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Original article

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Morbidity and mortality burden of COVID-19 in rural Madagascar: results from a longitudinal cohort and nested seroprevalence study

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Abstract

Introduction: Three years into the pandemic, there remains significant uncertainty about the true infection and mortality burden of COVID-19 in the World Health Organization Africa region. High quality, population-representative studies in Africa are rare and tend to be conducted in national capitals or large cities, leaving a substantial gap in our understanding of the impact of COVID-19 in rural, low-resource settings. Here, we estimated the spatio-temporal morbidity and mortality burden associated with COVID-19 in a rural health district of Madagascar until the first half of 2021.

Methods: We integrated a nested seroprevalence study within a pre-existing longitudinal cohort conducted in a representative sample of 1600 households in Ifanadiana District, Madagascar. Socio-demographic and health information was collected in combination with dried blood spots for about 6500 individuals of all ages, which were analysed to detect IgG and IgM antibodies against four specific proteins of SARS-CoV-2 in a bead-based multiplex immunoassay. We evaluated spatio-temporal patterns in COVID-19 infection history and its associations with several geographic, socio-economic and demographic factors via logistic regressions.

Results: Eighteen percent of people had been infected by April-June 2021, with seroprevalence increasing with individuals' age. COVID-19 primarily spread along the only paved road and in major towns during the first epidemic wave, subsequently spreading along secondary roads during the second wave to more remote areas. Wealthier individuals and those with occupations such as commerce and formal employment were at higher risk of being infected in the first wave. Adult mortality increased in 2020, particularly for older men for whom it nearly doubled up to nearly 40 deaths per 1000. Less than 10% of mortality in this period would be directly attributed to COVID-19 deaths if known infection fatality ratios are applied to observed seroprevalence in the district.

Conclusion: Our study provides a very granular understanding on COVID-19 transmission and mortality in a rural population of sub-Saharan Africa and suggests that the disease burden in these areas may have been substantially underestimated.

Keywords: SARS-CoV-2 seroprevalence, COVID-19 risk factors, COVID-19 mortality, sub-Saharan Africa.

Key Messages

- Three years after the start of the COVID-19 pandemic, the disease burden in areas that are traditionally most vulnerable—rural populations in the developing world—is still unclear.
- Despite low density and connectivity, about 1 in 5 people were infected by the period of April-June 2021 in a rural district of Madagascar. COVID-19 primarily spread along the transportation network and transmission was shaped by socio-economic level.
- Adult mortality substantially increased in 2020, particularly for older men, but the majority of excess mortality during this period could not be directly attributed to COVID-19 deaths.
- The true burden of COVID-19 in poor rural areas of sub-Saharan Africa may be larger than previously recognized.

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Introduction

Soon after the initial outbreak of COVID-19 in Wuhan, China, scientists estimated the key epidemiological properties that determined the spread and impact of the disease[1–3] and, as the pandemic progressed, the role of age, gender and comorbidities as risk factors became clearer.[4] However, key aspects of the epidemic remained uncertain for areas of the world where high-quality data were not available, leading to considerable debate about the expected burden in these areas.[5,6] This was especially pronounced in the World Health Organization (WHO) Africa region. At the onset of the pandemic, there was substantial concern that the region could be especially vulnerable for the same reasons it suffers from high burdens of other infectious diseases: low access to health care and limited capacity for treatment.[7,8] As the pandemic continued, low rates of reported cases and deaths corresponded to a growing chorus suggesting that perhaps Africa was at lower risk for COVID-19 morbidity and mortality due to genetic, environmental or immunological differences.[9,10]

Three years on, there remains significant uncertainty about the true infection and mortality burden of COVID-19 in Africa. Low capacity for routine testing has undermined the use of government statistics for understanding COVID-19 spread. Recent seroprevalence studies have demonstrated that patterns of infection in Africa are consistent with patterns observed elsewhere, ranging between 40% and 60% positivity by mid-2021.[11] In fact, sub-Saharan Africa could be the region with the highest infection rates globally.[12] However, high-quality representative studies in Africa continue to be rare. Studies tend to rely on opportunistic data collection, such as from blood donors,[13–18] health care workers[19–24] or patients coming to health facilities for reasons other than COVID-19,[25–30] none of which are representative of the general population. Where population-representative studies do exist, they tend to be conducted in national capitals or large cities.[11]

A second challenge is estimating the mortality burden associated with COVID-19 in Africa from existing sources. Indeed, the limited availability of vital registration records means that deaths are not systematically reported,[31] and higher rates of background mortality in these populations can obscure the characterization of excess deaths from COVID-19.[32] In addition, the indirect death toll of an epidemic can be substantial due to health system disruptions, lower access to health care and other factors, as observed during the 2014–15 Ebola epidemic.[33,34] Consequently, estimates of the COVID-19 mortality burden for the African Region vary widely: whereas the WHO estimates that less than 500 000 died from COVID-19 in 2020–21,[35] other studies suggest this number could be three to four times higher.[12,36,37] All these challenges are exacerbated in poor, rural areas of sub-Saharan Africa, where there is virtually no population-representative information on COVID-19.

Here, we take advantage of a pre-existing longitudinal cohort study in a representative sample of a rural district of Madagascar to estimate the morbidity and mortality burden associated with COVID-19 in this population until April/June of 2021. We evaluated patterns in SARS-CoV-2 infection history across space and time in the context of several socioeconomic and demographic factors. This resulted in the most granular data on COVID-19 for a rural population of the WHO Africa Region that we are aware of.

Methods

Survey data collection and serological analyses

The study was conducted in Ifanadiana, a rural health district of approximately 200 000 people in South-eastern Madagascar (see the Supplementary Material, Section S1, available as Supplementary data at *IJE* online for details). A seroprevalence survey was added to an existing longitudinal cohort study initiated in 2014 (the Ifanadiana Health Outcomes and Prosperity longitudinal Evaluation, or IHOPE)[38] to obtain demographic, health and socioeconomic information from a representative sample of 1600 households in Ifanadiana District over time. Questionnaires in the cohort were mostly adapted from the Demographic and Health Survey.[39] The Madagascar National Institute of Statistics (INSTAT) was responsible for data collection, survey coordination, training and oversight. The main goal of the IHOPE cohort was to evaluate the impact of a health system strengthening (HSS) intervention, so a two-stage sample stratified the district by the HSS intervention's initial catchment area. Eighty clusters, half from each stratum, were selected at random from enumeration areas mapped during the 2009 census, and households were then mapped within each cluster. Twenty households were selected at random from each cluster.

Four waves of data collection have been conducted in 2014, 2016, 2018 and 2021, in which the original 1600 households were revisited. Response rates were about 95% for each wave.[38] Individual face-to-face interviews were conducted with all women aged 15 to 49 years and men aged 15 to 59 years (usual residents or visitors). Data collected in the questionnaires included, among others: household composition (size, genders, age); indicators of socioeconomic status (education, employment, household durable assets); and adult, maternal and child mortality. To learn more about the impact of COVID-19, the 2021 wave of data collection (22 April to 20 June) included, for each household member, questions on COVID-related symptoms in the previous 6 months. For all consenting individuals of all ages, a dried blood spot (DBS) was obtained by finger prick using a single-use lancet needle by trained nurses, with one to five DBS collected on Whatman 903 Protein Caver Card filter papers. To ensure the safety and avoid transmission of COVID-19 during the survey, field protocols were adapted based on guidance from the SMART initiative.[40] This included: frequent testing of survey teams before, during and after the survey; initial quarantine of all teams on site before beginning the survey; immediate quarantine of any COVID-positive staff from the survey; use of masks and other protective equipment for survey teams and participants during interviews.

Using methods previously described,[41] DBS samples were processed using a multiplex bead assay on the Luminex platform (MagpixTM) for antibodies against four antigens of SARS-CoV2: Spike S1, Spike S2, Spike RBD and NP. Magnetic beads (Luminex, MagplexTM MC100XX-01) were coupled to these four antigens using xMAP Antibody coupling kit (Luminex, 40–50016). Cut-off limits for determining positive antibodies for SARS-CoV-2 were estimated based on receiver operating curve (ROC) characteristics for the median fluorescence intensity (MFI). Further details on serological analyses are available in the Supplementary Material, Section S1 (available as Supplementary data at *IJE* online).

Data analyses

Seroprevalence of recent and past SARS-CoV-2 infections

To obtain seroprevalence estimates, we carried out two sets of analyses. First, normalised and protein-corrected values of MFI for

each of the eight SARS-CoV-2 antibodies were compared with their corresponding positivity threshold[42] to determine whether the sample was positive for that particular antibody. Second, to reduce the number of dimensions of the serological data and obtain discrete consistent groups, we used k-means clustering to classify individuals' sero-positivity. Data were visually inspected for outliers, and 134 individuals (outside the range of mean + 3 SD) were removed. We preconditioned the data via a principal components analysis to reduce its dimensionality,[43] and used the first two principal components, which explained over 60% of the variance, in the subsequent cluster analysis. We then estimated the optimal number of clusters via the average silhouette width. We used the Hartigan–Wong algorithm to perform k-means clustering using 999 starting sets of centroids.

Clusters were assigned meaningful sero-positivity labels based on the component loadings of the initial principal components analysis and the clusters' locations relative to these loadings (Figure 1). IgG and IgM antibodies had a strong positive effect on the first principal component (PC1), representing the gradient between overall positive and negative sero-positivity. The second principal component had strong positive loadings for IgM antibodies, which are indicative of recent infection,[44] and therefore represented the difference between past and recent infections.

Trends and factors associated with SARS-CoV-2 seroprevalence

Statistical analyses were carried out to understand local patterns of COVID-19 seroprevalence, including geographical trends and associations with socioeconomic and demographic factors (see the Supplementary Material Section S1, available as Supplementary data at *IJE* online for details). Associations were modelled individually using univariate and multivariate logistic regressions in generalized linear models. Sampling weights that adjusted for unequal probability of selection due to stratification and non-response were calculated. Estimates (totals, proportions, odds ratios) were obtained using survey commands available in R-package *survey* and applicable sampling weights.[45] All analyses were done for each individual serological marker and for the composite indicators. All analyses were performed with R software, version 4.2.1,[46] and R packages 'survey', 'gstat', 'rgdal', 'stats' and 'ggplot2'.

Excess mortality and infection fatality rates associated with SARS-CoV-2

Adult mortality was estimated from the IHOPE cohort using the synthetic life-table method for DHS surveys.[47] First, 6-year averages of adult mortality per 1000 population, split by age group and sex, were estimated for each wave of the cohort in order to obtain robust estimates over time that are comparable to standard DHS methods, before and after the COVID-19 epidemic. Because the period for these 6-year estimates overlaps, mortality rates per year were then estimated from the 2021 wave of the cohort only, for the 10 years prior to the survey. From this, a 10-year average was estimated (2012–21) and excess mortality occurring in the years 2020 and 2021 was estimated as the difference between these years' mortality and the 10-year average. Not all excess mortality in 2020–21 can be assumed to be directly the result of COVID-19 deaths. To estimate expected excess mortality associated directly with SARS-CoV-2 infections in our population, infection fatality rate (IFR) estimates per year of age (including lower and upper bounds for these estimates) were obtained from a recent study by the COVID-19 Forecasting Team.[48] These age-specific IFR values were then combined with the observed age-specific number of SARS-CoV-2 cases and age

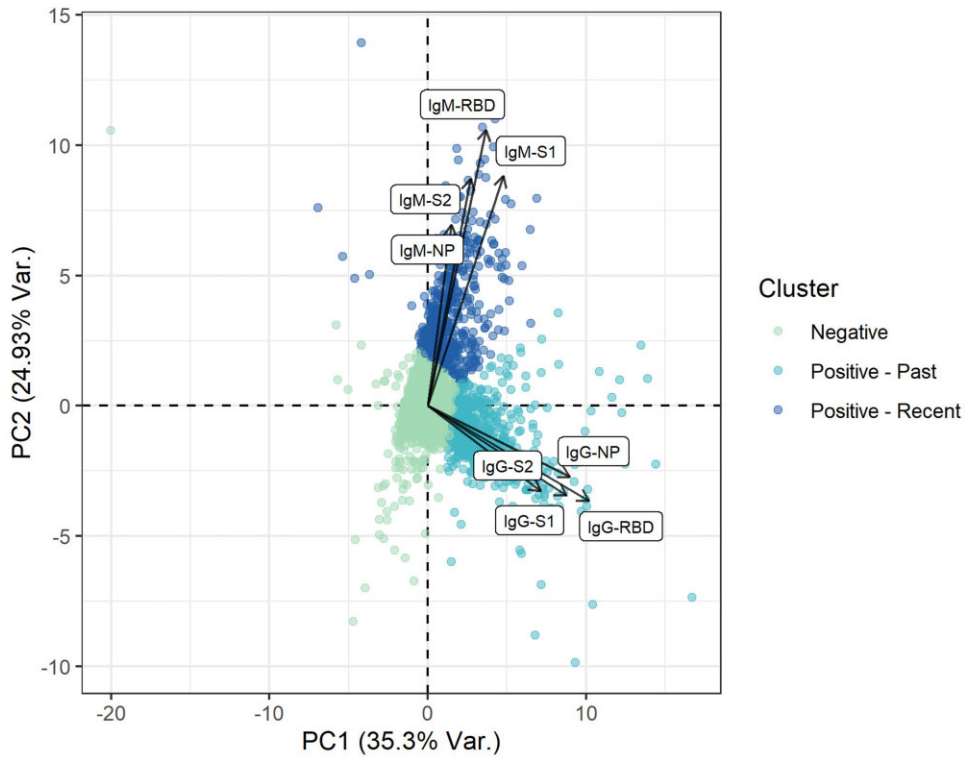

Figure 1 K-means clustering of SARS-CoV-2 seroprevalence. Results are based on normalized mean fluorescence intensity values for eight antibodies against SARS-CoV-2 (names in white boxes). Colours represent the three clusters obtained, plotted along the axes of the first two components of a principal components analysis that explained over 60% of the variance in these antibodies. PC1: first principal component; PC2: second principal component; Var: variance; RBD: Spike receptor-binding domain protein; S1: Spike S1 protein; S2: Spike S2 protein; NP: nucleocapsid protein

Table 1 Characteristics of individuals included in the COVID-19 seroprevalence survey

Variable	Observation	Percentage
Demographic factors		
Sex		
Female	3323	51.2%
Male	3173	48.8%
Age (years)		
0–5	1069	16.5%
>5–15	2109	32.5%
>15–30	1386	21.3%
>30–60	1646	25.3%
>60–100	285	4.4%
COVID-19 symptoms within previous 6 months[a]		
Not reported	5837	89.9%
Reported	659	10.1%
Geographical factors		
Distance to main town (km)		
0–5	2358	36.3%
>5–10	2074	31.9%
>10–15	2064	31.8%
Distance to main road (km)		
0–5	1531	23.6%
>5	4964	76.4%
Distance to secondary road (km)		
0–5	1704	26.2%
>5	4791	73.8%
Socioeconomic factors		
Household wealth		
Q1 (poorest)	1297	20.0%
Q2	1334	20.5%
Q3	1334	20.5%
Q4	1277	19.7%
Q5 (wealthiest)	1253	19.3%
Occupation (15+ years old only)		
Agriculture	2353	82.0%
Daily worker	177	6.2%
Commerce	156	5.4%
Formal employment	83	2.9%
Others	102	3.6%
Attends school (ages 5–14 years only)		
No	377	20.9%
Yes	1423	79.1%
Total	**6496**	**100.0%**

[a] Includes any of the following symptoms: fever, cough or respiratory problems, headache, fatigue or pain in muscles/joints, diarrhoea or nausea, loss of smell, sore throat. See Table 2 for details on each.

distribution in our population to obtain an expected excess mortality by age group. Observed excess mortality in our cohort was then compared with expected excess mortality.

Results

SARS-CoV-2 seroprevalence trends and associated factors

Overall, 6496 individuals were included in the seroprevalence analyses, nearly half of whom were children under 15 years (Table 1). Only one out of 10 individuals reported COVID-related symptoms in the 6 months prior to the survey (Table 2), with the most common symptoms being fever (6.5%) and respiratory problems (4.1%). District seroprevalence ranged from 5.1% (Spike RBD) to 43.8% (Spike S2) for IgG antibodies, and from 7.2% (Spike S1) to 17.9% (Spike S2) for IgM antibodies. Positivity to Spike S2, which is less specific to SARS-CoV-2, was higher than to any of the other markers, both for IgG and IgM antibodies. After clustering via principal components analysis and k-means, overall seroprevalence was

Table 2 Reported COVID-19 symptoms in the previous 6 months and estimated COVID-19 seroprevalence, all ages ($n = 6496$)

COVID-19 symptom (past 6 months)	Percentage (95% CI)
Fever	6.46 (5.3–7.6)
Cough or respiratory problems	4.1 (3.2–5.0)
Headache	3.9 (2.9–4.8)
Fatigue or pain in muscles/joints	3.3 (2.5–4.0)
Diarrhoea or nausea	1.1 (0.8–1.5)
Loss of smell	0.8 (0.4–1.2)
Sore throat	0.4 (0.2–0.6)
SARS-CoV-2 serological marker	**Seroprevalence (95% CI)**
IgG	
Spike S1	13.0 (10.6–15.4)
Spike S2	43.8 (40.9–46.7)
Spike RBD	5.1 (3.8–6.3)
Spike NP	16.4 (14.1–18.7)
IgM	
Spike S1	7.2 (6.3–8.2)
Spike S2	17.9 (15.8–19.9)
Spike RBD	9.1 (7.9–10.3)
Spike NP	9.1 (7.5–10.7)
Composite (k-means clustering)	
Infected vs healthy	18.0 (15.9–20.1)
IgG predominance (past infection)	10.1 (8.3–11.9)
IgM predominance (recent infection)	7.9 (6.5–9.2)

RBD: Spike receptor-binding domain protein; S1: Spike S1 protein; S2: Spike S2 protein; NP: nucleocapsid protein

estimated to be 18%, with 10.1% having a predominantly IgG response suggestive of past infection and 7.9% having a predominantly IgM response suggestive of recent infection.

The spatial distribution of past infections suggests that COVID-19 cases during the first wave accumulated predominantly in proximity to the paved road, with the exception of a few clusters in remote areas in the north of the district where seroprevalence reached nearly 30% (Figure 2). In contrast, recent infections during the second wave were more evenly distributed, with lower prevalence in clusters located along the main road. Similarly, the factors associated with SARS-CoV-2 seroprevalence varied substantially between past and recent infections (Figure 3 and Table 3). In both recent and past infections, seroprevalence was similar for males and females, increased with age and decreased with distance to a major town. However, seroprevalence for past infections was higher for individuals in the wealthiest household quantiles and those whose occupation was not agriculture, with opposite associations for recent infections. In addition, seroprevalence of recent infections doubled for individuals reporting a COVID-19 symptom in the previous 6 months compared with those not reporting symptoms, whereas no association was observed for past infections. Spatial distributions and associations for each of the eight SARS-CoV-2 serological markers are available in the Supplementary Material, Section S2 (available as Supplementary data at *IJE* online).

Mortality and infection fatality rates associated with COVID-19

Estimates of 6-year adult mortality in the 2021 wave of the IHOPE cohort collection were higher than in all previous waves of data collection (Figure 4). The largest increase was observed for older men (35–49 years), who experienced a nearly 100% increase in mortality in reports from the 2021 cohort wave as compared with the 2018 cohort wave (from

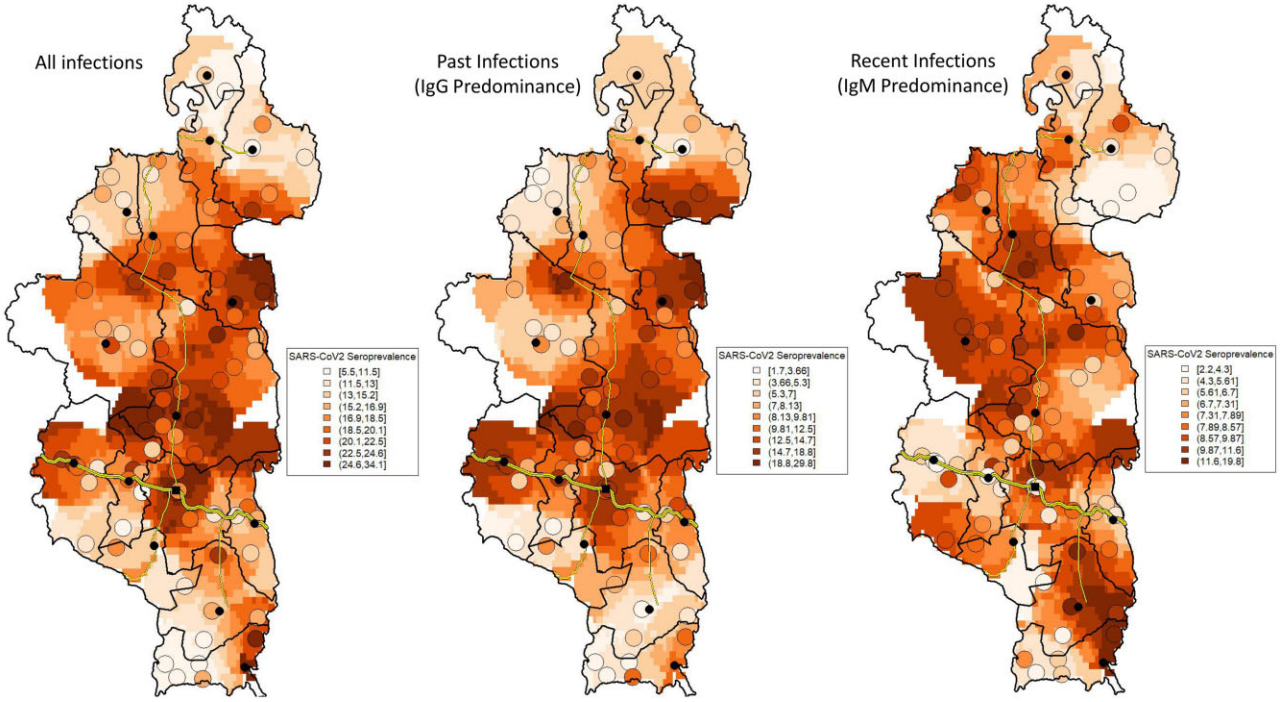

Figure 2 Spatial distribution of SARS-CoV-2 seroprevalence in Ifanadiana District. From left to right, maps show seroprevalence of all infections, past infections and recent infections based on k-clustering analyses, with colours ranging from light (low seroprevalence) to dark orange (high seroprevalence). Average seroprevalence and location of each of the 80 clusters in the survey are represented by circles and the rest of the raster is based on inverse distance weighted interpolation. Location of major towns is represented by black points and the district capital is represented by a black square

10.6 to 19.3 per 1000). For women and young men, mortality had been declining in the previous three waves, and then increased by 20–50% in the 2021 cohort wave to a level higher than baseline. Similar trends were observed using data from the 2021 cohort wave only, where analyses of annual mortality rates showed that mortality in 2020 was substantially higher than average, especially for older men (Figure 4). However, these time series were more stochastic given lower sample sizes and, for women and young men, the peak in mortality observed in 2020 was not higher than other peaks observed in previous years. Overall, excess mortality for 2020–21 was estimated at 1.61 per 1000 for individuals 15–34 years and 4.82 per 1000 for individuals 35–49 years. Given previously estimated COVID-19 IFRs[48] and observed seroprevalence by age group, expected excess mortality associated directly with COVID-19 infections in our cohort would be 0.05 (range 0.04–0.08) per 1000 for individuals 15–34 years and 0.54 (range 0.39–0.85) per 1000 for individuals 35–49 years (Table 4). This suggests that if IFRs in Ifanadiana were consistent with those previously estimated, only 3.1% (range 2.5–5.0%) and 8.9% (range 6.5–14.1%) of observed excess mortality for individuals 15–34 years and 35–49 years, respectively, would be directly associated with COVID-19 deaths.

Discussion

Nearly 3 years after the start of the COVID-19 pandemic, the burden of the disease in areas that are traditionally most vulnerable—rural populations in the developing world—is still unclear. SARS-CoV-2 seroprevalence surveys have been essential for understanding COVID-19 transmission, but quality studies have rarely focused on rural areas of sub-Saharan Africa.[11,48] Using a population-representative cohort and a nested seroprevalence survey of nearly 6500 people of all ages, we provide a fine-scale account of COVID-19 spread and burden in a rural district of Madagascar during its first two epidemic waves. Our results suggest that despite low density and connectivity in the majority of the district, about one in five people had been infected by April/June 2021. COVID-19 primarily spread along the only paved road during the first wave, and then spread along secondary roads during the second wave to more remote areas. Adult mortality increased in 2020, particularly for older men, but the majority of excess mortality during this period could not be directly attributed to COVID-19 deaths, given previously estimated IFRs. This suggests that for populations living in rural, low-resource settings, COVID-19 could have significant health impacts, either because of higher IFRs than previously estimated or because of substantial indirect impacts on health care.

Our results reveal that seroprevalence was lower in this rural district of Madagascar than in the nearby city of Fianarantsoa (1 h 30 min drive), where seroprevalence was about 20% by November 2020[14] and over 40% by February/June 2021.[49] This is consistent with studies conducted in other African settings, which found that seroprevalence tends to be lower in rural populations.[11,50–56] The lower seroprevalence in Ifanadiana could also be explained by delays in epidemic spread within Madagascar. April/June 2021 was the middle of the second epidemic wave according to national data, but the vast majority of reported cases come from Antananarivo due to diagnostic challenges in the rest of the country.[8] Given that there was a lag of nearly 2 months between the first epidemic wave in Antananarivo and in other cities,[14] it is possible that our data collection occurred at the beginning of the second wave in Ifanadiana, which could explain the low rates of IgM seroprevalence. However, our study revealed substantial heterogeneity in the spatio-

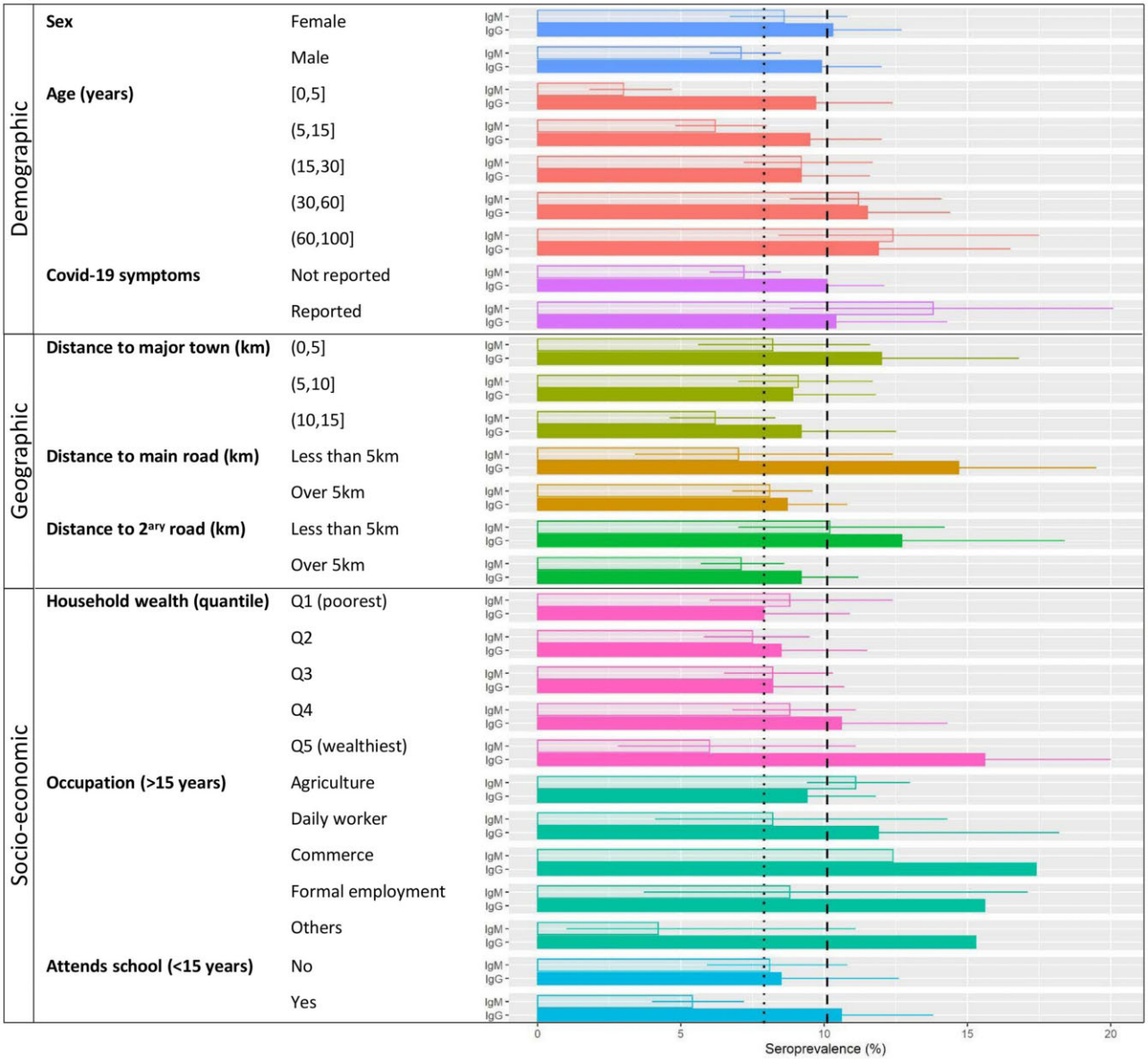

Figure 3 Factors associated with SARS-CoV-2 seroprevalence in Ifanadiana District. Horizontal bars show average seroprevalence per group, split into past infections (IgG, filled colour bars) and recent infections (IgM, translucent colour bars), with 95% confidence intervals as whiskers. Vertical lines represent average seroprevalence in Ifanadiana for past infections (dashed) and recent infections (dotted). Four confidence interval limits (Occupation variable) were removed to improve visualization of results

temporal patterns of these rural infections. Populations in the district living within 5 km to roads or large towns had comparable seroprevalence rates to those from urban Fianarantsoa,[14,49] which highlights the major role played by population density and road connectivity in the spread of COVID-19 even in rural areas of the developing world, where both factors are significantly lower than average.

Seroprevalence rates differed across demographic and socioeconomic groups in our population. Seroprevalence in both epidemic waves increased with age, especially for those older than 30, but risk did not differ between men and women. Although associations with demographic factors can be context-specific and vary across settings, a recent review of seroprevalence studies found similar average trends for Africa.[11] Socioeconomic factors also modified individuals' risk of infection in this largely impoverished population, where the primary occupation is subsistence agriculture. Wealthier individuals and those with certain occupations such as commerce and formal employment were at higher risk of being infected in the first wave but at lower risk of being infected in the second. It is well known that individuals with high social connectivity are at higher risk of infection and can contribute disproportionately to the spread of diseases such as COVID-19.[57–59] Whereas research on at-risk occupational activities in Africa has mostly focused on health care workers,[24] a better understanding of the role played by other socioeconomic groups with high mobility and social connections could open new possibilities for disease control.[60] The inverse relationship found from the first wave to the second for some risk factors (geographical, socioeconomic) was unexpected given low seroprevalence rates. We found that rates of cluster seroprevalence during the second wave were consistently lower when seroprevalence during the first wave was 30–50% (Supplementary Figure S10, available as

Table 3 Logistic regression results for associations with SARS-CoV-2 seroprevalence in past and recent infections, based on IgG and IgM predominance

Variable	IgG (past infection)		IgM (recent infection)	
	Univariate odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Univariate odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Demographic factors				
Sex (ref. female)				
Male	0.95 (0.79–1.14)	—	0.82 (0.64–1.05)	—
Age in years (ref. 0–5)				
>5–15	0.97 (0.74–1.28)	—	2.17 (1.32–3.58)**	2.24 (1.36–3.71)**
>15–30	0.94 (0.71–1.24)	—	3.32 (1.97–5.59)***	3.45 (2.04–5.85)***
>30–60	1.21 (0.9–1.62)	—	4.12 (2.51–6.75)***	4.07 (2.48–6.68)***
>60–100	1.25 (0.86–1.81)	—	4.64 (2.38–9.04)***	4.34 (2.22–8.48)***
COVID-19 symptoms (ref. not reported)				
Reported	1.03 (0.75–1.42)	—	2.05 (1.35–3.12)**	1.91 (1.26–2.91)**
Geographical factors				
Distance to health centre (ref. 0–5 km)				
>5–10 km	0.72 (0.44–1.17)	—	1.12 (0.72–1.75)	1.21 (0.86–1.7)
>10–15 km	0.74 (0.45–1.23)	—	0.75 (0.47–1.18)	0.74 (0.52–1.05)†
Distance to main road (ref. 0–5 km)				
>5 km	0.55 (0.37–0.81)**	0.7 (0.49–1.02)†	1.18 (0.64–2.19)	—
Distance to secondary road (ref. 0–5 km)				
>5 km	0.69 (0.43–1.11)	—	0.67 (0.45–1)†	0.62 (0.44–0.86)**
Socioeconomic factors				
Household wealth (ref. Q1 poorest)				
Q2	1.07 (0.74–1.57)	1.04 (0.71–1.53)	0.84 (0.56–1.27)	0.79 (0.53–1.17)
Q3	1.03 (0.69–1.54)	0.97 (0.65–1.45)	0.93 (0.64–1.35)	0.84 (0.6–1.17)
Q4	1.37 (0.89–2.12)	1.23 (0.79–1.92)	1 (0.65–1.54)	0.83 (0.56–1.22)
Q5 (wealthiest)	2.14 (1.4–3.27)***	1.69 (1.14–2.52)*	0.67 (0.31–1.44)	0.48 (0.26–0.88)*
Occupation, individuals aged 15+ years (ref. Agriculture)[a]				
Daily worker	1.31 (0.78–2.18)	—	0.71 (0.39–1.31)	—
Commerce	2.03 (1.13–3.67)*	—	1.14 (0.22–5.97)	—
Formal employment	1.78 (1.04–3.07)*	—	0.77 (0.36–1.65)	—
Others	1.74 (0.87–3.47)	—	0.35 (0.12–1.01)†	—
Attends school, children aged 5–14 years (ref. No)[a]				
Yes	1.29 (0.8–2.06)	—	0.65 (0.44–0.97)*	—

[a] Variable only applicable to a population subgroup, not included in multivariate analyses.
† $P<0.1$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Supplementary data at *IJE* online), which suggests that in this rural context with a potentially lower COVID-19 effective reproduction number, a seroprevalence level of 30–50% could have offered some level of protection, at least in the initial epidemic progression of the second wave.

In this poor rural setting where mortality rates were already high prior to the pandemic, our results suggest that the COVID-19 epidemic was associated with a substantial increase in adult mortality. The increase observed for Ifanadiana was similar to that found in Sudan's capital,[61] where a 67% rise was observed. However, about three-quarters of deaths in the Sudan study were among individuals aged 50 years or older, a vulnerable population group that was not assessed here due to study design limitations. The excess mortality in individuals aged 15–49 years in Ifanadiana was substantially higher than what could have been expected based on observed seroprevalence and known infection fatality rates for these age groups in other parts of the world.[48] COVID-19 is known to have indirect impacts on mortality, such as through the effects on health care access and health system disruptions,[62,63] and these effects could have been larger here than in other settings.[64] Interestingly, the only other outlying year for mortality in this 10-year period was 2017, when Madagascar's largest plague epidemic in recent history occurred, even though no plague deaths were reported from this area.[65] For both 2017 and 2020, it is also possible that other factors unrelated to COVID-19 were associated with higher mortality in this period. For instance, lower precipitation in 2016 due to El Nino Southern Oscillation led to lower agricultural yields and higher rice prices in 2017, which could have affected rural populations' nutrition and illustrates the complexity of attributing excess mortality to specific events. Importantly, the lack of seroprevalence studies in rural areas of the developing world means that global age-specific IFRs used here as reference[48] could be substantially underestimated for our population, given higher rates of poverty and

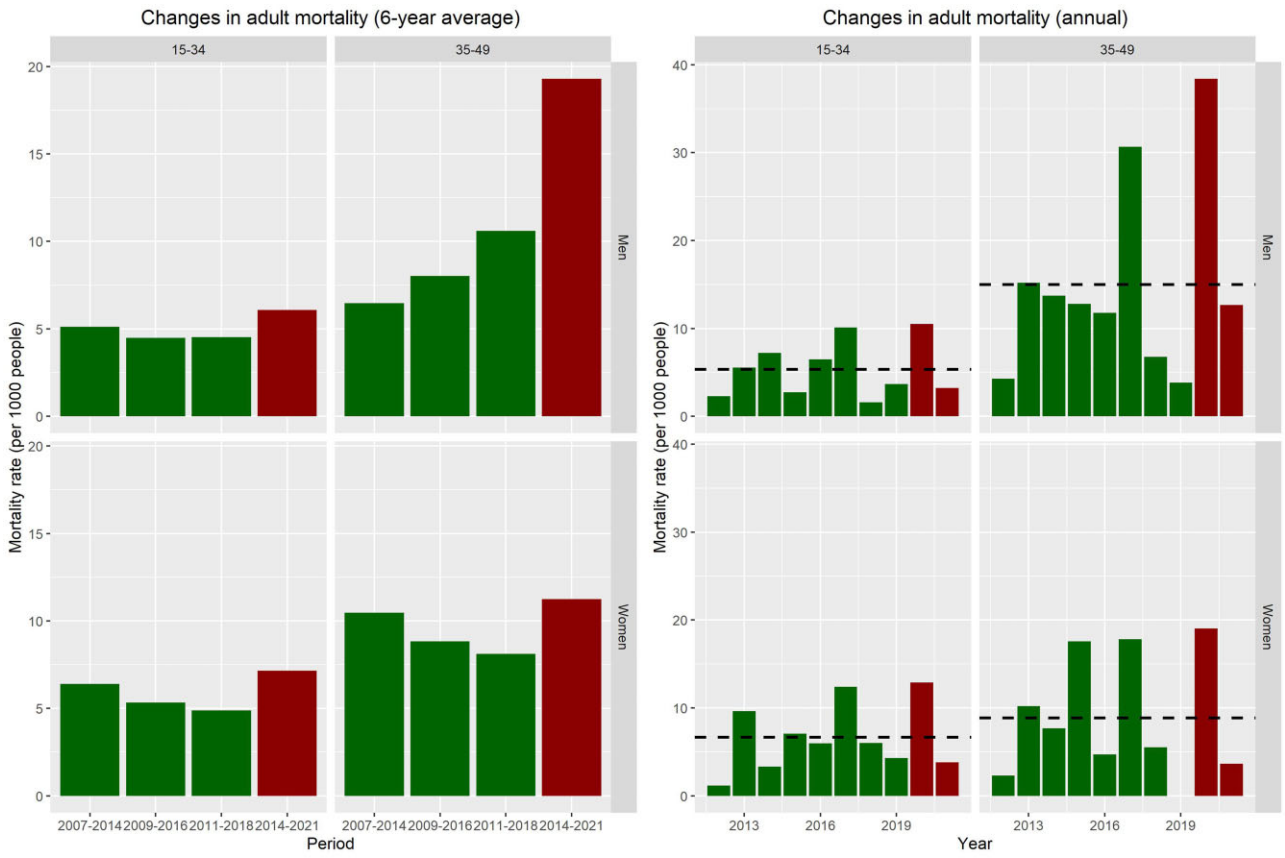

Figure 4 Trends in adult mortality rates in the IHOPE cohort, Ifanadiana District. Graphs show mortality per 1000 people before (green) and during (red) the COVID-19 pandemic. Left panels show changes in mortality rates across survey waves using the 6-year average prior to each survey wave. Right panels show changes in mortality rates per year in the 10 years prior to the 2021 survey. Dashed lines are the 10-year average for the period for each age and sex group. Note that the 2021 survey was conducted in April/June, so mortality estimates for this year only comprise part of the year

Table 4 Estimation of excess adult mortality and percentage that could be directly attributed to COVID-19, given observed seroprevalence and known infection fatality ratios by age

Age group	Seroprevalence (%)	Observed excess mortality 2020–21 (per 1000)	Expected excess mortality directly from COVID-19 infections (per 1000)	% of excess mortality directly attributed to COVID-19 infections
15–34 years	32.7	1.61	0.05 (0.04–0.08)	3.1 (2.5–5.0)
35–49 years	35.8	6.04	0.54 (0.39–0.85)	8.9 (6.5–14.1)

lower rates of health care access. It is plausible that our population had significantly higher IFRs than global estimates and that the direct contribution of COVID-19 to excess mortality was higher than reported here.

Our study had several limitations. First, although we used robust clustering methods to classify infections into past and recent, there is uncertainty around such classification. For instance, IgM titres can remain high in past symptomatic infections and IgG NP titres can increase early in the infection.[41,66] However, the facts that the first epidemic wave occurred nearly a year previously and that results are consistent with known patterns of COVID-19 spread (e.g. initial spread along better-connected populations) suggest that potential misclassification biases had little impact. It is also possible that lower sensitivity of the assay for IgM markers (Supplementary Table S1, available as Supplementary data at *IJE* online) could have resulted in a slight underestimation of recent infections, meaning that both the overall seroprevalence and the direct contribution of COVID-19 to excess mortality could have been higher than reported here. Second, low sample sizes for analyses of adult mortality could have affected our estimates of excess mortality, particularly because annual rates in the 10-year period were not stable and had considerable uncertainty (Supplementary Table S2, available as Supplementary data at *IJE* online). Despite this, results of these analyses were consistent with 6-year averages (which include a much larger sample size but overlap over time). Third, our survey mirrored a DHS design, where mortality estimates are based on information for siblings provided by men and women of reproductive age. As a result, even though individuals older than 50 years are the most likely to be affected, we could not assess the impact of COVID-19 on this age group due to low sample sizes. Fourth, the survey was conducted in the middle of the second wave of COVID-19, which prevented us from fully capturing the impact of this wave. This could also have affected the trends observed if the timing of the survey in different parts of the district had an impact on their corresponding seroprevalence, but complementary

analyses suggest this was unlikely (Supplementary Figure S9, available as Supplementary data at *IJE* online). Fifth, fear and stigma around COVID-19 could have biased participants' responses and resulted in an underestimation of COVID-19 symptoms in the previous 6 months. For instance, rates of illness reporting in children had been decreasing in previous survey years, but this decrease was particularly acute for respiratory infections in 2021 (Supplementary Figure S11, available as Supplementary data at *IJE* online). Finally, as is the case with any local-scale survey, the results of this study do not necessarily represent the COVID-19 situation in other parts of Madagascar or sub-Saharan Africa.

Conclusion

In conclusion, our study provides an unusually detailed picture of COVID-19 morbidity and mortality in a poor rural setting of sub-Saharan Africa, with important implications for similar settings. It suggests that the disease burden in these areas may have been substantially underestimated. Given known vulnerabilities to other infectious diseases, combined with the fragility of their health systems, more attention and quality research are needed to better understand the true burden of COVID-19 in poor rural areas of sub-Saharan Africa and to devise appropriate responses to this and future pandemics.

Ethics approval

The study was approved by the Madagascar National Ethics Committee and Harvard Medical School institutional review board, including amendments for changes in 2021. All adults (aged ≥15 years) provided oral informed consent for the in-person interview and written informed consent for biological sample collection. Parents or guardians provided written consent for biological sample collection from children <15 years of age, and children ged 7–14 years provided written assent separately.

Data availability

Data are available upon request to the address [research@pivotworks.org].

Supplementary data

Supplementary data are available at *IJE* online.

Author contributions

Conceived and designed the experiments: A.G., A.C.M., K.E.F., M.H.B., M.S. Performed laboratory analyses: L.T.R., M.S. Performed statistical analyses: A.G., M.E. Contributed reagents/materials/data/analysis tools: A.G., R.J.L.R., A.C.M., M.R., S.A., L.T.R., M.S. Wrote the initial draft of the manuscript: A.G., M.H.B. Revised the manuscript and accepted it in its final form: A.G., L.T.R., R.J.L.R., M.E., A.C.M., K.E.F., L.F.C., G.C., B.R., M.R., S.A., S.P., R.H., M.H.B., M.S.

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Conflict of interest

Some authors are current or former employees of institutions discussed in this article, including the non-governmental organization Pivot and the Government of Madagascar. These affiliations are explicitly listed in the article.

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