diagnosis in India is complicated by the varied distribution of both monoinfection and mixed infections. Microscopy and rapid diagnostic tests (RDTs) are appropriate tools for diagnosis in low-resource settings, and yet they fail to detect low-density and mixed malaria infections that are detected by molecular methods.[2] This poor performance is mainly because of the dominance of one species of malaria parasite over others in mixed infections, since microscopy usually misses the low-density parasitaemic species.[3]

Detection of malaria parasites with deleted *hrp*-2[4] and of afebrile malaria[5] are the most recent challenges for the malaria elimination programme in India. Most people with afebrile malaria do not seek diagnosis and treatment, and ultimately serve as a reservoir for malaria, unknowingly transmitting it to others. To identify mixed infections, low-density parasitaemia, and infections from *hrp*-2-deleted parasites, new diagnostic devices or devices with improved sensitivity are greatly needed.[6] Additionally, in remote areas, microscopists often do not manage to identify different stages of the malaria parasites in blood smears because of inadequate training and insufficient experience. Studies report that more than a quarter of malaria cases are missed by microscopy.[7] However, microscopy-based identification can be improved by the development and use of locally annotated databases of images of the malaria parasite, with platforms using machine learning algorithms such as ParaSight. Moreover, new point-of-care diagnostic devices should be validated to strengthen the diagnosis of malaria in remote or tribal areas, such as the recently developed, battery-operated magneto-optical device, and the DNA aptamer-based device. Using such devices will improve diagnosis and delay the development of drug resistance. Furthermore, RDTs are susceptible to high temperature and humidity. In India, the temperature can rise to 45°C or more in the malaria preparatory months (May–June) in endemic areas of the country. The transport, storage, and use of RDTs in high-temperature and high-humidity settings might affect their performance. The poor performance of RDTs is mainly due to damage to nitrocellulose membranes, bound monoclonal antibodies, and environmental temperatures.[8]