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## Spatial analysis of the distribution of intestinal nematode infections in Uganda

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(Accepted 15 July 2004)

### SUMMARY

The spatial epidemiology of intestinal nematodes in Uganda was investigated using generalized additive models and geostatistical methods. The prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* was unevenly distributed in the country with prevalence greatest in southwest Uganda whereas hookworm was more homogeneously distributed. *A. lumbricoides* and *T. Trichiura* prevalence were nonlinearly related to satellite sensor-based estimates of land surface temperature; hookworm was nonlinearly associated with rainfall. Semivariogram analysis indicated that *T. trichiura* prevalence exhibited no spatial structure and that *A. lumbricoides* exhibited some spatial dependency at small spatial distances, once large-scale, mainly environmental, trends had been removed. In contrast, there was much more spatial structure in hookworm prevalence although the underlying factors are at present unclear. The implications of the results are discussed in relation to parasite spatial epidemiology and the prediction of infection distributions.

### INTRODUCTION

Renewed commitment to global parasite control has created an impetus to attain comprehensive data on infection distribution and intensity. Once survey data have been collected they can be integrated in a geographical information system (GIS) for mapping and analysis, thus helping guide available resources to be most rationally and cost-effectively deployed. For example, the African Programme for Onchocerciasis Control (APOC) has effectively used GIS to visualize priority areas for mass distribution of ivermectin and estimate the number of individuals to be treated [1]. Knowledge of the spatial distribution of parasitic

species can also improve our understanding of the spatial scale at which variation occurs and underlying epidemiological factors [2–5]. With this realization plus advances in statistical modelling approaches and geographic tools, such as geographical information systems and remote sensing [6], the study of spatial dynamics of infectious disease is ever increasing. Despite this, few nationwide data are available for intestinal nematode species in Africa, with the exception of a 1987 survey undertaken in Cameroon [7, 8]. In addition, to our knowledge, there have been only two geostatistical analyses of intestinal nematodes [9, 10]. The present study was carried out to describe the epidemiology and the spatial distribution of intestinal nematodes (*Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms, *Ancylostoma duodenale* and *Necator americanus*) in Uganda. A further aim was to investigate both large-scale and small-scale spatial

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patterns of infection, thereby shedding additional light on the ecology of intestinal nematodes.

## SUBJECTS AND METHODS

Between 1998 and 2002, cross-sectional surveys of schoolchildren were carried out to determine the geographical distribution of helminths. No previous mass treatment had been undertaken in any of the schools or communities. Survey locations were selected on the basis of ecological differences in rainfall and temperature. These ecological zones were identified using a combination of expert opinion and ecological zone mapping [11]. Collected samples were examined in duplicate within 30 min using the Kato-Katz method. Participation was voluntary and had been approved by the school committee. Mass treatment of the whole school with albendazole was undertaken where the prevalence of infection exceeded 50% of the study group and specific treatment given to infected individuals otherwise. The geographical location of each school was recorded in the field by means of a global positioning system (Magellan Systems Corp., San Dimas, CA, USA), and spatial data were displayed in ArcView (Version 3.3, ESRI, CA, USA).

To identify potential large-scale environmental correlates of large-scale infection patterns, the study investigated measures of temperature and rainfall. Estimates of land surface temperature were derived from the Pathfinder Advanced Very High Resolution Radiometer (AVHRR) Land dataset and processed using standard procedures outlined by Hay [12]. Estimates of annual total rainfall were obtained from the Spatial Characterization Tool [13]. The selection of these variables was *a priori* on published studies on the role of temperature and humidity influencing the free-living stages of nematode species (see ref. [14] for a review). To allow for the potential non-linear relationships of infection prevalence to these environmental variables, generalized additive models (GAMs) were used to fit non-parametric functions to the relationships between the response and predictors [15]. Terms were fitted using a locally weighted regression smoother to summarize the relationship between predictors and the response. Because of the marked skewness in the raw data, prevalence ( $p$ ) estimates were logarithmically transformed:  $\ln(p+0.5)$ .

We used semivariogram analysis to investigate the spatial structure and spatial autocorrelation in the survey data [16]. The semivariogram estimates how

data are related with distance and direction, and presents the semivariance of the pairs of observations that are separated by the same distance; thus, describing how similar observations are at different separation distances (see work by Srividya et al. [4] and Gyapong et al. [5] for recent applications to filariasis). If there is spatial autocorrelation in the data, values are typically low and the semivariance increases with separation distance. Levelling out of the semivariogram indicates lack of spatial autocorrelation. The value where the semivariogram level out is called the sill and the separation distance at which the sill is attained is called the range, which is arbitrarily defined to be that distance at which 95% of the sill is reached. Where the semivariogram is extrapolated to cross the  $y$ -axis is called the nugget, which represents the micro-scale variation or measurement error. In order to provide a continuous description of the covariance structure, various spatial models (spherical or exponential) were fitted to empirical variogram points using nonlinear least squares [17]. All the analyses were carried out using S-Plus 2000 (Mathsoft, WA, USA) and its spatial analysis module, S+SpatialStats [17].

## RESULTS

Data were available for 13 378 children from 191 schools in 39 of the 56 (69.6%) districts in Uganda. The age range of the study population was 5–21 years (mean 10.9 years) and the male to female ratio was 1.08:1. The mean number of children in each school was 69 (range 27–142). The overall prevalence of *A. lumbricoides* was 5.3% (range 0–66.7% by school), *T. trichiura* was 3.8% (0–45.0% by school), and hookworm was 51.6% (0–94.4% by school). Overall, 54.8% of the children were infected with *A. lumbricoides*, *T. trichiura* or hookworm.

Figure 1 shows the geographical distribution of each species by school and demonstrates that the prevalence of *A. lumbricoides* and *T. trichiura* was greatest in southwest Uganda, with moderate prevalences occurring along the northern shores of Lake Victoria and an absence of transmission in the north and northeast of the country. By contrast, hookworm was more widespread throughout the country, although prevalence was lower in northeastern regions. These distributions are likely to reflect both large-scale spatial trends as the result of climatic factors, and small-scale spatial trends as the result of local spatially variable factors.

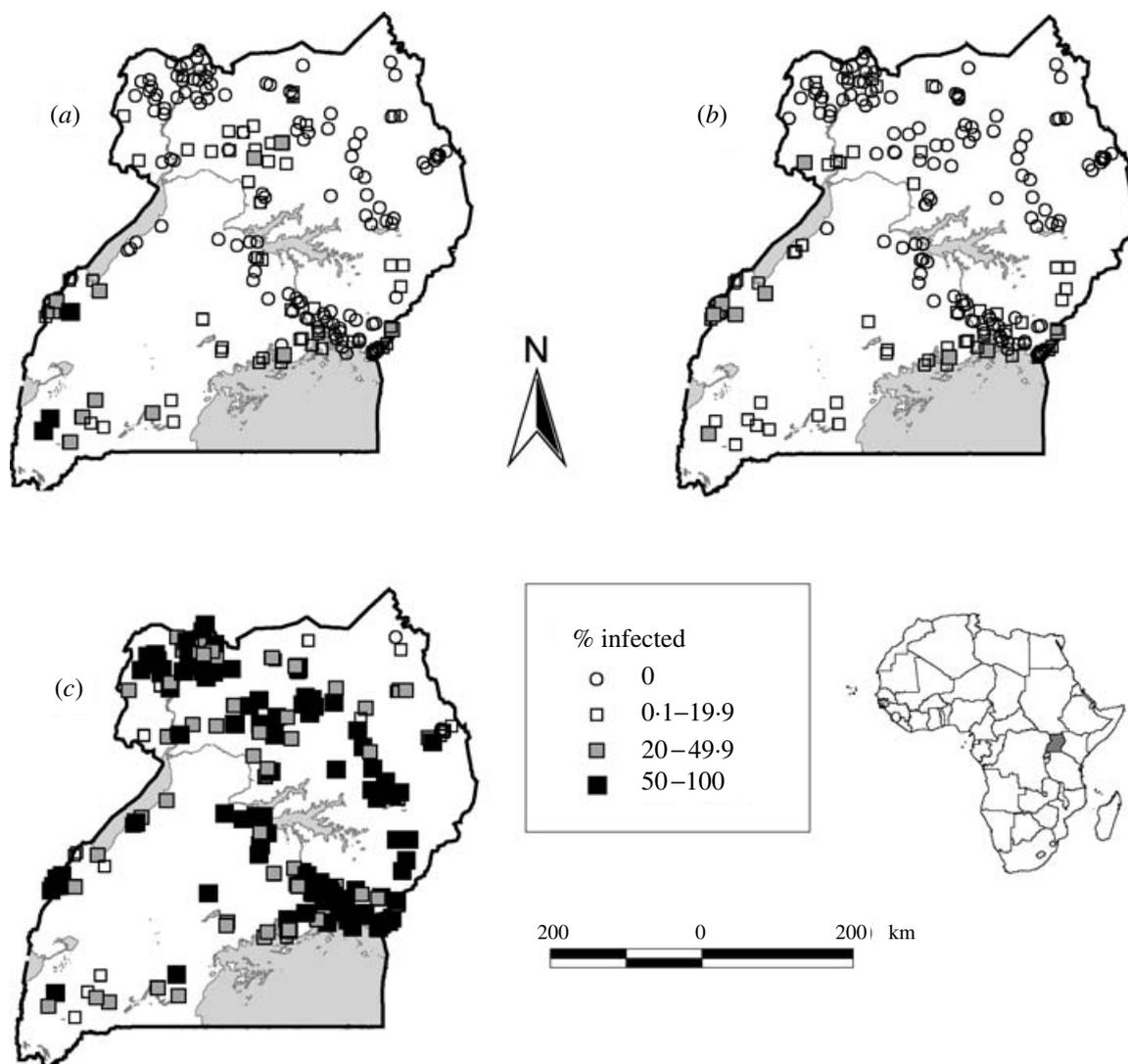
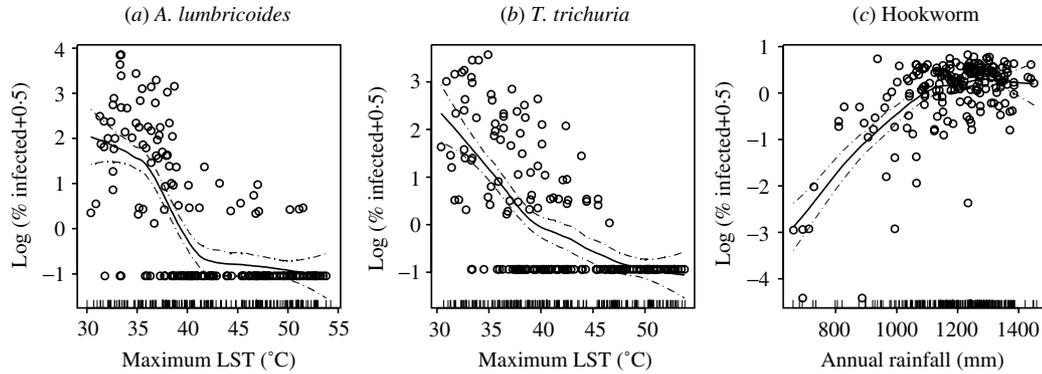


Fig. 1. Geographical distribution of (a) *A. lumbricoides*, (b) *T. trichiura*, and (c) hookworm in 191 km schools in Uganda.

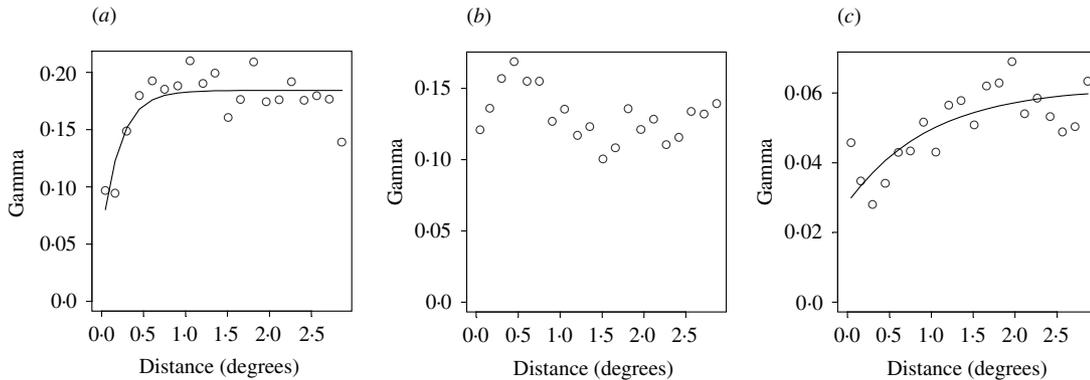
To investigate the large-scale spatial trends and the role of climatic factors, Figure 2 shows the relationships between species log prevalence and maximum land surface temperature (LST) and rainfall. GAMs were fitted separately between each species log prevalence and each of the climatic variables. In this analysis, there was a significant nonlinear relationship between maximum LST and *A. lumbricoides* log prevalence ( $F=5.7$ , D.F. = 1, 2.7,  $P=0.0002$ ) and *T. trichiura* log prevalence ( $F=7.2$ , D.F. = 1, 2.7,  $P=0.001$ ), but no significant relationship with hookworm log prevalence ( $F=2.3$ , D.F. = 1, 2.7,  $P=0.08$ ). These trends indicate that prevalences of *A. lumbricoides* and *T. trichiura* were low (transformed estimated prevalence is  $<5\%$ ) in areas where maximum LST exceeded  $38-40^\circ\text{C}$  (Fig. 2a,b), whereas hookworm was prevalent throughout the thermal range

found in Uganda (data not shown). There was also a significant nonlinear relationship between annual total rainfall and hookworm log prevalence ( $F=5.1$ , D.F. = 1, 3.4,  $P=0.001$ ) (Fig. 2c). While there was a significant relationship between rainfall and *A. lumbricoides* log prevalence ( $F=5.7$ , D.F. = 1, 3.4,  $P<0.0001$ ) and *T. trichiura* log prevalence ( $F=7.2$ , D.F. = 1, 3.4,  $P=0.001$ ), there was no clear trend in the relationship (data not shown).

To investigate the small-scale spatial structure of infection patterns, semivariograms were estimated on the basis of logarithmically transformed prevalence. Initial analysis indicated that the semivariograms failed to rise to an upper bound, suggesting the presence of a large-scale trend, shown above to reflect in part climatic factors. To remove this apparent trend and ensure an isotropic spatial process, GAMs were



**Fig. 2.** Relationships between log prevalence of *A. lumbricoides*, *T. trichiura*, and hookworm and climatic variables. Each plot shows the relationship of the fitted function to the response variable. Points represent observed relationship and solid lines represent fitted generalized additive model to the log prevalence data using maximum land surface temperature (LST) and total annual rainfall.



**Fig. 3.** Omnidirectional semivariograms and best-fitted lines of exponential spatial models for de-trended log prevalence data based on generalized additive model residuals of longitude, latitude, rainfall and maximum land surface temperature from 191 schools in Uganda for (a) *A. lumbricoides*, (b) *T. trichiura* and (c) hookworm. Parameter values of the fitted spatial model for *A. lumbricoides* were range=0.2194271, sill=0.1276312, nugget=0.0565912; and for hookworm were range=0.983877, sill=0.03317698, nugget=0.02842463. No spatial model could be developed for *T. trichiura* because of the lack of spatial structure in the semivariogram values. Directional semivariograms did not differ from the omnidirectional variograms and, therefore, an isotropic spatial process was assumed, and omnidirectional variograms presented.

fitted to log prevalence using survey locations (longitude and latitude), temperature and rainfall as predictors, and the resultant residuals were used to estimate the semivariogram [4, 17]. The species-specific semivariograms following removal of large-scale spatial trends and best-fitting lines of spatial models are shown in Figure 3. There was an apparent lack of spatial correlation or structure for *T. trichiura* across all separation distances (Fig. 3*b*). This so-called ‘pure nugget effect’ [16] indicates that there is considerable spatial variability in log prevalence and that once large-scale, principally environmental, trends are removed there is no spatial structure in the distribution of *T. trichiura*. This also indicates that the environmental variables, namely temperature, explained most of the spatial pattern in the data. The sample variogram and fitted spatial model for *A. lumbricoides*

showed somewhat more spatial structure than for *T. trichiura* but also demonstrated much spatial variability at relatively short distances and there was a lack of spatial autocorrelation at approximately 33 km, as evidenced by the estimated range of 0.2° (Fig. 3*a*). In contrast to *A. lumbricoides* and *T. trichiura*, the variogram for hookworm showed considerable spatial structure up to a range of 1.1°, approximately 123 km, even after removing large-scale trend effects (Fig. 3*c*). This result also indicates that factors other than rainfall are influencing the spatial structure of hookworm prevalence.

**DISCUSSION**

Knowledge of the spatial epidemiology of parasitic infection is important from the viewpoints of both

planning surveys and scientific understanding of parasite transmission and epidemiology [2, 3, 5]. This study represents one of the first detailed investigations of the spatial epidemiology of intestinal nematodes on a national level. The use of geostatistical and mapping techniques, in combination with remote-sensing satellite sensor and interpolated climate surfaces, enabled the investigation of both large-scale and small-scale spatial patterns in Uganda. It is important to note that data were collected over a relatively short time period (4 years) and that no previous large-scale treatment programmes have been undertaken, thus reducing potentially confounding secular effects.

An important finding of this study is that large-scale environmental factors influence large-scale infection patterns. In particular, temperature was significantly associated with the prevalence of *A. lumbricoides* and *T. trichiura* such that prevalence was generally <5% in areas where maximum LST exceeded 36–37 °C. By contrast, hookworm occurred throughout the thermal range found in Uganda (30.3–50.8 °C). This species-specific difference in thermal limits is similar to that found elsewhere in Africa. In Cameroon, for example, prevalences of *A. lumbricoides* and *T. trichiura* are shown to be greatest in the southern equatorial zone and lowest in the so-called Sahara and Sudan tropical zones [7], where maximum LST exceeds 37–40 °C [18]. Extreme thermal conditions are also suggested to prevent transmission of *A. lumbricoides* and *T. trichiura* in Chad [19]. Hookworm, by contrast, occurs throughout Cameroon [8] and much of Chad [19] suggesting hookworm has wider thermal distribution limits than either *A. lumbricoides* or *T. trichiura*.

The biological explanation for these findings can be based on an understanding of the parasites' life-cycles. The life-cycles of intestinal nematodes are direct. Eggs are produced by mature adults growing in their human host and are shed in human faeces. After a period of development in the external environment, infection with *A. lumbricoides* or *T. trichiura* occurs via ingestion of infective ova and infection of hookworm larvae typically occurs through penetration of the skin. The microclimatic requirements for embryonation and survival of nematode ova and larvae, principally in terms of temperature and moisture, have been extensively studied under laboratory conditions [20–24]. These studies show that rates of embryonation and survival are temperature-dependent and optimum conditions have been defined between 28 and 32 °C, with development of *A. lumbricoides*

and *T. trichiura* arresting between 5 and 38 °C [20, 21], and development of hookworm larvae ceasing at 40 °C [22]. The apparent ability of hookworm to survive hotter conditions can be explained in part by its ability to find suitable thermal and moisture conditions. In particular, whereas the ova of *A. lumbricoides* and *T. trichiura* are non-motile, hookworm larvae stages have limited motility and, therefore, although high surface temperatures may cause ova to die from desiccation, hookworm larvae can migrate downwards into the soil, thereby avoiding desiccation [25].

In addition to temperature, soil moisture and relative atmospheric humidity are known to influence the development and survival of ova and larvae: higher humidities are associated with faster development of ova; and at low humidity the ova of *A. lumbricoides* and *T. trichiura* do not embryonate [23, 24]. Humidity is inversely correlated with temperature, such that high temperatures are associated with humidities too low for embryonation. In addition, since rainfall is positively correlated with atmospheric humidity it is unsurprising that annual rainfall has been shown to be associated with prevalence patterns. Studies from West Africa suggest that a minimum of 1400 mm annual rainfall is necessary for the prevalence of *A. lumbricoides* to exceed 10% [14, 26]. In Uganda, by contrast, prevalence greater than 10% is observed in areas with an annual rainfall between 1000–1400 mm. This apparent regional difference may reflect differential moisture retention of varying types of vegetation and land-use that occur in East and West Africa. The observed association between hookworm prevalence and rainfall corroborates earlier findings from East Africa [27]. These observational studies are interpretable by work by Nwosu & Anya [28] who found that the abundance of hookworm larvae available in the environment is related to a derived wetness index.

Thus, our observed statistical relationships are consistent and interpretable with the known biology of intestinal nematodes, and provide justification for using climate variables in identifying large-scale distributional limits of transmission. The finding that a few climatic factors influence the boundaries of transmission has been demonstrated for other parasites and their vectors. For example, satellite-derived maximum monthly temperature appeared to determine alone the distribution of the tsetse fly, *Glossina morsitans* (vector for African trypanosomiasis) at the edge of its continental range [29].

The impact of other factors, such as variability in human behaviour, including personal hygiene, as well as differences in sanitation and socioeconomic status, are undoubtedly important in influencing local transmission patterns but we suggest are unlikely to influence the large-scale limits of transmission in a manner similar to climatic factors. Rather such non-climatic factors will influence small-scale patterns, evident within environmentally determined, distributional limits. Such a suggestion is confirmed by our application of geostatistical techniques [16] which indicated that once large-scale trends – so-called first-order effects – were removed, *T. trichiura* prevalence in Uganda indicated no small-scale spatial structure and that *A. lumbricoides* prevalence showed much smaller-scale spatial dependency than hookworm prevalence. These findings confirm the suggestion that climatic variables influence large-scale trends in prevalence of *A. lumbricoides* and *T. trichiura*, but that there exists considerable local stochastic spatial variation, due presumably to unobserved differences in personal hygiene, sanitation and socioeconomic status.

Consequently, large-scale distributions of *A. lumbricoides* and *T. trichiura*, therefore, may be predicted on the basis of remotely sensed environmental factors, as has been shown previously for Cameroon [18], but that small-scale distributions may not. This is because these species showed little or no spatial structure at local scales. The ability to predict large-scale but not small-scale patterns has recently been emphasized for *Schistosoma haematobium* [11]. In contrast, hookworm prevalence showed considerably more spatial correlation structure, so-called second-order effects. Why there is this spatial structure in hookworm prevalence is at present unclear, but there appears to be locally important spatial structuring factors. Mindful of the central importance of scale [30, 31], further investigations at varying spatial scales are required to better understand spatial structure of hookworm and underlying epidemiological factors. The prediction of hookworm spatial patterns has not previously been possible on the basis of large-scale environmental factors [18], but the spatial structure evidenced here suggests that prediction may be possible if underlying epidemiological factors can be identified and mapped. Previous study in South Africa, for example, found a strong association between patterns of hookworm infection and soil type and satellite sensor-derived normalized difference vegetation index (NDVI) [9].

Alternatively, spatial interpolation methods such as kriging to estimate hookworm prevalence at any point from the observed data may be possible on the basis of spatially detailed stratified hookworm samples. For filariasis, the World Health Organization proposes a sampling grid of 25 × 25 km for the rapid mapping of filariasis antigenaemia prevalence [5, 32], although this has been questioned [4]. The present results suggest that a similar sampling design could usefully be adopted for hookworm, but that different sampling frames are ideally required for *A. lumbricoides* or *T. trichiura* if spatial variability in prevalence is to be adequately captured. However, this is clearly offset against the practical convenience of combining surveys of intestinal species.

The current study, which forms part of ongoing work to better define the distribution of intestinal nematodes in Africa, has highlighted the role that GAMs and geostatistics can play in investigating both large- and small-scale spatial infection patterns. Such an approach can usefully be applied to other settings, as well as other parasitic species and contribute towards improving our understanding of the spatial ecology of parasites and the contribution this knowledge can have for the sampling plans of future surveys.

## ACKNOWLEDGEMENTS

We thank the Division of Vector Control technicians and our data manager Jackson Rwaheru for their hard work and enthusiasm, and extend sincere thanks to the schoolchildren, teachers and community members who participated in the study. Fieldwork received financial support from the Danish Bilharziasis Laboratory, World Health Organization, World Food Programme and the Wellcome Trust [through its provision of an Advanced Training Fellowship to S.B. (062692)]. The satellite data used in this study include data produced through funding from the Earth Observing System Pathfinder Program of NASA's Mission to Planet Earth in cooperation with the National Oceanic and Atmospheric Administration. The data were provided by the Earth Observing System Data and Information System (EOSDIS), Distributed Active Archive Center (DAAC) at Goddard Space Flight Center which archives, manages and distributes this dataset. We thank Simon Hay for processing these data. Finally, we thank the Director General of Health Services for giving us permission to publish the results.

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