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Advances in epidemiology survey methodology and techniques in schistosomiasis

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Quantitative techniques are now recognized to contribute to the validity and comparability of data from epidemiological studies in schistosomiasis. These methods have been developed and tested in field investigations in areas where Schistosoma mansoni *is endemic and, to a lesser extent,* S. haematobium *endemic areas. Carefully planned epidemiological investigations using standardized and quantitative methods have contributed to our understanding of the relationships between intensity of infection and morbidity, as well as to the development of improved control strategies relevant to these areas. This article reviews the newer parasitological techniques, methods of morbidity assessment, and data analysis procedures employed in current epidemiological studies in schistosomiasis, as well as the analytical questions involved in research on the epidemiology of schistosomiasis.*

Recent advances in the methodology of epidemiological research on schistosomiasis are based on a growing recognition of the acceptability of quantitative techniques in the field.

After *Schistosoma mansoni* and *S. haematobium* egg counts were shown to be relatively constant in the same individual over short periods of time (*1*, *2*), egg-counting techniques were recommended for use in epidemiological studies on these parasitic infections. Later the relationship between intensity of infection and morbidity of *S. mansoni* infection was assessed by quantitative stool examinations (*3*). Since that time, the World Health Organization (*4*, *5*) has repeatedly advocated the use of quantitative methods in all aspects of epidemiological studies on schistosomiasis.

In general, successful control programmes for schistosomiasis have been based on sound epidemiological studies using standardized, if not quantitative, methods (*6*). Quantitative techniques have a measurable variability and thus the inherent differences in their application from one area to another by different personnel can be taken into account. This increases the accuracy of epidemiological measurements such as prevalence and incidence, and also improves confidence in the validity of conclusions drawn from these data.

In spite of the inherent difficulties of field investigations of schistosomiasis, such efforts are to be encouraged, in order to:

(*a*) promote further understanding of the relationships between the intensity of infection and morbidity (*3*);

(*b*) provide reliable baseline data for intervention studies in the areas of chemotherapy (*7*, *8*), malacology (*9*, *10*), and sociology (*11*, *12*);

(*c*) provide precise information on which to base control strategies (*6*).

METHODOLOGY AND TECHNIQUES

Organization of epidemiological studies

The advent of effective and safe oral chemotherapeutic agents and other improved tools for successful control of schistosomiasis enlarges the scope of epidemiological studies beyond descriptive academic exercises. And since all epidemiological studies in endemic areas should contribute to the understanding and improvement of control strategies, close collaboration should be encouraged between those carrying out research and governmental agencies concerned with schistosomiasis control in endemic areas.

The selection of populations for epidemiological studies

Data derived from epidemiological studies of entire large communities in Ghana (*10*) and St Lucia (*6*), and from a systematic sample in Qalyub, Egypt (*13*) have appeared in the literature recently and have formed the basis for testing and refining combined control strategies.

The growing awareness of the focality and different methods of schistosomiasis transmission emphasizes

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the need for studies in carefully mapped communities in which an accurate census has been taken. The study of such small communities in endemic areas has the advantages of allowing examination of the entire population and facilitating short-term follow up. Epidemiological studies have been reported in small communities where *S. mansoni* is endemic, such as in Brazil (*14*, *15*), Ethiopia (*16*), Kenya (*17*), Puerto Rico (*18*), Sudan (*19*), and Uganda (*20*).

Similar studies in areas with endemic *S. haematobium* have been reported from Egypt (*21*), Gambia (*22*), and the United Republic of Tanzania (*23*, *24*). Longitudinal studies on urinary schistosomiasis in small populations in Nigeria (*25*) and Zanzibar (*24*) have been published. Epidemiological studies of *S. japonicum* using quantitative techniques have been reported from Indonesia (*26*) and the Philippines (*27*).

Schoolchildren, who usually represent the age groups of highest intensity of infection (*28–30*), often provide convenient study populations. Appropriately designed studies in schoolchildren may provide data on the overall prevalence and incidence of schistosomiasis (*31*).

Studies of individuals with special exposure characteristics, such as migrants who have left an endemic area (*32*, *33*), residents of non-endemic areas who have become infected in an endemic area and returned to their original residence (*34*), or individuals with constant re-exposure (*35*), afford important opportunities to study the natural history of schistosomiasis.

Quantitative parasitological techniques

Quantitative techniques of parasitological examination have, in recent years, replaced qualitative procedures in most community studies of schistosomiasis because of the useful additional information provided by quantification of egg output (*36*). Although parasitological techniques are often selected because of personal preference and available laboratory support, there is a need for more objective criteria on the basis of which the investigator can select the most appropriate methods to accomplish a specific objective.

S. mansoni *infections.* The filtration-ninhydrin-staining technique for *S. mansoni* egg counts (*37*) and its modifications for field use (*38*, *39*) have been used in population studies in Brazil (*15*), St Lucia (*40*), and Uganda (*20*). Stool specimens fixed and stored in merthiolate/formaldehyde solutions may be transported from the field to a central laboratory. Egg counts obtained by this method are usually lower than those obtained by other quantitative methods (*41–43*).

The Kato thick-smear technique (*44*), with various modifications to measure specified amounts of faeces (*45*, *46*) and to stain *S. mansoni* eggs, has been used increasingly because of its relative ease, speed of performance, and utility under field conditions. In populations with high egg outputs, examination of a single 50-mg specimen provides a good estimate of prevalence and intensity of infection. The technique, however, lacks sensitivity and is therefore less useful in lightly infected populations, except where the objective is only to detect individuals with relatively high egg outputs (*47*, *48*). Several studies have shown that the egg count per gram of faeces obtained by the Kato technique is higher than that obtained by the other techniques (*43*, *49*, *50*).

Concentration procedures, such as the formol–ether technique, although generally considered the most sensitive, have not been used widely in population studies because of their relative complexity and the need for a centrifuge. However, in recent years, a modified Ritchie formol–ether concentration technique (*47*) has been used successfully in studies of lightly infected populations in Egypt (*13*) and Puerto Rico (*18*), and has the advantage that the faecal specimens to be transported and stored are already fixed.

S. haematobium *infections.* New quantitative methods for counting *S. haematobium* eggs use the Sedgwick-Rafter chamber (*51*) or Millipore (*51*) or Nucleopore filters (*52*), but have rarely been used in population studies. In recent community-based studies, filter-paper techniques were used to examine either 10-ml (*22*, *23*) or 5-ml (*12*) urine specimens.

Quantitative miracidial hatching tests for *S. haematobium* and *S. mansoni* have been recommended for longitudinal evaluation of the efficacy of chemotherapy (*53*), but have not yet been used in epidemiological studies or compared with quantitative egg counts in populations.

S. japonicum *infections.* For quantitative determination of *S. japonicum* infection in the field, the merthiolate–iodine–formaldehyde concentration (MIFC) method has been most widely used. In the Philippines, Blas (*54*) has examined its limitations and has noted that examination of the sediment from two consecutive centrifugations of a single sample are necessary for 95% recovery of the total egg count. The Kato thick-smear technique has also recently been used as a quantitative measure of *S. japonicum* infection (*26*, *27*), while in China, a qualitative miracidial hatching method (*55*) has been used for detection of *S. japonicum* infection.

Immunological techniques

An immunodiagnostic test that would quantify the level of *Schistosoma* infection, discriminate between

active and past infection, and be as sensitive as available parasitological techniques would be most valuable. The available immunological techniques do not fulfil these criteria.

Skin testing. The intradermal test has obvious appeal for use in population studies of schistosomiasis because of its economy and potential for mass application. Unfortunately, the results from the intradermal tests currently available correlate poorly with infection and disease and consequently are of limited value (*30*, *56*).

Serological techniques. Ideally, serodiagnostic tests for use in epidemiological studies or in control programmes should have a slightly higher sensitivity than routine stool examinations, in order to increase the proportion of infected persons detected. Such an immunodiagnostic test would also be useful in endemic areas of low prevalence and low intensity of *Schistosoma* infection.

Numerous serodiagnostic tests for schistosomal infections are available but none possesses the specificity or sensitivity necessary for use in epidemiological studies (*57*). High initial indirect fluorescent and indirect haemagglutination antibody titres to *S. mansoni* whole-worm antigen were found in a longitudinal study in individuals whose *S. haematobium* egg counts decreased over time (*58*).

The circumoval precipitin (COP) test, although originally described for the diagnosis of *S. mansoni*, has been used most frequently as a qualitative serodiagnostic technique for *S. japonicum* infection. The preparation and storage of the antigen has not been standardized and so reproducibility cannot be ensured. Recently, the sensitivity of the COP test was shown to approach 100% in individuals with *S. mansoni* egg counts greater than 10 eggs per gram (*59*).

The major limitation of serodiagnosis in schistosomiasis is the non-availability of purified antigens rather than the test system itself. In the future, purification and characterization of *Schistosoma* antigens should provide specific antigens necessary for improved immunodiagnostic tests.

The enzyme-linked immunosorbent assay (ELISA) with crude whole-worm antigens for detection of antibodies was relatively insensitive, but the sensitivity increased when an ultracentrifuged whole-worm antigen was used (*60*). Furthermore, the ELISA test using an anodic circulating antigen from *S. mansoni* had 100% sensitivity and high specificity (*61*). This same study showed that in persons less than 30 years of age and with less than 1265 eggs per gram of faeces, there was an excellent correlation between anti-circulating-antigen antibody titre and egg count.

Recently, a radioimmunoassay utilizing a labelled purified *S. mansoni* egg antigen was found to be highly sensitive and highly specific (*62*, *63*). As yet, the rate of false positive reactions to this test and its correlation with intensity of infection in an endemic population are unknown. This radioimmunoassay procedure uses filter-paper blood samples and has been semi-automated to handle large numbers of specimens.

Morbidity

Recent studies of the morbidity of *S. mansoni* infection in populations have provided important new information on the relationships between the intensity of infection and the occurrence of associated disease. Studies of heavily infected communities in Brazil, Kenya, and Uganda (*15*, *17*, *20*), moderately infected individuals in St Lucia (*28*), and lightly infected communities in Ethiopia and Puerto Rico (*16*, *18*) have clearly demonstrated positive correlations between egg output and morbidity. Those studies have been mostly cross-sectional in design, and longitudinal observations are needed, especially in lightly infected populations, to determine the consequences of infection.

The studies cited above have led to improved definition of the methodology and techniques required to assess morbidity in populations, but they have not been adequately standardized (*64*). General agreement now exists among most epidemiologists that liver size is best determined (in centimetres) by palpating the liver edge below the right costal margin at the midclavicular line (right lobe) and at the midsternal line (left lobe) during quiet breathing. Spleen size is best measured in the supine position and recorded in centimetres below the left costal margin. Spleen and liver consistency should also be noted. In malarious areas it is useful to assess the prevalence of malaria antibodies in order to attempt to determine their confounding effect on splenomegaly rates (*15*, *19*, *29*).

Using clearly defined methods for measuring liver and spleen size, several workers have shown that enlargement of these organs is related directly to intensity of infection with *S. mansoni* (*15*, *17*, *28*) and with *S. japonicum* (*27*). Other investigations of lightly infected populations have failed to demonstrate such a relationship (*16*, *18*). Qualitative assessment of symptoms and physical signs, such as the 'clinical gradient' (*65*) does not allow for standardized comparison between different observers and different studies.

Arterial blood pressure elevation has not been correlated with *S. mansoni* infection in a community (*15*). However, in a recent study, mean blood pressure in a community where *S. haematobium* was endemic was found to be higher than in a non-endemic community although there was no correlation with the intensity of infection (*66*).

Questionnaire techniques

The utility of information derived from morbidity questionnaires is somewhat limited. In order to be meaningful, the questionnaire should be administered in a reproducible manner, free from observer bias; pretested simple questions should relate to a specified time-frame (e.g., during the past month); and finally, an uninfected comparison group (with a similar distribution of age and sex groups), which may be derived from the study population or from a separate geographical area, must also be analysed (*18*).

The prevalence of a history of abdominal pain has been shown to increase in parallel with the intensity of *S. mansoni* infection (*16*, *17*, *28*). A history of blood in the stool (*16*, *18*, *19*, *20*) did not correlate with lower haemoglobin or haematocrit levels. Other symptoms, such as 'weakness', inability to work, and diarrhoea have not been shown to be related to infection with *S. mansoni.*

No questionnaire-derived information on the morbidity from *S. haematobium* infection in communities has been published recently.

Laboratory measurements

Recent studies in Brazil and St Lucia showed a significant association between intensity of infection and occult blood in the stool (detected with guaiac or Hematest[a]) (*15*, *28*) but in a Puerto Rican community this association was not found (*18*).

Higher *S. mansoni* egg counts have not been associated with decreased haemoglobin levels (*20*, *28*, *29*) except in Sudan (*19*). Relative or absolute eosinophilia has generally not been associated with chronic *S. mansoni* infection.

In one study, liver function tests (alkaline phosphatase (EC 3.1.3.1), serum aminotransferase, and bilirubin) were not associated with the intensity of infection with *S. mansoni* (*28*). Serum albumin levels have been found to be inversely related and serum globulin levels directly related to the intensity of infection (*28*, *29*).

High levels of proteinuria were found among members of a community infected with *S. mansoni* but were not correlated with the intensity of infection or high blood pressure (*67*).

In *S. haematobium* infection, the level of proteinuria determined by urinalysis reagent strips was related to the intensity of infection (*68*). The combined criteria of at least trace haematuria and 300 mg/litre proteinuria by these strips was very specific for detection of individuals with high egg counts.

Intravenous urography has been useful for clinical assessment of urinary tract disease associated with *S. haematobium* infection. The logistic and technical difficulties of carrying out this procedure in a population have limited such studies and few have been reported (e.g., *24*, *69*). No standardized method is available for recording radiographically visible urinary tract lesions due to *S. haematobium.* Isotope renography has been performed in hospitals but community studies have not been reported.

Sociological studies

Quantitative water contact studies performed as a component of carefully planned epidemiological investigations have been reported recently from St Lucia (*11*) and Lake Volta, Ghana (*12*). In both of these areas, prevalence and intensity of infection are more closely associated with frequency and duration of water contact than with age.

Mortality data

Meaningful data on mortality from schistosomiasis have not appeared since 1969 (*24*) even in countries with a relatively well-developed health infrastructure and a vital statistics recording system. Even under favourable conditions, useful mortality data can only be obtained with difficulty from longitudinal studies of large populations over prolonged periods of time.

Analysis of data

Few papers have been concerned with the analysis and interpretation of quantitative parasitological data from population studies (*70*), especially regarding comparability and variation of results with time. Computer hardware and software is now readily accessible throughout the world and has greatly expanded the field worker's capacity for data handling and analysis. The collaboration of epidemiologists and statisticians in designing epidemiological studies, and quantifying all epidemiological measurements are necessary to exploit the full potential of computer analysis.

Standardization of egg-counting techniques

Despite the acknowledged need, little progress has been made either in the standardization of egg-counting techniques or in the development of methods to improve the comparability of results from different techniques. Sensitivity, i.e., the probability of detecting at least one egg in a known positive specimen, is a function of (i) the amount of excreta examined; (ii) the number of eggs present; (iii) the proportion of total eggs detected; and (iv) the randomness of distribution of eggs in the faeces.

Sensitivity is a most important characteristic for comparison of results from different techniques and has a profound effect on prevalence, incidence,

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intensity, and other related indices.

Criteria for the selection of the parasitological technique that is most appropriate for a given study have not been well developed. In general, a technique should be sufficiently sensitive to minimize the proportion of false negative results, and thus, in populations with light infection, a relatively sensitive method is required (*47*). However, a selectively insensitive technique may be appropriate if it is desired to detect only those individuals with high egg output (*46*).

Stability of egg output

S. mansoni egg output has been shown to be stable for up to two years in different endemic areas, such as Brazil (*48*), St Lucia (*28*), and Venezuela (*1*). Nevertheless the variation between consecutive *S. mansoni* egg counts in the same specimen may be considerable (*39*, *43*) even though the distribution of eggs is random (*39*).

S. haematobium egg output has also been shown to be stable in Gambia (*71*) and in the United Republic of Tanzania (*23*, *53*).

Similar longitudinal studies on variance and stability of *S. japonicum* egg output have not been carried out. Wide variation in *S. japonicum* egg counts on the same specimen has been reported (*54*).

Prevalence

Although apparently simple, the measurement of prevalence, and especially changes in prevalence over time, require careful definition and critical analysis. The prevalence of infection detected in a population varies directly with the sensitivity of the diagnostic technique. In prevalence analysis, it is useful to present data of subgroups by standardized age and sex strata, although overall population prevalence is also a valuable measure of infection. It is essential to distinguish between changes of prevalence in the same group of individuals examined at different times and changes in a specified age group examined at different times. The consequences of failure to recognize the difference between the two prevalence measures is clearly pointed out in a valuable paper on methodology of control evaluation (*72*). Reports on the effect of molluscicide for control of *S. mansoni* in St Lucia (*9*, *40*) provide good examples of the use of prevalence figures to assess control.

Incidence

Incidence, as a measure of transmission in large communities, was first used by Farooq & Hairston (*73*) and relatively little work has since been published on this important subject. Recently, workers in St Lucia used the conversion: reversion ratio in addition to the traditional conversion from negative to positive stool examination as a measure of intervention effects on transmission control (*9*). Continued evaluation of the several alternative techniques for measuring incidence is required to determine which are most meaningful both for field studies and for assessing control programmes.

Intensity of infection

It is essential that pitfalls in measuring intensity of infection of populations are recognized and overcome. The detection and counting of *Schistosoma* eggs in heavily infected individuals do not present any statistical or practical problems (*74*). In such cases, a small sample of urine or stool is sufficient to classify the individual as infected or uninfected and if a quantitative method is used, the minimum number of eggs per gram can be estimated.

Transformation of egg count data for purposes of standardizing comparisons of large numbers of persons may be done by log, log + 1, or other power transformations.

Furthermore, the geometric mean egg output of the infected individuals in a community varies according to the sensitivity of the parasitological technique used. With a sensitive method, such as the formol–ether technique using 1 g of faeces, the lowest egg count can be 1 egg per gram (epg). In contrast, in a 20-mg thick-smear technique, results are multiplied by a factor of 50 to convert to an epg basis, and consequently, the lowest possible count is 50 epg. These examples illustrate one of the limitations of comparisons between different methods.

Although measurement of changes in the intensity of infection may be useful in assessing snail control programmes (*9*, *40*), it is again necessary to distinguish between changes in a cohort (same individuals) and changes in an index group (different individuals).

Laboratory control

The validity of egg counts in large-scale epidemiological studies has been a major concern (*40*). Recently, a quality control method based on missed positive specimens has been introduced in St Lucia (*75*). The limitations of this approach in areas of low prevalence are recognized and further studies are under way.

FUTURE PERSPECTIVES

Quantitative egg counting techniques are now accepted as an integral requirement for epidemiological studies in schistosomiasis. As experience

increases, fewer parasitological techniques are being used and this trend facilitates comparison between studies in widely separated geographical areas. A logical outcome of the acceptance of quantitative egg counting techniques has been renewed emphasis on sound epidemiological principles in the study design, e.g., precise definition of the study population and standardized measures of morbidity. This trend is further promoted by the advances in computer technology, now available in most endemic areas, which require that data be collected in a form that is easily prepared for computer analysis.

It is hoped that in the future, epidemiological research in schistosomiasis will become integrated into control programmes with long-term funding and governmental support, and will thus have a sound administrative basis for longitudinal research. Such efforts would make it possible to investigate problems related to schistosomiasis control and hence provide the information needed to assess and, if necessary, to modify control strategies. Closer collaboration between academic research programmes and governmental control agencies would aid in the achievement of this goal.

CONCLUSIONS

Specific gaps in our knowledge of the epidemiology of schistosomiasis have been highlighted. More epidemiological studies on *S. japonicum* using quantitative techniques are needed. Because of lack of appropriate field techniques, our understanding of the morbidity of *S. haematobium* in endemic areas is limited. Morbidity data in schistosomiasis is almost non-existent owing to the difficulties of diagnosing the disease and of obtaining reliable information over long periods in defined populations.

Before any new diagnostic tools can be tested in the field, schistosomiasis infection in the study population must be completely characterized by quantitative parasitological techniques in well-designed epidemiological studies. New immunodiagnostic tests may be able to detect, and distinguish between, current and past infections, and these may be simpler, requiring visual interpretation and not expensive laboratory equipment. Measurement of morbidity using newer techniques, such as ultrasound or radioisotopes, may become possible as field equipment is developed. The data obtained by these new techniques will have to be compared with those obtained using the standard techniques.

The use of quantitative techniques now offers the opportunity for the development of systematic and standardized analytical approaches to problems such as measurement of incidence, evaluation of sensitivity of egg counting techniques, and laboratory quality control. As pointed out in this review, little attention has been given in the past to these analytical questions, which should now be integrated into all epidemiological studies on schistosomiasis.

RÉSUMÉ

PROGRÈS DANS LES MÉTHODES D'ENQUÊTE ET LES TECHNIQUES APPLICABLES À L'EPIDÉMIOLOGIE DE LA SCHISTOSOMIASE

Les progrès récents dans les méthodes de la recherche épidémiologique sur la schistosomiase sont fondés sur une adoption croissante des techniques quantitatives dans les enquêtes sur le terrain. Or ces dernières sont nécessaires pour i) mieux comprendre la relation entre l'intensité de l'infection et la morbidité; ii) fournir des données de référence satisfaisantes pour les études d'intervention dans les domaines de la chimiothérapie, de la malacologie et de la sociologie; et iii) apporter des renseignements précis susceptibles de servir de base aux stratégies de lutte.

Théoriquement, les études épidémiologiques doivent être des efforts collectifs d'instituts de recherche ou d'établissements universitaires et d'organismes gouvernementaux s'occupant de la lutte contre la schistosomiase. Cette recherche doit être conçue de manière à faciliter l'acquisition de nouvelles connaissances et l'amélioration de la stratégie de lutte.

La population étudiée, qu'il s'agisse d'une vaste collectivité, d'un petit village ou d'une cohorte définie, doit être précisément décrite à l'aide de cartes dressées avec rigueur et de méthodes de recensement.

Les techniques parasitologiques quantitatives ont largement remplacé les procédés qualitatifs en raison des renseignements supplémentaires qu'elles apportent. En ce qui concerne l'infection à *Schistosoma mansoni*, les techniques de filtration usuelles, de concentration ou de frottis épais de Kato (avec carré de cellophane), sont actuellement recommandées en fonction des objectifs de l'étude et des conditions qu'on rencontre sur le terrain. Les techniques quantitatives de filtration pour *S. haematobium* sont bien standardisées. Quant aux méthodes quantitatives applicables à *S. japonicum*, elles demandent une évaluation plus poussée dans les conditions du terrain. D'autre part, les épreuves d'éclosion des miracidiums sont recommandées pour l'évaluation de la chimiothérapie.

Les techniques immunologiques existantes, y compris les épreuves sérodiagnostiques, ne possèdent pas la spécificité ou la sensibilité nécessaires pour servir dans des études épidémiologiques.

L'évaluation de la morbidité, bien qu'elle ne soit pas

uniformisée, comporte l'utilisation de questionnaires soigneusement élaborés et un examen physique avec mesure des dimensions du foie et de la rate (en centimètres). Les méthodes de laboratoire telles que chimie clinique, urographie intraveineuse et analyse d'urine n'ont été que rarement utilisées en raison des conditions difficiles qui existent sur le terrain, mais leur mise au point et leur amélioration sont encouragées.

Les aptitudes des agents travaillant sur le terrain à organiser des études épidémiologiques dont les données puissent être traitées et analysées sur ordinateur se sont grandement accrues. La standardisation des techniques de numération des œufs doit faire partie de la phase initiale de toutes les études épidémiologiques. La stabilité à court terme des numérations d'œufs chez les personnes infectées par *S. mansoni* ou *S. haematobium* a maintenant été bien établie.

Nombre de questions analytiques concernant la mesure précise de la prévalence, de l'incidence et de l'intensité de l'infection peuvent être élucidées par des études épidémiologiques bien conçues, s'appuyant sur des techniques parasitologiques quantitatives.

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