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Review Article

Spatial awareness in parasite immuno-epidemiology

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INTRODUCTION

Experimental studies using animal models have vastly improved our understanding of the host–parasite relationship in many parasitic diseases. Manipulation of a passive host under carefully controlled conditions and with increasingly sophisticated laboratory techniques benefits the reductionist approach, by allowing ever-finer details of the mechanism of interaction between host and parasite to be resolved.

However, it is well recognized that animal models may not accurately represent the situation regarding parasites and their human hosts. In building up our knowledge of the host–parasite relationship, it is therefore important to conduct basic research on human subjects as far as ethically and logistically possible.

Immuno-epidemiology studies of parasitic infections in humans presents numerous challenges beyond those found in the laboratory. One major obstacle to resolving the finer details of the host–parasite relationship in humans is that subjects are inherently more heterogeneous in many respects than laboratory-bred animals, and the sources of variation are difficult to quantify or control. In particular, it is almost impossible to control the tremendous amount of variation in the human population in terms of their demographic and genetic background, as well as history of exposure to the infection of interest, and other infections, all of which may profoundly affect the measured outcome. Instead, investigators need to collect sufficient information about important demographic and environmental factors so that the results of any analysis are appropriately adjusted for their influence. Practical and financial constraints often limit the amount of information collected, with the result that a large proportion of the variance in the measured responses remains unexplained. It is, however, becomingly increasingly recognized that the locality (rather than just the household) in which individuals live is a useful proxy for a considerable amount of information related to demographic, and environmental factors. Discussion of the use of geographical techniques in immuno-epidemiology is therefore both timely and warranted.

In this article we first summarize demographic and environmental factors that are known to affect infection patterns, morbidity and immune responses. We then discuss the particularly important contribution that co-infections have on the response to specific pathogens, and describe how collection of location data can facilitate estimating exposure to several pathogens within a single community. Finally, we outline the procedures for identifying and analysing variables with spatial structure. For discussions of remote sensing and larger scale application of Geographical Information Systems in control of parasitic infections the reader is referred elsewhere (1).

Demographic and genetic influences

Historically, immuno-epidemiological studies of human subjects involved selecting representative cohort members from a population, and stratifying data by age (or age group) and sex. These two demographic variables are well-recognized sources of variation in exposure and response that are both easily and accurately measured in the field. For example, in the field of schistosome immuno-epidemiology, it is often observed that infection intensity measured in terms of egg counts vary strongly by age and sex. Serological surveys have also observed that antibody isotype responses vary strongly with both age and sex (2–4). Human immune responses and regulatory mediators also vary with age in both healthy volunteers (5,6) and in response to parasitic protozoan (7,8) and helminth (9,10) antigens. A recent example of where both age and sex were important factors in affecting the relationship between immune responses and disease was observed in a study of *Schistosoma mansoni*

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infection in a fishing community in Uganda (11). Duration of residency in an area where infections are endemic has been implicated not only in the development of antibody response profiles (12), but also in the risk of presenting with periportal fibrosis by a given age (13).

The relative roles of genetic and environmental factors in the development of both immunity and risk of morbidity have been difficult to delineate in the past due to the problem of genetically related individuals often living close together. In one study of schistosomiasis, shared household (irrespective of genetic relatedness of occupants) was observed to account for 22% of the variance in *S. mansoni* egg counts (14) and clustering of malaria infections at the household level is common due to, among other things, variation in house construction and bed net use. Nonetheless, a number of studies have been able to identify genetic factors associated with susceptibility to infection and disease and immune responses to several parasitic infections, including malaria (15–17) and schistosomiasis (18–20).

Other environmental influences

Climatic and topographical factors have long been recognized as important determinants of the distribution of parasitic infections between communities. For example, the transmission potential of geohelminth infections is determined partly by surface temperature (21), altitude, soil type and rainfall (22,23). Schistosome infections are often referred to as being 'focally' transmitted, due to the patchy distribution of intermediate host snails (24) and water contact patterns that may vary amongst even culturally similar communities (25). The distribution and incidence of malaria infections between villages is limited by, among other things, altitude (26,27). Clustering of malaria infections in certain communities is also likely to be due to environmental restriction of *Anopheles* abundance (28).

Many environmental factors also operate on a finer scale, and it is possible to observe heterogeneity of exposure within communities for certain types of infection, particularly if the source of infection itself is contained within a small area. For example, a recent analysis of *S. mansoni* egg counts within a 10×4 km area of a village in Kenya revealed a clustering of egg counts at one end of the study area, probably due to the patchy distribution of *Biomphalaria* snails along the course of the local river (29). Elsewhere in Kenya, a geo-statistical analysis of *S. haematobium* infections within a community observed age-related clusters of infections within an 11 km^2 area around a pond with high numbers of *Bulinus* snails (30). Distance to vector breeding sites is also important. In Mozambique, for example, a significantly reduced risk of acute malaria was associated with living more than half a kilometre away, coinciding with a reduced prevalence of parasitaemia (31). This small-scale variation in exposure is reflected in other studies that have demonstrated a rapid change in malaria-related splenomegaly, but an increased risk of severe malaria, further away from known breeding sites (32).

Behavioural factors

Location within certain parts of a village may also indicate neighbourhood-level behavioural differences with regards to the risk of infection. For example, in communities living along Lake Albert, the two main tribes (Alur and Bagungu) often inhabit different areas within the same village. Differences in behaviour of the two tribes include the nature of water contact, with females of the Alur tribe reporting that they have less contact than females of the Bagungu tribe (MB, personal observations). Distance to a health centre is particularly important as it may strongly affect utilization of health resources (33,34), and treatment for chronic infections may not only have an impact on the level of infection, but may also result in a more vigorous immune response on challenge.

The influence of co-infections

Of all the factors that can modulate the immunological and inflammatory responses to a parasitic infection, the influence of co-infecting pathogens is particularly important. The catastrophic immuno-compromising effects of HIV infection on responses to other infectious agents are well documented, however, we are becoming increasingly aware of less dramatic immunological interactions between commonly occurring chronic infections that might influence human immune responses to a parasite of interest.

Apart from the overwhelming immuno-compromising effects of HIV infection, the immunological influence of one infectious agent on responses to another is difficult to demonstrate in humans. However, in *in vivo* experimental models the influence of parasite infections on immune responses to unrelated antigens (35) or to co-infecting pathogens is now well established.

Much of the theoretic framework for these immunological interactions between co-infecting parasites is based on the Th1–Th2 cytokine counter-regulation paradigm of immune control, with protozoan, as well as viral and bacterial, pathogens promoting Th1 responses and helminths inducing counter-regulating IL-4 associated Th2 responses. In mouse models it has been shown that protozoan infections such as *Toxoplasma gondii* can down-regulate Th2 responses against *Nippostrongylus brasiliensis*, allowing higher and prolonged nematode egg production (36). On the other hand, a pre-existing murine Th2 environment

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resulting from *S. mansoni* infection can suppress the IFNγ, TNFα and NO responses to a subsequent *Leishmania major* infection and impair the ability of macrophages to kill *L. major in vitro* (37). Similarly, *Fasciola hepatica* infection suppresses protective Th1 responses against *Bordella pertussis* (38), and persistent *Taenia crassiceps* cysticerci infection suppresses murine responses to *Trypanosoma cruzi* and increases susceptibility infection (39). *S. mansoni* infection can up-regulate Th2 responses in mice that are susceptible to chronic *Trichuris muris* infections and thereby facilitate the expulsion of co-infecting *Trichuris* (40). Co-infections can also synergize to cause increased morbidity; for example, *Toxoplasma gondii* and *S. mansoni* induce high plasma levels of TNFα, hepatocyte dysfunction and early death in co-infected C57BL/6 mice (41).

It is now becoming clear that differential recognition of different classes of pathogen by the innate immune system (42) and specific interactions with a number of regulatory mechanisms (43,44) underlie many of these inter-species immune modulatory effects of co-infections. However, the implications of such interactions for infection and disease at the human population level are yet to be systematically addressed, even though they could have a significant influence on immuno- and clinico-epidemiological data. The principle that parasite infections can modulate human immune responses against unrelated antigenic stimuli has been established by reports that helminth infections can affect human vaccine responses. Thus, *S. mansoni* infected individuals have impaired Th1 responses to vaccination with tetanus toxoid (45), and children born of mothers infected with *Wuchereria bancrofti* or *S. haematobium* have 26-fold lower IFNγ responses to BCG vaccination than children born of non-infected mothers (46). Viral infection can also switch human immune responses to helminth infections from Th2 to Th1. Thus, HTLV-1 infection decreases IL-5 and IgE responses in patients with strongyloidiasis (47) and schistosomiasis (48).

LOCATION AND PROXIES OF EXPOSURE

A common hypothesis in parasite immunology is that variation in the current level of infection is associated with variation in the measured immune response. The infection level is most commonly measured indirectly as faecal egg counts, or parasitaemia, depending on whether the parasite is a helminth or a protozoan species. It has, though, been evident for some time that current infection level is often not measured accurately in humans, and may not even be the most appropriate proxy of either recent or long-term exposure to infection. Someone living close to a source of exposure may not yield a high egg count or parasitaemia at time of sampling, despite being relatively highly exposed over a long period of time due to day-to-day fluctuations in egg count, or seasonal incidence in transmission. In such cases, the label 'uninfected' may be uninformative. Location itself may be a more reliable indicator of exposure than parasitological measurements, particularly if reference to a known source of infection can be made (e.g. distance to a river). Depending on the nature of the survey, it may be possible to proxy exposure more directly with serological or vector maps.

Serological mapping

The use of serology for individual diagnosis in areas where an infection is highly prevalent has limited scope for distinguishing between previous and current infection. However, it has been recently suggested that serology may be useful in the context of control (49), following many observations that commonly used diagnostic tests based on the detection of parasite ova or parasitaemia have low sensitivity (50,51). A positive antibody test at least indicates prior exposure. Although advocated for control purposes, the mapping of antibody responses adds value to basic research studies, in being able to confirm prior exposure in the absence of parasitological data demonstrating current infection. Antibody responses to parasite antigen can be mapped, and areas where exposed individuals live may be easily identified.

Serology data can be used to map the distribution of either antibody responses or circulating parasite antigen in individuals across a study area, and allow many infections to be mapped quickly and easily from a single sample survey. It is also possible to map toxins, such as aflatoxin, in order to examine the potential influence of environmental contaminants on the outcome of interest. The same sample can, of course, also be used to measure inflammatory markers, immunoregulatory molecules, physiological and disease markers, all of which can be mapped.

In relation to infections with distinct seasonality, serological data offer distinct advantages over parasitology. The sero-prevalence of an infection is less susceptible to dynamic fluctuations than the parasitologically derived infection prevalence. For example, we have observed in a study in Kenya that whereas malaria parasitaemia varied between 16% and 70% from dry to wet season, the prevalence of IgG3 response to schizont antigen was constant (S. Wilson, personal communication). Screening of a population using serological data can therefore be useful for identifying areas of relatively high exposure outside a transmission season. This latter aspect was recently used in another study in Kenya, where the IgG3 response to malaria schizont antigen was mapped across a 10×4 km study area. Blood samples were taken several months after the end of the transmission

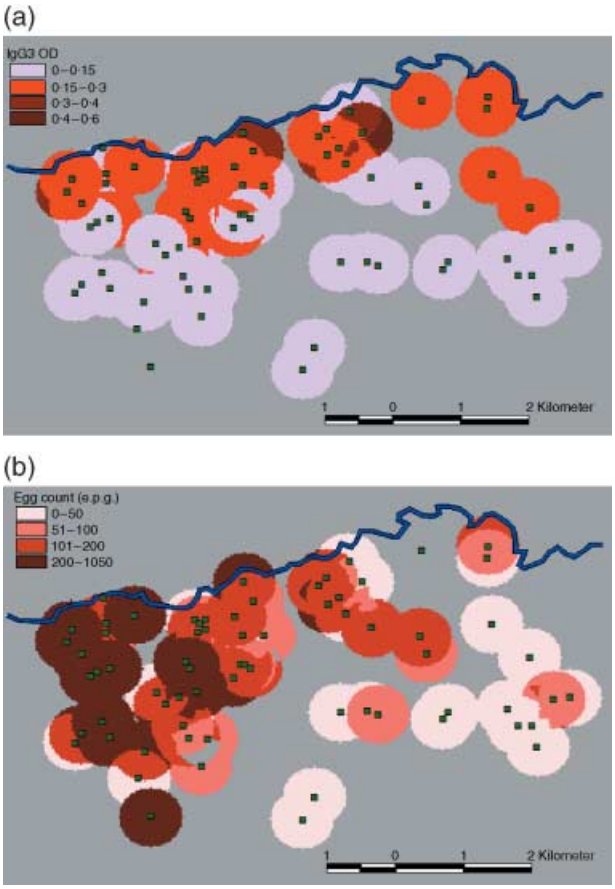


Figure 1 Neighbourhood chloropleth maps of childhood IgG3 response to *Plasmodium falciparum* schizont antigen (a) and *Schistosoma mansoni* egg counts (b) in a Kenyan village, adapted from Booth *et al*. (29). The maps use data averaged at the household level. Coloured circles of 0·5 km radius around each house give a partly smoothed surface, and are coloured according to the band in which the average egg count in that household falls. Where circles overlap, the data from houses within each circle are aggregated and then averaged. The colour of the overlapping area is then re-assigned, if necessary, to reflect this average value. These maps were used to identify areas of relatively high or low exposure to either or both infections.

season, yet there was a significant clustering of high IgG3 responses along the course of the local river (29). The spatial clustering of these responses is shown in Figure 1.

Vector mapping

One of the more extensive uses of spatial data in parasite epidemiology thus far has been mapping of vector species. Historically, the transmission intensity of parasitic infections was estimated at the level of whole communities or even larger administrative areas. Whilst this approach is relevant for allocation of resources in control efforts, it can lead to severe ecological bias if used for basic research by miscategorizing large numbers of individuals in terms of their level of exposure to infection. Introduction of GPS (Global Positioning System) technology to entomological surveys within single communities has allowed much finer resolution maps of vector distribution to be drawn (52–54). This type of mapping has great relevance in immuno-epidemiology, as the distribution of vectors or intermediate hosts may have a direct influence on the level of exposure. Furthermore, the estimation of exposure based on measurements of parasitic loads in vectors (e.g. sporozoite rates in mosquitoes), or intermediate hosts (e.g. cercarial shedding of snails) can be used to confirm that antibody measurements are not simply a measurement of prior exposure, but do also reflect more recent infection events.

Mapping of co-infections

Given that co-infections can have a profound effect on numerous outcomes, it is important to record during immuno-epidemiological studies who is infected with which specific combinations of pathogens. This has become a particularly important issue in relation to the apparent associations between malaria and helminth infections. It has been suggested that an underlying chronic helminth infection may increase the incidence of malaria, perhaps as a result of biasing the host immune response towards a susceptible Th2 phenotype (55); however, helminth infection may also concurrently protect against cerebral malaria (56) and malaria-associated hepatomegaly (57).

Parasitological surveys often record the presence or absence of multiple species of pathogen (58), and if location data are recorded for each individual, it is then possible to construct maps (see below). The limit of this approach is seen when using one diagnostic technique of differing sensitivity for different infections, which may give a misleading picture in terms of the prevalence of co-infections (59). It is also often not possible within a single parasitological survey to record the presence of each parasite that is endemic within a community, particularly if one or more infections have a distinct seasonal incidence. This presents a problem at the analytical stage unless a proxy measure of exposure to each endemic infection is available. It may therefore be worthwhile to map the likely geographical distribution of infection using proxy variables, such as serological or vector intensity data.

Mapping the distribution of, or exposure to, co-infections within a community facilitates determining whether an apparent association between two infections is due to increased susceptibility, as a result of immunological or other physiological interactions, or merely due to coincidental co-exposure to the two infectious agents. Conversely, if

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two infections rarely occur in the same individuals, mapping their geographical distribution may reveal a lack of overlap in geographical distribution of the two infections rather than the competitive exclusion from the host of one species by the other.

MAPPING IN PRACTICE

Any mapping exercise begins by recording the precise location (geo-referencing) of features of interest, such as houses of cohort members, positions of local health centres, known sites of exposure, sources of safe water (for example, water kiosks and boreholes). The equipment required is a hand-held GPS receiver and a computer for downloading the co-ordinates. Since the removal of 'selective availability' by the US government (www.igeb.gov/sa.shtml), it has been possible to obtain latitude and longitude values accurate to within a few metres, though the accuracy of the co-ordinates depends on the number of visible satellites, and it may be sometimes difficult to distinguish individual households that are close together. Alternatively, and more expensively, high-resolution satellite images can be used to identify the positions of houses from the air; the information being later linked to data from surveys on the ground (30).

Without any analysis, co-ordinates can be used to produce scale maps of a study area in programs such as Arcview™. Once the locations of cohort members' places of domicile have been recorded, any attributes that were recorded can also be displayed as point data. This elementary step can give the first indication that some variables are spatially clustered. Chloropleth maps are particularly useful in visualizing the spatial distribution of a particular variable using graduations in colour or tone across the study area (29).

Creating smooth maps that extend over areas without observations can be achieved using techniques that are based on the tenet that measurements taken closer together are more similar than those taken further away. The process of kriging is commonly used in mapping the geographical distribution of pollutants (60,61), but can be used for any variable exhibiting spatial clustering. Although there are several algorithms and variation in scope, kriging essentially allows the level of a variable in the study area to be estimated from data collected at any other two points. The technique was recently used to map the spatial distribution of *Echinococcus* infections in foxes (62), and has also been applied to map *Ascaris* infections in Brazil (63). Kleinschmidt and colleagues used this method for constructing a map of malaria in KwaZulu Natal (64), and the same approach has also been taken to map the geographical distribution of anopheline mosquitoes (65). In the field of immuno-epidemiology, kriging has potential in serological mapping of areas where no data are available, although the method may give conservative estimates as extrapolation beyond the limits of empirical measurements is not possible.

Identifying spatially clustered data within a study area

Although maps can give visual clues as to spatial clustering of a variable, inferences are severely limited unless the clusters are formally identified. One of the controversies in spatial epidemiology, though, has been in the definition of a cluster, as cases of infection or disease may be close together in either time or space by chance. Simply visualizing a cluster of a few cases may therefore be misleading. A particular problem associated with small area studies is 'data noise' or stochasticity: clusters may not be large enough to reach significance, or disease rates may be unstable. The 'action space' of individuals is also a potential problem, as individuals may move from place to place either as part of their daily routine or for other reasons. This could affect their short, medium and/or long-term exposure to a particular infection, whether vector borne (e.g. malaria, filarial worms) or indirectly transmitted (schistosomes, geohelminths). It is therefore important to record not only location, but duration of residence and movement patterns.

Several methods are in use to overcome the problem of identifying whether or not a variable exhibits spatial clustering (66). Some of these methods test for correlation amongst the number of cases in neighbouring areas (the Moran test), whereas others (so-called K-functions) are concerned with analysing the number of cases within a given distance from an arbitrary point. Such methods give a summary statistic indicating whether or not a particular variable is spatially clustered, but do not identify which records are clustered, nor do they pinpoint the location of a cluster. Another set of techniques seek to establish the presence of localized clusters of cases using 'moving window' algorithms. One of these, the Kulldorf scan (67), implemented in the SatScan program (http://satscan.org), has been recently used to locate clusters of malaria infections in the highlands of Kenya (27) as well as clusters of giardiasis in Ontario, Canada (68). Scanning algorithms typically work by superimposing circles of varying radius onto the study area, and comparing the number of cases inside the circle against those outside. These techniques are straightforward to implement, and are particularly useful in relieving problems associated with multiple-testing. They may also reveal secondary clusters, but assume that all clusters are spherical, and may therefore not be suitable for use where either landscape features or sources of exposure do not lend themselves to circular clustering of either infection, immune response or health parameters.

Analysis of clustered data

Geo-referencing within the context of a small-scale immuno-epidemiological study gives investigators opportunities to identify whether or not there are locally important spatially distributed factors associated with either parasitological, health or immune response parameters. If there is evidence of spatial clustering, this first means that the responses measured in different people may be more similar than would be expected by chance (in the same way that measurements amongst closely related individuals are more likely to be similar than amongst distantly related or unrelated individuals). Subsequent analysis may need to adjust for this dependency. Although complicating the analysis, the correct analysis of spatially clustered data can also add an extra dimension to the study and lead to novel inferences.

Common methods for accommodating covariates and confounding variables in epidemiology include Poisson, multiple linear, and logisitic regression. Ignoring spatial structure in these analyses can have the same effect as reducing the sample size – larger standard errors and uncertainty in whether or not a parameter estimate is truly significant at the given level of significance (69). Spatial regression techniques extend linear regression by incorporating the spatial correlation of the data into the model (reviewed by Waller & Gotway, 2004 (70)). One approach is to construct a regression model that includes outcome and predictors of interest. The residuals can be tested for spatial patterning, and the covariance structure modified to ensure that the estimates are adjusted for the spatial correlation. This approach was recently taken to identify factors associated with small-area heterogeneity in malaria incidence in South Africa (64).

SPATIO-TEMPORAL AWARENESS

One of the advantages of collecting geographical information during a study is that unless the environment changes during the study period, or there is movement of the population within or away from the area, it is possible to analyse associated data either prospectively or retrospectively. This facilitates the further analysis of existing data, or allows the impact of location on the outcome of an intervention to be estimated. For example, immuno-epidemiological studies of schistosomiasis have demonstrated that both humoral and cellular responses increase after praziquantel treatment, but there is considerable variation observed, much of which usually remains unexplained. Treatment may not only impact on the level of a particular infection, but also will have potentially unpredictable effects on immune responses against the parasite targeted by the specific treatment, with some responses increasing and others declining (71–73). These effects of treatment may even extend to modulating responses against co-infecting organisms.

Including location data into the analysis has the potential to explain part of the variance in both baseline and follow-up data. The value of retrospective geo-referencing of households in longitudinal studies was recently highlighted in a study of hepatomegaly in Kenyan children (74). During the three-year follow-up period, the locations of cohort members' houses were recorded. It was then possible to map baseline variation in serological and clinical measurements across the study area (29), and examine whether or not location affected the regression of organomegaly after praziquantel treatment. The results indicated that children living in an area of relatively high exposure to both infections not only had the highest level of organomegaly before treatment but also throughout the three-year follow-up. As malaria infections had not been treated, the inference was that a residual burden attributable to this infection remained (75). Further analysis will focus on the spatial distribution of changes in immune responses after praziquantel treatment.

CONCLUSIONS

There is considerable evidence in the literature that the place where someone involved in an immuno-epidemiological study lives is an important factor related to a number of demographic and environmental influences. Recording location using GPS equipment is a high value, low cost extension to immuno-epidemiological studies that can be applied either prospectively or retrospectively. The tools for obtaining spatial co-ordinates are portable, require little training, and are ideally suited for fieldwork. Although potentially adding an extra layer of complexity to data analysis, the acquisition of location data can be exploited in novel ways that can lead to observations that would otherwise remain hidden. One of the major potential benefits of being spatially aware lies in being able to consider a range of environmental variables as modifiers of the immune response. Location itself can also act as a proxy measure for exposure. More complex geo-statistical analysis has the potential to considerably strengthen the value of immuno-epidemiological studies of parasitic infections. Until recently, the techniques have been mainly employed in parasitology with a view to improving control prospects or identifying risk factors for infection. It is likely that future studies that have basic research components will incorporate geo-referencing as a matter of routine, given the low cost and high added value.

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