

Annual Incidence of Lassa Virus Infection in Southern Mali

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Abstract. Previously, we reported a high seroprevalence rate of Lassa virus antibodies in inhabitants of three villages in southern Mali where infected rodents have been demonstrated. Herein, we report a 1-year follow-up study in which we were able to collect a second blood samples from 88.7% of participants of the same cohort. We identified 23 seroconversions for IgG antibodies reactive against Lassa virus, representing an incidence of 6.3% (95% confidence interval = 3.8–8.8%). Seroconversion was frequently seen in preteenage children (12/23, 51.7%) and two household/familial clusters were identified. These results confirm active transmission of Lassa virus is occurring in southern Mali and appropriate diagnostic testing should be established for this etiological agent of severe viral hemorrhagic fever.

Lassa fever is an acute infection in humans caused by Lassa virus (LASV, family *Arenaviridae*, genus *Mammarenavirus*). Like most *Arenaviruses*, LASV is a rodent-borne pathogen and is maintained in nature in the common multimammate rat, *Mastomys natalensis*. Annually, as many as 300,000 LASV infections and 5,000 deaths occur in west Africa, primarily in Sierra Leone, Liberia, Guinea, and Nigeria where the virus is considered endemic.¹ The impact of LASV on the public health resources in these countries is significant with an estimated 10–30% of adult hospital admissions and 14% of all febrile disease attributed to LASV infection.^{2–4} Recent ecological studies conducted in response to the identification of sporadic cases of Lassa fever in other west African countries including Benin, Togo, Ghana, Cote d'Ivoire, and Mali suggest a larger region where LASV is circulating.^{5–11} As a result, it is likely that the overall burden of LASV infection across west Africa is underestimated.

In February 2015, we conducted a serosurvey of a total of 600 inhabitants in three villages in southern Mali (Soromba, Bamba, and Banzana) where infected rodents have been documented, though few cases of Lassa fever have been recognized.^{12,13} The results of the 2015 serosurvey demonstrated that village-specific prevalence rates in humans correlated with infection rates previously observed in rodent populations. In Banzana, where rodent prevalence rates were relatively low, 14.5% of study participants had detectable IgG antibodies against LASV, whereas in Soromba and Bamba, where up to 50% of sampled rodents had evidence of LASV infection, 41% and 44% of volunteers, respectively, were seropositive for LASV.¹² These findings confirmed a large proportion of people in this region have been previously exposed to LASV. To ascertain the annual incidence of LASV infections in these villages, we returned in February 2016 to collect and serologically analyze blood samples from the same individuals. Ethical approval for research on human subjects was obtained from the independent institutional research boards of the Faculty of Medicine and Pharmacy of

the University of Sciences Techniques and Technologies of Bamako, Mali, and the National Institutes of Health (NIH), United States. All research on samples from human subjects was conducted in accordance with the policies and regulations of the U.S. NIH and adhered to the principles of the Belmont Report (1979) (www.hhs.gov/ohrp/humansubjects/guidance/belmont.html).

We were successful in re-enrolling and sampling 532 (88.7%) of the original 600 volunteers, including samples from 365 individuals who were LASV seronegative 12 months prior. Village specific re-enrollment rates were 83%, 89%, and 94% for Soromba, Banzana, and Bamba, respectively. Written consent, enrollment, physical assessment, and blood sample collection into ethylenediaminetetraacetic acid-treated microtubes via finger prick was identical to our previous study.¹² Plasma was separated on site and immediately preserved in liquid nitrogen. At the conclusion of sample collection in the third village, samples were transported to a laboratory in Bamako for serological testing using the same methods as the year before. Plasma samples were tested for IgG antibodies reactive against LASV using a recombinant nucleocapsid-based enzyme-linked immunosorbent assay (ReLASV[®], Corgenix Medical Corporation, Inc., Broomfield, CO) as previously described.¹² The kits are produced under Corgenix Quality System and have been thoroughly evaluated for the detection of anti-LASV antibodies in patients at the Lassa fever ward of the Kenema Government Hospital in Sierra Leone (Branco, Boisen unpublished data). The 532 samples were initially screened at a 1:100 dilution. The results were compared with the previous year's data and samples, which demonstrated seroconversion were then titrated by 4-fold serial dilutions in parallel with the previous sample collected in 2015 for both IgG and IgM.

In total, 23 of the 365 previously negative individuals demonstrated seroconversion for IgG antibodies against LASV, representing an overall incidence of 6.3% (95% confidence interval [CI] = 3.8–8.8%). No samples tested yielded positive results for LASV IgM antibodies. Village-specific seroconversion rates were 4.8% (5/103, 95% CI = 0.67–8.93%) for Soromba, 4.2% (5/118, 95% CI = 0.58–7.82%) for Bamba, and 8.4% (13/115, 95% CI = 4.0–12.8%) for Banzana. Across the three sites, seroconversion rates were similar in

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TABLE 1
Location of residence, age, and sex of persons who seroconverted to Lassa virus in southern Mali, February 2015–2016

Village	Age* at follow-up enrollment (February 2016)	Sex	LASV ELISA result 2015	LASV ELISA result 2016 (titer)
Bamba	19	M	Negative	Positive (400)
Bamba	8	M	Negative	Positive (400)
Bamba	19 months	F	Negative	Positive (1,600)
Bamba	2	M	Negative	Positive (1,600)
Bamba	3	M	Negative	Positive (100)
Banzana	20	F	Negative	Positive (400)
Banzana	46	F	Negative	Positive (400)†
Banzana	13	F	Negative	Positive (400)†
Banzana	6	M	Negative	Positive (400)†
Banzana	55	F	Negative	Positive (≥ 6,400)
Banzana	61	M	Negative	Positive (400)
Banzana	17	F	Negative	Positive (400)
Banzana	5	F	Negative	Positive (1,600)‡
Banzana	2	F	Negative	Positive (1,600)‡
Banzana	41	F	Negative	Positive (1,600)‡
Banzana	24	F	Negative	Positive (1,600)‡
Banzana	10	M	Negative	Positive (1,600)‡
Banzana	17	M	Negative	Positive (400)
Soromba	66	F	Negative	Positive (400)
Soromba	4	F	Negative	Positive (400)
Soromba	8	M	Negative	Positive (1,600)
Soromba	8	M	Negative	Positive (100)
Soromba	21	F	Negative	Positive (100)

ELISA = enzyme-linked immunosorbent assay; F = female; LASV = Lassa virus; M = male.

*Unless otherwise stated age is depicted in years old.

† and ‡ indicates household/familial clusters of seroconversion to LASV.

males (5.5%, 10/183) and females (7.1%, 13/182). Interestingly, 52.2% (12/23) of the seroconversions occurred in children ≤ 13 years of age (February 2016 age range = 1.7–13 years). Also of note, 97% (162/167) of follow-up samples collected from individuals deemed seropositive in 2015 remained seropositive for LASV IgG antibodies.

It is well established that the majority of LASV infections are contracted in the household/peridomestic setting. In Banzana, 8 of the 13 seroconversions occurred in two separate household/familial compounds consisting of a male head of household with multiple wives and numerous children (Table 1). The first cluster of exposures included the husband's second wife along with two children from his first wife. In the second setting, the husband's second and third wives seroconverted along with two children from his second wife and a third child from his first wife. The remaining seroconversions documented here occurred as individual cases with no apparent epidemiological link other than villages of residence.

Unfortunately, the questionnaire administered during the follow-up sample collection was unable to identify any clinically relevant indicators of Lassa fever or risk factors in the 23 individuals with known recent LASV exposure. Similar to our earlier study, the majority of people surveyed reported multiple febrile episodes of undefined severity over the course of the previous 12 months and most individuals reported year-round rodent infestations within their homes, particularly in kitchens and sleeping areas.

Despite significant infection rates in peridomestic rodents as well as high seroprevalence rates in humans in this region, to date, the original case of Lassa fever documented in a young British man remains the only confirmed case contracted in Mali.¹⁰ Despite an apparent lack of clinically relevant disease and no documented outbreaks, our data demonstrates annual human exposure to LASV is occurring in southern Mali. At 6.3%, the incidence docu-

mented in this region of Mali is similar to that observed by McCormick and others in villages of eastern Sierra Leone, an area known to be hyperendemic for LASV and Lassa fever.⁴ Similar to the seminal work on Lassa fever in Sierra Leone conducted by McCormick, Johnson, and others,¹⁴ further prospective epidemiological studies are needed to better elucidate the clinical manifestations and severity of Lassa fever in this region of Mali as well as other regions in west Africa where LASV infects a large proportion of rodents but human cases of disease appear to be rare or absent. In addition, given the incidence of LASV infection documented here, this area of Mali may represent a suitable region to study the mechanism of protection from severe Lassa fever after exposure/infection.

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