Modelling human *Schistosoma mansoni* infection

the art of counting eggs in faeces

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the art of counting eggs in faeces

Het modelleren van *Schistosoma mansoni* eitellingen in faeces

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PREFACE

Human schistosomiasis (bilharzia) is one of the major parasitic diseases in the world. At least 200 million people are infected and many more are at risk. It is an important public health problem in many developing countries. The disease is caused by *Schistosoma* worms, which live in the perivesical (*S. haematobium*) or mesenteric (*S. japonicum* and *S. mansoni*) veins of the human host. This thesis deals with *S. mansoni*, which is endemic in most African countries and parts of South America (Brazil).

Thus far, aims to control schistosomiasis are mainly based on pragmatic and not very specific considerations. Mathematical modelling could be an aid for more rationally based planning of control strategies, but existing models are too simplistic in their assumptions and not very accessible to non-mathematicians. In a joint effort, the Department of Public Health of the Erasmus University Rotterdam and the Department of Parasitology of the University of Leiden started with the development of the SCHISTOSIM model for the epidemiology and control of schistosomiasis. Based on the successful experiences with the ONCHOSIM model for river-blindness, the 'microsimulation' approach was selected to mimic the complex dynamics of schistosome transmission. These dynamics depend on a multitude of interrelating factors, ranging from biological processes to human behaviour. In a control oriented model, moreover, many operational and economic factors must be considered to provide useful predictions of the efficacy and cost of various strategies. Input from several disciplines is essential. Under the umbrella of the EC network on schistosomiasis research, several groups (from North and South) have now joined the project. The aim is to integrate each group's specific knowledge in the development of a comprehensive SCHISTOSIM model. It is hoped to eventually identify the most cost-efficient control strategies to eliminate schistosomiasis as a public health problem, or at least to find an answer to the ancient question why and how schistosome populations maintain themselves, even under pressure of intense control efforts.

A crucial issue in the development of SCHISTOSIM, as for any self-respecting applied modelling effort, is testing model predictions against real-world observations. Over the past decades of schistosomiasis research a wealth of data have been collected, and many data bases are available for our project. However, already at first sight it became clear that the interpretation of epidemiological measurements, let alone detailed

comparison with model predictions, would be very complicated. Identification of individuals with *S. mansoni* infection is usually based on the demonstration of parasite eggs in faecal specimens. A 'smear' of faecal material is put under a microscope to detect and count possible *S. mansoni* eggs: the more eggs in the sample the more intensely the person is supposed to be infected. Infected persons can subsequently receive treatment with one of the available safe and effective drugs. The 'Kato-Katz thick-smear' technique (recommended by WHO) is the most widely applied screening method, as large numbers of samples can be examined within a short time. This technique, however, has the drawbacks that (lightly) infected individuals are easily missed. The considerable day-to-day fluctuation in egg counts further hampers the determination of the true individual infection status. Although the presence of variation in schistosome egg counts and the resulting misdiagnosis have been reported in several publications, little attention has been paid to finding formal and statistically valid descriptions of this variation.

In order even to consider developing a SCHISTOSIM, it was therefore necessary to construct a specific stochastic submodel which distinguishes the most important mechanisms that determine the observed variation in schistosomiasis egg counts. This exercise turned out to be much more complex and rewarding than merely unravelling the desired mechanisms. The structure of the egg count model allows to predict the worm burden distribution that corresponds with observed community egg counts, so that predictions of the underestimation of prevalences could be made. This thesis reports the results from modelling variations in faecal egg counts and the main consequences for schistosomiasis research and control.

The first part (and **Chapter 1**) of this thesis contains a general introduction to the transmission of human *S. mansoni* infection, its consequences, diagnosis and control, with particular reference to the importance of faecal egg counts. This part is concluded by an illustration of our approach to model the variation in egg counts.

The second part deals with the development and testing of the basic model. In **Chapter 2**, an overview is given of several phenomena that can influence epidemiological measurements of infectious diseases in general. The approach for modelling variation in measurement of *S. mansoni* infection, as described in more detail in the next chapters, is compared with a completely different method for *Giardia lamblia*, a parasite that multiplies within the human host. In **Chapter 3**, the construction of the statistical model for variations in *S. mansoni* egg counts, the estimation of its parameters and testing against existing data sets from Burundi and Zaire, is described. In **Chapter 4**, it is demonstrated that this model can adequately predict relations between prevalences after one and three measurements as observed in St. Lucia in 1975. The important parameter assumption for the association between individual worm (pair) burden and the corresponding mean egg count is extensively discussed in **Chapter 5**. It is shown that

several arguments imply that worm burdens in endemic areas are much higher than generally assumed.

In the third part, the model is applied to evaluate the accuracy of the Kato-Katz technique to detect and quantify *S. mansoni* infection. In **Chapter 6**, the relevance of missed infections is described and the number of undetected cases predicted. In **Chapter 7**, a practical pocket chart to infer 'true' prevalences from simple observed prevalences and mean egg counts in any endemic situation is presented. This chart has subsequently successfully been validated against new data sets from Burundi, Brazil and Surinam, as described in **Chapter 8**. In **Chapter 9**, the variation in measuring individual infection status is illustrated, followed by using the egg count model to estimate the number of repeated examinations requested for acceptable measurement of intensity of infection.

The implementation of the egg count model as a submodule in SCHISTOSIM is reported in the fourth part. In **Chapter 10**, an introduction to the microsimulation technique, using SCHISTOSIM as example, and an overview of the essential steps in the development of such a model, are given. **Chapter 11** contains a detailed description of input and output of the SCHISTOSIM model, with special reference to the impact of egg count variation for the results of a particular control setting in Burundi.

In the fifth and last part of this thesis (**Chapter 12**), the results of the preceding chapters are brought together in a general discussion of technical aspects of the stochastic model for variations in *S. mansoni* egg counts, more practical considerations in its use for estimating prevalences, and other possibilities for application of this and comparable submodels, especially within the context of the ongoing development of SCHISTOSIM.

Chapters 2-11 of this thesis have also appeared, or will shortly appear, as papers in scientific journals, and are included without modification. Because it was intended that each paper could be read in itself, some overlap is unavoidable. This was especially the case for Chapter 2, which partly reviews the earlier published Chapters 3, 4 and 6.

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PART I

General introduction

1

HUMAN *SCHISTOSOMA MANSONI* INFECTION

1.1 Introduction

Schistosomiasis is a parasitic disease caused by infections with flatworms (Trematoda, Platyhelminthes). Mammals and birds are the final hosts of these worms, and freshwater snails act as intermediate hosts. Three species are responsible for most of the schistosome infections in man: *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni*. The species differ in location within the human host, size and shape of the eggs, the number of eggs produced, and the species of snail host. The geographical distribution of schistosomiasis includes most developing countries in tropical areas, where hygienic conditions are poor and the climate is warm enough for the snail host to breed. Most African countries and some in the Middle East are endemic for *S. haematobium*, *S. mansoni* or both. *S. mansoni* is also present in Latin America, especially in Brazil. *S. japonicum* is confined to China and other countries of the Far East. In total, 200 million people are infected with schistosomiasis and 600 million are estimated to be at risk (WHO, 1993).

Figure 1.1 illustrates the transmission cycle of *S. mansoni*, responsible for intestinal schistosomiasis, and the species considered in this thesis. The adult worms are white-grey, 1 to 2 cm long, and they live in the intestinal veins where they feed on blood particles. They live in couples with the stouter male holding the longer but thinner female worm in a 'gynaecophoric canal'. The average lifespan is estimated at 3 to 5 years, but some may live up to 30 years (Wilkins, 1987). The female worm produces eggs with a characteristic lateral spine (see e.g. Figure 1.3a, further on) at a rate of several hundreds daily. The eggs penetrate through the intestinal wall, which takes several days to weeks, and after reaching the lumen they are excreted with the faeces. Many eggs get stuck in the intestinal wall, or are carried away with the blood stream and trapped in the liver. These eggs provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host.

The excreted eggs hatch if they come into contact with water, and release a ciliated mobile larva called 'miracidium'. Such larvae, which can survive up to two days, penetrate suitable snail hosts. For *S. mansoni* these belong to the *Biomphalaria* genus, of which *B. pfeifferi* (Africa) and *B. glabrata* (Latin America) are the predominant species. In the snail, asexual multiplication takes place and after four to six weeks of development,

hundreds of mobile larvae with a typical bifurcated tail are released per day. Shedding of these 'cercariae' can continue for months. Infection of the human host occurs when he/she is exposed to infested water, for instance during bathing, swimming or when fording a stream. Cercariae, which survive up to three days, can pierce the human skin within seconds. They loose their tail and the young parasites (called 'schistosomula') migrate with the bloodstream via the lungs to the liver, where they mate and mature into adult worms in about one month. These pairs migrate to the intestinal veins, where in a total of 4 to 7 weeks after infection they start producing eggs.

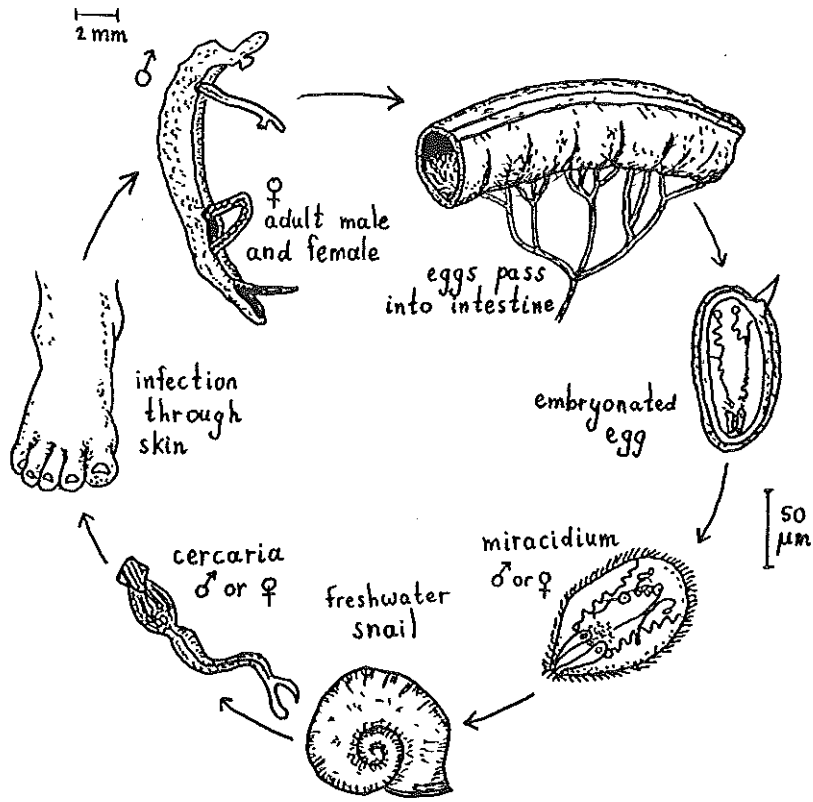


Figure 1.1 Transmission cycle of *Schistosoma mansoni*. After Barnes (1980).

The consequences of schistosome infection are very diverse. The penetration of cercariae through the skin can provoke a rash, called cercarial dermatitis or 'swimmers' itch'. During development into adult worms a feverish syndrome called 'Katayama fever' may occur. This acute form of schistosomiasis is mainly seen in people infected for the first time. Chronic schistosomiasis is due to prolonged accumulation of eggs trapped in the tissues and the subsequent inflammatory reactions. In the intestinal wall, these give rise to

(bloody) diarrhoea, abdominal discomfort and colicky pains. In the liver, fibrotic reactions will develop, leading to hepatomegaly (liver enlargement), fibrotic lesions and ultimately occlusion of the portal system. The ensuing portal hypertension leads to splenomegaly, ascites, and oesophageal varices which may result in fatal haemorrhages. Generally, it is assumed that the severity of intestinal schistosomiasis depends on the intensity and duration of infection. Immunological and genetic factors are also thought to play a role, but these are still largely unknown.

The local distribution of *S. mansoni*, as of other schistosomes, is typically a focal phenomenon, with transmission depending on the presence of water bodies (rivers, ponds, lakes) with the specific intermediate hosts, hygienic conditions and human water contact behaviour. New foci can suddenly appear after modifications of the environment, like dams and irrigation systems. The distribution of infection within endemic communities is generally widespread, with measured prevalences often ranging from 20% to over 90% (Gryseels, 1989). Generally, only a few people carry heavy infections and develop disease, whereas many have lighter infections with light or no symptoms at all. Prevalences and intensities of infection normally show a typical convex shaped curve with a peak at the age of 10 to 15, and a decrease in adults (Figure 1.2). It is still not clear to what extent this pattern is due to differences in water contact, the gradual development of immunity or other age-related factors (Bradley, 1972; Warren, 1973; Gryseels, 1994).

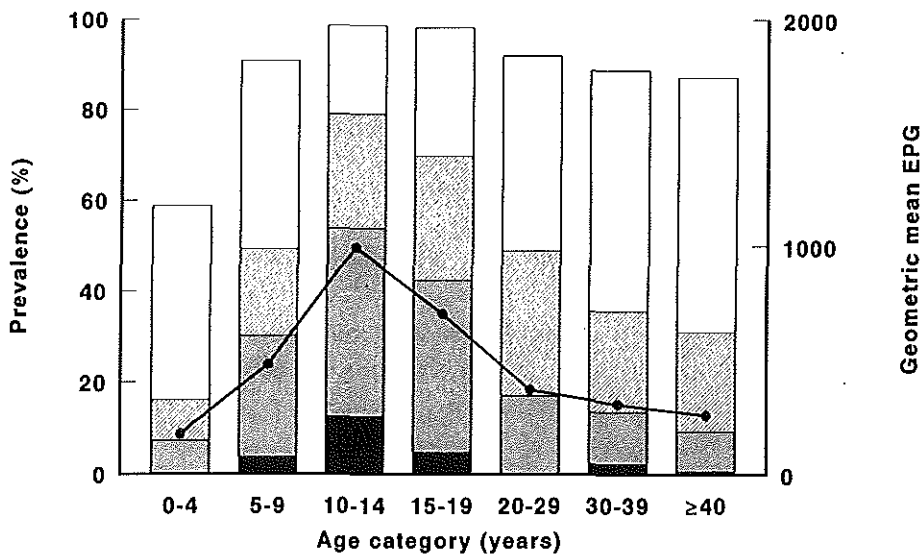


Figure 1.2 Prevalence and egg count distribution of *Schistosoma mansoni* relative to age of the human host (n = 1215) in Tshamaka (Maniema, Zaire). Solid bars = eggs per gramme of faeces (EPG) > 4000; narrow-hatched bars = EPG > 1000; wide-hatched bars = EPG > 400; open bars = EPG > 0. The line shows the geometric mean EPG. With kind permission of Dr. A.M. Polderman.

The different ways to control schistosomiasis can be considered according to the location they aim at in the transmission cycle. Currently, chemotherapy is the predominant way of control. The currently used drugs praziquantel and oxamniquine effectively kill most *S. mansoni* worms, thereby drastically reducing egg output. Moreover, chemotherapy generally reduces pathology or at least terminates its development. Both drugs cause few side-effects, are effective in a single dose, but are still relatively expensive. Sanitation by the construction of latrines targets at preventing schistosome eggs reaching the environment. In addition with health education, this is potentially the best way for durable control. For the time being, in many endemic areas latrines are not widely available. They are not consistently used and difficult to maintain, so the impact on transmission is believed to be negligible. Reduction of water contact by the construction of foot bridges or by the provision of safe water for drinking and washing, will reduce the chance of cercariae infecting the human host. Here also, costs and maintenance cause a problem. Until modern drugs became available, the use of chemical molluscicides to kill the snail host was the most important strategy for control. However, costs are high, and only specialized teams can effectively apply the molluscicides. Furthermore, shortly after control, snails reinvade the water bodies, so that molluscicide must be repeated forever. Finally, some partly successful attempts have been made to influence the presence or abundance of snails by biological control (e.g. introducing competitor snails, or crayfish as a predator) or by environmental control (e.g. lining of canals, or increasing velocity of water).

1.2 Public health impact

The impact of infection with *S. mansoni* as a public health problem must principally be considered in terms of the presence and seriousness of its clinical consequences. Mortality is usually very low, though in some studies a considerable number of deaths attributed to schistosomiasis mansoni have been reported (Ongom & Bradley, 1972; Ongom *et al.* 1972; Williams, Hayes & Smith, 1986). Due to the uneven distribution of infection in the population and the many years with silent or mild consequences until chronic morbidity develops, the public health significance of schistosomiasis is thought to be underestimated. As WHO (1993) states, if 10% of those millions infected have serious clinical disease and another 50% mild symptoms, schistosomiasis certainly is a serious public health problem, which justifies extensive actions to control the disease.

A major problem is, however, the fact that morbidity related to *S. mansoni* is difficult to assess, both for the patient and clinically. For an infected individual, the intestinal symptoms (abdominal discomfort, diarrhoea) especially will be a reason for visiting a health centre. Merely the presence of infection poses no problem and chronic consequences are usually felt only at a very advanced stage, when it is often too late for

effective treatment. On the other hand, morbidity due to *S. mansoni* infection is difficult to distinguish clinically from consequences of other infectious diseases: amoebic and bacillary dysentery also result in bloody diarrhoea and, for example, malaria may also cause liver enlargement. It was therefore felt that in operational situations one should not specifically focus at measuring morbidity. Instead, WHO (1985) recommended to aim at assessing the presence of the parasite (especially in children, who show most intense infection), followed by proper treatment to reduce the intensity of infection. This was advocated by the following rationale: (1) having a heavy infection now will lead to severe morbidity later, and (2) chronic (liver) pathology is irreversible and should therefore be prevented now. Moreover, (3) treatment of infection has economic benefits as impaired educational and physiological performance have been related to the presence of the *S. mansoni* parasite (e.g. Fenwick & Figenschou, 1972; Awad El Karim *et al.* 1980). As a consequence, development of better and easily applicable diagnostic techniques was supported, in combination with the development of safe and effective drugs. This has lead to the current acceptance and widespread use of the 'Kato-Katz thick-smear' technique for the detection of *S. mansoni* infection (see next section), and praziquantel as the drug of choice for the subsequent treatment.

Ever since the early use of faecal smears for measuring schistosome infections, we have known that the individual counts vary considerably from day to day. Therefore, as extensively demonstrated in this thesis, many infected persons will remain undetected and several repeated measurements are necessary for accurate assessment of individual infection status. In addition to this, there are important controversies that further cloud the reasoning outlined above. (1) Reviews of several community studies have shown that in different endemic countries with comparably high levels of infection, the public health consequences of schistosomiasis can vary drastically (Gryseels, 1989; Gryseels & Polderman, 1991). (2) Many individuals with intense infection at early ages never develop morbidity later in their lives. (3) Recent ultrasound studies have shown that certain stages of liver damage can be reversed after treatment with praziquantel (Hatz, 1993). (4) Although cure rates after use of praziquantel can be impressive (70% - 100%), population treatment is usually followed by reinfection to pre-control levels within a few years (Gryseels, 1990). (5) Several studies (e.g. Bell *et al.* 1973; Collins *et al.* 1976; Van Ee & Polderman, 1984) did not succeed at all in finding an association between *S. mansoni* infection and detrimental effects on educational and physiological performance.

Obviously, in the search for the best way to control schistosomiasis, many difficult but fascinating questions still have to be answered. The topic of this thesis, to better interpret diagnostic results in human *S. mansoni* infection, is just a first step. However, as research and interventions are basically evaluated through such egg counts, the outcome of our study will also be relevant for elucidating other existing knowledge gaps and controversies in schistosomiasis epidemiology and control.

1.3 Parasitological diagnosis of *S. mansoni* infection

Diagnosis plays a key role in decision making in the individual and community-based management of schistosomiasis. Whether or not to treat an individual, the planning of large scale interventions, cure rates after treatment, evaluation of the effect of control measures are all based on diagnostic tests. For *S. mansoni*, direct parasitological diagnosis by demonstrating and counting parasite eggs in faeces has been the predominant approach, although users have always realized the considerable variation in results.

There are four basic points in the diagnosis of schistosome infection. (1) In contrast to protozoan infections like malaria, schistosome worms do not multiply in the host. The infection status is the result of accumulation of consecutive infections. Consequently, besides the mere presence of infection, the degree of severity of infection is relevant for diagnosis, with individuals with most intense infections having the highest risk of developing morbidity (see also previous section). Diagnostic techniques should therefore preferably provide quantitative results (Mott & Cline, 1980). (2) Due to the relationship between egg count and the number of worms harboured, sensitivity generally depends on the intensity of infection (Goddard, 1977; Ruiz-Tiben *et al.* 1979; Sleigh *et al.* 1982). Studies to evaluate the sensitivity of a diagnostic test therefore depend on the endemic situation considered. (3) Obviously, under field conditions, any test should be easy and rapid to perform, without the need for skilled personnel and expensive equipment. The balance between sensitivity/reproducibility and practical applicability of any test will determine its operational use. (4) Finally, individual schistosome worms cannot be observed as such, in contrast with intestinal parasites that live inside the gut. Hookworms, for example, are expelled after treatment and can subsequently be counted. Evidence about performance of a test to indicate the presence of schistosomes in humans can therefore only be obtained indirectly by animal models and possibly by human post mortem studies.

It is from research on hookworm that counting eggs in stool samples was introduced to study the distribution, epidemiology and public health aspects of human intestinal parasites. Stoll (1923) introduced dilution egg counting as an alternative to the laborious method of counting worms passed after treatment. He showed that day-to-day variation was relatively low and investigation of only a small sample of stool was representative when dealing with groups of people (Stoll, 1924). In their review of the use of the 'Stoll dilution' method, Earle and Doering (1931) discuss several phenomena that are still the topic of debate, also for schistosomiasis: are size and consistency (formed, mushy) of stool important for the results of faecal tests? Is there any density dependence (decreasing egg production per worm as worm burdens increase) in the association between the number of worms harboured and the number of eggs produced? The problem of how to account for the undue influence of a few unusually high egg

counts in the population, led to the introduction of the geometric mean for epidemiological studies on parasites. For example, Teesdale, Fahringer & Chitsulo (1985), Fulford (1994), Gryseels & De Vlas (1996) and Engels *et al.* (1996) have only recently discussed these topics.

Scott (1931) was the first to suggest use of egg counts by the Stoll dilution method for the epidemiology of human *S. mansoni* infection as well. In a still unsurpassed study, he collected complete stools from three individuals during a whole month, and could conclude that day-to-day variability in egg counts was sufficiently low to allow this method to be used in studies on schistosomiasis (Scott, 1937). He furthermore demonstrated that eggs per volume of stool are less variable than expressed per day or per complete stool. The Stoll technique entails dilution of 3 g of faeces in 42 cc sodium hydroxide solution, stirring vigorously, and investigating 0.075 or 0.15 cc drops on a slide by means of a microscope. The effective amount of stool examined was therefore 5 or 10 mg.

Detection of schistosome eggs for individual diagnosis was however already known. For example, Fülleborn (1921) described a hatching technique so that miracidia escaping from the eggs could be seen with a hand lens. Fouad (1929) applied the fact that schistosome eggs do not float in concentrated salt water and can be found in the deposit, and Khalil & Salah El Din (1930) introduced the sensitive rectal swab method. However, all three methods did not provide quantitative results and were quite impracticable for routine examination. At that time, the most commonly applied method for rough demonstration of *S. mansoni* infection was the 'direct smear', again first described for hookworm research (Smillie, 1921). A small smear of stool is emulsified in a drop of water on a slide and examined with a microscope (Figure 1.3a). Though relatively simple, the technique lacks sensitivity. Only a limited amount of faeces (about 2 mg) leaves the suspension transparent. Some improvement could be achieved by removing gross debris through sieving, and standard densities were obtained by use of a photoelectric meter (Beaver, 1949).

A major step forward in the parasitological diagnosis of *S. mansoni* was due to Kato & Miura (1954), who introduced the faecal 'thick-smear' technique. This method enables examination of faecal samples that are much larger than before, because glycerine is used for the clearing of faecal material. The general procedure is as follows: press a fresh faecal sample of a known weight (up to 50 mg) between a microscope slide and a cellophane coverslip that has been soaked for one day in a 50% glycerine solution (possibly with some malachite green for staining). The smear must stand for a few hours until the faecal film becomes transparent, after which it can be scanned for eggs with a microscope (Figure 1.3b). Not until WHO (1965) got to know about the general satisfaction with its use in *S. japonicum* control programmes in Japan, did a description of this 'Kato cellophane faecal thick-smear' technique become available in the English language

(Komiya & Kobayashi, 1966). Since then, several improvements have been applied to make the method more practical, but the basic idea remained the same.

Martin & Beaver (1968) came up with the idea to first strain some grammes of faecal material through a wire cloth or sieve in order to remove large particles of debris and undigested fibre, and then scrape the desired amount of stool with an applicator stick from the cloth or sieve onto the slide. They could furthermore improve the quality of the film by inverting the preparation after covering with the cellophane coverslip, and pressing it down against a flat, absorbent surface until the smear covered an area 20 to 25 mm in diameter. This size is convenient for efficient systematic screening with a microscope. Layrisse, Martinez & Ferrer (1969), and later Teesdale & Amin (1976a), used a calibrated syringe to ensure a correct volume of stool. However, a more practical solution to do this, was the application of a cardboard template (1.37 mm thick) with a 6 mm hole in the centre, as described by Katz, Chaves & Pellegrino (1972). After putting faeces in the hole with an applicator stick (or tooth pick), levelling off the sample and removing the template, a 39 mm^3 'plug' (measured at about 43.7 mg) is left on the slide. Although this technique is based on volume, the results are generally expressed in eggs per gramme faeces (EPG) based on the estimated weight of the smear. This was basically how the 'Kato-Katz thick-smear' technique was born. Teesdale & Amin (1976b) subsequently reported that instead of using a glycerine-impregnated cellophane coverslip, a thick glass coverslip with a previously placed 50% glycerine drop worked equally well. Later, Peters *et al.* (1980) demonstrated a significant reduction in processing time by using templates for 20 mg. These so-called 'thin-smear' or 'quick Kato' technique resulted in smears that cleared within 15 minutes, whereas several hours up to one day were needed for 50 mg templates. They furthermore preferred using stainless steel material to the disposable cardboard templates.

At present, different commercial kits are available, consisting of nylon or stainless steel sieves, cellophane coverslips with or without pre-stained malachite green, plastic or wooden spatulas and plastic or stainless steel templates with different sizes of holes. The most commonly applied standards at the moment are single (or duplicate) 25 mg slides (Polderman *et al.* 1985) and 41.5 mg slides (Katz, Chaves & Pellegrino, 1972), which correspond with multiplication factors 40 (20) and 24 to express counts in EPG. The Kato-Katz technique is currently widely adopted as a compromise for field work on *S. mansoni* infection, and recommended by WHO (1985, 1993). It indeed allows rapid screen-and-treat operations, the material is relatively cheap and slides can be stored for weeks to enable quality control. The Kato-Katz faecal smear further allows detection of eggs from *Ascaris*, *Trichuris* and *Taenia*, and to a lesser extent from hookworm (which dissolve after a few hours).

Nevertheless, measurements with the Kato-Katz technique are still subject to considerable variation, and some of the infected persons will certainly be missed after

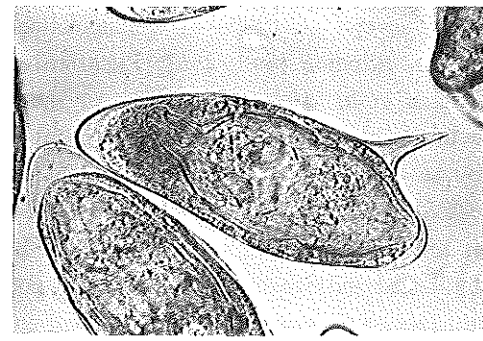
screening based on a single examination (see Chapters 3 and 6 of this thesis for several references). A simple calculation shows that a burden of 40 worm pairs - assuming 150 eggs per worm pair per day passed into the faeces and a daily faecal production of 150 g - results in an average of 1 egg per 25 mg sample, so that - if eggs are randomly distributed in the stool, i.e. Poisson - there is a 37% chance of finding no eggs. Some clustering of eggs in the faeces will even increase this chance. Repeated sampling can to a large extent compensate for this lack of accuracy (Barreto *et al.* 1978; Engels, Sinzinkayo & Gryseels, 1996), but the required number of repetitions is difficult to assess and depends on the situation considered.

There has therefore been a continuous need to develop alternative, more sensitive techniques to detect human schistosome infection. Other direct parasitological techniques to demonstrate eggs in faeces mainly aim at concentrating eggs from much larger faecal samples (up to several grammes) by sedimentation (Ritchie, 1948; Blagg *et al.* 1955; Shidham, 1991), filtration (Bell, 1963; Pitchford & Visser, 1975) or a combination of both (Polderman *et al.* 1994). Sensitivity of these methods is in most cases at least comparable to the smear methods or even (much) better, as is reported in these articles and also by several other authors (e.g. Knight *et al.* 1976; Jordan, Bartholomew & Peters, 1981; Sleigh *et al.* 1982; Schutte *et al.* 1994). However, the applicability in field situations is limited because the procedures are rather tedious and laborious, and usually require complicated and expensive apparatus and potentially hazardous chemical reagents. Furthermore, loss of material often leads to semi-quantitative results (Knight *et al.* 1976; Polderman *et al.* 1994). Investigating the ability of eggs to hatch is especially important as far as detection of viable eggs is concerned, as only these are able to do so. Since Fülleborn (1921), hatching procedures have been improved (Weber, 1973; Xu & Dresden, 1986) but still provide only qualitative results and are not very practical for field use. *S. mansoni* eggs can further directly be demonstrated by examination of biopsies of the rectal mucosa (Badran *et al.* 1955). Though considered to be very sensitive (Da Cunha, 1982) this invasive technique is obviously not acceptable for routine application (Figure 1.3c).

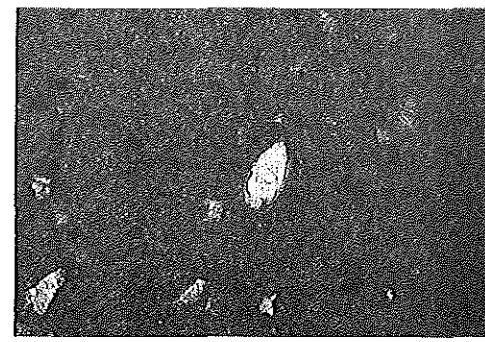
Indirect methods to demonstrate *S. mansoni* infection based on clinical and immunological characteristics, are also numerous. The difficulty of clinical diagnosis has already been mentioned in the previous section. The non-invasive ultrasound technique, also mentioned earlier, is able to demonstrate specific liver pathology. It can even be applied in community-based surveys since portable ultrasound scanners became available (Hatz, 1993). Immunodiagnostic methods measure the human antibody response to schistosome antigens, or the presence of parasite antigens in serum or urine. Though most immunological tests for demonstration of antibodies appear to be rather sensitive and specific, they cannot differentiate between past and present infection, and provide no information on the intensity of infection (Ruiz-Tiben, 1979; Mott & Cline, 1980; Ruppel

et al. 1990). A more promising approach is the detection of the specific circulating antigens excreted by schistosomula, adult worms or eggs, especially since results can be expressed in terms of concentrations. Van Dam (1995) has given an overview of the immunological and biomedical aspects of gut-associated circulating antigens, and Van Lieshout (1996) studied the application of CAA (circulating anodic antigen) and CCA (circulating cathodic antigen) detection in diagnosis and epidemiology of human schistosome infections. Interestingly enough, evaluation of these techniques is still basically carried out by comparison with Kato-Katz faecal egg counts, and is thus also subject to their limitations (Van Lieshout *et al.* 1995b).

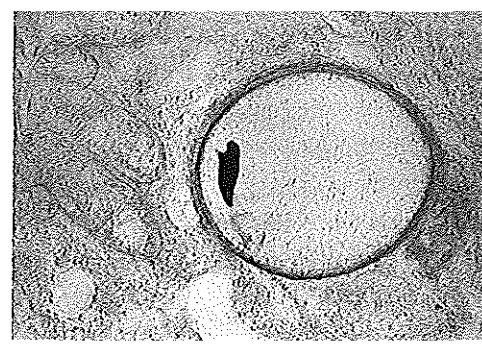
Figure 1.3 Eggs of *S. mansoni*. Sizes of eggs vary from 100 to 170 μm. With kind permission of Dr. A.M. Polderman.



(a) In a fresh faecal smear the morphological characteristics of the large laterally spined eggs are clearly visible. Note that the shape and the details of the miracidium. The flame cells and the cilia covering the epithelial plates can be seen actively moving within the egg shell.



(b) In a Kato-Katz faecal smear, normally examined at low power, the characteristic details do not show up very clearly but the shape of the egg shell and some of the internal structures can be readily recognized amidst the faecal debris.



(c) Eggs that do not manage to successfully pass the intestinal wall get stuck within the tissues. They die, calcify, and are encapsulated by fibrous tissue. The picture shows a spheric fibrous reaction around a black (calcified) egg in the intestinal mucosa.

1.4 The general concept of modelling egg count variation

The main objective of the research contained in this thesis is to improve the interpretation of *S. mansoni* egg counts obtained by the Kato-Katz technique. To this end, a statistical model that distinguishes the main sources of variation is presented. With this model we try to demonstrate how many infections are missed after screening, and how many repeated measurements would be necessary for accurate screening of populations, in any endemic situation. As part of the comprehensive SCHISTOSIM model, the consequences of shortcomings in diagnosis can be compared with other aspects of *S. mansoni* epidemiology and control.

Our search is for a statistical model that describes the distribution of *S. mansoni* egg counts as observed in stool samples from members of an endemic community. The model should account for the (large) differences *between* individual infection status and the (large) *within*-individual variation in repeated egg counts. The series of graphs in Figure 1.4 shows our approach to construct such a model. For the sake of clarity, we have selected simplistic parameter values. We refer to Chapter 3 for more technical details and realistic parameter values.

The main source for differences between individual egg counts are their respective worm burdens. From observations on e.g. hookworm and *Ascaris* infection (Anderson & May, 1991b), we know that the spread of worms among individuals in a host population generally can be described by an 'aggregated' probability distribution. This means that most people harbour no or only a few worms and that many worms are aggregated in only a few heavily infected persons. Figure 1.4a shows that whereas 11% of the individuals harbour no worms at all, 8% have a burden of more than 125. This high variation is thought to be the result of great differences in human water contact behaviour, immune status and susceptibility. More aggregation means a longer tail of the distribution in combination with more persons harbouring no worms. Minimum aggregation corresponds with all individuals having exactly the same probability of acquiring new infections.

Mating of male and female worms will also influence the observed distribution of egg counts between individuals. Schistosomes live in pairs, and female schistosome worms are assumed only to mature and produce eggs when mated (Erasmus, 1987). If individuals harbour unequal numbers of male and female worms, certain worms will not be paired. Lightly infected persons even have a high chance to harbour worms of only one sex, and thus no pairs at all. Assuming a sex ratio of 1:1, our example now shows that 20% of all individuals have no worm pairs and thus show no eggs (Figure 1.4b). The corresponding mean worm pair burden equals 18, slightly lower than the 20 which would be expected if all worms were mated. This underlying distribution of worm pair burdens is used as the determinant of between-individual egg count variation.

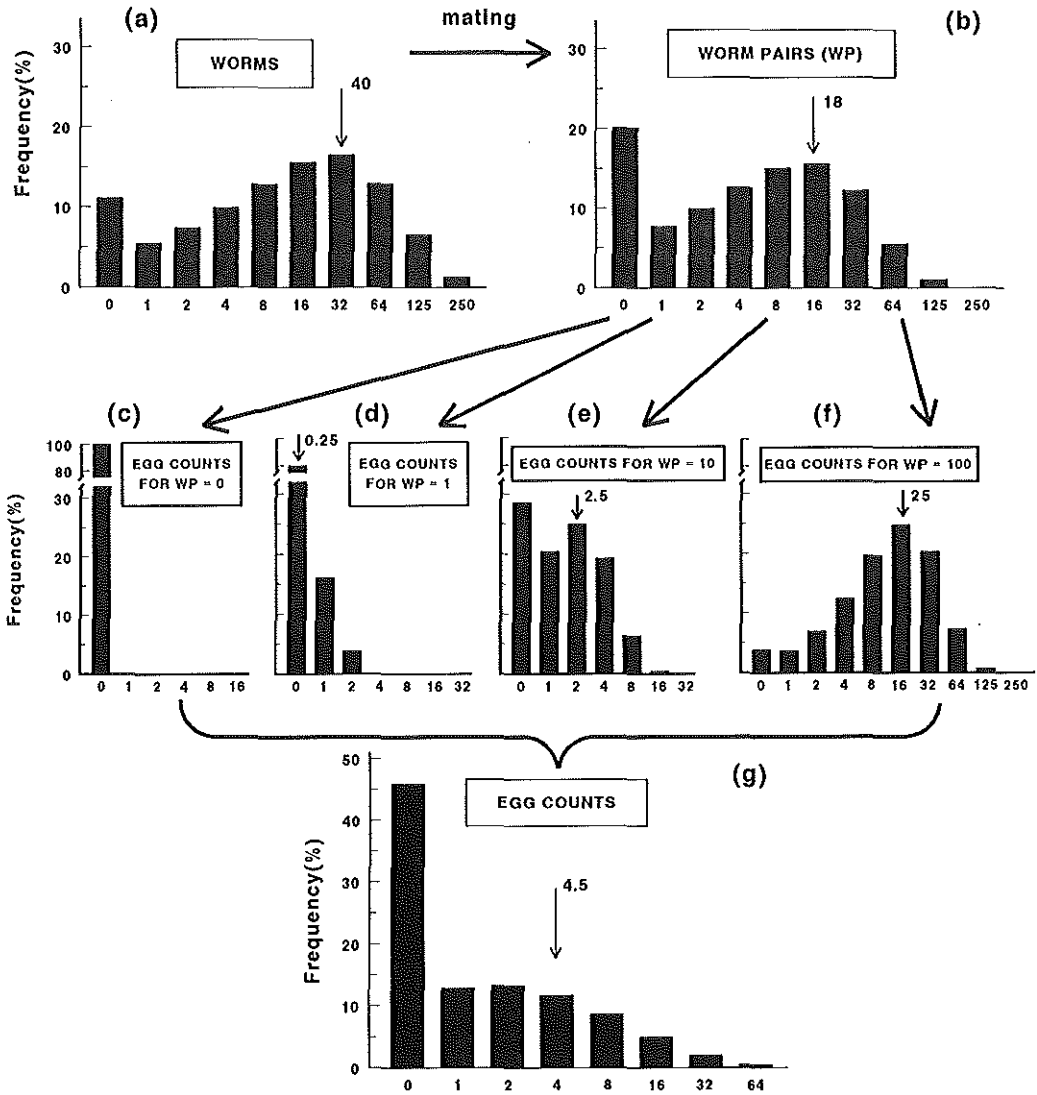


Figure 1.4 Graphical illustration of the model for variations in *Schistosoma mansoni* egg counts: (a) assumed underlying distribution of individual worm loads in the population, (b) distribution of individual worm pair (WP) loads after mating of male and female worms, (c) respective distributions of egg counts in faecal samples given a burden of 0, 1, 10, or 100 WP, (d) resulting distribution of egg counts in the population after a single survey. On the x-axis, the lower limits of intervals are indicated. The arrows show the arithmetic means of each distribution. This example represents very simplistic parameter values, Chapters 3, 5 and 12 provide more realistic values. Technical note: in this example we have used a negative binomial distribution with mean $M = 40$ and aggregation parameter $k = 0.5$ for worm burdens, and, after assuming $h = 0.25$ eggs/sample/WP, a negative binomial distribution with mean h*WP and aggregation parameter $r = 1.0$ for the egg counts of an individual with burden WP.

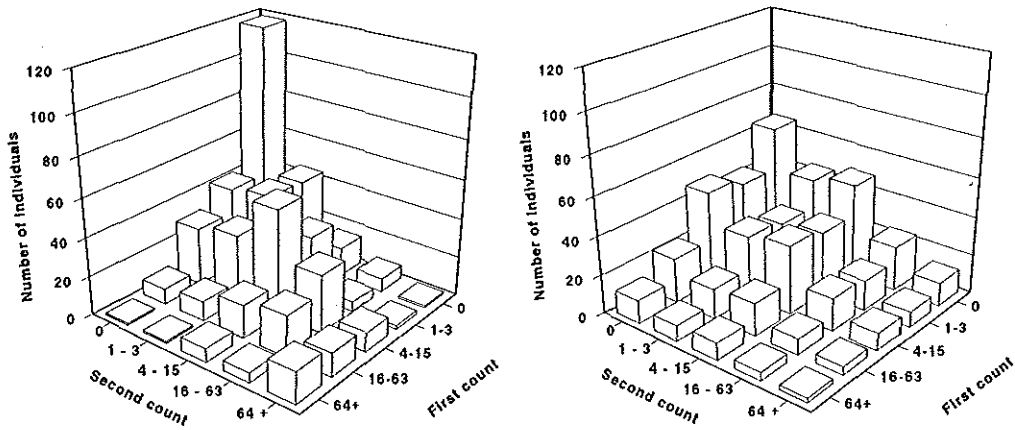
The next step is the relationship between worm pair (WP) burden and the corresponding average egg count per sample. In Chapter 5, an overview is given of empirical and theoretical evidence on this important topic. In the current example, we assume egg counts of an individual to be linearly dependent on his/her worm pair burden by a 0.25:1 relationship. Thus, 1 WP will correspond with on average 0.25, 10 WP with 2.5, and 100 WP with 25 eggs per sample. Within-individual variation is subsequently introduced by the distribution of repeated egg counts on different days for each given number of worm pairs. Also here we use an aggregated distribution, which means that many examinations (of a moderately infected person) will result in zero counts but a few very high counts are also possible. This within-individual variation in repeated egg counts is in fact the combination of day-to-day variation in stool production or schistosome oviposition, and the variation of taking small samples from the same stool specimen. Obviously, 0 WP will always correspond with only 0 egg counts, provided there are no 'false positives' (Figure 1.4c). Also harbouring 1 WP will in this example mostly lead to no eggs detected (80%) (Figure 1.4d). Loads of 10 WP (Figure 1.4e) and 100 WP (Figure 1.4f) show a high variation in corresponding measurements, sometimes zero counts but usually resulting in positive measurements.

The combination of all respective egg count distributions for 0, 1, ..., 10, ..., 100, ... WP (Figures 1.4c-f) times the probability of having these WP loads (Figure 1.4b) results in the ultimate distribution of egg counts in the population, as represented by Figure 1.4g. The latter distribution can be tested against data from community surveys. Reversely, we can now estimate the underlying distribution of worm pair burdens for a given set of observed egg counts. More in particular, we can study the extent of 'false negative' diagnosis (see also Chapter 6). It appears that 46% of individuals with zero counts (Figure 1.4g) actually corresponds with an underlying proportion of 20% really without worm pairs (Figure 1.4b). The 'true prevalence' of individuals with at least one worm pair would therefore be 80%, and thus considerably higher than the single survey prevalence of 54%. In this case, the sensitivity would thus be $54/80 \approx 2/3$.

Figure 1.5 gives a typical example of *S. mansoni* egg count data with two repeated measurements from each person. In order to describe such data, one could try to use the observed overall probabilities of each count to calculate the expected number of individuals in each cell (like for contingency tables). The 6.3% probability of showing a high count ($\geq$ 64), would then mean that only $0.063 \times 0.063 = 0.4\%$ show 2 high counts. From the figure, it is however immediately clear that such a simple model never works. Indeed, repeated counts from an individual are to some extent related to each other, i.e. by his/her burden of infection, therefore leading to more individuals on the diagonal than expected from such an oversimplified model.

The approach in Figure 1.4 seems more promising to describe repeated measurements of *S. mansoni* infection, for individual egg counts are linked by the

assumed underlying worm pair distribution. In the following chapters, we will demonstrate the appropriateness of this approach to describe single and repeated *S. mansoni* egg counts, and the insights that emerged from this exercise.



(a) Observed data **(b) Predicted by oversimplified model**

Figure 1.5 (a) Epidemiological data as observed in a *S. mansoni* population survey, compared with (b) predictions from an oversimplified model. The data concern the first two repeated examinations in a series of nine, from 567 inhabitants ($\geq$ 5 years) of Gihungwe (Burundi). The survey has been described by Gryseels, Nkulikyinka & Engels (1991). The predictions are based on assuming complete independence between individual repeated measurements.

PART II

Modelling variation in egg counts

2

STATISTICAL MODELS FOR ESTIMATING PREVALENCE AND INCIDENCE OF PARASITIC DISEASES[1]

Summary

The estimation of prevalence and incidence of parasitic infections is considered. As the detectability of such infections is not 100% and may furthermore depend on their intensity, statistical methods are often required to arrive at meaningful results. It appears to be essential to distinguish between parasites that multiply within the (human) host and those that do not. An overview of some models discussed in the literature is presented. These models can indeed be used in assessing detectability of infection, and they indicate that observations may lead to considerable misinterpretation of 'true' prevalences and incidences.

2.1 Introduction

Parasitic infections constitute one of the most important health problems of man in developing countries, where infection with one or more types of parasites is practically universal. Malaria, the most important parasitic disease, infects approximately three hundred million people each year and kills over two million individuals annually, especially children. About two hundred million individuals suffer from schistosomiasis (bilharziasis). Other important parasitic diseases include filariasis, onchocerciasis, trypanosomiasis, leishmaniasis and amoebic disease. In addition, infections with various other types of helminths, e.g. hookworm, roundworm, a variety of tapeworms, whipworm, etc. are extremely common (Manson-Bahr & Bell, 1987; Warren & Mahmoud, 1990).

The formulation and validation of epidemiological hypotheses and models of parasitic diseases is clearly important for public health purposes, like the planning and evaluation of interventions. Ideally, prevalence and incidence of infection in a population

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[1] De Vlas SJ, Nagelkerke NJD, Habbema JDF & Van Oortmarssen GJ (1993). Statistical models for estimating prevalence and incidence of parasitic diseases. *Statistical Methods in Medical Research* **2**, 3-21. Re-used with permission of Edward Arnold.

can be estimated through classical one stage or multi stage survey methods. The prevalence, i.e. the fraction of individuals infected, can be estimated from simple cross-sectional surveys. The estimation of incidence, i.e. the rate by which individuals become infected, usually requires follow-up studies.

However, the study of the epidemiology is complicated by the fact that both presence and intensity of infection are not easily observed. In malaria, for example, the usual diagnosis of disease depends on the detection of trophozoites in blood smears. Especially in light infections or in infections partly suppressed by antimalarials, these can easily be missed on examination. For the study of enteric infections, e.g. hookworm, ascariasis, schistosomiasis, amoebiasis and giardiasis, the ascertainment of infections relies almost exclusively on the detection of eggs or cysts excreted in the faeces. These eggs and cysts are not always present in stool samples so that false negative diagnoses are very common (Barreto *et al.* 1978; Seo, 1981; Hall, 1982; Warren & Mahmoud, 1990; De Vlas & Gryseels, 1992). The occurrence of false negatives will not only lead to the underestimation of prevalence, but also, in follow-up studies, to the overestimation of both cure rates and the rate of reinfection of disease (Walter & Irwig, 1988). Furthermore, in follow-up studies incidence may be influenced by loss and re-gain of infection between successive counts as well. Even more complicated is the estimation of the parasite burden. For a given parasite load, intra-individual and inter-individual variation in egg (or cyst) excretion can be substantial (Scott, 1937; Barreto *et al.* 1978; Hall, 1982; Anderson & Schad, 1985; Teesdale, Fahringer & Chitsulo, 1985; De Vlas *et al.* 1992). An additional source of variation is introduced through the process of sampling and inspecting stools (Martin, 1965; Hall, 1982).

Although antibody tests (e.g. ELISA tests) may in the future overcome the problem of the qualitative diagnosis of the disease, these tests are still too complicated and costly for clinical and screening purposes in many parts of the world. Furthermore, these techniques will never give reliable estimates of the parasite load of an individual, so that for quantitative estimates of infections one will always have to resort to counting eggs, cysts, etc.

The variability in observations from these traditional methods make them unreliable for direct use. Conclusions about what happens on an individual level are inevitably probabilistic. Thus, advanced statistical methods are essential for proper inferences on the dynamics of disease. In order to make proper inferences on the epidemiological properties of parasitic diseases, one needs to model the relationship between the individuals' state of infection (presence of infection, number of parasites) and the observations.

In this chapter we shall explore statistical models and methods to interpret data with the aim of estimating prevalence, including intensity and incidence of infection.

2.2 Models and methods, some general considerations

Models and methods for statistical analysis of data on infectious and parasitic diseases naturally depend strongly on study design, epidemiological aspects (the nature of the observations collected and the population affected by the disease), and on the features of the disease one wants to model. In the following paragraphs we will consider each of these aspects in more detail.

2.2.1 Aspects of study design

In order to estimate the prevalence, intensity and incidence of a disease in a population one needs to collect data from a sample, as examination of the entire population is only possible with small populations, e.g. villages. Prevalence and intensity can normally be estimated from cross-sectional studies, whereas for the direct estimation of incidence follow-up studies are required. Unlike clinical trials, for example, the design of such prevalence and incidence studies has received little attention under conditions which prevail in many developing countries. Many studies report results of samples which are not necessarily representative for a population. Often several arbitrary villages are taken to represent a whole area or country. Many diseases with intermediate hosts are very sensitive to local ecological circumstances. The occurrence of infections can therefore be highly focal. Thus even for randomly selected villages there may be very strong clustering of infection, which brings about a strong design effect (Kish, 1965). This effect is often ignored in the analysis of survey data, leading to inflated precision of estimates. The effective sample size is frequently closer to the number of clusters (villages, households within villages) than to the number of examined individuals.

If n primary sample units (PSU) are selected and m individuals sampled per PSU (from a total of M per PSU; and assuming all PSU to be of the same size) are examined, and the cost C of the study is

$$C = c_1.n + c_2.nm$$

with c_1 and c_2 cost factors related to selecting a PSU and examining an individual respectively, then, for fixed C, the optimal value of m is

$$m_{opt} = \frac{S_2}{\sqrt{S_1^2 - S_2^2/M}} \sqrt{c_1/c_2}$$

where S_1 is the variance among primary unit means, S_2 variance among individuals within primary units (Cochran, 1977).

It is tempting for many researchers to try to maximize the naive sample size, i.e. the total number of individuals, and hence to take a large number of individuals from a relatively low number of villages. Such a small value of n and a large value of m suggest that the costs of examining an extra individual are very small compared to selecting an extra PSU. It is doubtful whether this is always the case.

2.2.2 *Epidemiological aspects*

Many bacterial and viral infections tend to occur in the form of epidemics, i.e. there is a large variation in the incidence of infections. Examples are the ordinary influenza, the current AIDS epidemic, etc. Consequently, the proportion of infected individuals, i.e. the *prevalence*, also tends to vary considerably with time. Most parasitic diseases do not display such epidemic behaviour, but are constantly present in a population, i.e. the infection is *endemic*.

Sometimes the assumption of stability, i.e. infection and recovery rates constant for a sufficiently long period in the past, is essential for modelling efforts with the aim of estimating prevalence and incidence of infection. For non-endemic disease, prevalence and incidence vary with time and are not stable parameters of the epidemic process, which makes them difficult to interpret or to use for the evaluation of interventions. To extract more relevant, time-invariant, parameters, e.g. the basic reproduction rate, one usually needs to model the transmission cycle of the parasitic disease fully, something we do not aim to do in this chapter.

Prevalence only refers to presence or absence of disease. Similarly, incidence refers to the acquisition of infection *per se*. For some parasitic diseases this is the only relevant information that can be measured. However, for many parasitic diseases the *intensity* of infection is an important additional parameter. The population distribution of parasite numbers specifies both prevalence and intensity. We shall use the relevance of intensity to classify the models in this chapter.

Knowledge about the three epidemiological parameters (prevalence, incidence and intensity) is obtained through measurements (observations) on individuals. When intensity is not a relevant feature of the disease, measurements can be limited to the detection of the presence or absence of infection, i.e. simple binary recordings. When intensity is important one may decide whether to measure or not, depending on the availability, costs and quality of diagnostic techniques.

Variation in measurement outcomes (of the same individual) can reflect both lack of reproducibility (reliability) due to various sources of measurement error, and variation in the individual state of infection (presence, intensity) due to gain and loss of individual parasites or the infection *per se*.

2.2.3 Some features of parasitic infection relevant for modelling

Important features on the *population level* are the ecology of the parasite, for example, the distribution of the number of parasites among a host population, the rate of acquisition of new parasites and the rate of loss of parasites. These issues depend on the level of infection in populations, and are therefore different for each set of data.

On the *individual level* the following important features should be considered: the relationship between the state of infection of an individual and the sign and symptoms used for measuring infection, thus the relationship between the number of parasites and the mean production of eggs or cysts; the presence of acquired immunity and mating; the reproducibility of measurements, i.e. the variation in counts between different measurements from the same individual (assuming the parasite load has not changed). These properties are in fact equal for each set of data, and should therefore be obtained from literature or be inferred from additional data.

Intensity

Intensity is the number of parasites carried by a host. This number can be the result of multiplication of the parasite within the (human) host, or determined by the number of ingested parasites. For instance, diseases like malaria, amoebiasis, giardiasis (and many other protozoan infections) can be caused by a single infection, whereas in most diseases caused by helminths, the severity depends on the frequency of infections. For protozoan infections, there is often no point in trying to include the intensity of the infection, or the parasite load, as important and epidemiologically pertinent aspects of the model.

In helminthic infections the parasite burden is not only very important clinically, but should be incorporated into any epidemiologically adequate description or model of the spread of the disease.

Dynamics of the disease

The dynamics of the disease, i.e. the rate and mechanism by which infections are acquired and lost, should be considered when building a model which describes the epidemiological properties of an infectious disease. In part, these dynamics are to be inferred from the data instead of assumed; the rate of obtaining new infections, the force of infection or infection pressure, is different for each population. Therefore, in modelling a disease it is necessary to make assumptions about the likelihood of changes in the state of infection of an individual between successive observations. In most infections some prior information is available on the lifespan of the parasite. For instance many helminths survive for several years and it is therefore reasonable to consider the state of infection to be constant between successive egg counts, provided these are at most several months apart. Also the duration of infection may depend on the parasite load. An

infection with a single worm lasts for the lifetime of that worm, whereas in severe infections the infection lasts as long as the longest living worm.

For example, if the survival distribution of the parasites were exponential with hazard rate λ, and the survival times of parasites were mutually independent then, in the absence of new infections, the duration of infection with n parasites would be

$$S(t) = 1 - [1 - e^{-\lambda t}]^n$$

If knowledge of the dynamics (rate of loss and acquisition) of disease is important, repeated measurements will normally be necessary. The intervals between successive observations of the same individual should be determined, *inter alia*, by the expected rate of change of infection.

In this chapter, we consider estimation only in stable endemic situations. Control measures taken in the population usually disturb such situations. Then acquisition and loss of infection are not in balance and vary with time. Prevalence, incidence and intensity of infection no longer parameterize the epidemic process, requiring more elaborate methods and models for its study. Normally various other aspects of parasitic diseases have to be incorporated into the model. In such 'full' epidemiological transmission models, intermediate hosts play among others one important role. However, as we only consider models for the interpretation of statistical data with the objective of estimating incidence and prevalence of infection, these intermediate hosts can be left out of the model.

Imperfection of measurement method

As the study of the actual prevalence and intensity of infection depends on the method used to measure these quantities, the error properties of the measurement method are crucial for any modelling effort. If infection is only observed in terms of presence or absence of disease, these (error) properties are the sensitivity and specificity of the test.

The classical method of treating sensitivity α, i.e. the probability of detecting infection among those infected, and specificity β, i.e. the proportion of detected negatives among uninfected individuals, of a test is by treating these properties as intrinsic, fixed, characteristics of the test. Under this simplifying assumption the real prevalence p can be calculated immediately from the observed prevalence p^*, through

$$p = \frac{p^* + \beta - 1}{\alpha - 1 + \beta}$$

The assumption of constant sensitivity and specificity is, however, usually untenable for parasitic diseases as the probability of detection depends on the intensity of disease (Goddard, 1977).

Measurements of incidence are particularly sensitive to measurement errors, as the process of measurement errors is superimposed upon the process of true fluctuations in the presence of disease. If intensity is an aspect of infection one intends to measure, then the description of the relationship between real and observed intensity will become more complex. For cross-sectional studies the measurement properties are fully specified by the probability distribution of the outcome, conditional on the real intensity. For repeated measurements the reproducibility of the test should be taken into account. Consecutive measurements on the same individual, of say the number of eggs or cysts per stool sample, are subject to day-to-day variations in stool excretion and consistency.

Host immunity or other infection limiting mechanisms

If the life cycle of the parasite is not influenced by density dependent mechanisms, the reproductive rate R of a parasite (the number of fertile female offspring from one fertile female parasite) will always equal the basic reproductive rate R_0, i.e. the reproductive rate in an uninfected population. Then, no stable endemic situation can occur, and the whole host population will be swamped with parasites if $R_0 > 1$; whereas in stable endemic situations each fertile female worm should yield the offspring of one new fertile female worm, i.e. the reproductive rate R is one. This is contrary to all epidemiological observations, in which the usual finding is a rapid build-up of the degree of infection during childhood, followed by a stable, or even declining, intensity of infection (Anderson & May, 1985a). Density dependence occurs when the procreative success of the parasite is a function of the parasite load. This is the case when the probability of reinfection diminishes with the parasite burden, or when the production of eggs per worm decreases with the total worm load. While immunity plays a crucial and proven role in many protozoan infections (malaria), this role is less established in some helminthic infections (Behnke, 1987; WHO, 1987; Hagan, 1992).

If density dependence is brought about by a non-linear relationship between the parasite load and the observations (number of eggs in stool), then this relationship should be taken into account for estimating intensity.

Mating

Another potentially relevant feature of most helminthic diseases is the impact of 'mating'. The probability that a female worm is mated depends on the sexual habits of the parasite (e.g. monogamy), on the average worm load and on the male:female ratio (Macdonald, 1965; May, 1977). Especially when the level of infection is low, mating plays an important role: people with only a few worms have a high chance of containing only worms of one sex, and thus no pairs.

For the situation when the sex ratio is 1:1, and mating is monogamous, the distribution of the number of mated females x, given a population of n worms, is

$$\binom{n}{x}\left(\frac{1}{2}\right)^{n-1} \qquad \text{if } x = 0, 1, \ldots, \left[\frac{n}{2} - 1\right]$$

$$\binom{n}{x}\left(\frac{1}{2}\right)^{n} \qquad \text{if } x = \frac{n}{2}$$

Macdonald (1965) calculated that in this case the chance of a female worm, from an even-total worm load n (or odd load $n+1$), to be mated is

$$1 - \frac{n!}{\left(\frac{n}{2}!\right)^2 2^n}$$

If parasites are polygamous, such that one male will fertilize all females, the formula for the probability that there are female worms, all of which are mated, will be

$$1 - \left(\frac{1}{2}\right)^{n-1}$$

Mating is also one of the processes that should be taken into account when relating the number of parasites to the observations of intensity. Even if the relationship between egg counts and the number of hosted fertile female worms is perfectly linear, there is still some non-linearity in the relationship between the total number of worms and the egg excretion. Furthermore, when estimating prevalence and incidence, it must be considered that infection could be veiled because the worms did not mate. However, detection by antibody-based serological techniques could reveal these monosexual infections.

Additional information

Sometimes only qualitative information is collected, for example, only the presence or absence of disease (eggs, cysts) is registered. When quantitative information is available in the form of eggs per sample or eggs per gram faeces, the size of the stool sample and information on the total stool excretion collected are important. Small samples give a large variation in repeated measurements from the same individual.

In some diseases, deworming such that the worms can be counted is possible. Then diagnosis can be made on the basis of the presence of worms in the stool after deworming (Anderson & Schad, 1985) and the uncertainty because of variations in measurements are circumvented.

Worm counts may also be available from autopsy studies and these can provide the prevalence and intensity of infection in a (selective) sample of the population. Then, (stochastic) relationships between worm load and egg counts can be estimated (Cheever,

1968; Cheever *et al.* 1977). In other diseases this is not possible, and the relationship can only be estimated indirectly, for example by means of statistical models.

2.3 Statistical models for infections where multiplication occurs within the human host: examples

When multiplication occurs within the human host, the number of parasites may vary between successive observations. Variations between such observations do not necessarily reflect the variation in measurement for a fixed state of infection, so that it is often not possible to distinguish several grades of infection. Then diagnosis can only be in terms of presence or absence of disease. However, a complicating factor is the wide variation in intensity of disease among populations. While the first attack of malaria usually leads to very high levels of parasitaemia, and is thus easily detectable in blood smears, established or repeat infections in partially immune individuals are often hard to measure (Nedelman, 1988). This leads to the conclusion that test properties, like sensitivity and specificity, are population parameters and should be established for each population separately. This aspect should be treated as such in adequate statistical models.

Aron (1982) fully recognizes the problem of poor detectability of malaria infection in partially immune individuals. To obtain prevalence estimates from *repeated* observations she assumes a beta distribution of the detectability in the population. Consequences for the estimation of recovery rate and infection rates are explored, but no method for such estimation is presented.

Nedelman (1988) used data from the Garki project (Molineaux & Gramiccia, 1980) to analyze how misdiagnosis affects the estimated prevalence of malaria. Three different double-sampling models that take into account the fallibility of experts are derived and applied. These models incorporate information about the density of parasites in the blood to varying degrees. Model 1 ignores the effect of density of infection on detection properties. This choice leads to the classical diagnostic test theory already mentioned. Model 2 and 3 take into account the effect of the density of parasites on the probability of detection. The error in the estimation of prevalence is quantified; and its dependence on calendar time, age, prevalence and density is investigated. Prevalence and average density are discovered to be good predictors of the error, thereby vitiating Model 1. Implications for the double-sampling models for the design of epidemiological surveys are investigated.

Although Nedelman's model provides an adequate description of the problems concerning the estimation of malaria prevalence, it is less relevant for some enteric protozoal infections, e.g. *Giardia lamblia*, where low detectability can be caused by the absorption of cysts before excretion, so that some stools are completely free of cysts and poor detectability cannot be ascribed to the role of fallible experts. Furthermore,

Nedelman did not address the estimation of the dynamics of the disease, i.e. the infection and cure rate of the disease.

These problems can be circumvented by formulating a model which explicitly incorporates all the above mentioned parameters, i.e. detectability, recovery rate and infection rate. Naturally, the formulation of such a model requires an adequate description of the mechanisms of acquisition and clearance of infection.

Bekessy, Molineaux & Storey (1980) argued the use of a Markov model for the description of this mechanism. The probability of loss or gain of infection is independent of the duration of infection or lack of infection. Of course, this probably only holds true for partly immune individuals. In a Markov model, with recovery rate r and infection rate h the prevalence equals $h/(h+r)$.

In follow-up studies the transition probabilities between successive observations a time interval t apart, from non-infected to infected and vice versa, denoted by α and ß respectively, are given by

$$P_{01}(t) = \frac{h}{r + h} (1 - e^{-(r+h)t})$$

$$P_{10}(t) = \frac{r}{r + h} (1 - e^{-(r+h)t})$$

Values of h and r can easily be calculated from P_{10} and P_{01}. If diagnosis of infection were perfect, the values of h and r could easily be estimated from repeated observations on the same individual. Imperfect detectability complicates matters, and naive estimators of h and r would be seriously biased in that they overestimate the true values.

Nagelkerke, Chunge & Kinoti (1990) apply Bekessy's model to a situation with imperfect detectability in the study of the dynamics of *Giardia lamblia* infection among children in Kenya. For a group of these children weekly stool samples were collected and examined for the presence of cysts of *Giardia*. The result of the jth examination of the ith child ($i = 1, \ldots, n; j = 1, \ldots, m_i$) is given by X_{ij} ($X_{ij} = 1$ and $X_{ij} = 0$ denote observed presence and absence of the parasite, respectively). The true state, infected or not, is given by ξ_{ij}.

They assume constant detectability ν, and the same recovery and infection rate for all children. The likelihood of ν, α, ß given $\{ X_{ij} \}$ is

$$\prod_{i=1}^{n} pr(X_{i1}, \ldots, X_{im_i}; \nu, \alpha, \beta) = \prod_{i=1}^{n} \left[pr(X_{i1}; \nu, \alpha, \beta) \prod_{j=2}^{m_i} pr(X_{ij} \mid X_i^{(j-1)}; \nu, \alpha, \beta) \right]$$

where $X_i^{(j-1)}$ denotes $(X_{i1}, \ldots, X_{ij-1})$

with

$$pr(X_{i1} = 1) = \nu \frac{\alpha}{\alpha + \beta}$$

and

$$pr(X_{ij} = 1 \mid X_i^{(j-1)}) = \nu[(1 - \rho_{ij-1})\alpha + \rho_{ij-1}(1 - \beta)]$$

where

$$\rho_{ij} = pr(\xi_{ij} = 1 \mid X_{i1}, \ldots, X_{ij}; \nu, \alpha, \beta).$$

Now ρ_{ij} can be calculated recursively as follows

$$\rho_{ij} = \begin{cases} \dfrac{(1-\nu)[(1-\alpha-\beta)\rho_{ij-1}+\alpha]}{1-\nu[\rho_{ij-1}(1-\alpha-\beta)+\alpha]} & (X_{ij} = 0) \\ 1 & (X_{ij} = 1) \end{cases}$$

with

$$\rho_{i1} = \begin{cases} \dfrac{(1-\nu)\alpha}{(1-\nu)\alpha + \beta} & (X_{i1} = 0) \\ 1 & (X_{i1} = 1) \end{cases}$$

Both a partial likelihood based on the second product in the likelihood, and a full likelihood, were used for estimation. Both yielded similar results, e.g. a detectability of *Giardia* of approximately 90%. Standard maximum likelihood estimates of the standard errors of estimates were also derived.

A goodness-of-fit test based on the concordance of successive pairs was also derived and applied. This yielded an acceptable goodness-of-fit. Nevertheless, it is clear that the assumptions made in the model, e.g. constant detectability, can only be approximately true. The effect of violations of the assumptions on the conclusions drawn from the model, e.g. of the Markov property of true transitions, is something deserving further study.

2.4 Statistical models for infections where multiplication occurs outside the human host: examples

In many helminthic diseases the parasite does not multiply within the human host. Examples of such infections are ascariasis, schistosomiasis, onchocerciasis, hookworm, filariasis and other helminthic diseases. Each parasite in the body is the result of a separate infection. This makes the intensity of infection a relevant aspect of the disease, necessary for the interpretation and modelling of the data. On the other hand, due to the relatively long lifespan of these parasites, influences of gain and loss of worms during repeated measurements (with intervals less than several months) can be ignored for most helminthic diseases.

Early attempts to interpret epidemiological data were often limited to the prevalence of disease *per se*. In some studies the relation between observed prevalence after one measurement and the 'true prevalence' through examination of repeated measurements was investigated (Barreto *et al.* 1978; Hall, 1981). However, these results are only pertinent to the data that have been used, as effects of intensity of infection are not taken into account. When detection of disease depends on (egg) counts, sensitivity of the measurements depends on the number of eggs excreted and thereby on the intensity of infection. In communities where infection is common, the average parasite burden is likely to be high, resulting in a relatively high sensitivity.

Goddard (1977) recognized this problem. He felt that higher disease prevalence would correspond to a higher intensity of infection. He explored the relationship between observed and actual prevalence, when presence or absence of disease is established by means of the detection of eggs in stool samples. He considered the relationship between prevalence of disease and sensitivity of detection of schistosomiasis when diagnosis is based on the detected presence or absence of eggs in stool samples. As the average intensity of infection increases with the prevalence, and the probability of finding eggs depends on the worm load, it is reasonable to assume that the sensitivity increases with prevalence. He modelled, *inter alia*, the probability of a false negative outcome as a negative exponential function F of the prevalence P

$$F(P) = Ce^{-aP}$$

and explored the effects of this relationship on the measurement of prevalence and its consequences on the expectations of control programmes. He did not actually estimate the parameters in his model. Neither did he apply his model to real data so that the consequences of his results have not been tested in practice.

As sensitivity depends on the intensity of infection, any satisfactory model for the relationship between observed and actual prevalence should incorporate the relationship

between intensity and prevalence into the model. However, as Goddard (1977) stated, this relationship is not yet satisfactorily resolved.

One of the major issues in modelling this relationship is that of the distribution of worm numbers in the host population. This distribution depends on the transition dynamics of infections, i.e. the rate of acquisition and loss of parasites. A major problem in identifying those transition dynamics is that these transitions are usually not observable. However, some information can be obtained by observing the increase of prevalence with age, or by observing the uptake of infections by uninfected individuals.

For parasites that do not multiply within the human host, the state of infection of an individual in an endemic situation can be modelled by the immigration-death process (Bartlett, 1966; Cox & Miller, 1977). If all individuals were exposed equally to infection, the distribution of parasites in individuals of the same age would be Poisson distributed, i.e. the probability of having n parasites is

$$pr(n) = \frac{\theta^n e^{-\theta}}{n!}$$

The mean intensity, and variance, of infection is θ, while the prevalence equals $1 - pr(n = 0) = 1 - e^{-\theta}$. However, data obtained from deworming and autopsy studies clearly show overdispersion, i.e. the variance exceeds the mean, and the Poisson distribution does not constitute an adequate description of reality (May, 1977; Bradley & May, 1978; Anderson & May, 1985a).

A commonly used model for the relationship between intensity and prevalence of infection is the negative binomial. This distribution can be derived if one assumes the population to be *heterogeneous* in infection risk. If the risk in the population has a gamma distribution, and for a given risk the actual number of parasites has a Poisson distribution, then the number of parasites is negative binomial distributed (Johnson & Kotz, 1969).

For this distribution,

$$pr(n) = \begin{bmatrix} k+n-1 \\ n \end{bmatrix} \left[\frac{k}{M+k}\right]^k \left[\frac{M}{M+k}\right]^n$$

Each negative binomial distribution NB(M, k) is characterized by the mean number of infections M and an aggregation parameter k. For several diseases, for which direct measurements of worm burdens are available, the negative binomial appears to be an appropriate distribution to describe variation in worm numbers among individuals of a population: for *Ascaris lumbricoides* in man (Seo, Cho & Chai, 1979), for hookworm in man (Anderson & Schad, 1985), for *Schistosoma mansoni* in mice (Bradley & May, 1978), for *Wucheria bancrofti* in man (Hairston & Jackowski, 1968). The appropriateness of this distribution can be tested by conventional goodness-of-fit techniques.

Similar arguments may lead to a negative binomial model for the number of eggs sampled from an individual in repeated measurements. As the number of stools per person is mostly limited, a combined test for several individuals can be used. Assuming the index of aggregation (r) for the distribution of repeated egg counts equally for each individual, the variance (V_i) of the negative binomial distribution for an individual i with mean count m_i is

$$V_i = m_i + \frac{m_i^2}{r}$$

So that, for large m_i,

$$\log(V_i) = 2\log(m_i) - \log(r)$$

For a collection of replicated measurements from different individuals, plotting $\log(V_i)$ against $\log(m_i)$ can reveal whether the negative binomial distribution is adequate (Figure 2.1).

Anderson & Schad (1985) have been able to measure the egg production per worm for 84 individuals from West Bengal who were infected with hookworm. They found that egg excretion for a given worm load varies strongly, so that, within individuals, quite disparate worm loads cannot be distinguished by means of single egg counts. In order to explore this variation further, log variances of egg counts of 19 different individuals were plotted against log means. The best fitting slope of the regression line, $\beta = 1.982$, supports using a negative binomial distribution for repeated egg counts of one individual (Figure 2.1a).

For *Schistosoma mansoni* infection a similar conclusion can be derived from the data of Barreto *et al.* (1978) (Figure 2.1b). Note however that the departure from the linear relationship, which is expected for low values of the mean and indeed present in these data, is not observed in the data from Anderson & Schad (1985) (Figure 2.1a).

When the distribution of the number of fertile female parasites x among individuals can be modelled by means of a certain (discrete) distribution $P_X(x)$, and the number of eggs y in a sample for a given value of x can be modelled by a distribution $P_{Y|X}(y|x)$, the observed distribution of eggs is then given by the compound distribution

$$P_Y(y) = \sum_x P_{Y|X}(y|x) \cdot P_X(x)$$

If not the relationship between the number of fertile females and the egg load, but the total number of worms n and the egg production *after mating of worms* is important, then the relationship becomes more involved. As the number of fertile (i.e. mated) females depends on both the sex ratio and the sexual habits (monogamy or polygamy) of the

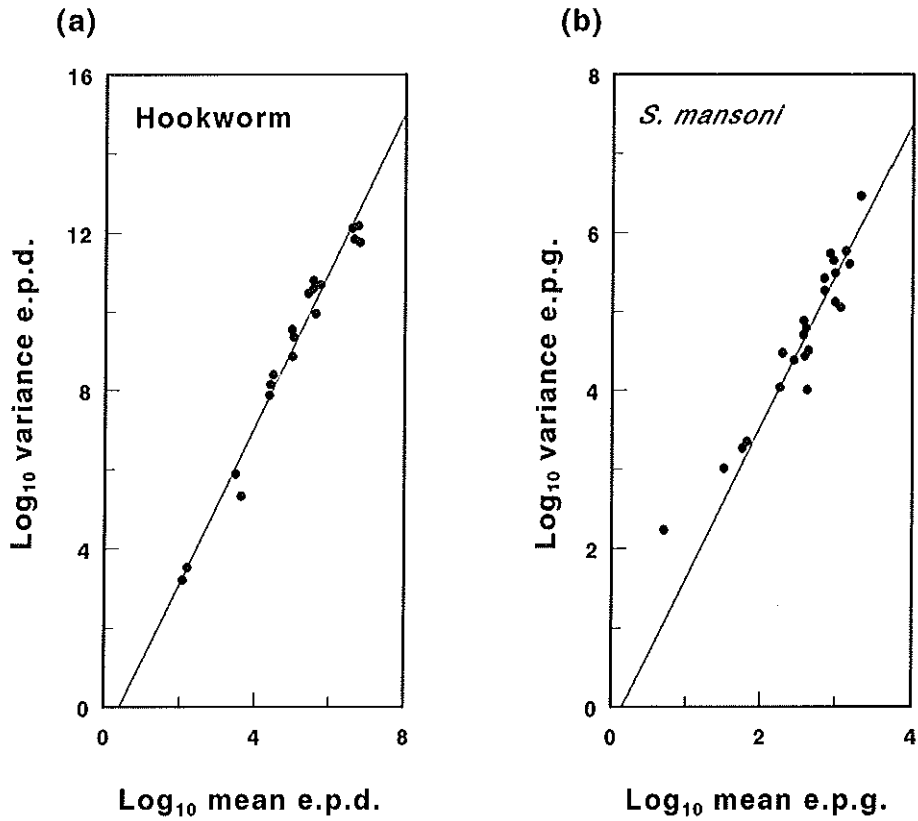

Figure 2.1 Day-to-day variability in egg output from individuals infected with (a) hookworm and (b) *Schistosoma mansoni*. Indicated are relationships between the logarithms of the variance (V) and mean (m) egg counts for individual patients (dots). The lines are the best fitting linear models $\text{Log}_{10}(V) = \alpha + ß\text{Log}_{10}(m)$. All counts were based on approximately 50 mg of faeces. Hookworm data are from Anderson & Schad (1985) and concern egg counts (expressed in number of eggs per day, e.p.d.) of 19 patients who were examined on 11 to 59 consecutive days, with $\alpha = -0.838$ and $ß = 1.982$. Schistosomiasis data were obtained from Table 2 in Barreto *et al.* (1978) and concern egg counts (in number of eggs per gram faeces, e.p.g.) of 23 patients who were examined on 10 to 18 different days: $\alpha = -0.287$ and $ß = 1.917$ (considering only e.p.g.s larger than 100). The slopes of both lines are highly suggestive of a negative binomial for the distribution of repeated measurements from one individual.

parasite, additional information is required to formulate a model $P_{X|N}(x|n)$ for the distribution of the number of fertile females x for a given number of parasites n. If the distribution of the number of parasites is given by $P_N(n)$, the distribution $P_X(x)$ is given by

$$P_X(x) = \sum_n P_{X|N}(x|n) \cdot P_N(n)$$

As each individual with at least one worm pair could show a positive egg count, when sufficient repeated measurements were performed, the probability of having at least one worm pair, $1 - P_X(x = 0)$, can be assumed to indicate the 'true prevalence' of infection in a population. Comparison of true and observed prevalence reveals the proportions of false negatives. False positive diagnosis, due to confusion of foreign faecal material with eggs, is not very likely to occur, and is therefore not considered here.

Hence, the sensitivity of detecting infection in a population can now be described by

$$\alpha = \frac{1 - P_Y(y = 0)}{1 - P_X(x = 0)}$$

which can be calculated from known (or assumed) distributions $P_{Y|X}(y|x)$, $P_{X|N}(x|n)$, and $P_N(n)$.

The empirical evidence that both the distribution of the number of worms between individuals and the distribution of egg counts within an individual for a given worm load in case of *Schistosoma mansoni* infection can be adequately modelled by negative binomial distributions was exploited by De Vlas *et al.* (1992). They considered the distribution of eggs per sample conditional on the number of fertile female worms x, assuming a linear relationship between the mean egg count $y^* = h.x$ and x. A linear relationship is proper for describing the observed egg counts under the assumption that density dependence (of egg excretion) is negligible.

The relationship between worm load and mean egg count for schistosomiasis has been examined by Medley & Anderson (1985). They used autopsy data to establish the number of worms, and concluded that density dependence, e.g. by the power function $y^* = ax^b$, is important. However, Wertheimer *et al.* (1987) in a similar study contradict this finding and conclude that egg excretion per worm is constant. They attributed the former contrary findings to giving undue weight to outlier observations. Keymer & Slater (1987) draw attention to several difficulties in concluding density dependence from parasite fecundity data.

The assumption of linearity greatly simplifies the formulation of a model for the number of eggs in stool samples. Assuming the distribution of worm loads between individuals and egg counts within individuals negative binomial NB(M, k) and NB($h.x$, r),

respectively, and under the additional assumption of monogamous mating, the distribution of egg counts in the population is

$$P_Y(y\,;\,M,\,k,\,h,\,r) \;=\; \sum_{x=0}^{\infty} \left\{ \frac{\Gamma(r+y)}{\Gamma(r)\,\Gamma(y+1)} \left[\frac{r}{hx+r}\right]^r \left[\frac{hx}{hx+r}\right]^y \cdot \right.$$

$$\left. \sum_{n=2x}^{\infty} \frac{\Gamma(k+n)}{\Gamma(x+1)\,\Gamma(n-x+1)\,\Gamma(k)} \left[\frac{k}{M+k}\right]^k \left[\frac{M}{2(M+k)}\right]^n \cdot \left[1+\mathrm{I}_{n\neq 2x}\right] \right\}$$

with parameters as described above.

For single observations per individual the parameters M, k, h, r are not identifiable, as the variation in egg counts can either be attributed to the variation in worm loads or to the variation in egg counts for a given worm load.

In order to identify all parameters, *repeated* observations on each individual are required. For multiple observations per individual the joint distribution of w observations is

$$P_{Y_1,Y_2,\ldots,Y_w}(y_1,\,y_2,\,\ldots,\,y_w\,;\,M,\,k,\,h,\,r) \;=$$

$$\sum_{x=0}^{\infty} \left\{ \left[\prod_{i=1}^{w} \frac{\Gamma(r+y_i)}{\Gamma(r)\,\Gamma(y_i+1)}\right] \left[\frac{r}{hx+r}\right]^{wr} \left[\frac{hx}{hx+r}\right]^{\sum_{i=1}^{w} y_i} \cdot \right.$$

$$\left. \sum_{n=2x}^{\infty} \frac{\Gamma(k+n)}{\Gamma(x+1)\,\Gamma(n-x+1)\,\Gamma(k)} \left[\frac{k}{M+k}\right]^k \left[\frac{M}{2(M+k)}\right]^n \cdot \left[1+\mathrm{I}_{n\neq 2x}\right] \right\}$$

To explore the value of their model for the estimation of the worm load distribution in practical applications, De Vlas *et al.* (1992) used data from Buhandagaza (Burundi). In total 435 individuals were examined 5 times each, with 3 months intervals (Gryseels, Nkulikyinka & Engels, 1991). All egg counts are based on examination of approximately 50 mg faeces.

Parameters M and k determine the distribution of the worms in the population and thus actually describe its state of infection (of a certain age). Their values must be estimated for each population separately. Parameters h and r actually have a biological interpretation and should, ideally, be equal or similar in each population. The value of r was estimated by maximum likelihood estimation from the Buhandagaza data.

Values of h, i.e. the expected number of eggs per sample per worm pair, were found in the literature, as this is the parameter which has been studied best. These published values, however, range from 0.34 and 0.14 eggs/worm-pair/sample, based on human autopsy data (respectively: Cheever, 1968; Cheever *et al.* 1977) to 0.05 based on experiments on mice, monkeys, hamsters, etc. (Loker, 1983). Maximum likelihood was also applied to estimate the value of h and its confidence interval (De Vlas *et al.* 1992). Acceptable values of h covered a wide range and seemed not to have a lower bound. The upper bound of approximately 0.20, albeit it was somewhat smaller than Cheever's (1968) autopsy values, seems to be of the same order of magnitude.

One of the drawbacks of such modelling approaches is that the robustness of the estimation of the (mean) worm load hinges crucially on the correctness of the distributional assumptions. For schistosomiasis much work has been done and the negative binomial distribution, although not strictly proven, is sufficiently adequate to be used in practice, and gives estimates compatible with directly estimated eggs/worm-pair/sample values. The assumption that egg production per worm pair is not density dependent (of course, other density dependent mechanisms can exist) is still disputed for schistosomiasis. However, the values De Vlas *et al.* (1992) found for worm loads under this assumption are very high already compared with the results from autopsy (Cheever, 1968; Cheever *et al.* 1977) and, therefore, assuming non-linearity would lead to unacceptably high worm loads. Moreover, incorporating the assumption of density dependence in their model did not improve the goodness-of-fit significantly. In fact, estimates of the expected number of eggs per sample per worm pair (h) should preferably be obtained from direct measurement, such as deworming experiments. For several helminths, e.g. hookworm, *Ascaris lumbricoides*, and *Opisthorchis viverrini*, it has cogently been demonstrated that h decreases with worm load, i.e. the relationship is non-linear (Anderson & May, 1985a; Ramsay *et al.* 1989). Density dependence in egg excretion vitiates the use of one parameter h for the relationship between eggs and worms.

It appeared that for schistosomiasis the incorporation of mating did not influence the results of fitting the model to the data. This is intuitively clear given the obtained low value of egg production/worm-pair/sample: starting from a production of about 0.05 eggs per fertile female worm per sample, one should harbour about 20 female worms, and thus at least 40 worms to show a considerable chance of a positive egg count. These worm loads imply that almost all worms are mated (according to Macdonald's (1965) formula).

Patients infected with for example *Onchocerca volvulus* harbour much lower numbers of worms. When worms are monogamous, the impact of mating will be much more significant for this disease.

Furthermore, in order to apply this approach to other parasitic diseases one should be aware of mechanisms which could influence the shape of the distributions. A high

probability of dying from the infection will influence the tail of the (negative binomial) distribution of the number of worms per individual, and therefore suggests the use of a truncated distribution.

This method of analyzing egg count data gives an insight into the underlying distribution of the worm and worm pair load among the individuals of a population (of a certain age). The values of parameters M and k enable one to make inferences on the intensity and spread of actual infection. The predicted 'true prevalence' can be simply inferred from the distribution of worm pair loads, by

$$1 - P_X(x=0;\, M,\, k) \;=$$

$$1 - \sum_{n=0}^{\infty} \frac{\Gamma(k+n)}{\Gamma(n+1)\,\Gamma(k)} \left[\frac{k}{M+k}\right]^k \left[\frac{M}{2(M+k)}\right]^n \cdot \left[1 + I_{n\neq 0}\right] \;=$$

$$1 + \left[\frac{k}{M+k}\right]^k - 2\left[\frac{2k}{M+2k}\right]^k$$

De Vlas & Gryseels (1992) have applied this approach for *S. mansoni* infection and showed that even multiple egg counts may leave a large proportion of (low) infected persons undetected. On the basis of the estimated values of h and r originating from the analyses by De Vlas *et al.* (1992), a graph can be constructed which shows for three values of k the relationship between observed and true prevalences (Figure 2.2). Low values of k are associated with high variations in worm loads, indicating that the worms are concentrated in a heavily infected part of the population. This leads to a high sensitivity of detecting infection, as indicated in Figure 2.2 by the small difference between observed and true prevalences. On the other hand, high values of k correspond with very poor sensitivity and means that when detecting 20% positives, in fact all individuals may be infected.

Although projected true prevalences only indicate the presence of infection in the whole population and do not reveal whether particular individuals are infected or not, this result is worth considering: persons with low worm numbers may have severe pathology and, when not treated, are a potential reservoir for reinfection after selective chemotherapy based on egg counts.

2.5 Discussion

The estimation of prevalence and incidence of infection is not a trivial matter. In addition to the 'classical' problems of study design one has to deal with the complication of poor detectability of the parasite, i.e. the sensitivity of the methods of detection is low.

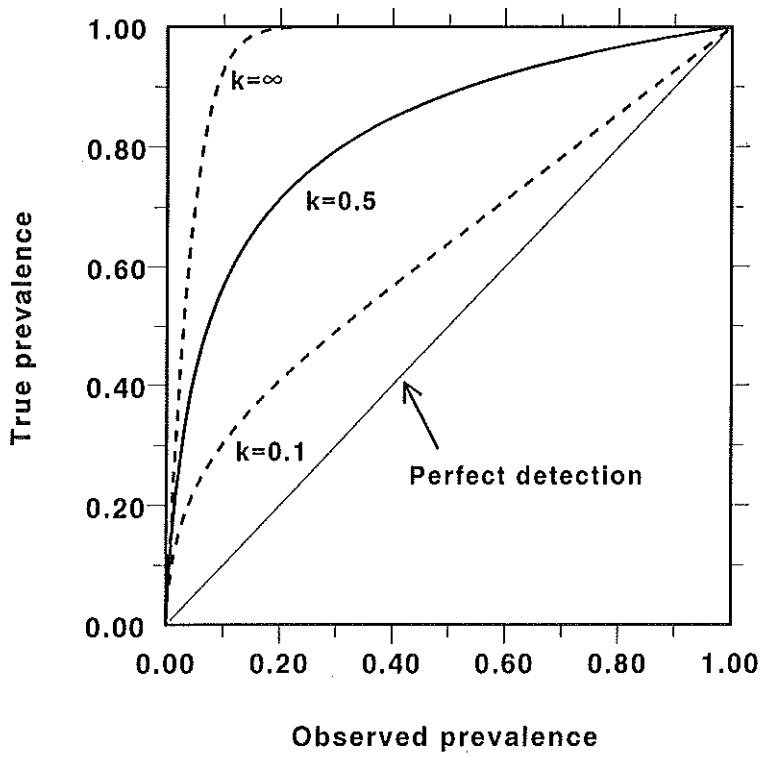

Figure 2.2 Relation between true and observed *Schistosoma mansoni* prevalence from examining one stool sample per individual. The chart is constructed on the basis of the model described in the text: true and observed prevalences are projected from the assumed distributions of worm pair loads and egg counts, respectively. The values of h and r are assumed 0.05 and 0.87, respectively (see De Vlas *et al.* 1992). The curves are obtained by combining all possible values of the mean (M) and three values of the index of aggregation (k) of the worm distribution, and calculating the corresponding values of true and observed prevalences. Especially in communities with high values of k, prevalences will be considerably underestimated if examining only one stool sample per individual. Figure from De Vlas & Gryseels (1992).

Furthermore, this detectability may not only depend on the test method, but also on the parasite load of the host or (immune) status of the population. Under these circumstances 'naive' estimators are misleading and may give rise to questionable policy measures. To arrive at correct conclusions more complex methods of inference are called for. These methods will almost always require some form of statistical modelling of the relationship between observation and the underlying real situation. Some simplification of reality by reducing the true complexity is always necessary. For example for protozoan parasites which multiply within the human host the parasite load is usually not a useful quantity. On the other hand for many helminths any model which ignores the parasite load is unrealistic.

Although modelling often yields estimates of infection prevalence, incidence and intensity which appear realistic, effective empirical checks are difficult to obtain. Indirect estimates which depend on the correctness of models are therefore inevitably speculative. Furthermore, many models either need assumptions about nuisance parameter values that have not been measured directly by experiments, or those model parameters have to be estimated from the model together with the parameters of interest. Robustness studies, goodness-of-fit studies and similar calculations should therefore always be part of the statistical analysis. Real progress can only come from improved detection methods which obviate the use of indirect estimates. Such improvements are not simple, though. While antibody detection tests (e.g. by ELISA) may constitute an improvement for some parasites, e.g. malaria, for other diseases less can be expected from such technology. Such tests pose certain problems with regard to specificity, because antibodies can be present in patients who have lost their infection several years ago, or can be due to cross-reactivity or monosexual infections (Ruppel *et al.* 1990).

The statistical methods described in this chapter are mainly illustrated by means of the diseases schistosomiasis and giardiasis. Although several assumptions are tailored towards these diseases, the methods are in fact also applicable to other (related) parasitic diseases. Some of the assumptions may be violated by control interventions as well. For schistosomiasis, intervention certainly disturbs (the shape of) the distribution of the worm numbers, even when the rate of reinfection after intervention is relatively low. For giardiasis one has to realize that the rate of infection can no longer be treated as constant, but reflects the rate of reinfection of the population. In view of these biological difficulties, statistical models remain an indispensable tool for the estimation of prevalence and incidence in the near future. It is therefore essential that experiments are designed and carried out to validate model assumptions and to enable direct estimations of model parameters. Knowledge of many crucial aspects, e.g. the distribution of egg production per worm, is still scanty and needs improvement.

3

A MODEL FOR VARIATIONS IN SINGLE AND REPEATED EGG COUNTS IN *SCHISTOSOMA MANSONI* INFECTIONS[1]

Summary

Faecal egg counts are often used to measure *Schistosoma mansoni* infection, but the considerable variation between successive counts complicates their interpretation. The stochastic model described in this chapter gives a description of observed egg counts in a population and can be used as a tool to gain an insight into the underlying worm load distribution. The model distinguishes between two sources of variation in egg counts: (1) variation caused by the difference in worm load between individuals, and (2) the variability of egg counts for an individual with a given worm load. Empirical data, single and repeated measurements, from surveys in five villages in Burundi and Zaire have been used to fit and validate the model. We have discussed possible mechanisms that explain the differences in estimated values between the villages. The model indicates that the expected number of eggs in a stool sample per *S. mansoni* worm pair is lower than suggested by autopsy data and that, possibly as a consequence of immunity, the inter-individual variation in worm loads decreases with age.

3.1 Introduction

In studies of *Schistosoma mansoni* infections, faecal egg counts are used to measure the intensity of infection. Several studies have demonstrated that egg load is correlated with severity of disease (Cheever, 1968; Chen & Mott, 1988), at least on the community level (Gryseels & Polderman, 1991). The Kato thick smear, based on examination of a calibrated amount of faeces, is the most widely applied method to determine faecal egg counts in field surveys (Katz, Chaves & Pellegrino, 1972; WHO, 1985).

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[1] De Vlas SJ, Gryseels B, Van Oortmarssen GJ, Polderman AM & Habbema JDF (1992). A model for variations in single and repeated egg counts in *Schistosoma mansoni* infections. *Parasitology* **104**, 451-460. Re-used with permission of Cambridge University Press.

Epidemiological studies show a considerable inter-individual variation in egg counts. Generally, a small number of individuals excrete a large proportion of the eggs in an endemic community (Bradley, 1972). The inter-individual variation in egg counts is thought to reflect the variation in worm loads, caused by differences in exposure to contaminated water and/or differences in immune status (Warren, 1973; Butterworth *et al.* 1985; Wilkins, 1987).

However, repeated egg counts in one individual also vary considerably. Several authors have shown that, because of this variation, a large fraction of infected individuals will remain undetected if only one examination is performed (Barreto *et al.* 1978; Polderman, 1979; Gryseels, Nkulikyinka & Engels, 1991). Teesdale, Fahringer & Chitsulo (1985) found that drier stools from the same person can produce up to seven times the counts of wet ones. A review of several processes which can cause day-to-day variation in individual egg counts has been given by Hall (1982).

We have developed a stochastic model in which a distinction is made between the variation in worm loads between individuals and the variation in egg counts within individuals. This model has been tested against empirical data from five villages in Burundi and Zaire.

For a first fit, the quantification of the parameter describing the relationship between the individual worm load and faecal egg counts has been based on the autopsy data obtained by Cheever (1968). However, as these data probably do not reflect the situation in a normal endemic community (Cheever *et al.* 1977), we have also tested the model with other values.

3.2 Materials and methods

3.2.1 Epidemiological data

We used egg counts from pre-control community surveys of *S. mansoni* in 5 villages in Burundi and Zaire, with varying levels of endemicity (Table 3.1). The respective areas and populations have been described in detail by Gryseels, Nkulikyinka & Engels (1991), Polderman & De Caluwé (1989) and Polderman *et al.* (1985).

All egg counts were based on examination of approximately 50 mg faeces. Duplicate 25-28 mg Kato thick smears had been prepared from a fresh stool sample of each individual, and were examined by two microscopists; the results were also submitted to various other quality control mechanisms (Polderman *et al.* 1985; Gryseels & Nkulikyinka, 1988). The sum of these two egg counts is considered in our study.

In the Burundi villages 3-5 successive surveys were performed with intervals of about 3 months. The age of a person at the first survey is used in this analysis.

Table 3.1 Characteristics of the regions where the survey data come from.

Location	Country	Year	Number of Individuals	Prevalence	Number of Surveys
Buhandagaza	Burundi	1984	435	0.38	5
Gihungwe	Burundi	1984	525	0.60	3
Kizina	Burundi	1984	216	0.30	3
Mungembe	Zaire	1988	950	0.75	1
Tshamaka	Zaire	1979	1079	0.87	1

3.2.2 Model structure

The model makes a distinction between two components of variation: the difference in worm load between individuals and, for an individual with a given worm load, the variability of the number of eggs found in Kato slides.

The assumption that adult schistosomes are randomly distributed among the human population, with all individuals having the same chance of obtaining new infections, would result in a Poisson distribution for the number of worms per person (Macdonald, 1965; Nåsell & Hirsch, 1973). However, variation in exposure, acquired immunity, and susceptibility will result in heterogeneity in the rate of infection. As a result, the worms will be concentrated in a part of the population. If the heterogeneity is characterized by a gamma probability distribution, a negative binomial distribution for the number of worms per person will result (Johnson & Kotz, 1969). Bradley & May (1978) discussed theoretical reasons and empirical evidence to support the use of the negative binomial distribution in modelling the number of schistosomes per individual.

The negative binomial distribution is described by the mean and an aggregation parameter (in the distribution of the number of worms per person denoted by M and k, respectively). The smaller the value of k, the more the worms are concentrated in a small, highly infected part of the population. Higher values mean that all members of the population have about the same chance of being infected, and thus approximating a Poisson distribution.

The other component of variation that we consider is the fluctuation in the number of eggs found in Kato slides for 1 infected individual. Individual variation in egg counts has been analyzed before by Barreto *et al.* (1978). Taking into account the original counts (eggs/sample instead of eggs/gram faeces), their results show that the variance in egg

counts is higher than the mean for each individual. Figure 2 from Barreto *et al.* (1978) reveals that, at high mean values, the slope of the log(variance) as a linear function of the log(mean) approximates the value 2, which is a property of the negative binomial distribution (Johnson & Kotz, 1969). These findings support the use of a negative binomial distribution also to describe this *intra*-individual variation.[2] We consider the expected egg count to be a linear function of the number of worm pairs/person. In other words, the expected number of eggs/sample/worm pair (h) is assumed to be independent of worm load. The index of aggregation in the distribution of egg counts is denoted by parameter r.

In order to get a correct relationship between the number of worms and the number of worm pairs, we include *mating* in the model. The probability that a female worm is mated depends on the sexual habits of the parasite, and on the average worm load (Macdonald, 1965; May, 1977). Especially when the level of infection is low, mating plays an important role: people with only a few worms have a high chance of containing only female or only male worms, and thus no pairs. We assume schistosomes to be monogamous, and the overall ratio of male to female worms in man to be 1:1. In the model the type of worm pairing is in the terminology of May (1977) the so-called 'Case I' (males and females distributed together). 'Case II' (males and females distributed separately) applies to circumstances where transmission is low and is probably not plausible for our data which represent rather highly endemic situations. Therefore the possibility of clustered infections of one sex is neglected in our model.

Two of the four parameters described above, the egg production/worm pair (h) and the variation in egg counts within individuals (denoted by r), have a biological interpretation. We assume their values to be constant for each village and age. Since in Buhandagaza 5 surveys were performed, this data set is most suitable for precise estimation of these parameters. The data from the other villages will be used for validation of the model. In order to compare the degree and variation of infection between different populations we only make use of the estimated underlying worm load distributions (defined by their mean M and aggregation parameter k).

Many epidemiological reports about schistosomiasis show that prevalence and intensity of schistosome infections in a population are age-related (e.g. Anderson & May, 1985a; Wilkins, 1987). To account for this effect we have subdivided the data into 4 age classes (5-9 years, 10-19 years, 20-39 years, and 40 years and older). Increase of egg counts between successive surveys was apparent only in the age group 0-4 (Gryseels, Nkulikyinka & Engels, 1991), which was therefore excluded. First, we will perform estimations of parameters for the age classes separately (Model A). Next, we assume that the aggregation parameters k and r are independent of age, and investigate whether

[2] See also Figure 2.1b of this thesis.

simplified models are sufficient for describing the data. Two different assumptions for the age-dependency of the mean worm load (M) are studied: M is also independent of age (Model B), and M is different for each age class (Model C).

Cheever (1968) published autopsy data of 103 hospitalized patients in Brazil. We have used the results of 44 cases to determine a baseline value for the expected number of eggs/stool sample/worm pair (h). Only individuals who contained more than 10 worm pairs have been considered. Other cases were not taken into account for lack of egg counts, or incomplete autopsy (Medley & Anderson, 1985). The median number of eggs/worm pair/g faeces (6.8) multiplied with the amount of faeces/sample (0.05 g) yields a baseline value of 0.34 for h.

Cheever *et al.* (1977) also published autopsy data from patients who had died in an Egyptian hospital. They found an average number of 2.8 eggs/worm pair/g faeces, which suggests a considerably lower value for h of 0.14. Other quantifications for the egg production/worm pair are based on experiments on non-human mammals like hamsters, mice, and monkeys. In a review by Loker (1983) an average number of approximately 150 eggs passed in excreta/female *S. mansoni* worm/day is reported. For an average production of 150 g faeces/day (Davenport, 1961; Brooks, 1985), this would imply an h value of $(150/150) \times 0.05 = 0.05$. We will investigate for which h the model fits the data best, with special reference to the three values described above.

3.2.3 Mathematical formulation

The model is formulated in terms of the probability to obtain specific egg counts/sample. The resulting egg count distribution, governed by the four parameters M, k, h, and r, is a combination of the distribution of the number of worm pairs/individual and the egg count distributions for each given number of worm pairs. The worm pair distribution results when the mating process is applied to the distribution of the number of worms per individual. Figure 3.1 gives a schematic representation of the model.

For a person we define $P(N = n; M, k)$, the probability of having n worms, originating from a negative binomial distribution with mean M and index of aggregation k. For an individual with given worm load n, $P(X = x \mid N = n)$ represents the probability of having x worm pairs. If n_m and n_f represent the number of male and female worms ($n_m + n_f = n$), then $x = min(n_m, n_f)$ is considered to be the number of worm pairs; n_m and n_f are binomial distributed with parameters $p = \frac{1}{2}$ and n. The probability $P(Y = y; hx, r)$ of finding y eggs in a stool sample from a person with x worm pairs follows a negative binomial distribution with mean hx and index of aggregation r. Parameter h represents the expected number of eggs in a stool sample/worm pair.

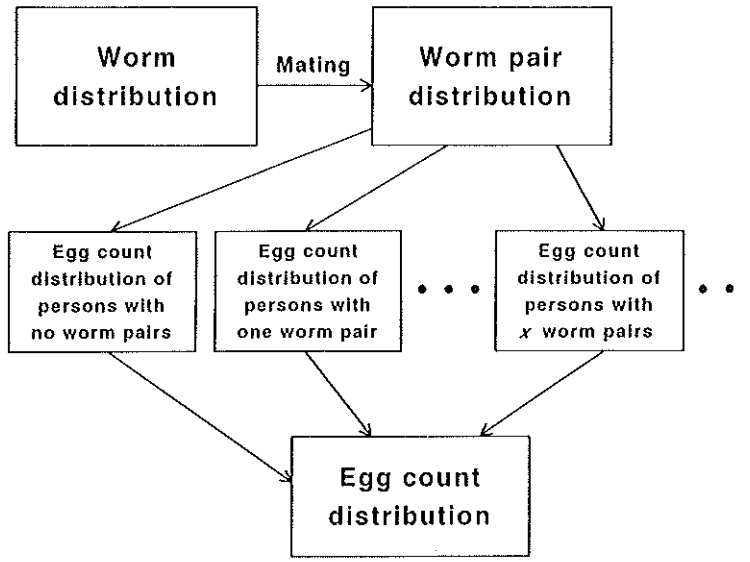

Figure 3.1 Schematic representation of the model. See the text for explanation.

The overall distribution of the number of eggs per sample (y) is represented by

$$P(Y{=}y; M, k, h, r) = \sum_{x=0}^{\infty} \left\{ P(Y{=}y; hx, r) \,.\, \sum_{n=2x}^{\infty} \left[P(X{=}x \mid N{=}n) \,.\, P(N{=}n; M, k) \right] \right\}$$

Appendix 3.1 gives a detailed description of the formula.

The model can simply be extended if more than 1 egg count/person is performed. For example, the distribution of counting y_1, y_2, y_3 eggs in 3 samples taken at the same time can be represented by

$$P(Y_1{=}y_1, Y_2{=}y_2, Y_3{=}y_3; M, k, h, r) =$$

$$\sum_{x=0}^{\infty} \left\{ P(Y_1{=}y_1; hx, r) \,.\, P(Y_2{=}y_2; hx, r) \,.\, P(Y_3{=}y_3; hx, r) \,. \right.$$

$$\left. \sum_{n=2x}^{\infty} \left[P(X{=}x \mid N{=}n) \,.\, P(N{=}n; M, k) \right] \right\}$$

Assuming that the number of worm pairs x present in an individual does not change within a short period of time, we can also use this formula for successive surveys.

3.2.4 Estimation and testing procedures

The parameters are estimated by maximizing the likelihood. The maximization is performed by means of a downhill simplex method (Press *et al.* 1990). The confidence limits are based on the estimated information matrix (Cox & Hinkley, 1974). In order to compare different models the *Akaike Information Criterion* (*AIC*) is used. This is a measure which describes the degree of agreement between the model and the data, and is defined by $-2 \times$ (*maximum log likelihood of the model*) $+ 2 \times$ (*number of free parameters of the model*). The model with the smallest *AIC* is considered to be the most appropriate. A difference of *AIC*'s (denoted by ΔAIC) larger than 3.84 is considered to be significant. For further details see Sakamoto, Ishiguro & Kitagawa (1986).

Chi-square tests, with 10 egg count categories, are performed to test the goodness-of-fit. In case of repeated measurements we specifically account for all successive counts by means of more-dimensional chi-square categories. In order to have enough individuals in each category, in some cases 7 or only 4 categories are used. In each chi-square category all permutations of repeated counts have been combined. In Appendix 3.2 we illustrate the model and the methods of testing by means of a data set considering two egg counts from 100 individuals.

3.3 Results

Table 3.2 shows the results of fitting the model, with the pre-set value $h = 0.34$, to the four age classes of Buhandagaza separately (Model A). As expected, the estimated value of M almost equals the mean egg count divided by $h/2$; the minor differences are mainly caused by the impact of mating. Children of age 10-19 have the highest worm load. Parameters k and r (index of aggregation in the distribution of worms and egg counts, respectively) both appear to have the lowest value for the youngest age group, and thus indicate the highest inter- and intra-individual variation in egg counts for children of age 5-9.

Next, we have tested the model with values of k and r being equal for all four age categories. Table 3.2 shows that the fit improves only if we account specifically for an association between the mean worm load and age, as should be expected. Compared to Model A, a constant value of M for all ages (Model B) considerably worsens of the fit ($\Delta AIC = +11.4$), while assuming different M values for each age class (Model C) improves the fit significantly ($\Delta AIC = -6.1$). The four values of M hardly differ from those estimated in Model A. The combined values for k and r are of the same magnitude as the estimations for each age class separately.

Table 3.2 Results of fitting the model to the Buhandagaza data (435 individuals). For different assumptions the table indicates the estimated values of the mean number of worms (M), the index of aggregation in both the worm distribution (k) and the egg distribution within each individual (r), and their 95% confidence limits. The fit improves (as shown by the AIC) by assuming k and r to be independent of age, compared with considering them different for each age class (Model A). Model B and C assume parameter M to be independent and dependent on age, respectively. Parameter h is preset at 0.34 in Models A-C. Model D, with assumptions about M, k, and r as in Model C, represents the results for the best fitting value of h (0.05), see Figure 3.2.

Model	Assumptions about M	Assumptions about k and r	Number of estimated parameters	Average count	M	k	r	AIC
A	Different for each age class	Different for each age class	12	4.6	26.5 (± 10.6) *	0.38 (± 0.14) *	0.67 (± 0.21) *	6642.8
				7.2	47.5 (± 15.0)	0.52 (± 0.16)	0.91 (± 0.20)	
				2.7	18.5 (± 5.5)	0.49 (± 0.16)	0.80 (± 0.23)	
				2.6	18.3 (± 6.1)	0.44 (± 0.15)	0.99 (± 0.31)	
B	Constant for all ages	Constant for all ages	3	4.1	26.8 (± 4.6)	0.43 (± 0.07)	0.85 (± 0.12)	6654.2
C	Different for each age class	Constant for all ages	6	4.6	25.6 (± 9.4) *	0.46 (± 0.08)	0.85 (± 0.12)	6636.7
				7.2	48.6 (± 16.2)			
				2.7	18.7 (± 5.6)			
				2.6	18.3 (± 6.0)			
D	Different for each age class	Constant for all ages	6	4.6	161 (± 44) *	0.33 (± 0.05)	0.87 (± 0.12)	6626.3
				7.2	309 (± 117)			
				2.7	111 (± 36)			
				2.6	109 (± 39)			

* Age class 5-9 (91 individuals), 10-19 (102 ind.), 20-39 (135 ind.) and 40+ (107 ind.), respectively.

We have applied the best fitting model so far (C) to find an alternative value for h by estimating it from the Buhandagaza data (Figure 3.2). It appears that the fit is much better for lower values than the baseline value 0.34. By decreasing h the *AIC* rapidly declines to a minimum value when h reaches 0.05, after which it steadily rises to a slightly (not significantly) higher saturation value. Values smaller than $h = 0.004$ have not been investigated, because the corresponding mean worm loads would exceed several thousands.

A very low egg production can imply that, because of the corresponding high number of worms/individual, mating has little impact. We have found that for h smaller than 0.18, the results do not differ significantly from a model which does not specifically include the process of mating.

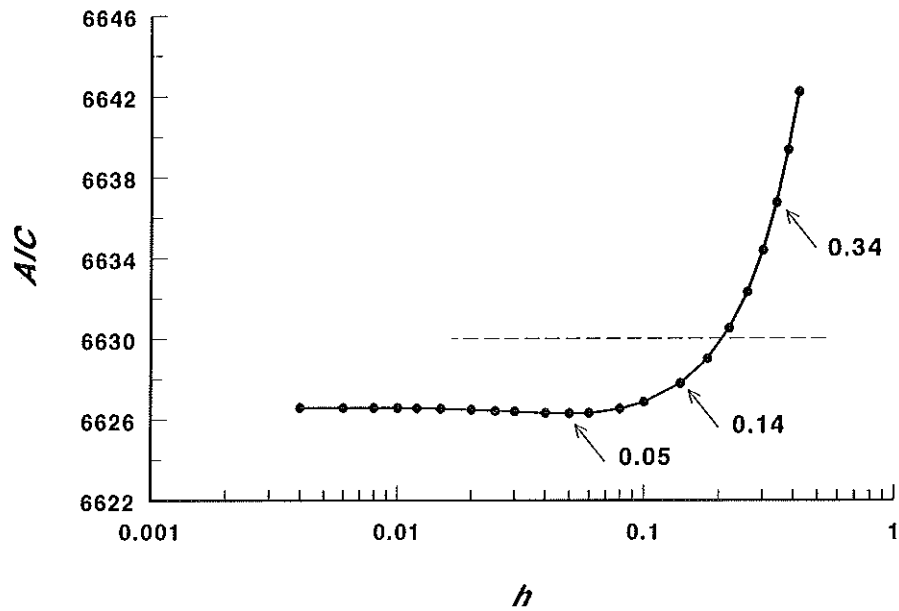

Figure 3.2 Goodness-of-fit for different values of the expected number of eggs in a stool sample/worm pair (h). The lowest *AIC* means that the corresponding value for h provides the best fit. In this case, where the degrees of freedom do not change, differences in *AIC* equal differences in deviance. Table 3.2 gives the corresponding estimated values of k and r, both assumed to be independent of age, at $h = 0.34$ (Model C) and $h = 0.05$ (Model D). In the figure three values are given that are based on empirical studies. The values 0.34 and 0.14 are calculated from autopsy data on men in Brazil (Cheever, 1968) and Egypt (Cheever *et al.* 1977), respectively. The value 0.05 is based on experiments with non-human mammals (reviewed by Loker, 1983). The dashed line indicates the level below which the results do not differ significantly from the best estimate.

For the best fitting value of h (Model D) the corresponding estimations for M, k, and r are also shown in Table 3.2. Compared to Model C the four values of parameter M have increased according to the ratio of $h = 0.34$ and $h = 0.05$. Parameter r concerns only the variation in egg counts within each individual and is hardly affected by the value of h. On the other hand, k has decreased significantly because the declining importance of mating requires more variation in the underlying worm load distributions, in order to be in accordance with the observed inter-individual variation in egg counts.

We have used the obtained values for h (= 0.05) and r (= 0.87) from the Buhandagaza data, and fitted the model with free parameters M and k to the data from the other villages. For both repeated (Gihungwe and Kizina) and single measurements (Mungembe and Tshamaka), the model provides a good description of the data (Table 3.3). Only the oldest age class in Tshamaka shows a significant difference ($P < 0.05$) between observed and predicted egg counts. Parameter M differs much between villages and age classes. As $M \times h/2$ approximates the average egg count, the value of M is almost entirely dependent on the value of h. Table 3.3 therefore gives the results for $h = 0.14$, one of Cheever's estimates (Cheever *et al.* 1977) and within the confidence limits (Figure 3.2), as well. The values of M changed less than would be expected on the basis of the ratio 0.14:0.05 and the values of k increased, both because of the higher impact of mating for $h = 0.14$. In these villages, the fit for $h = 0.05$ is not clearly superior to the fit for $h = 0.14$.

In all villages the estimated value for parameter k is lowest for the age group 5-9 years, which suggests that the inter-individual variation is highest for this age group. Fitting a model with a separate k value for the youngest age group and one k value for all the other age groups yielded a statistically superior (in terms of *AIC*) fit to both a model with only one k value for all ages and to a model with different k values for all age groups in the villages Gihungwe, Kizina, and Mungembe. However, as the choice of this model (two k values) is highly data driven, the conclusion that the youngest age group has the lowest k value should be considered as merely tentative and needs confirmation.

3.4 Discussion

Analysis of *S. mansoni* egg counts by means of a stochastic model that distinguishes inter- and intra-individual variation, enables us to get insight into the distribution of the worm load in the population. This distribution actually reflects the degree and variation of infection, rather than the observed egg counts. The model is very suitable to describe repeated measurements because successive egg counts for an individual are linked by a hypothetical number of worm pairs. Appendix 3.2 illustrates that our model is superior to simple models that do not assume some kind of inter-dependence between repeated measurements from the same individual.

Table 3.3 Validation of the model. Estimations of the mean (M) and index of aggregation (k) of the distribution of the number of worms/individual are shown. The results are presented for $h = 0.05$ and $r = 0.87$ (the best fitting values for the Buhandagaza data, Table 3.2), and for $h = 0.14$ and $r = 0.86$ (within the confidence limits, Figure 3.2), respectively. The goodness-of-fit is represented by the P value of the chi-square test (with D.F. degrees of freedom).

				$h = 0.05$ and $r = 0.87$			$h = 0.14$ and $r = 0.86$		
Data (age)	Individuals	Average count	D.F.	M	k	Goodness-of-fit	M	k	Goodness-of-fit
Gihungwe									
5-9	84	27.0	4	1194	0.45	0.14	440	0.48	0.18
10-19	124	29.7	7	1242	0.67	0.064	459	0.71	0.066
20-39	171	4.9	7	184	0.64	0.68	70	0.74	0.63
40-	146	5.2	7	190	0.54	0.091	72	0.62	0.11
Kizina									
5-9	31	2.6	1	112	0.23	0.81	43	0.27	0.74
10-19	38	3.8	4	149	0.64	0.52	58	0.74	0.58
20-39	89	2.4	4	92	0.33	0.98	36	0.40	0.99
40-	58	2.1	4	83	0.45	0.65	33	0.55	0.61
Mungembe									
5-9	190	29.6	7	1235	0.28	0.29	449	0.30	0.25
10-19	212	43.3	7	1876	0.56	0.059	685	0.59	0.068
20-39	271	31.5	7	1250	0.67	0.58	458	0.71	0.56
40-	277	16.8	7	690	0.53	0.34	256	0.57	0.35
Tshamaka									
5-9	215	47.6	7	1947	1.38	0.58	716	1.43	0.58
10-19	249	76.4	7	3048	10.95	0.11	1110	12.27	0.10
20-39	312	28.6	7	1132	2.51	0.054	418	2.70	0.057
40-	303	21.1	7	845	2.14	0.019	315	2.31	0.021

It must be noted that the parameter dealing with the variability of egg counts for an individual (r) depends on the quantity of faeces examined, and on several processes causing fluctuations of individual egg counts within a given period. The value 0.87 determined in this study, based on examination of 50 mg of faeces and an interval between surveys of three months, can therefore not automatically be used for data of other studies. For example, a preliminary analysis of a smaller data set of daily repeated egg counts over a 7 day-period from 73 individuals in Burundi resulted in a value for r of 1.56. As could be expected, variations of individual egg counts are more important from month to month, than from day to day. This can be explained by, among others, variations in the consistency of the faeces caused by nutritional and other factors, and also by slight variations in individual worm loads.

Apart from being dependent on the type of data, estimations could also be dependent on our choice of the negative binomial distributions for both sources of variation. Especially the best fitting value for the production of eggs/sample/worm pair (parameter h) could be different if alternative frequency distributions were used. The robustness of the estimates is not studied in further detail in this chapter. The credibility of the negative binomial distributions used in the model is supported by results presented earlier in literature (see *3.3.2 Model structure*).

It is reassuring that the order of magnitude of h derived from our model is similar to that found from direct observations on worm loads. From our analysis of the Buhandagaza data, we conclude that h values below 0.20 are consistent with these data (Figure 3.2). The value based on the Brazilian autopsy data (Cheever, 1968) is not within this range, but the value from a similar study in Egypt (Cheever *et al.* 1977) does not significantly differ from the best estimate. We expect that an estimation of h based on still more samples per individual would result in a smaller range for h. In both autopsy data papers it was argued that the high numbers of eggs/worm pair found in the faeces could be due to concentration of the faeces in terminal patients. Another factor they considered was the low fibre content of the diet of their patients, whereas the diet of the African communities concerned in this study consists for a large part of fibre-rich cassava. The best value for h we obtained corresponds in fact rather to a mean value based on experiments on several non-human mammals (Loker, 1983). This value ($h \approx 0.05$) suggests a daily production/*S. mansoni* worm pair of 150 eggs passed in excreta.

Table 3.3 shows very high worm loads for certain data sets. Starting from $h = 0.05$, 10 to 19-year-old children in Tshamaka have a mean number of about 3000 worms, which is almost as high as the highest number of worms recovered from one individual (3669 worms from a 4-year-old child) in the Brazilian autopsy study (Cheever, 1968). Further, these are means from an overdispersed distribution, so that a significant proportion should harbour extremely high worm burdens. The most extreme situation (Mungembe 10 to 19-year-olds: $M = 1876$ and $k = 0.56$) suggests that about 1% of all

individuals would harbour more than 10 000 worms. We realize that the estimated worm loads depend largely on the assumed value for h, as shown in Table 3.3. Nevertheless, we think that such high worm loads are a distinct possibility: transmission in the Maniema villages (Mungembe and Tshamaka) is known to be extremely intense; in one focus, mice immersed for only 60 minutes were shown to harbour up to 350 worms (Gryseels & Polderman, 1987).[3]

In this model, we have assumed h to be constant with the worm load. Medley & Anderson (1985), however, suggested that the egg production/female worm decreases as the number of worm pairs/individual increases. This would lead to even higher worm loads in high endemic populations than we have calculated now. On the other hand, Wertheimer *et al.* (1987) concluded that egg production of schistosomes is density-*in*dependent. In a preliminary analysis we extended the model by assuming a density-dependent relationship between the number of eggs in a sample and the number of worms/person, but the fit with the Buhandagaza data did not improve significantly. Therefore, we have concluded that assuming parameter h to be independent of the worm load is sufficient for the present data. Another density-dependent mechanism could be parasite-induced host mortality. However, decompensated portal hypertension, which is the main cause of death from schistosomiasis infection, is not reported for the areas where our data come from (Gryseels & Polderman, 1987; Gryseels, 1988).

Acquired immunity can not only result in density-dependent egg production, but could also influence the aggregation of infection. In all villages, including Buhandagaza, the variation in the number of worms/individual is highest for the 5-9 age group (indicated by the low values of k), probably due to the low level of immunity. It can be supposed that in younger age groups the inter-individual variation of exposure is not yet (fully) countered by the development of immunity (Butterworth *et al.* 1985). Thus, besides leading to the well-known drop in rates and intensities of infection in adults, immunity may also explain the reduced inter-individual variation indicated by higher values of k.

The results for children in the 0-4 age group are not presented in this study because of the reason described earlier. Indeed, the fit in the case of the repeated measurement data from Gihungwe was poor because of a considerable increase in prevalence of infection between successive surveys. Furthermore, the low prevalences of infection prevented us from accurate estimation and testing. In all cases, however, the value obtained for k appeared to be considerably smaller than the corresponding value for the 5-9 age group. This once more suggests slow development of immunity in children,

[3] See Table 5.1 for more details about this experiment.

though the lower value of k in Gihungwe may also be partly due to the trend in mean worm load.

The k values for Mungembe and Tshamaka, both highly endemic villages in Maniema, are quite different. This may be a reflection of the ecological and sociological aspects of both foci: Tshamaka is an artificial miners' village in which the entire community is exposed to intense transmission. Mungembe is a traditional community with a variety of occupations (fishermen, farmers, etc.), in which exposure to infection is less homogenous (Polderman *et al.* 1985; Polderman & De Caluwé, 1989).

The assumption that egg count heterogeneity between individuals is only due to the distribution of worm burdens may be too simplified, because there could also be systematic differences in egg output that derives from variations in processes other than worm numbers. These processes, like faecal output and consistency, may especially vary between age groups, and can therefore easily be incorporated in the model by assuming different values of h for each age group. This aspect needs further investigation.

The best fitting model shows that mating needs not to be included in a model which describes egg counts. The estimated number of 0.05 eggs in a stool sample/worm pair implies that one should have at least 20 worm pairs or 40 worms to have a reasonable chance of showing a positive egg count. It has been calculated that a worm load of 40 means that as much as 88% is mated (Macdonald, 1965). Further applications of our model can be done with a simpler version which does not incorporate the process of mating. The underlying worm pair distribution can just as well be replaced by a simple negative binomial distribution for the number of female worms/individual.

In this article we have tried to show the usefulness of the model for studying single and repeated measurement data. This model may be used as a tool to orient studies of the underlying mechanisms leading to the variation in egg counts. Because the model deals with an underlying worm pair distribution, it may also be very useful for comparing the observed proportion of zero counts with the estimated actual proportion of non-infected individuals, with other words, the fraction of 'false negatives' in surveys based on single or repeated stool examinations. In a subsequent article we will describe how the model can indeed be applied to that end.

Acknowledgements

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Appendix 3.1

Model formulation

Here we present, in detail, the formula of the model which is described in the text. The probability to show y eggs/sample is defined by

$$P(Y=y;\ M,\ k,\ h,\ r) = \sum_{x=0}^{\infty} \left\{ \frac{\Gamma(r+y)}{\Gamma(r)\ \Gamma(y+1)} \left[\frac{r}{hx+r}\right]^{r} \left[\frac{hx}{hx+r}\right]^{y} \cdot \right.$$

$$\left. \sum_{n=2x}^{\infty} \frac{\Gamma(k+n)}{\Gamma(x+1)\ \Gamma(n-x+1)\ \Gamma(k)} \left[\frac{k}{M+k}\right]^{k} \left[\frac{M}{2(M+k)}\right]^{n} \cdot \left[1 + I_{n\neq 2x}\right] \right\}$$

The symbols are defined as follows:

- y = number of eggs/sample,
- x = number of worm pairs/individual,
- n = number of worms/individual,
- M = mean number of worms/individual,
- k = index of aggregation in the distribution of the number of worms/individual,
- h = expected number of eggs/sample/worm pair,
- r = index of aggregation in the egg count distribution for each individual.

Furthermore, $\Gamma(.)$ represents the gamma function, and $I_{n\neq 2x}$ denotes the indicator function which equals 1 if $n \neq 2x$, and 0 otherwise.

Appendix 3.2

Illustration of the model in the two-dimensional case

The first and second egg count of 100 persons, randomly sampled from the Gihungwe age group of 20 years and older, are used to illustrate the model. The contingency table shows (in normal typeface) the number of persons in each of nine combinations based on the three egg count classes: no eggs, a few (1-5), and many (≥ 6) eggs in one sample. Six persons belong to the combination of no eggs and many eggs in each of both samples, demonstrating the considerable variation in egg counts within individuals.

Second count	First count: 0	1-5	≥6	Total
	26	12	4	42
0	**26.8**	**11.4**	**3.2**	**41.4**
	16.8	*15.0*	*9.2*	*41.0*
	12	15	10	37
1-5	**11.4**	**15.2**	**8.3**	**34.9**
	15.0	*13.3*	*8.2*	*36.5*
	2	9	10	21
≥6	**3.2**	**8.3**	**12.3**	**23.8**
	9.2	*8.2*	*5.1*	*22.5*
	40	36	24	100
Total	**41.4**	**34.9**	**23.8**	**100**
	41.0	*36.5*	*22.5*	*100*

Next, we fit the model (with $h = 0.05$, and $r = 0.87$) to these data, and obtain $M = 220$ and $k = 0.49$. Using the model with these estimated values, we are able to calculate the number of individuals for all nine combinations (in **bold**). For all categories the table shows a very good agreement between observed and predicted values.

The predictions for permutations of each combination of egg count classes do not differ. For example, the predicted number of individuals having the first measurement no eggs in the sample and the second measurement 1-5, and the number of individuals with the first measurement 1-5 eggs but the second without eggs, both equal **3.2**. This is a consequence of the assumption that the worm load of a person does not change within successive surveys. In testing the goodness-of-fit we have therefore combined permutations of repeated measurements to reduce the number of chi-square categories. This example yields a P value = 0.83, based on $\chi^2 = 0.89$ with D.F. = 3 (five free categories, and two estimated parameters), indicating a very good fit.

However, in case of three egg counts per individual the number of chi-square categories would still be very high. Therefore, for the Gihungwe and Kizina data in Table 3.3 we have further reduced this number by systematically combining certain categories. In this example, this reduction would result in the combination of the categories with the first measurement no or 1-5 eggs, and ≥6 the other. This would mean a P value = 0.70, still indicating a very good fit. Combination of permutations and the systematic reduction result in the three-dimensional case in 4, 7, and 10 chi-square categories if we distinguish 2, 3, and 4 egg count classes, respectively.

Predictions based on a simple model that assumes the results of successive measures to be independent of each other, are also presented in the table (*italic* typeface).

For example, the observed probability of showing no eggs equals 0.41 (the average of 40 out of 100 individuals from the first count, and 42 the from second count) and yields the expected number of $0.41 \times 0.41 \times 100 = 16.8$ individuals with two negative samples. This is much lower than the 26 we have observed. Also the combinations of both counts with 1-5 eggs and both counts with ≥ 6 eggs, are underestimated by means of this model. On the other hand, the infrequently observed combination of no eggs and many eggs in each of both samples is now far overestimated. We clearly see that interdependence between successive egg counts from the same individual, incorporated in our model by means of an underlying worm load, is essential to describe this kind of data well. The obtained P values of 0.001 and 0.008, with the reduction in chi-square categories, both indicate a significant difference between observed and predicted values and thus a poor fit.

4

VALIDATION OF A MODEL FOR VARIATIONS IN *SCHISTOSOMA MANSONI* EGG COUNTS[1]

Screening for infections with *Schistosoma mansoni* in endemic communities is generally based on the microscopical examination of stools (Mott & Cline, 1980). Though useful in many aspects, many light infections may be missed, and prevalences underestimated. Examination of more than one stool sample improves the estimation, but is in practice not always feasible. Obviously, it would save effort, time and money if standard relationships between the outcome of single and multiple examinations could be established (Sleigh *et al.* 1982).

Jordan *et al.* (1975) collected data from 8 communities in St. Lucia and related prevalences from one and three stool examinations (Figure 4.1). The equation they derived, using polynomial regression, is, to our knowledge, the only reported attempt to formalize such a relationship. Though appropriate for describing this particular situation, it was not based on underlying mechanisms of egg count variability and cannot therefore be readily applied to other endemic situations or be extrapolated to prevalences from more than 3 examinations.

Recently, we have constructed a model to describe repeated egg count data (De Vlas *et al.* 1992). The model takes 3 sources of variation into account: variation in individual worm loads, variation in worm pair numbers for a given worm load, and variation in egg counts for a given number of worm pairs. In a given endemic community, the egg count distribution can be characterized by only 2 parameters: the mean M and index of aggregation k of the underlying negative binomial worm distribution.

We have used this model to predict a general relationship between prevalences obtained by a single and 3 stool examinations. Figure 4.1 shows the resulting curves assuming high, moderate and low aggregation; the value used for moderate aggregation (k

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[1] De Vlas SJ, Van Oortmarssen GJ & Gryseels B (1992). Validation of a model for variations in *Schistosoma mansoni* egg counts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 645. Re-used with permission of the Royal Society of Tropical Medicine and Hygiene.

= 0.5) was the median obtained from data sets from several villages (De Vlas *et al.* 1992). As our predictions have been made independently from the observations by Jordan *et al.* (1975), we can now check our model. All 8 observations of Jordan *et al.* (1975) were situated between the 2 lines obtained for high and low levels of aggregation. Even more strikingly, the curve based on their equation coincides almost exactly with our curve for moderate aggregation. This strongly supports the validity of our model and, hence, its usefulness to predict multiple survey prevalences for any set of survey data. It also reinforces the conclusion, based on the same model, that 'true prevalences' may be considerably underestimated, even by multiple egg counts (De Vlas & Gryseels, 1992). Clearly, it would be desirable to validate the model further with other data sets. Field experiments to this purpose would be most welcome.

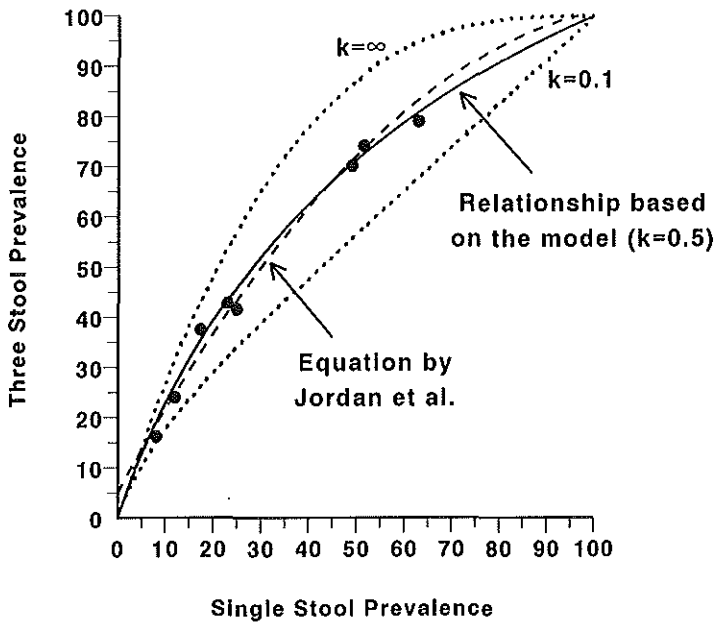

Figure 4.1 Relationship between the prevalence of *S. mansoni* infection determined by examining a single stool and three stools per person. Dots represent the empirical observations from eight communities in St. Lucia by Jordan *et al.* (1975). The dashed line represents the best fitting polynomial equation they found to relate prevalences based on one (x) and three (y) stool examinations: $y = 4.987 + 1.72754x - 0.0077x^2$. The solid line represents the results predicted from our model for moderate aggregation ($k = 0.5$), and is constructed by calculating both prevalences for all values of M (see De Vlas *et al.* 1992, for details of the model). The dotted lines show limit values assuming very low ($k = \infty$) and very high ($k = 0.1$) aggregation, respectively.

5

WORM BURDENS IN SCHISTOSOME INFECTIONS[1]

Summary

Schistosomiasis, caused by fluke worms of *Schistosoma* spp, is one of the most common tropical diseases. Despite decades of research and progress towards the control of the disease, many aspects of the dynamics of infection and immunity remain unresolved. There is, in fact, not even an approximate measure of how many worms are harboured by infected humans. Epidemiological, mathematical and biomedical arguments indicate that individual worm burdens in endemic areas number hundreds to thousands of adult schistosomes, instead of the few to dozens generally assumed on the basis of available autopsy data. As here discussed, this hypothesis has important consequences for research and control, as many constants in schistosomiasis research have to be reconsidered.

5.1 Introduction

In contrast to intestinal worms such as *Ascaris* and *Trichuris*, which are expelled after treatment and which can then be counted, direct quantification of *Schistosoma mansoni* in endemic situations is impossible. In control programmes and epidemiological studies, intensity of infection is estimated indirectly by counting the number of *S. mansoni* eggs in calibrated faeces samples, which are usually converted into eggs per gramme faeces (EPG). Surprisingly little effort has been made to relate EPGs to worm numbers, and to estimate actual worm burdens in endemic situations.

Quantification of worm burdens would greatly improve our understanding of the dynamics of schistosome populations, host immunity, morbidity and control. The regulation of schistosome populations is still fundamentally debated: for some authors, transmission is the main regulatory level (Warren, 1973), for others the host (Bradley, 1972). The answer has profound implications for control: should we concentrate on destroying snails or on developing vaccines? Epidemiological models that could test the

[1] Gryseels B & De Vlas SJ (1996). Worm burdens in schistosome infections. *Parasitology Today* **12**, 115-119. Re-used with permission of Elsevier Science Ltd.

various hypotheses against field data depend strongly on variables representing schistosome numbers (Woolhouse, 1991, 1992a; Gryseels, 1996).

Acquired immunity, developed over years of exposure, is believed to determine individual levels of susceptibility to (re)infection (Hagan, 1992; Maizels *et al.* 1993), and vaccine development has therefore received high priority (Capron *et al.* 1994; Bergquist, Hall & James, 1994). However, without a measure of worm burdens, the protective effect of a vaccine will be difficult to evaluate, especially if it would also trigger anti-fecundity immunity (Capron *et al.* 1994). Obviously, anti-infection and anti-fecundity effects cannot be distinguished on the basis of faecal egg counts.

Population chemotherapy has reduced morbidity in many endemic countries, so that transmission control now becomes the next objective (WHO, 1993). Whether interruption or even reduction of transmission can be pursued via chemotherapy or vaccination depends crucially on pre-intervention worm burdens. Indeed, if these were in the range of hundreds to thousands, even a 99% effective drug or vaccine would leave a few worms in most infected people. The remaining light infections, often not detectable by stool examination, will then act as a continued reservoir for transmission (De Vlas & Gryseels, 1992). Without additional interventions which disturb the ecologically established balance between humans and parasite populations, the latter will (after cessation of chemotherapy) inevitably re-establish their initial levels.

5.2 Commonly assumed knowledge and its consequences

The average productivity of mated female *S. mansoni* worms is about 300 eggs per day, of which about 50% will reach the faeces (WHO, 1985; Rollinson & Simpson, 1987; Jordan, Webbe & Sturrock, 1993). This assumption is based mainly on experiments in mice, hamsters, baboons and chimpanzees (Loker, 1983; Cheever *et al.* 1994), and - perhaps questionably - extrapolated to humans. For a daily faecal production of about 150 grammes per human (Brooks, 1985), a simple calculation shows that EPG and worm pair (WP) burdens would have a 1 : 1 relationship. A 'common' egg count of 200 EPG would then correspond with a burden of 200 worm pairs and at least 400 worms. Counts of 30 000 - 40 000 EPG, such as seen in some high-endemicity areas (Gryseels & Polderman, 1987; Stelma *et al.* 1993), would at first sight imply incredible numbers of worms.

However, individual counts are subject to marked variations (Barreto *et al.* 1978; De Vlas *et al.* 1992). We have developed a model for individual variations in worm loads and egg counts, which, after extensive fitting, testing and validating, allows the prediction of the corresponding distributions of WP burdens (De Vlas & Gryseels, 1992; De Vlas, Van Oortmarssen & Gryseels, 1992; De Vlas *et al.* 1992). Figure 5.1 shows the consequences of this model on the population level, when a 1.0 EPG per WP ratio is assumed, for three typical endemic situations where we have worked over the past decade (Gryseels

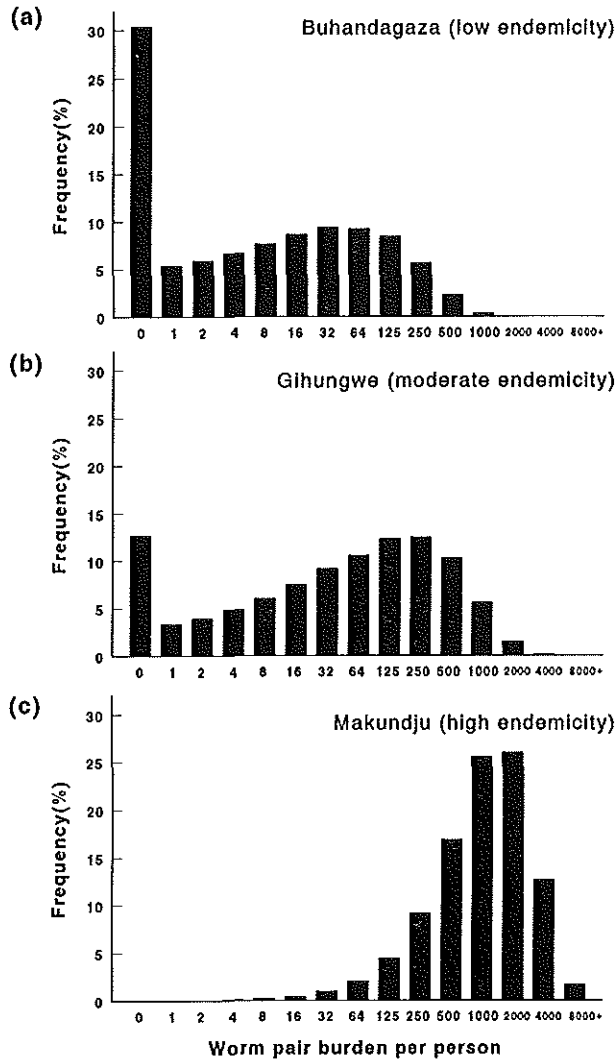

Figure 5.1 Predicted distribution of *Schistosoma mansoni* worm pairs among individuals from three different endemic communities in Burundi and Eastern Zaire: Buhandagaza, low endemicity (38%, 80 EPG) (a); Gihungwe, moderate endemicity (61%, 149 EPG) (b); and Makundju, high endemicity (96%, 791 EPG) (c). The values between parentheses represent the observed prevalences (%) and geometric means of positive egg counts (EPG), respectively. These observations were based on 50 mg Kato-Katz thick smears (Katz, Chaves & Pellegrino, 1972). On the x-axis, the lower limits of the predicted worm pair burden intervals are indicated. Predictions are obtained after fitting a model for egg count variation (De Vlas & Gryseels, 1992; De Vlas, Van Oortmarssen & Gryseels, 1992; De Vlas *et al.* 1992) to the observed data. The distribution of individual worm pair burdens is characterized by a negative binomial distribution of total worm burdens with mean (M) and aggregation parameter (k), and assuming monogamous mating with a male:female ratio equal to 1:1. The estimated parameter values are for Buhandagaza $M = 147$, $k = 0.23$, for Gihungwe $M = 561$, $k = 0.33$ and for Makundju $M = 4288$, $k = 1.2$.

& Polderman, 1987; Gryseels, Nkulikyinka & Engels, 1991). In all three villages, worm burdens over 100 (>50 WP) would be abundantly present. Also burdens over 1000 worms are a common phenomenon: even in the low-endemicity village of Buhandagaza, three percent of the population would harbour more than 1000 worms, and in the moderately endemic focus of Gihungwe, this would be 17%. In the intense-focus Makundju, eight percent of the population would harbour as many as over 10 000 worms.

5.3 Hard Evidence

It is quite remarkable that these consequences of handbook knowledge are in such flagrant conflict with the only available (and widely cited) direct measurement of worm burdens in humans. In 1968, Cheever published the results of perfusion and dissection of 197 cadavers of people who had died of different causes in a hospital in Salvador da Bahia, Brazil, of which 103 were infected with *S. mansoni* (Cheever, 1968). Worms were collected and counted, and faecal and tissue egg counts were determined from each cadaver. Figure 5.2 summarizes his findings with respect to faecal egg counts and WP loads. The majority of the cases had <100 WP. Only ten cadavers harboured >100 WP; the highest number was 1608 (3669 worms) found in a four-year-old child who had died from ulcerative colitis.

Evidently, this autopsy series does not represent a normal endemic community, particularly with regard to the higher range of worm burdens. Most terminal patients belonged to older age groups, in which infections are generally less intense (WHO, 1985, 1993; Rollinson & Simpson, 1987; Jordan, Webbe & Sturrock, 1993). Because of their illness and hospitalization, they probably had not been exposed to transmission for a considerable time anyway. Furthermore, most subjects came from around Salvador, a (sub)urban area with low transmission. It is therefore safe to assume that worm burdens in more typical, rural endemic communities will be considerably higher than those reflected in Figure 5.2.

Nevertheless, the autopsy studies could help to clarify the relationship between worm pairs and faecal egg counts. Figure 5.2 shows a significant positive correlation, although individual ratios of EPG per WP vary strongly. From the data, the overall EPG per WP ratio can be calculated to be 5.5 (with 95% confidence limits 3.9-7.9), significantly higher than the 1.0 assumed above. However, there are a number of reasons why this autopsy relationship may not be valid in natural endemic situations as well. First, a worm recovery rate of 100% could not possibly be achieved (Cheever, 1968, 1986); in six cadavers with positive tissue-egg counts, for example, no worm pairs were recovered. Second, by definition the study population consisted of terminally ill hospital patients, mostly with chronic diseases, in whom faeces production is usually reduced (Cheever, 1968). The stools from the cadavers indeed contained less debris and required

less concentration than those of live patients (Cheever, 1968). This factor would probably be even more important in comparison to patients from a rural endemic setting, where diets are usually rich in fibres. Third, Cheever corrected the cadaver EPG count for mushy and liquid stools with a factor 1.9 to 2.6, a correction which is usually not made in field observations. Without this correction the ratio would be 3.4 EPG per WP (with 95% confidence limits 2.4-4.9).

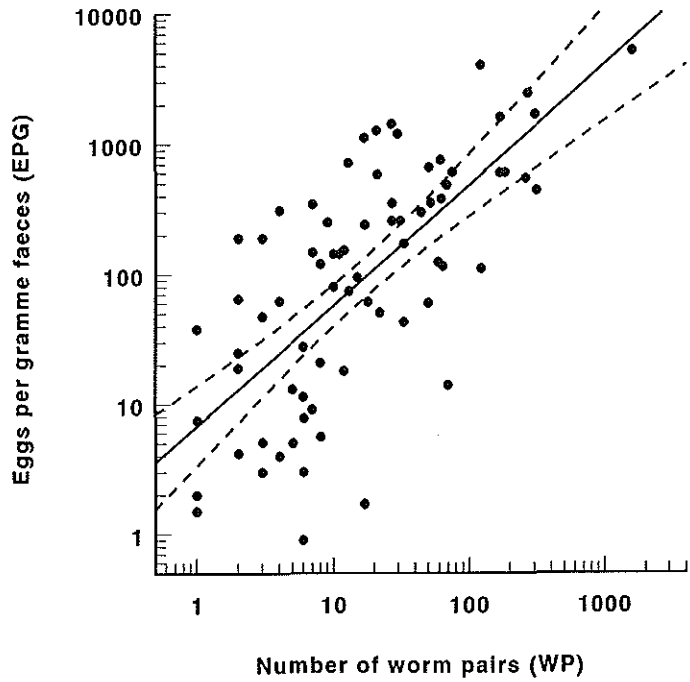

Figure 5.2 Relation between eggs per gramme faeces (EPG) and number of *Schistosoma mansoni* worm pairs recovered by autopsy from 74 out of 103 infected cadavers described by Cheever (1968). In total, 29 cases were excluded for lack of egg counts (16), no worm pairs recovered (7), use of antimonial drugs before investigation (3) or incomplete autopsy (3). In nine cases, zero egg counts in infected cadavers were assigned a value of one-half the minimum number of eggs that could have been detected by the procedures used; these differ as EPGs were based on examination of variable amounts of faecal material (50-200 mg). Log-transformations of both EPGs and worm pair burdens clearly satisfy normality and homoscedasticity conditions to apply regression. The straight line represents the best fitting equation for the relationship between number of worm pairs (WP) and EPG = $6.83.WP^{0.92}$, with 95% confidence limits (dashed lines). The slope 0.92 suggests a slight density dependence in fecundity, but the trend is far from significant ($P > 0.25$). We can therefore calculate a single value for egg production per worm pair, EPG per WP = 5.5 (within 95% confidence limits 3.9-7.9) at the geometric mean of 16 WP. In the text, we discuss the representativity of these values for endemic situations.

The ratio of EPG per WP in normal endemic situations is thus most probably several factors lower than that derived from the autopsy studies. Cheever (1968) himself noted that the EPG per WP ratio in his autopsy series was unusually high, as compared to mice. From a later autopsy study on *S. haematobium* patients in Egypt (Kamel *et al.* 1977; Cheever *et al.* 1977), in which also many light infections with *S. mansoni* were encountered, an EPG per WP of 2.8 was calculated (without correction for stool consistency), but these data were much less complete than the Brazilian set (De Vlas *et al.* 1992).

A complication in relating egg counts to worm numbers is that in humans with high worm loads, female worms may become less fertile. Some authors saw evidence in the Brazilian autopsy data for such density-dependent egg production (Anderson & May, 1982; Medley & Anderson, 1985). However, they excluded nine infected individuals with zero egg counts from their analysis and based their statistical analysis on individual EPG per WP ratios, which is a questionable approach. Their conclusion was therefore challenged by both Cheever (1986) and, based on a re-analysis including the Egyptian autopsy data, by Wertheimer *et al.* (1987). Keymer & Slater (1987) previously drew attention to pitfalls in deriving density dependence from parasite fecundity data in general. Figure 5.2 shows that, with a log-transformation, conditions for linear regression can be met, which is not the case with EPG per WP ratios. As the slope does not differ significantly from 1.0, density dependence cannot be confirmed from the Brazilian autopsy data. Density dependence might still exist for worm burdens higher than shown in Figure 5.2, but worm loads corresponding to high EPGs would then even be higher.

5.4 Anecdotal Evidence

Goldsmith *et al.* (1967) reported the results of extracorporeal blood filtration of living patients during splenectomy. Worm pair counts of the three cases (67, 383 and 69) were highly inconsistent with egg counts (125, 100 and 1100 EPG, respectively). If anything, they show that low EPGs can be found in people with high worm burdens. Worm counts were certainly underestimated with this method, as it is unlikely that all worms were passing through the shunt or withheld by the filter mesh (Goldsmith *et al.* 1967).

There are very little data on the cercarial challenge an individual in an endemic community is actually confronted with. The results of the few available natural mice exposure experiments vary widely. In St Lucia, Sturrock (1973) found only a few infections in over 11 000 naturally exposed mice, illustrating the erratic character of transmission in such low endemicity areas. In foci with continuous, intense transmission such as Makundju, quite different observations have been made. Mice exposed for a few hours in natural waters in which women and children dwelled for hours daily, contracted dozens to hundreds of worms (Table 5.1). It is thus quite probable that, in such

Table 5.1 Results of mice exposure and perfusion experiments in the intense focus of Makundju (Maniema, Zaire).[a]

Duration of exposure (min)	Total no. of mice	Dead/agony[b]	Without WP	Mean no. of WP[c]
16	8	0	2	19 ± 11
60	8	2	0	82 ± 35
120	9	5	1	38 ± 27

[a] Data from Gryseels & Polderman (1987).
[b] Mice with severe agony or which had already died could not be properly perfused.
[c] The mean number of worm pairs (WP) recovered from only those mice that harboured at least one worm pair (within 95% confidence limits) is indicated. The maximum number of worms recovered was 344 in one hour.

conditions, individuals are challenged by hundreds of cercariae per day and carry thousands of worms. A simple comparison of blood volumes between humans and mice makes clear, moreover, that five WP in mice is, relatively, a heavier infection than 5000 WP in adult humans.

5.5 Conclusions

There is a discrepancy between commonly assumed EPG per WP ratios and those found in autopsy series. There are good reasons to believe that the EPG per WP ratio of about 1:1 is a realistic starting point. This would imply that endemic worm counts are much higher than generally assumed (Figure 5.1).

However, egg counts cannot straightforwardly be translated into worm burdens. The extensive within-individual variation in schistosome egg counts (Barreto *et al.* 1978; De Vlas *et al.* 1992) results in a wide range of corresponding worm burdens. Furthermore, this range is dependent on the characteristics of the endemic situation. In an area of low endemicity, a high EPG is likely to be due to an incidental peak in egg excretion of this individual, whereas in an intense focus a high egg count more probably reflects a genuine high worm burden. Based on the model outlined above, we have calculated the range of worm burdens corresponding to given EPGs in different endemic situations (Table 5.2). In a low-endemicity community such as Buhandagaza, the possible range corresponding with 1000 EPG has 1000 WP as an upper limit. However, in a high-endemicity village such as Makundju 1000 EPG may reflect up to 5000 WP. On the other hand, in such an intense focus, negative egg counts most likely correspond to positive worm burdens. Of course, these predictions assume satisfaction of modelling conditions,

Table 5.2 Predicted range in *Schistosoma mansoni* worm pair (WP) burden for a given egg per gramme faeces (EPG) count in three different endemic situations.[a]

EPG	Buhandagaza (low endemicity)[b]	Gihungwe (moderate endemicity)	Makundju (high endemicity)
0	0 - 72	0 - 192	11 - 2977
100	22 - 427	28 - 863	83 - 3553
400	86 - 727	108 - 1423	222 - 4220
1000	183 - 1032	238 - 1977	422 - 4947
10000	942 - 2563	1326 - 4662	1972 - 8778

[a] See Figure 5.1, Gryseels & Polderman (1987) and Gryseels, Nkulikyinka & Engels (1991) for epidemiological characteristics.

[b] Indicated are 90% ranges; i.e. 5% chance that WP is below and 5% that WP is above this range. The calculations are based on a model for egg count variation (De Vlas & Gryseels, 1992; De Vlas, Van Oortmarssen & Gryseels, 1992; De Vlas *et al.* 1992), assuming a productivity of 1.0 EPG per WP.

like perfect negative binomial distributions of egg counts and worm burdens, and no disturbances by, for example, migration or chemotherapy interventions (De Vlas *et al.* 1992).

There are good reasons to believe that worm numbers in endemic communities are significantly higher than is usually perceived. Clearly, fundamental questions in the dynamics of schistosome infections remain unanswered, hampering the development of epidemiological models, and consequently of tools and strategies for control. Hopefully, new techniques for the assessment of worm burdens, such as antigen detection assays (Deelder *et al.* 1994), will improve our diagnostic and epidemiological toolbox. Meanwhile, we have to resort to statistical and mathematical approaches to solve, or at least to identify, such crucial gaps in our knowledge.

Acknowledgements

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PART III

Accuracy of egg counts

6

UNDERESTIMATION OF *SCHISTOSOMA MANSONI* PREVALENCES[1]

Summary

Field methods used for detecting *Schistosoma mansoni* infection miss a certain proportion of the infections. Prevalences of infection appear to be far underestimated by faecal screening, with important consequences for control and research. In this chapter, we investigate how the number of undetected infections can be statistically inferred from population surveys.

6.1 Introduction

Most control programmes and epidemiological studies on *Schistosoma mansoni* infection are based on the detection and quantification of parasite eggs by faecal thick-smear methods, such as the Kato-Katz technique (Katz, Chaves & Pellegrino, 1972; Mott & Cline, 1980). These methods allow the examination of large numbers of samples within a short time, and provide useful quantitative epidemiological information. However, in operational conditions it is generally not possible to examine more than one stool sample per individual. Repeated examinations or the application of more sensitive methods show that infections, especially light ones, may then be missed (the so-called 'false negatives') and prevalences underestimated (Jordan *et al.* 1975; Barreto *et al.* 1978; Ruiz-Tiben *et al.* 1979; Mott & Cline, 1980; Sleigh *et al.* 1982; Polderman *et al.* 1985; Da Cunha, Cancado & De Rezende, 1987; Barreto, Smith & Sleigh, 1990; Ruppel *et al.* 1990; Gryseels, Nkulikyinka & Engels, 1991).

6.2 The relevance of missed infections

Light infections, missed after screening, are not considered a crucial problem to morbidity control, as it is assumed that they are not associated with severe pathology (Jordan & Webbe, 1982; WHO, 1985). Nevertheless, it can be useful to estimate the

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[1] De Vlas SJ & Gryseels B (1992). Underestimation of *Schistosoma mansoni* prevalences. *Parasitology Today* **8**, 274-277. Re-used with permission of Elsevier Science Ltd.

proportion of missed infections for several reasons. First, the relationship between egg counts and morbidity is less straightforward than generally assumed (Gryseels & Polderman, 1991). Autopsy data show that the relationship between worm load and faecal egg counts is variable, particularly in cases with severe pathology, such as pipe stem fibrosis, where egg counts may be low or even negative (Cheever, 1968; Kamel *et al.* 1977). More recently, this has also been demonstrated in community-based ultrasound studies (Homeida *et al.* 1988a). Second, infections that remain undetected and untreated may be partly responsible for the persistence of transmission after population chemotherapy, a major problem in many control programmes (Polderman & De Caluwé, 1989; Webbe & El Hak, 1990; Butterworth *et al.* 1991; Gryseels, Nkulikyinka & Engels, 1991). Third, the sensitivity of detection decreases as the intensity of infection decreases; the proportion of missed infections increases after treatment, leading to an overestimation of cure rates (Goddard, 1977; Da Cunha, Cancado & De Rezende, 1987). False negatives obscure the results of selective chemotherapy by showing up as positives at the subsequent screening round (Gryseels, Nkulikyinka & Engels, 1991). Fourth, persistent light infections may be crucial for the maintenance of concomitant immunity and thus also for the understanding of acquired resistance and the development of possible vaccine strategies (Hagan, 1992; Woolhouse, 1992b; Gryseels & Polderman, 1992). In sero-epidemiological surveys, 'false positive' humoral reactions in parasitologically negative individuals cannot be properly interpreted; they may be due to light infections but also to either cross-reactions or past infections (Polderman & Deelder, 1977; Ruiz-Tiben *et al.* 1979; Mott & Dixon, 1982; Ruppel *et al.* 1990).

6.3 How many infections are missed by faecal egg counts?

Several authors have tried to estimate and to improve the sensitivity of egg counts by repeating stool examinations (Jordan *et al.* 1975; Barreto *et al.* 1978; Ruiz-Tiben *et al.* 1979; Mott & Cline, 1980; Sleigh *et al.* 1982; Polderman *et al.* 1985; Teesdale, Fahringer & Chitsulo, 1985; Da Cunha, Cancado & De Rezende, 1987; Barreto, Smith & Sleigh, 1990; Ruppel *et al.* 1990; Gryseels, Nkulikyinka & Engels, 1991). Most of these studies have been limited to three stool samples. On the basis of empirical data from eight communities, Jordan *et al.* (1975) have related prevalences estimated by a filtration method in a single stool (x) to those obtained from examining three stools (y). The formula they obtained using polynomial regression

$$y = 4.987 + 1.72754x - 0.0077x^2$$

is only applicable for the comparison of results from one and three stools. For every other combination, new empirical data must be gathered and processed. Furthermore, it is

not known how accurate the estimation of the 'true prevalence' with three or more examinations really is. How many more infections would show up after additional examinations? It would clearly be useful to have a generally valid estimation of true prevalences on the basis of conventional stool surveys.

The classical assumption of sensitivity and specificity being intrinsic, constant characteristics of a diagnostic test (McClish & Quade, 1985; Walter & Irwig, 1988) is not applicable for most helminthic infections. The probability of detecting parasite eggs in the stools of an individual depends on the intensity of his infection (Teesdale, Fahringer & Chitsulo, 1985; Gryseels, Nkulikyinka & Engels, 1991). Relationships between the sensitivity of stool examinations and prevalences, shown for example by Jordan *et al.* (1975), are in fact due to the relation between prevalence and intensity of infection (Goddard, 1977; Walter & Irwig, 1988). Goddard (1977) recognized that sensitivity varies with the level of endemicity, and modelled the probability of a false negative outcome as a negative exponential function of the prevalence. Exploring the consequences on the interpretation of the effects of chemotherapy, he demonstrated that cure rates are overestimated when detection of infection is imperfect. However, his model was not tested with empirical data and lacked a satisfactory relationship between prevalence and intensity of infection. In order to quantify underdiagnosis, we would need an approach that takes into account this relationship and, more generally, the underlying mechanisms of egg count variations in the population.

Recently, a stochastic model has been developed which incorporates the distributions of worms and worm pairs in the population, as well as the variability of egg counts in stools samples from an individual with a given worm load (De Vlas *et al.* 1992). The model thus explicitly distinguishes inter- and intra-individual variation in egg counts, and implies a consistent statistical relationship between prevalence and intensity of infection according to a negative binomial distribution. Empirical data based on single and repeated faecal egg counts from endemic communities in Zaire and Burundi have been used to test and validate this model (Polderman *et al.* 1985; Gryseels, Nkulikyina & Engels, 1991; De Vlas *et al.* 1992). Mathematical details of the model are found in Appendix 6.1.

By making inferences about the worm and worm pair distributions, the model can be applied to analyze the underestimation of prevalences in any surveyed population. The *true* prevalence of active infection is defined here as the proportion of individuals with at least one worm pair, and is calculated from the (estimated) distribution of worm pair numbers (Eqn 1, Appendix 6.1). The *observed* prevalence, defined here as the proportion of individuals that would show at least one positive egg count in a number of samples, is projected from Eqn 2. People who have no or monosexual infections will always show negative egg counts, i.e. true negatives. However, as a result of the intra-individual variation in egg counts, negative counts can also be found in samples from people with

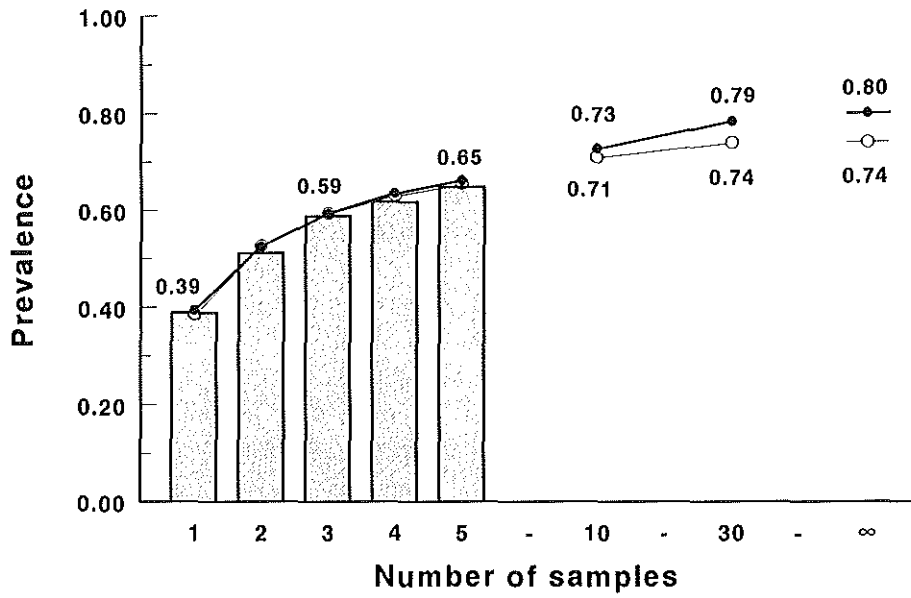

Figure 6.1 Prevalences of *S. mansoni* infection over successive surveys. The data concern 131 inhabitants of the village of Buhandagaza in Burundi, aged 20 and older, of whom five consecutive egg counts in 50 mg Kato slides (Katz, Chaves & Pellegrino, 1972) were obtained (Gryseels, Nkulikyinka & Engels, 1991). Bars indicate the observed proportion of positively diagnosed individuals. The model (Appendix 6.1) has been fitted to all quantitative egg counts of each individual. The two curves represent predicted prevalences, shown for two values of the expected number of eggs per sample per worm pair: $h = 0.05$ (●) and $h = 0.14$ (○). The value $h = 0.05$ was obtained by maximum likelihood estimation from the complete Buhandagaza data (De Vlas *et al.* 1992) and equals the mean value based on experiments in several non-human mammals (Loker, 1983). The corresponding values of mean (M) and index of aggregation (k) of the negative binomial distribution of worm numbers are $M = 78$ and $k = 0.40$. The value $h = 0.14$ approximates the results of an Egyptian autopsy study (Cheever *et al.* 1977), and is within the confidence interval from the Buhandagaza data set; the corresponding parameter values are $M = 29$ and $k = 0.46$. The index of aggregation for the negative binomial distribution of repeated individual egg counts (r) is 0.87 (De Vlas *et al.* 1992). The model predictions agree very well with the observed prevalences. The true prevalence is theoretically obtained by examining an infinite number of samples. The results differ slightly for the two values of h, but in both cases the true prevalence would be about twice as high as the prevalence measured in a single survey.

low numbers of worm pairs, i.e. false negatives. The difference between true and observed prevalence is the proportion of false negatives in a population survey.

Figure 6.1 shows an application of the model for selected data from Burundi. The prevalence measured in a single stool survey was 39%, increased to 59% after three surveys and to 65% after five. The agreement between measured and projected prevalences after one to five surveys shows that the model fits the data very well. Further extrapolation with the model predicts that, in such an endemic situation, the true

prevalence would be between 74% and 80%. Thus, only about half of all infected individuals would have been detected if only one survey had been done.

Figure 6.2 demonstrates the consequences of the model in a more general way, by showing the relationship between the prevalence and the proportion of false negatives. The relation with intensity is incorporated in the value of k. The graphs show that, particularly in low-prevalence areas, the underestimation of prevalences can be extremely important. An observed prevalence of 20% from a single survey would actually (for k = 0.5) correspond to a true prevalence higher than 70%. As may be expected, the proportion of false negatives decreases after examining multiple stools, but a considerable number of infections would still show up after more than three egg counts; even after 30 surveys, previously undetected infections may be found (Figure 6.1).

The consequences of the underestimation of prevalences for the interpretation of control results are shown by the following example. A prevalence reduction from 40% to 20%, based on one sample per individual, appears considerable. Assessment of the true prevalences, assuming moderate overdispersion (k = 0.5), indicates a much less spectacular reduction from 85% to 71%. The proportional prevalence reduction in this case would thus be 14% instead of 50%. It may be argued that the negative binomial distribution at the basis of these projections may not be valid shortly after population treatment. However, reduction of worm loads in heavily infected individuals would decrease overdispersion, giving a higher value of k, and would result in an even lower sensitivity of detection (Figure 6.2).

6.4 Concluding remarks

We have attempted to formalize the obvious point that single or even multiple faecal egg counts reveal only a proportion of the infections with *S. mansoni* in an endemic community. More sensitive parasitological methods, such as elaborate concentration techniques or rectal biopsies, are not very suitable for field work. Indirect techniques such as haematuria dipsticks, which have shown their merit in surveys and control of urinary schistosomiasis (Taylor, Chandiwana & Matanhire, 1990), are unfortunately not available for schistosomiasis mansoni. Conventional immunodiagnostic assays to detect antibodies are not specific enough to provide a valid alternative, although antigen detection may be a more promising approach (De Jonge *et al.* 1988).

Previous attempts to determine underdiagnosis mathematically were not very satisfactory as they were either specific for particular data sets or did not make allowance for underlying egg count variation. Our approach shows that statistical methods can make a valuable contribution to the quantification of true prevalences. The results reveal that underestimation of prevalences, even by repeated egg counts, may be surprisingly large. Although our results provide no 'gold standard', comparative graphs such as those in

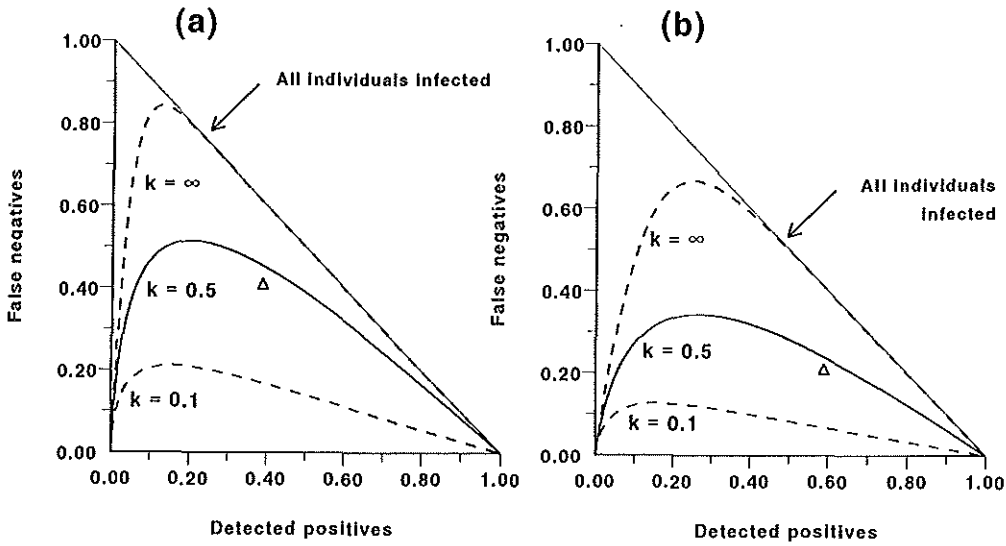

Figure 6.2 The relationship between the proportion of false negatives and the observed *S. mansoni* prevalence, i.e. proportion of detected positives, after examining one (a) and three (b) stool samples per individual. The chart is constructed on the basis of the model (see Appendix 6.1) by using combinations of all possible values for the mean (M) and three values for the index of aggregation (k) of the negative binomially distributed worm numbers. The values used for h and r are 0.05 and 0.87, respectively (see Figure 6.1). The values used for k are ∞, 0.5, and 0.1. If k approximates infinity, all individuals have the same chance of being infected, resulting in a Poisson distribution of the number of worms per individual. Lower values of k indicate heterogeneity among individual worm burdens, due to variations in exposure, acquired immunity and susceptibility to infection (Bradley & May, 1978). The worms are then concentrated in a heavily infected part of the population, resulting in a higher diagnostic sensitivity. The values 0.5 and 0.1 are the median value and the lower limit of k, respectively, obtained from several sets of data (De Vlas *et al.* 1992). For $k = 0.5$, for example, an observed prevalence of 20% in a single survey (one stool sample per individual) corresponds to a proportion of 52% of false negatives, and a true prevalence of 72%. An observed prevalence of 20% in three repeated surveys corresponds to 33% false negatives, and a true prevalence of 53%. The triangles represent the predictions from Figure 6.1.

Figure 6.2 may help field workers to estimate at least the order of magnitude by which they underestimate prevalences by conventional screening methods.

Our findings have substantial implications for the control of *S. mansoni*, that can also be extended to *S. japonicum*. Control programmes based on screening and selective chemotherapy will inevitably leave a large number of infected people untreated. Some of these may have severe pathology, and a potential reservoir for reinfection will always

remain untouched. Both considerations support the case for non-selective mass treatment (Gryseels & Polderman, 1991). Furthermore, the evaluation of control results from estimations of prevalence may lead to unwarranted optimism, as such estimates are inherently biased by a reduction of diagnostic sensitivity. In epidemiological and immunological research, true 'negative controls' in endemic areas cannot be reliably selected on the basis of stool examinations. Defining and modelling resistance on the basis of 'absence of infection' should thus be treated with great caution (Hagan, 1992). Foolproof assessment of the sensitivity and specificity of antibody-based serological assays will probably never be possible. Clearly, the development of more sensitive and specific diagnostic tools for the detection of *S. mansoni* infection deserves high priority.

Acknowledgements

We would like to thank G.J. van Oortmarssen, A.M. Polderman, J.D.F. Habbema, L. Bonneux, A.M. Deelder and K.E. Mott for their contributions. The field work in Burundi was supported by the Belgian Cooperation Agency (ABOS), and the WHO/UNDP/World Bank Special Programme for Training and Research in Tropical Diseases (TDR).

Appendix 6.1

A model for variations in S. mansoni *egg counts*

We consider three sources of variation responsible for the egg count distribution in a population:

(1) variation in exposure, acquired immunity, and susceptibility; the resulting probability for a member of the population of having n worms is indicated by $P_N(n)$.

(2) mating of male and female worms; the probability of having x worm pairs for an individual with worm load n is indicated by $P_{X|N}(x|n)$.

(3) variation in repeated egg counts from the same individual, which depends on several processes such as day-to-day fluctuations of egg production, the amount and consistency of stools, etc.; the probability of counting y eggs in a stool sample from an individual harbouring x worm pairs is represented by $P_{Y|X}(y|x)$.

The distribution of the number of worm pairs in the population is a mixture of (1) and (2) and is expressed by:

$$P_X(x) = \sum_n P_{X|N}(x|n) \cdot P_N(n) \qquad (1)$$

The joint distribution of egg counts y_1, y_2, ..., y_w in w consecutive stool samples is now described by:

$$P_{Y_1, Y_2, \ldots, Y_w}(y_1, y_2, \ldots, y_w) = \sum_x \left[\prod_{i=1}^{w} P_{Y_i|X}(y_i|x) \right] . P_X(x) \qquad (2)$$

In *S. mansoni* infection, theoretical and empirical considerations support the use of the negative binomial distribution for describing both the variation in worm numbers and the individual variation in faecal egg counts (Bradley & May, 1978; De Vlas *et al.* 1992). We consider schistosomes to be monogamous with a male:female ratio of 1:1, and we therefore use a binomial distribution with $p = 0.5$ for describing the mating process. Under the assumption that egg excretion is not density dependent, a linear relationship between worm pair load, x, and the mean number of eggs per sample is used (De Vlas *et al.* 1992).

The model now enables the estimation of the worm and worm pair distribution in the population, $P_N(n)$ and $P_X(x)$, respectively, on the basis of actual egg counts, which provides insight into the 'true prevalence'.

7

A POCKET CHART TO ESTIMATE TRUE *SCHISTOSOMA MANSONI* PREVALENCES[1]

Summary

In the previous chapter, we have explored the extent and implications of the underestimation of *Schistosoma mansoni* prevalences, as measured by faecal screening. In the current chapter, we present a practical and generally applicable chart to infer 'true' prevalences from simple egg count surveys.

7.1 Introduction

Commonly applied screening methods for *Schistosoma mansoni* infections leave a large number of infections undetected (De Vlas & Gryseels, 1992). Repeated egg counts in consecutive samples can improve sensitivity, but require considerable additional efforts and expenses (Jordan *et al.* 1975; Barreto *et al.* 1978; Gryseels, Nkulikyinka & Engels, 1991). It would be useful to dispose of a practical statistical tool to derive more accurate prevalence estimations from a simple single-stool survey. The proportion of missed infections in a given endemic situation can be estimated by means of a stochastic model of egg-count variations (De Vlas & Gryseels, 1992; De Vlas *et al.* 1992). However, the parameters of the model have to be re-estimated for each particular setting. Field workers will obviously wish to avoid this mathematical hurdle and use simple statistics, such as measured prevalences and mean egg counts, to estimate the real number of infected individuals.

7.2 Linking field statistics to model parameters

Our egg-count model is based on two sources of variation: those between individuals due to differences in the number of worms or worm pairs harboured; and those within

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[1] De Vlas SJ, Gryseels B, Van Oortmarssen GJ, Polderman AM & Habbema JDF (1993). A pocket chart to estimate true *Schistosoma mansoni* prevalences. *Parasitology Today* **9**, 305-306. Re-used with permission of Elsevier Science Ltd.

individuals due to the variability of egg excretion and its measurement. Testing of several data sets indicated that the parameters concerning within-individual egg count variation are independent of the endemic situation (De Vlas *et al.* 1992). The two parameters of the assumed negative binomial distribution (Bradley & May, 1978; De Vlas *et al.* 1992) for worm loads then suffice to characterize observed egg counts in a population for a certain age group: the mean individual worm load, M, and the index of aggregation, k. Rather than a random distribution of worms, the negative binomial supposes aggregation of infection in part of the population; the lower the value of k, the more aggregation. Each value of M and k can be translated into a hypothetical endemic situation with a specific prevalence and mean egg count. Conversely, each combination of observed prevalence and mean egg count corresponds to a unique combination of M and k, which can be derived from the model (see Appendix 7.1).

7.3 A practical pocket chart

By calculating the corresponding values of M and k numerically for all combinations of a given prevalence and geometric mean of positive egg counts, we have now constructed a chart (Figure 7.1) that makes it possible to project a 'true prevalence' from any observed data set. This chart allows the field worker to circumvent M and k: by plotting the observed prevalence against the geometric mean egg count of positive individuals, the projected true prevalence can be derived from the dashed contour lines. For example, the chart indicates that an observed combination of a 60% prevalence and (geometric) mean egg load of 200 eggs per gram faeces (EPG) implies that 85% of all individuals would carry at least one worm pair. Observation errors are most significant in areas with low prevalences and egg counts. For a prevalence of 20% and a mean egg load of 40 EPG, for example, the true prevalence could be as high as 60%.

It must be noted that the chart is based on the conventional examination of one sample of 50 mg of stools, but can be adapted for other numbers and sizes of samples. The chart should be applied separately for different age groups, as the level and aggregation of worm loads change with age (De Vlas *et al.* 1992). The model covers only the areas within the solid lines. The upper left corner combines very high prevalences with very low mean egg counts, which would not satisfy the generally accepted negative binomial distribution for worm loads (Bradley & May, 1978; Anderson & May, 1991a). However, such observations could result from control measures in which only heavily infected persons are treated. In general, the chart is not applicable after population chemotherapy, during a variable period that depends on the type of intervention and the level of re-infection. The lower right corner combines low prevalences with very high mean egg counts. This combination indicates unlikely extreme aggregation ($k < 0.05$) (De Vlas *et al.* 1992). Nevertheless, it could temporarily result from large-scale

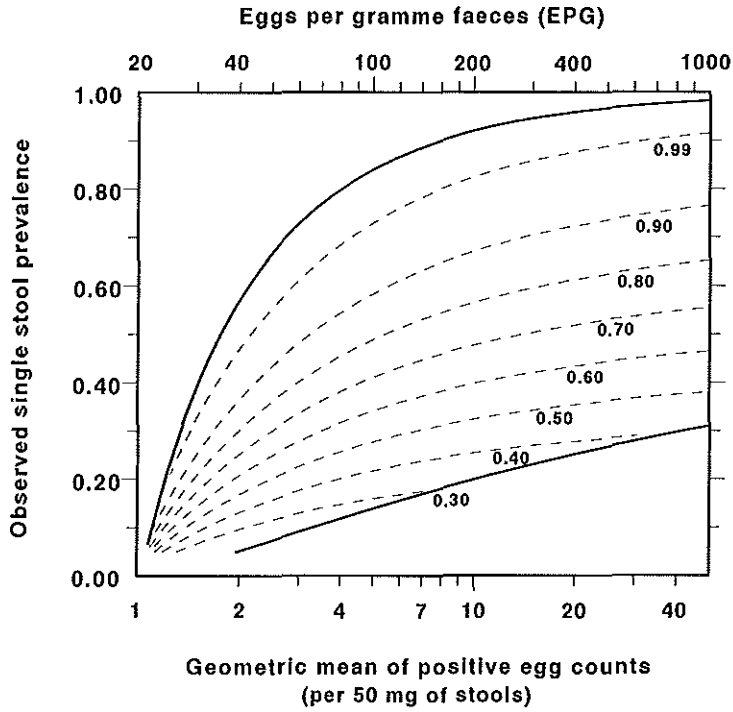

Figure 7.1 Pocket chart to estimate true *Schistosoma mansoni* prevalences. For each combination of observed prevalence and geometric mean of positive egg counts, the estimated proportion of individuals that harbour at least one worm pair can be read from the broken contour lines. The model on which the predictions are based applies to pre-control situations, and holds only between the solid lines. The chart is based on egg counts obtained in 50 mg stools, and should preferably be applied for separate age groups (e.g. five-year age classes in children, and 10-20-year age classes in adults).

immigration of uninfected individuals into a sparsely populated area of high endemicity. Data from 'mixed' populations (e.g. in schools which host children from villages with strongly different endemicity levels) should be handled with some caution. One should also be aware that the results of the chart in the lower left side could be different due to the possibility of cercarial single-sex clustering (May, 1977), which the model does not account for. Though available data from the literature do fit the model projections quite well (De Vlas & Gryseels, 1992; De Vlas, Van Oortmarssen & Gryseels, 1992; De Vlas *et al.* 1992), the chart would benefit from further validation in the field (e.g. by data from repeated egg counts, or serological surveys as compared to single stool examinations). All contributions in this respect would be welcomed.

This easily applicable 'pocket chart' can be a useful tool for several purposes:

- Public-health workers responsible for control can obtain a close approximation of the real number of infected people, which can be considerably underestimated by con-

ventional surveys. Though most missed infections are light and may have little consequence for morbidity and its control, their detection and treatment may become more important when control of infection *per se* and/or control of transmission is envisaged.

- For the allocators of funds, the chart may help to resolve the question of when it becomes more cost-effective to apply indiscriminate mass treatment, rather than selective treatment based on screening.
- For researchers, insight into the real proportion of people infected is indispensable to model the dynamics of transmission and of human immunity, the mechanisms of which are still poorly understood (Hagan, 1992; Gryseels & Polderman, 1992).
- For the evaluation of new diagnostic methods, the chart may help to estimate the acceptable proportion of false positives, and thus to some extent provide a 'statistical gold standard'.

The chart presented here has been developed for *S. mansoni* infection. Provided that suitable descriptive distributions can be found, the exercise could also be extended to other schistosome species, and to many other parasites of which the diagnosis is based on the detection of eggs or larvae in excreta, blood or tissues.

Appendix 7.1

Calculation of the true prevalence of Schistosoma mansoni

Assuming monogamous mating between male and female worms, the probability of having at least one worm pair, and thus also the true prevalence, equals:

$$1 - \textit{proportion of individuals without infection} - \textit{proportion with single-sex infection} =$$

$$1 - \left[\frac{k}{M+k}\right]^k - \sum_{n=1}^{\infty} \frac{\Gamma(k+n)}{\Gamma(n+1)\,\Gamma(k)} \cdot \left[\frac{M}{M+k}\right]^n \cdot \left[\frac{k}{M+k}\right]^k \cdot \left[\frac{1}{2}\right]^{n-1} =$$

$$1 + \left[\frac{k}{M+k}\right]^k - 2\left[\frac{2k}{M+2k}\right]^k$$

in which M and k are the mean and index of aggregation, respectively, of the negative binomial distribution of worm burdens n ($n = 1, 2, \ldots$) and $\Gamma(.)$ indicates the gamma function.

8

VALIDATION OF A CHART TO ESTIMATE TRUE *SCHISTOSOMA MANSONI* PREVALENCES FROM SIMPLE EGG COUNTS[1]

Summary

Schistosoma mansoni egg counts by faecal examination vary considerably and are not very sensitive, so prevalences are underestimated. The distribution of egg counts can adequately be described by a stochastic model which distinguishes variation in counts between persons and variation in repeated counts within a person. Based on this model a pocket chart has been developed which predicts the proportion of individuals harbouring at least 1 *S. mansoni* worm pair - the 'true prevalence' - from a simple single survey prevalence and geometric mean egg count (using common duplicate 25 mg Kato-Katz smears). The current chapter describes the validation of this chart by comparing predicted true prevalences with prevalences observed after 5-7 repeated Kato-Katz faecal examinations (Burundi), by examination of a large quantity of stool using the Visser filter (Brazil) or a selective sedimentation-filtration method (Surinam). Because 5-7 repeated examinations do not suffice to measure all infections, predictions have been made of the cumulative proportion positives over 5-7 surveys - the 'approximate true prevalence' - as well. After dividing the data into age groups, 12 different subsets were considered for validation. In all 12 cases, predicted true prevalences (or approximate true prevalences for the Burundi data) agree well with those observed. The overall agreement depends only slightly on the assumed relationship between worm numbers and mean egg counts, with a good fit for a productivity between 0.8 and 4.4 eggs per gramme faeces (EPG) per worm pair (WP). This interval includes the most plausible value from the literature, i.e. 1.0 EPG/WP, which has been applied in the initial pocket chart. These findings support the validity of the chart to predict true prevalences for a wide range of productivity assumptions, and reinforces the applicability of its underlying stochastic model to describe egg count variation. However, as predictions appear to vary importantly when using only

[1] De Vlas SJ, Engels D, Rabello ALT, Oostburg BFJ, Van Lieshout L, Polderman AM, Van Oortmarssen GJ, Habbema JDF & Gryseels B (1997). Validation of a chart to estimate true *Schistosoma mansoni* prevalences. *Parasitology* **114** (in press). Re-used with permission of Cambridge University Press.

part of the data, it is also concluded that the pocket chart never compensates for limited validity of initial single survey prevalences and geometric means in consequence of small sample sizes.

8.1 Introduction

Detection and quantification of human *Schistosoma mansoni* infection is mainly based on counting eggs in stools. The faecal thick-smear technique (Kato & Miura, 1954; Katz, Chaves & Pellegrino, 1972) is widely accepted as the best diagnostic tool for use in the field (WHO, 1993). Slides prepared from templates with 20-30 mg of stools can already be screened after 15 minutes (Peters *et al.* 1980), and if necessary be followed by treatment on the spot. Since no alternatives for such an easily and rapidly applicable quantitative method are available as yet, the Kato-Katz technique is also the method of choice for population-based research and intervention studies on *S. mansoni*. Its lack of sensitivity (especially in detecting light infections), however, hampers interpretation of research results and evaluation of control programmes (De Vlas & Gryseels, 1992). Moreover, common knowledge on human worm burdens and *S. mansoni* egg production is conflicting, causing complications in relating egg counts to the number of worms actually harboured (Gryseels & De Vlas, 1996).

Recently, a stochastic model for egg count variation has been developed which incorporates the distributions of worms and worm pairs in the population, as well as the variability of egg counts in repeated stool samples from an individual with a given worm pair load (De Vlas *et al.* 1992). The model thus explicitly distinguishes inter- and intra-individual variation in egg counts, and relates egg counts to worm burdens. Empirical data based on single and repeated faecal egg counts from several endemic communities could be described with only a few parameters. On the basis of this model a practically applicable pocket chart to predict true *S. mansoni* prevalences has been constructed (De Vlas *et al.* 1993a). This chart uses 2 measures from field observations: (1) the prevalence from 50 mg single stool surveys (duplicate 25 mg Kato-Katz smears), and (2) the corresponding geometric mean egg count of detected positive individuals. By plotting the observed prevalence against the geometric mean, one can predict the true prevalence from the contour lines (Figure 8.1). The pocket chart is based on 2 hypotheses: (1) the underlying model for egg count variation can adequately be explained by only these 2 simple field measures, and (2) the true prevalence can properly be predicted from this model. In the original paper, researchers were invited to test the validity of this chart by comparing predicted true prevalences from the chart with observed true prevalences after using more sensitive techniques (De Vlas *et al.* 1993a). The current chapter describes the experiences so far.

8.2 Materials and methods

8.2.1 Epidemiological data

The data come from 4 population surveys in 3 different countries: Burundi, Brazil and Surinam. 'Simple' prevalence and geometric mean EPG (eggs per gramme faeces) for positive individuals were based on examination of approximately 50 mg of faeces from a single stool sample. Duplicate 25 mg Kato-Katz faecal samples were prepared as described by Katz *et al.* (1972) and Polderman *et al.* (1985). More reliable prevalences for validation of the pocket chart (the 'observed true prevalence') were obtained after repeated Kato-Katz surveys, or by the additional examination of much larger amounts of stools using filtration techniques as described below. All individuals excreting eggs received treatment by praziquantel (40 mg/kg). In Brazil, this was 60 mg/kg for patients < 15 years and 50 mg/kg for patients ≥ 15 years.

In Gihungwe (Burundi), repeated surveys were carried out on 7 occasions (days 1, 3, 5, 8, 10, 32, 37) in a study population of 200 individuals (100 adults and 100 children). Follow-up was almost complete, only 17 individuals missed one survey. The observed true prevalence is calculated as the cumulative proportion of positives after all 7 (or 6) measurements. The details of study-design and further outcomes are described by Engels, Sinzinkayo & Gryseels (1996).

In Buhandagaza (Burundi), 5 repeated surveys were performed with intervals of about 3 months (Gryseels & Nkulikyinka, 1988; Gryseels, Nkulikyinka & Engels, 1991). This data-set has previously been used to estimate parameters of the egg count model (De Vlas *et al.* 1992). For the present validation of the pocket chart, we only use those 231 persons (out of 435) with complete follow-up. The observed true prevalence is calculated by the accumulation of all 5 surveys.

In Sabará, Minas Gerais state (Brazil), the Visser method was used for detecting all *S. mansoni* infections in a random sample of 141 (out of 347) school children. The Visser filter enables examination of several grammes of faecal material (Pitchford & Visser, 1975; Schutte *et al.* 1994). For the current study, faecal samples were calibrated at around 1 g. Specimens to be filtered were formalinized, and eggs were stained with acid fuchsin on filter paper (Bell, 1963). Use of this highly sensitive method left only 1 positive individual using the Kato-Katz method (from the same stool sample) undetected. This lightly infected person, who only showed 1 egg, was included for the observed true prevalence.

In Catharina Sophia, district of Saramacca (Surinam), the sedimentation-selective-filtration (SSF) technique was used as a sensitive technique. Approximately 2-3 g of stools from each of 205 subjects were investigated. SSF is comparable to the Visser filter, but includes several steps of washing and rinsing. Probably this had lead to some 'loss of

eggs', because 2 Kato-Katz surveys (1 from the same and 1 from an additional stool sample) showed another 16 individuals positive in addition to the 70 detected by SSF. See Polderman *et al.* (1994) for a description of the SSF technique and further details of this study. The observed true prevalence is assumed to be the combination of positives from SSF and both surveys.

The 4 data-sets were divided into age categories of about 50 individuals, with the condition of at least 20 of them being positive after 1 survey in order to guarantee a reliable estimate of the geometric mean EPG among positives. In total, 12 different subsets resulted for validation of the pocket chart (Table 8.1). In cases with repeated examinations - i.e. Gihungwe (7), Buhandagaza (5), and Catharina Sophia (2) - we have made predictions of the true prevalence using the single survey prevalence and geometric mean of each survey separately, and then considered the average result for validation. This was done in order to diminish the effect of variation in initial measurements of single stool prevalence and geometric mean of positives due to the low number of individuals investigated. However, for the Gihungwe data we will also evaluate what would have happened in case only 1 survey had been available.

8.2.2 Model structure and validation

All 'true' prevalences inferred from the pocket chart (Figure 8.1) have been based on an existing stochastic model which distinguishes variation in *S. mansoni* egg counts *between* individuals due to differences in the number of worm pairs harboured, and *within* individuals due to the variability of egg counts (De Vlas *et al.* 1992). The model is essentially based on 4 parameters: the mean M and aggregation parameter k of the underlying negative binomial distribution of individual worm burdens, the relationship between egg counts and worm burdens, and the aggregation parameter r of the negative binomially distributed egg counts within an individual. The smaller the value of k, the more the worms are aggregated in a small, highly infected part of the population. Similarly, a smaller value of r means that eggs are less homogeneously distributed over the subsequent stool samples and corresponds with more variation in repeated measurements. The relationship between egg counts and worm burdens has been assumed 1.0 EPG/WP (see also Gryseels & De Vlas, 1996). The aggregation in repeated individual egg counts r was earlier estimated at $r = 0.87$ (De Vlas *et al.* 1992), and has also been assumed fixed for all further calculations.

This leaves only M and k as 'free' parameters to describe a particular endemic situation (for a specific age group). The pocket chart assumes that the prevalence and geometric mean EPG among positive individuals can adequately determine M and k, and thereby used to explain the whole model. The chart was constructed by first calculating the values of M and k that correspond to each combination of prevalence and geometric

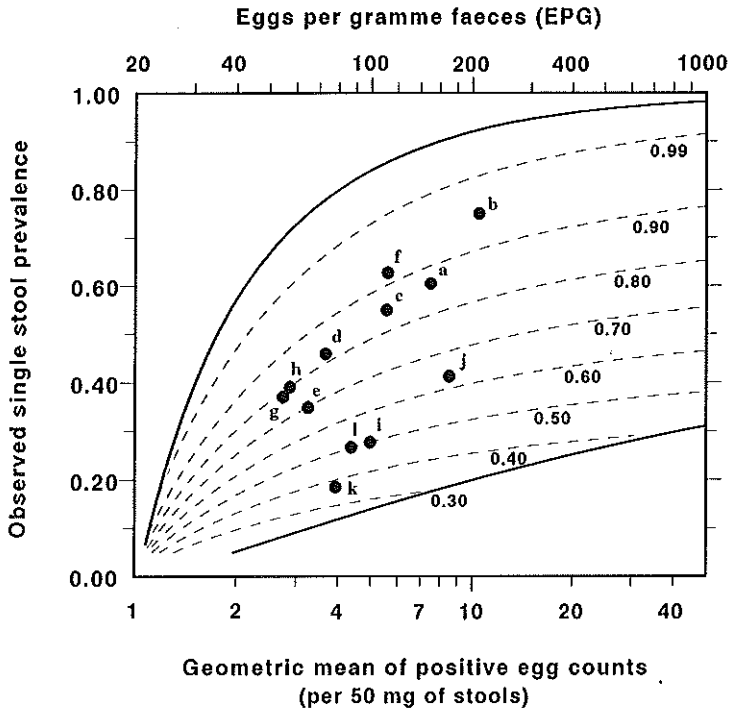

Figure 8.1 Pocket chart to estimate true *Schistosoma mansoni* prevalences. By plotting observed single survey prevalence against the geometric mean of positive egg counts, the proportion of individuals with at least 1 worm pair (i.e. the true prevalence) can be predicted from the dashed contour lines. The model which underlies the predictions only applies for pre-control situations and is only defined between the solid lines (De Vlas *et al.* 1993a). The dots indicate the 12 different subsets that are used for validation of the chart, with indices referring to Table 8.1.

mean, and then predicting from these parameters the proportion of individuals with at least 1 worm pair (i.e. the true prevalence). Mathematical details of the model and the pocket chart can be found in previous papers (De Vlas *et al.* 1992, 1993a, b).

Validation of the pocket chart occurs through comparison of predicted true prevalences with the observed true prevalences using the more sensitive approaches described above. Overlap of 90% confidence intervals, a high correlation and no systematic differences between observed and predicted true prevalences are criteria for a good fit. We use 90% intervals rather than the standard 95% in order to diminish the chance of unwarranted acceptance of the chart. The mathematical background of the calculation of confidence intervals and the deviance are given in Appendix 8.1. We employ the deviance *dev* as an overall goodness-of-fit criterion to investigate alternative assumptions of both fixed parameters: the EPG/WP productivity and the aggregation r in repeated individual egg counts.

Table 8.1 Results of the pocket chart for predicting true *Schistosoma mansoni* prevalences from simple egg counts, validated through comparison with results from more sensitive parasitological methods. Geometric mean EPGs (of positives only) and simple observed prevalences (x 100%) were obtained from community surveys based on examination of about 50 mg Kato-Katz thick smears per individual. Predicted true prevalences represent percentages of individuals with at least 1 worm pair as can be read from the pocket chart using these two measures (Figure 8.1). 'True' prevalences were empirically obtained by using more sensitive methods: repeated Kato-Katz surveys and/or filtration methods (see last column). For adequate comparison of model predictions with the observations from repeated surveys, additional predictions have been made of the cumulative proportion positive after the actual number of measurements (viz. 7 for Gihungwe and 5 for Buhandagaza). In the text these are referred to as 'approximate true prevalences'. Validation of the pocket chart is based on the comparison of the predicted (approximate) true prevalences with the observed prevalences from the sensitive method (both in **bold** typeface). Figure 8.2 gives a graphical representation of the agreement. All intervals are 90% CI.

Focus (country)/ age category	No. of individuals	Geometric mean EPG	Observed single survey prevalence	Predicted true prevalence from the pocket chart	Predicted prevalence after actual number of measurements	Observed prevalence with sensitive method	Sensitive method
Gihungwe (Burundi)							
a 5-8	48	150	60.4	86.4 ± 3.3	**81.0** ± 3.2	**83.3** ± 7.4	
b 9-16	52	209	75.0	95.4 ± 1.8	**92.2** ± 2.2	**92.3** ± 5.3	7 repeated
c 18-33	49	111	54.9	84.7 ± 3.7	**78.4** ± 3.6	**77.6** ± 7.9	surveys
d 34+	51	73	45.9	82.1 ± 5.1	**73.9** ± 4.7	**68.6** ± 8.4	
Buhandagaza (Burundi)							
e 5-9	55	65	34.9	70.0 ± 7.6	**57.7** ± 5.5	**69.1** ± 8.9	
f 10-19	45	112	62.7	91.7 ± 3.7	**84.7** ± 4.0	**84.4** ± 7.4	5 repeated
g 20-39	63	55	37.1	79.5 ± 7.2	**65.4** ± 5.9	**66.7** ± 8.2	surveys
h 40+	68	57	39.1	79.9 ± 6.0	**66.9** ± 5.3	**63.2** ± 7.6	
Sabará (Brazil)							
i 11-13	47	99	27.7	**49.9** ± 13.5	——	**48.9** ± 9.3	Visser filter
j 14-18	80	171	41.3	**63.3** ± 7.4		**55.0** ± 6.0	+ 1 survey
Catharina Sophia (Surinam)							
k 1-20	100	79	18.5	**37.2** ± 6.5	——	**38.0** ± 6.3	SSF method
l 21+	105	87	26.7	**49.9** ± 6.0		**45.7** ± 6.0	+ 2 surveys

8.3 Results

Table 8.1 gives an overview of the results for the 12 data sets considered. Single stool prevalences and geometric mean EPGs varied considerably between the communities and different age groups. A substantial part of the pocket chart is thereby covered (Figure 8.1). The corresponding true prevalences as predicted from the chart are given in Table 8.1, and can be compared with the observed prevalences from the sensitive methods.

Although the observed and predicted 'true' prevalences in most cases overlap, the pocket chart systematically overestimates all 8 observed prevalences from Burundi (Table 8.1). This is due to the fact that the repeated Kato-Katz faecal examinations do not result in detection of all (lowly) infected individuals. The 5 repeated surveys in Buhandagaza and the 7 in Gihungwe still showed new infections in the last survey (De Vlas & Gryseels, 1992; Engels *et al.* 1996), so that it was very likely that more infections would have been detected in further surveys. Thus, the cumulative proportion of positive individuals only approximates and undoubtedly underestimates the true prevalence, and is not adequate for comparison with predictions from the chart. We have therefore added to Table 8.1 a column with the predicted prevalence for the actual number of measurements: 7 for Gihungwe and 5 for Buhandagaza. These 'approximate true prevalences' can be derived from single survey data on prevalence and geometric mean in much the same way as the 'true prevalences' have been estimated. 'Pocket charts' that predict the cumulative proportion positive after a specific number of surveys can be obtained from the first author on request. Note that, hypothetically, the original pocket chart (Figure 8.1) predicts the cumulative proportion positive after an infinite number of repeated measurements. Henceforth, we will apply the predicted approximate true prevalences for comparison with the Burundi data. The validation of the pocket chart is now based on the agreement of the predicted (approximate) true prevalences and the observed prevalences using the sensitive methods (both in **bold** typeface, Table 8.1).

Figure 8.2 shows that for all 12 situations confidence intervals of the observed and predicted (approximate) true prevalences clearly overlap. Although the confidence intervals of observations and predictions are quite large, in 10 cases the agreement between point estimations is striking with differences between observation and prediction $\leq 5\%$, or just 2-4 persons. Plotting observed and predicted prevalences against the corresponding single survey prevalences shows that there was no relationship with the level of endemicity (Figure 8.2). There was furthermore no predominance in over- or underestimation of true prevalences, nor a relationship with age. The agreement, however, depended largely on the variation in initial measurements of single stool prevalence and geometric mean. The minimum and maximum predictions in Gihungwe differed dramatically if only 1 of the 7 surveys would have been used (Table 8.2). Without detracting from the overall statistical validation, this demonstrates that the chart is less informative

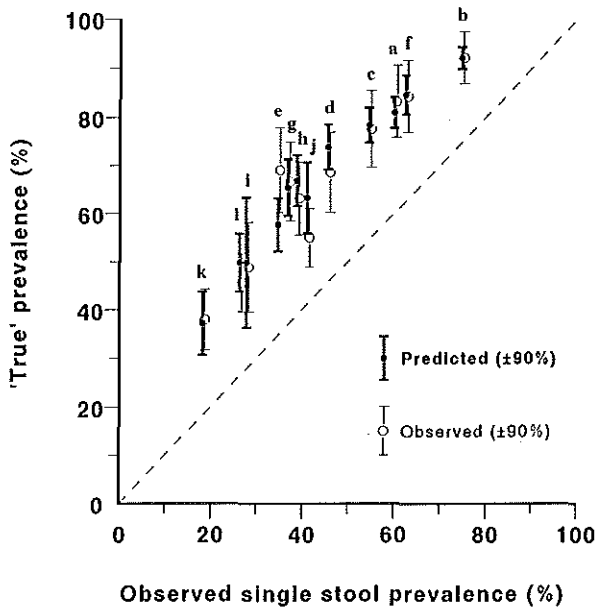

Figure 8.2 Graphical representation of the comparison between observed and predicted (approximate) true prevalences plotted against the corresponding single survey prevalences. The indices correspond with the 12 subsets in Table 8.1. Intervals represent 90% CI.

for operational situations where examinations on only a few persons are available. Note that the corresponding (much wider) confidence intervals in Table 8.2 in 7 out of the 8 cases still overlap with the observed prevalences in Table 8.1.

All predictions were based on a relationship between worm pair numbers (WP) and faecal egg counts (EPG) as 1.0 EPG/WP. At first sight, the results for alternative productivity assumptions would not differ much, as for most of the 12 data sets there would still be a good agreement between observation and prediction if a 5 times lower or 5 times higher productivity had been assumed. However, a productivity of 0.2 EPG/WP resulted in a systematic overestimation (Figure 8.3a) and a productivity of 5.0 EPG/WP in a systematic underestimation (Figure 8.3c) of (approximate) true prevalences, respectively, whereas for the initial assumption of 1.0 EPG/WP all 12 dots are evenly distributed around the dashed line (Figure 8.3b). This suggests that, based on this agreement of predicted and observed true prevalences, an optimal estimate for productivity can be found between both extremes. Figure 8.4 shows the joint goodness-of-fit, expressed by the deviance (see Appendix 8.1), as a function of the productivity of *S. mansoni* worms. The best fit was obtained for 2.0 EPG/WP (*dev* = 9.88), with a broad 95% confidence interval ranging from 0.8 to 4.4 EPG/WP. This interval includes the value 1.0 EPG/WP which was assumed in the pocket chart. A separate estimate for the relationship between worm burdens and egg counts according to age of the human host,

Table 8.2 Ranges (90% CI) in predicted cumulative proportion individuals positive for *Schistosoma mansoni* infection after 7 and an infinite number of repeated stool examinations, i.e. the true prevalence, based on the observed single stool prevalence and geometric mean EPG of positive individuals. For each of 4 age groups in Gihungwe village (Burundi), those 2 surveys (out of the 7 performed) have been selected that result in the lowest and the highest predictions.

Age category	No. of individuals	Geometric mean EPG	Observed single survey prevalence	Predicted prevalence after 7 surveys	Predicted true prevalence	
5-8	48	220	50.0	59-73	63-80	Lowest
	48	134	66.7	81-97	86-100	Highest
9-16	52	280	75.0	85-96	89-99	Lowest
	51	181	76.5	88-100	93-100	Highest
18-33	49	170	46.9	57-72	61-80	Lowest
	49	88	59.2	77-96	84-100	Highest
34+	51	96	37.3	48-67	53-77	Lowest
	50	75	54.0	72-96	79-100	Highest

showed a higher estimate for adults (3.0 EPG/WP) than for children (1.6 EPG/WP), but this extension of the model did not lead to a significant improvement of the fit (*dev* = 9.48; so $\chi^2_{DF=1} = 0.40$, $P \approx 0.5$). Figure 8.4 also shows that assuming a larger value for r, or less variation in repeated individual egg counts, did not alter the results significantly, although the best fitting productivity appeared to be somewhat lower (1.6 EPG/WP).

8.4 Discussion

This study tests whether true *S. mansoni* prevalences can be predicted from a pocket chart which uses only 2 simple field measures: the single survey prevalence and geometric mean among positives. In an earlier study we have already demonstrated that the underlying stochastic model for egg count variation can explain the relationship between prevalences from single and 3 stool examinations using an independent data source (Jordan *et al.* 1975; De Vlas, Van Oortmarssen & Gryseels, 1992). The current research is an extension in as much that now the model's ability to estimate (approximate) true prevalences has been tested.

Justification of the chart and model by measuring cumulative prevalences after repeated stool examinations will necessarily be incomplete, because some infections are probably still being missed. Seven consecutive stool examinations is, however, at the limit of

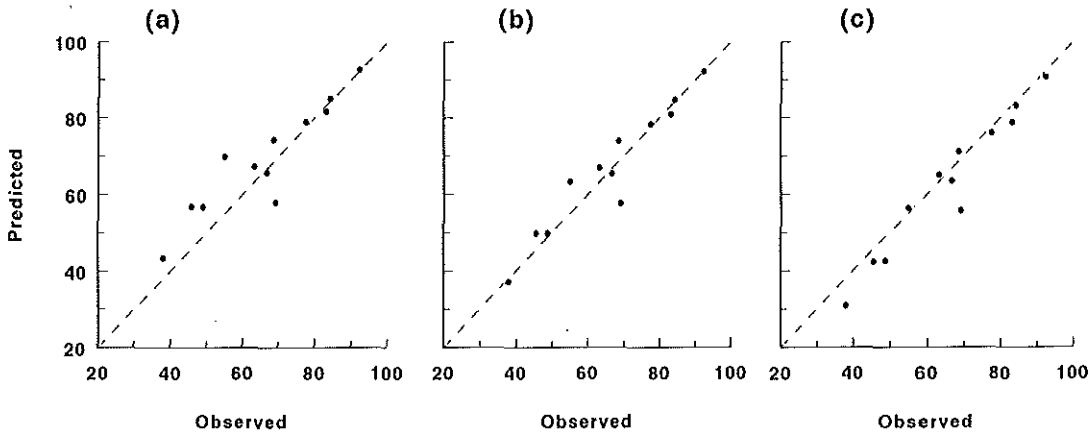

Figure 8.3 Relationship between observed and predicted (approximate) true prevalences for 3 alternative values of the productivity of *Schistosoma mansoni* worms: 0.2 (a), 1.0 (b) and 5.0 (c) eggs per gramme faeces (EPG) per worm pair (WP). Each dot represents a data set. Ideally, all dots would be positioned on the dashed line, leading to a deviance equal to 0. The value 1.0 EPG/WP is the most plausible value from the literature and has been used in the original pocket chart.

what can reasonably be obtained from community studies. Much longer periods between repeated examinations could avoid this problem but might, especially for young children, violate the initial model assumption of individual worm burdens not changing over time (De Vlas *et al.* 1992). The 3 month period between repeated examinations in Buhandagaza might therefore explain the relatively poor fit for 5-9 year olds. Without showing a clear trend, the prevalences for this group fluctuated considerably over time (between 29 and 44%). Furthermore, delaying treatment of positives until the end of a long series of examinations is unacceptable for ethical reasons. In this respect, the data set from Gihungwe, 200 persons examined at 7 occasions over a 1-month period with almost no loss to follow-up, can be considered as one of the most valuable sets ever collected.

Filtration of large amounts of stool also offers no guarantee that all infected individuals will be found, as empirically demonstrated by the detection of some additional infections by the Kato-Katz surveys. Theoretically, one can expect a few very light infections still being missed by examination of 1 g of faecal material (as by the Visser filter), given the assumed one-to-one relationship between individual worm pair burdens and EPGs (Gryseels & De Vlas, 1996). The more qualitative SSF method starts from 2 to 3 g of faecal material, but leads to significant loss of eggs as demonstrated by the mean EPG count which can be 10 times lower than by the Kato-Katz method (Polderman *et al.* 1995).

Taking into account these considerations, we conclude that the high level of agreement between observations and predictions strongly supports the validity of the pocket chart to predict true levels of *S. mansoni* infection, and its underlying model to describe variations in egg counts. Still, the cumulative proportion positive after 5 (Buhandagaza) and 7 (Gihungwe) repeated measurements only approximates the desired true prevalences, and thus provides only partial evidence. Comparable field experiments (in other endemic areas) would therefore still be welcome, especially if the endemic situation corresponds with a combination of prevalence and geometric mean that is located at parts of the pocket chart not yet covered. Determination of circulating antigens can provide another sensitive technique for testing the pocket chart. In the same Surinam community, the prevalence as predicted from the pocket chart was found in concordance with the results from immunodiagnosis by detection of the circulating antigens CAA and CCA (Van Lieshout *et al.* 1995a). However, more research is necessary to find out to what extent the false negatives by the Kato-Katz method were compensated by false positive individuals due to cross-reactivity reactions.

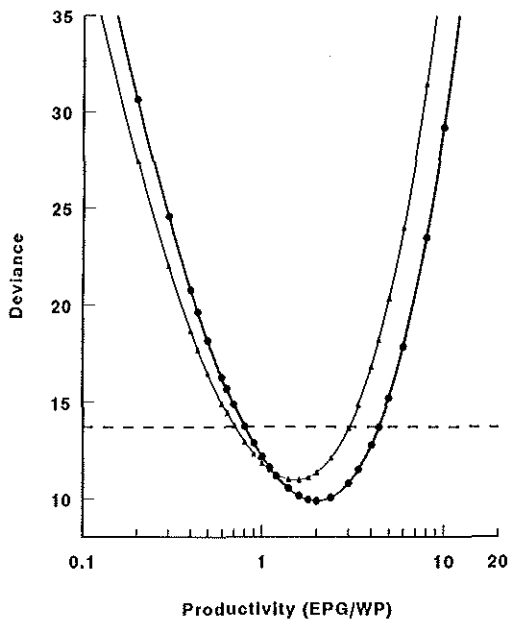

Figure 8.4 Deviance for different values of the productivity in eggs per gramme faeces (EPG) per *Schistosoma mansoni* worm pair (WP). The lowest deviance indicates the value for which the predicted (approximate) true prevalences fit the observed prevalences best. The horizontal dashed line is 3.84 (i.e. $\chi^2_{1;5\%}$) above the curve minimum and indicates the 95% confidence interval for the best estimated productivity. The dots indicate results for the aggregation in repeated individual egg counts as has been assumed in the pocket chart (r = 0.87), the triangles represent the calculations for a less intense aggregation (r = 1.0).

The broad range of adequate values for productivity of *S. mansoni* worms demonstrate that the pocket chart is not very sensitive to the assumed relationship between worm pair burdens and mean egg counts. This means that the current chart can still be considered valid in case future evidence would point out that other, not too different, values are more likely. On the population level, indeed, variability in productivity might exist because of differences in *S. mansoni* strains or immunity levels. The presence of some density dependence in worm fecundity will also leave the current chart unaffected. In general, a lower productivity corresponds with a higher mean worm burden which, in turn, coincides with a higher individual probability of harbouring at least 1 worm pair and hence a higher true prevalence. Given the wide range in productivity, it is nevertheless reassuring that the best estimate of 2.0 EPG/WP is of the same order of magnitude as the initially assumed value of 1.0 EPG/WP. This is in contrast with a productivity of 5.0 EPG/WP, as can be estimated from autopsy data (Cheever, 1968), which is outside the interval. It has earlier been pointed out that a ratio of 1.0 EPG/WP corresponds with individual worm burdens numbering up to thousands or even tens of thousands in areas of moderate to high endemicity (Gryseels & De Vlas, 1996). In contrast to egg productivity, the aggregation parameter r is more important for describing community data with repeated individual measurements and therefore predicting true prevalences. Its value depends on the duration between successive surveys and, for example, the schistosome species involved (De Vlas *et al.* 1992). Re-analysis of the data originally used for fitting the underlying egg count model revealed that missing values could have resulted in an overestimation of the level of aggregation in repeated examinations, and therefore in an underestimation of r (unpublished observations).[2] A value of $r = 1.0$, which seems to be more accurate, however, hardly influences the general validation of the pocket chart.

The applicability of the pocket chart to estimate, or at least approximate, true prevalences is hereby proven to be statistically valid, and very robust to the main assumptions on parameter values. This does, however, not certify that the chart is always reliable. In operational conditions field researchers will principally apply the pocket chart based on only 1 survey, and for example in Gihungwe the predictions would diverge considerably if only 1 out of the 7 surveys had been used. In 3 age groups confidence intervals of minimum and maximum predictions would not even overlap! This is clearly due to the fact that prevalence and geometric mean are not reliable if only based on a single examination of 50 individuals. For practical purposes, field researchers should therefore convince themselves that the number of investigated individuals is at least high enough for an accurate measurement of single survey prevalence and geometric mean to be used in the pocket chart. The true prevalence can obviously never be more trustworthy than the measurements used to estimate it.

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[2] This point is extensively discussed in section 12.3.

A fully validated pocket chart for obtaining the real number of infected people is a helpful tool for several purposes, for example, to decide whether indiscriminate mass treatment or selective treatment based on screening should be carried out, or to provide a 'statistical gold standard' for new diagnostic methods (De Vlas *et al.* 1993a). Obviously, the chart only acts on the population level and cannot reveal whether particular stool-negative individuals are infected or not. Based on the same underlying model for egg count variation, similar charts can be developed which provide predictions of the number of repeated surveys necessary to leave only a small proportion of infected persons undetected. In combination with careful cost considerations, such charts will provide an even more practical starting point for planning chemotherapy interventions based on screening.

Acknowledgements

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Appendix 8.1

Confidence intervals and goodness-of-fit

Statistical comparison of observed and predicted 'true' prevalences is based on the individuals negative after 1 stool examination, because only they can show up as positives using the more sensitive technique. A proper confidence interval of the proportion P_w positive after w repeated surveys (with P_w approximating the true prevalence if $w \to \infty$) therefore depends on the observed prevalence after one survey P_1. If N represents the total number of individuals in the data set, then from the number of individuals negative after one survey, $n = N(1-P_1)$, a proportion $p_{obs} = (P_{w,obs}-P_1)/(1-P_1)$ is observed to be positive using additional surveys or filtration. Similarly, a proportion $p_{pred} = (P_{w,pred}-P_1)/(1-P_1)$ is predicted to be positive if the pocket chart is used. An approximate 90% confidence interval of p_{obs} simply equals $p_{obs} \pm u_{0.05}\sqrt{\{p_{obs}(1-p_{obs})/n\}}$. Multiplication of the interval with $(1-P_1)$ results in the desired 90% confidence interval of the observed prevalence using the sensitive technique:

$$P_{w,obs} \pm 1.645 \frac{\sqrt{(P_{w,obs} - P_1) \cdot (1 - P_{w,obs})}}{\sqrt{(1 - P_1) \cdot N}}$$

Calculation of the variation of p_{pred} is more complex, since the egg count model is used with the (correlated) geometric mean EPG as a second statistic. The jackknife re-sampling technique has been used to provide a confidence interval. By leaving out 1 individual at a time from the complete data set and determining the corresponding p_{pred}, a confidence interval can be obtained (Efron, 1982). Let $p_{(i)}$ be the ith jackknife replication of p_{pred} (i.e. from the data set with individual $(i)=1...N$ removed), then from the pseudo-values $\tilde{p}_{(i)} = Np_{pred} - (N-1)p_{(i)}$ the 90% confidence interval of p_{pred} can be estimated as:

$$\tilde{p}_{(.)} \pm t_{0.05,N-1} \sqrt{\sum (\tilde{p}_{(i)} - \tilde{p}_{(.)})^2 / [(N-1)N]}$$

with $\tilde{p}_{(.)}$ the mean of all $\tilde{p}_{(i)}$. Multiplication of the interval with $(1-P_1)$ again gives the desired 90% confidence interval of the predicted true prevalence $P_{\infty,pred}$ or approximate true prevalence $P_{w,pred}$.

Thus, the probability distribution of $x = N(P_w - P_1)$ positive individuals in a sample of $n = N(1-P_1)$ individuals negative after 1 survey is binomial with probability p_{pred}. The likelihood L of observing x infected individuals is therefore

$$L = p_{pred}^{x}(1-p_{pred})^{(n-x)} \{\text{terms not in } p_{pred}\}$$

where the 'terms not in p_{pred}' are the combinational factors concerning the order of observations. The deviance, *dev*, between the $-2 \log L$ function for all data sets and the best possible model, i.e. with $p_{obs,(j)} = x_{(j)}/n_{(j)}$ as probability for each subset $(j)=1...12$, is a joint indicator of the goodness-of-fit and equals

$$dev = -2 \cdot \sum_{1}^{12} \{ x_{(j)} \log(p_{pred,(j)}) + (n_{(j)} - x_{(j)}) \log(1 - p_{pred,(j)}) - x_{(j)} \log(x_{(j)}) - (n_{(j)} - x_{(j)}) \log(n_{(j)} - x_{(j)}) + n_{(j)} \log(n_{(j)}) \}$$

Test of significance and confidence intervals can be based on analysis of *dev*, with differences in *dev* between 2 hierarchical models following a χ^2 distribution.

9

ACCURACY OF INDIVIDUAL INTENSITIES OF *SCHISTOSOMA MANSONI* INFECTION DETERMINED BY STOOL EXAMINATION[1]

Summary

Although it is generally accepted that multiple faecal smears have to be examined for an accurate classification of the individual status of infection with *Schistosoma mansoni*, firm recommendations on this matter have never been given. In a group of infected people, up to 10 repeated stool examinations were performed with the Kato-Katz method. Individual egg load estimations after each of these examinations were compared with the available gold standard (overall individual mean egg count). By means of a mathematical model, these empirical findings were further extrapolated for up to 25 repeated examinations and compared with the expected 'real' infection status. This combined approach allowed to explore and quantify the precision levels of intensities of infection as measured by coprological examination. Several aspects of research and control of schistosomiasis may need to be reassessed in view of these findings.

9.1 Introduction

During the last decades, the Kato-Katz method has become a general field method for the coprological diagnosis of *Schistosoma mansoni* and intestinal helminth infections. However, the number of *S. mansoni* eggs in a given quantity of stool of infected people can show considerable fluctuations, both within one specimen and between specimens collected on different days (Teesdale & Amin, 1976a, 1976b; Hall, 1981, 1982; Teesdale, Fahringer & Chitsulo, 1985; Polderman *et al.* 1985; Barreto, Smith & Sleigh, 1990; De Vlas & Gryseels, 1992; De Vlas *et al.* 1992; Engels, Sinzinkay & Gryseels, 1996). Although some authors have shown that an 'on the spot' diagnosis with the Kato-Katz method is appropriate for most operational purposes (Barreto, Smith & Sleigh, 1990; Engels, Sinzinkayo & Gryseels, 1996), it is generally agreed upon that multiple smears

[1] Engels D, De Vlas SJ & Gryseels B (1997). Accuracy of individual intensities of *Schistosoma mansoni* infection determined by stool examination (submitted for publication).

are necessary for an accurate classification of the individual infection status. But firm recommendations on how to approach optimal accuracy have never been given. In the field, a multitude of different quantitative variants of the Kato-Katz method have been used in studies where accurate determination of the individual infection status was required: Hiatt & Gebre-Medhin (1977) in Ethiopia have used single 25 mg slides, Gryseels (1988) in Burundi duplicate 25 mg slides and Arap Siongok *et al.* (1976) and Smith, Warren & Mahmoud (1979) in Kenya duplicate 50 mg slides, all from 1 stool specimen per individual and with the aim to investigate the relationship between morbidity and intensity of infection. Sukwa, Bulsara & Wurapa (1986) in Zambia, Polderman and De Caluwé (1989) in Zaire and Stelma *et al.* (1994) in Senegal used duplicate 25 mg slides from 2 stool specimens and Salih, Marshall & Radalowicz (1979) in Sudan 50 mg slides from 3 stool specimens for the same purpose. Doehring-Schwerdtfeger *et al.* (1990) have used five 25 mg slides from 1 stool specimen, Homeida *et al.* (1988b) in Sudan triple 25 mg slides from 4 stool specimens and Richter *et al.* (1992) in Brazil five 41.5 mg slides from 3 stool specimens to relate intensity of infection to ultrasound findings. Butterworth *et al.* (1984) have used duplicate 25 mg slides from 3 stool specimens in immunological studies in Kenya. By analyzing up to 10 repeated examinations, both within single specimens and on specimens collected on different days, and by extrapolating these results by means of mathematical modelling we have tried to reach some practical conclusions on this matter.

9.2 Population and methods

In a group of 200 people, duplicate 25 mg Kato-Katz slides (50 mg of stool) were examined on 7 consecutive days (days 1-3-5-8-10-32-37). One hundred and fifty (150) of these people, 81 children (5-17 years old) and 69 adults (18-70 years old), were able to supply complete sets of stool specimens for examination and were infected with *S. mansoni*. All lived in an area where *S. mansoni* was endemic since many decades (Gryseels, 1991). None of them showed a systematic pattern of evolution in their egg count over the study period (not published). There are thus no reasons to believe that their infection status could considerably have changed during that interval of time. In a selected subgroup of 19 people (10 children and 9 adults), 3 extra stool specimens were examined (on days 28-31-35) by means of 10 duplicate 25 mg Kato-Katz smears. The aim of the study was to compare individual egg loads after repeated stool examinations with the maximum available information (gold standard - GS). This individual gold standard was the overall mean egg count of each subject. Three levels of precision were used to judge the accuracy of individual egg load estimations in comparison with these gold standards: GS $\pm$ 50%, GS $\pm$ 30% and GS $\pm$ 10%. Values falling on the limits of these intervals were included.

9.3 Empirical results

Table 9.1 shows, for the subgroup of 19 people, the percentage of individual egg loads meeting these levels of precision after 1 up to 10 repeated examinations. Three operational variants of the Kato-Katz method were compared: the examination of duplicate slides on different days, single slides on different days and multiple slides on a single stool specimen. The way in which the gold standard was calculated attributes more weight to day-to-day than to intra-specimen variation, which is in accordance with recent conclusions in the literature (Barreto, Smith & Sleigh, 1990; Engels *et al.* 1996). Day-to-day multiple sampling thus provided the highest percentages of 'accurate' estimates. When deviations of 50% and 30% of the gold standard are considered, daily single slides gave estimations of individual intensities which were of comparable accuracy as those made by duplicate slides. As an increasing number of samples from one specimen was examined, the accuracy showed a fluctuating pattern with hardly any upward trend. This means that egg counts observed in one whole stool specimen are not necessarily a good reflection of the real infection status. Multiple sampling from one stool will only provide a better estimation of the mean egg count in that stool, without becoming a better estimation of the person's real intensity of infection. The egg load in faeces that day might just be deviating due to e.g. variation in daily stool size and consistency.

Even when duplicate slides were performed on different days, quite a number of repeated measurements were necessary before most individual estimates approached the GS. After 3 repeated examinations, 74% (14/19) of the individual values lay within GS $\pm$ 50%, with all of them reaching this precision after at least 6 repeated examinations. A precision level of GS $\pm$ 30% was reached by 53% (10/19) of the individual egg loads after 3 repeated examinations. The remaining values then still lay within a wide range of deviation (up to 94%) from the GS. Two thirds (13/19) of the values reached the same level of precision after 5 repeated examinations, with the remaining ones lying within deviations of up to 69% of the GS. All individual egg load estimations reached this level of accuracy after at least 8 repeated examinations. With this number of examinations, only 63% (12/19) of the individual values lay within GS $\pm$ 10%.

These figures reflect the chronological sequence of the empirical observations. Alternative sequential patterns were tested by considering all possible permutations of the sequence of the different examinations. The number of these possible sequential permutations for 1 up to 10 repeated examinations were, respectively, 10-45-120-210-252-210-120-45-10-1. Figure 9.1 shows the mean, minimum and maximum figures calculated in this way for day-to-day sampling with duplicate slides. For the sake of simplicity and comparison with subsequent graphs, the gold standard used in this graph was the mean individual outcome of 10 duplicate slides. This means that, of the ten slides examined on days 28-31-35, only the first 2 slides were considered.

Table 9.1 Accuracy of individual intensities of *Schistosoma mansoni* infection estimated by different operational variations of the Kato-Katz method after repeated examinations in a group of 19 people.*

Tested value	Method of repetition	Amount of stool examined	Percentage of individual cumulative egg counts lying within the tested value after...									
			Exam 1	Exam 2	Exam 3	Exam 4	Exam 5	Exam 6	Exam 7	Exam 8	Exam 9	Exam 10
GS±50%	Between days	50 mg	53	58	74	89	84	100	100	100	100	100
	Between days	25 mg slide A	53	58	63	79	79	89	95	95	100	100
	Between days	25 mg slide B	32	47	53	63	68	74	89	100	100	100
	Within spec. 1	25 mg	42	53	37	47	53	37	53	47	47	53
	Within spec. 2	25 mg	37	58	58	58	58	58	53	47	53	53
	Within spec. 3	25 mg	42	53	47	47	47	63	58	53	53	47
GS±30%	Between days	50 mg	32	37	53	63	68	77	84	100	100	100
	Between days	25 mg slide A	37	26	37	53	53	63	63	79	84	79
	Between days	25 mg slide B	16	32	32	37	47	58	63	79	79	74
	Within spec. 1	25 mg	26	26	21	21	21	21	21	37	37	32
	Within spec. 2	25 mg	26	21	32	42	47	37	32	37	37	32
	Within spec. 3	25 mg	21	26	21	26	21	32	21	21	16	16
GS±10%	Between days	50 mg	0	32	16	26	32	32	42	63	58	89
	Between days	25 mg slide A	5	11	26	5	11	16	32	32	32	47
	Between days	25 mg slide B	5	5	5	16	16	21	21	32	37	32
	Within spec. 1	25 mg	11	11	5	5	0	5	11	11	16	16
	Within spec. 2	25 mg	5	0	5	16	16	11	26	11	16	11
	Within spec. 3	25 mg	5	0	5	0	5	16	0	0	11	11

* The Gold Standard (GS) used here is overall mean egg count for each individual in the duplicate slides examined on days 1-3-5-8-10-32-37 and the mean/50mg of stool of the 10 slides examined on days 28-31-35.

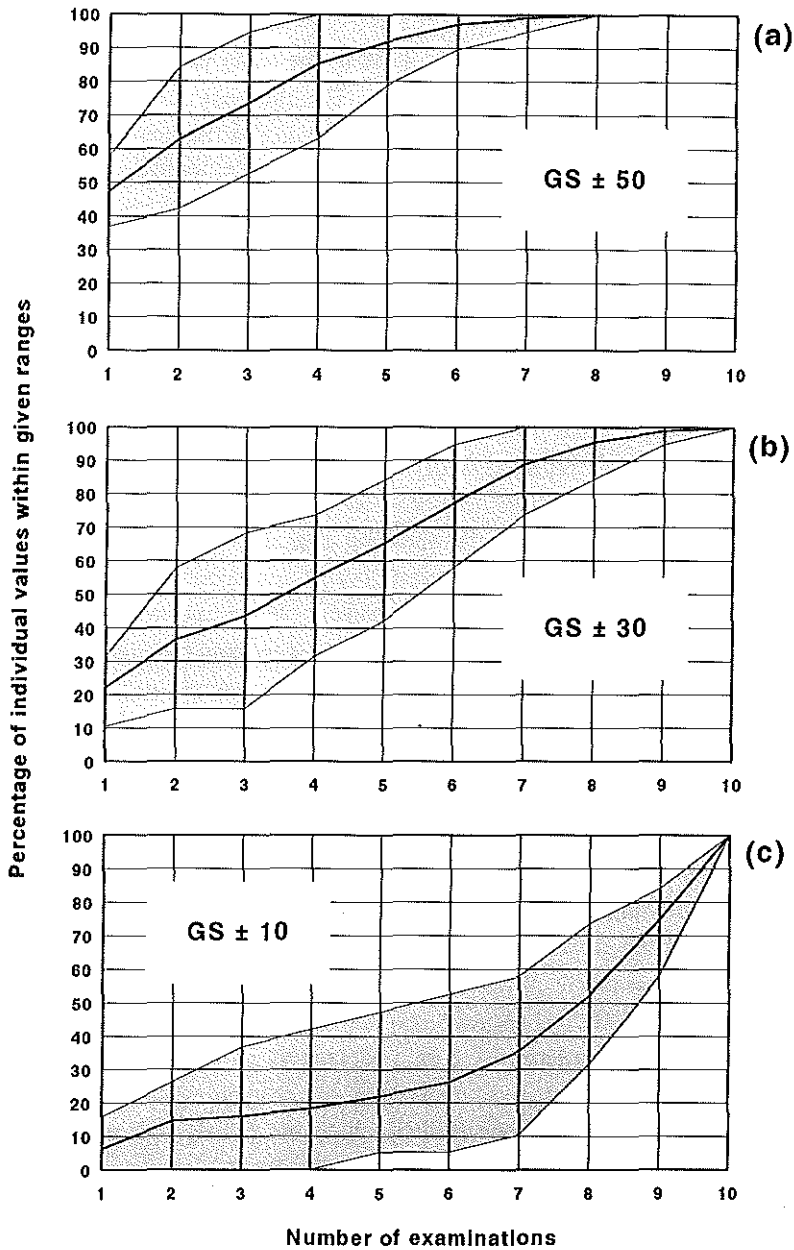

Figure 9.1 Percentages, in the subgroup of 19 people, of individual cumulative mean egg counts lying within 3 tested levels of precision, defined in relation to the gold standard (GS), after up to 10 repeated examinations on different days. The GS used in this graph was the overall mean egg count for each individual in the duplicate 25 mg Kato-Katz slides examined on days 1-3-5-8-10-32-37 and in the first 2 slides examined on days 28-31-35. The grey areas show minimum-maximum ranges; the bold lines indicate mean values.

The percentages shown in Figure 9.1 are consistent with those in Table 9.1. For the levels of precision GS ± 50% and GS ± 30%, the mean values in the graph are even very close to the figures shown in Table 9.1. The min-max range is obviously wider around 4-5-6 repeated examinations because of the higher number of possible permutations. Towards the right of the graph, lines are artificially 'pulled' to the 100% level, because the definition of the gold standard was limited to information of (only) 10 repeated examinations. This is particularly visible for the precision level GS ± 10% after 6-7 examinations.

9.4 Modelling extrapolation

In order to extrapolate what the accuracy would be beyond 7 or 10 repeated stool examinations and how well repeated stool examinations would approach this outcome, an existing statistical model was used (De Vlas *et al.* 1992). This model distinguishes 2 sources of variation in egg counts: the variation caused by the difference in worm pair (WP) load between individuals and the variability of egg counts for an individual with a given WP load. It is characterized by 4 parameters: M (= the mean number of worms per individual); k (= the index of aggregation in the distribution of the number of worms per individual); h (= the expected number of eggs per sample per WP) and r (= the index of aggregation in the egg count distribution for each individual). The value of h was set to be 0.05. This value is based on a ratio of EPG/WP of 1.0 (Gryseels & De Vlas, 1996) and an examined quantity of stool of 50 mg.

The model was first tested on its ability to reproduce individual mean egg counts as they were empirically observed in the complete data set (200 people, 7 repeated examinations, 17 missing values). Given the preset value for h, the best fit of the model to these data was obtained with the following parameter values: $r = 1.23$; $k = 0.37$ and an estimation of the parameter M per age category: 537 for the 5-8 years old; 939 for the 9-16 years old; 260 for the 18-33 years old and 131 for the 34-or-more years old. The whole data set was thus described by 6 free parameters (r, k and 4 age-related values of M). Monte Carlo simulation was then used to generate data sets with the same characteristics as the empirically observed one (200 individuals with each a GS based on 7 repeated measurements, 17 missing values). Figure 9.2 shows both observed and simulated mean, minimum and maximum percentage of individual values lying within GS ± 30% in this data set after each of the 7 examinations. Figure 9.2a shows the empirical data and Figure 9.2b those predicted by the model. Both parts of Figure 9.2 match remarkably well. Up to 5 repeated examinations, the values of the mean percentage are similar to those in Figure 9.1b. The interval between minimum and maximum values is smaller because of the higher accuracy due to the increased number of individuals and the smaller number of possible permutations (respectively 7-21-35-35-21-7-1 for 1 up to 7

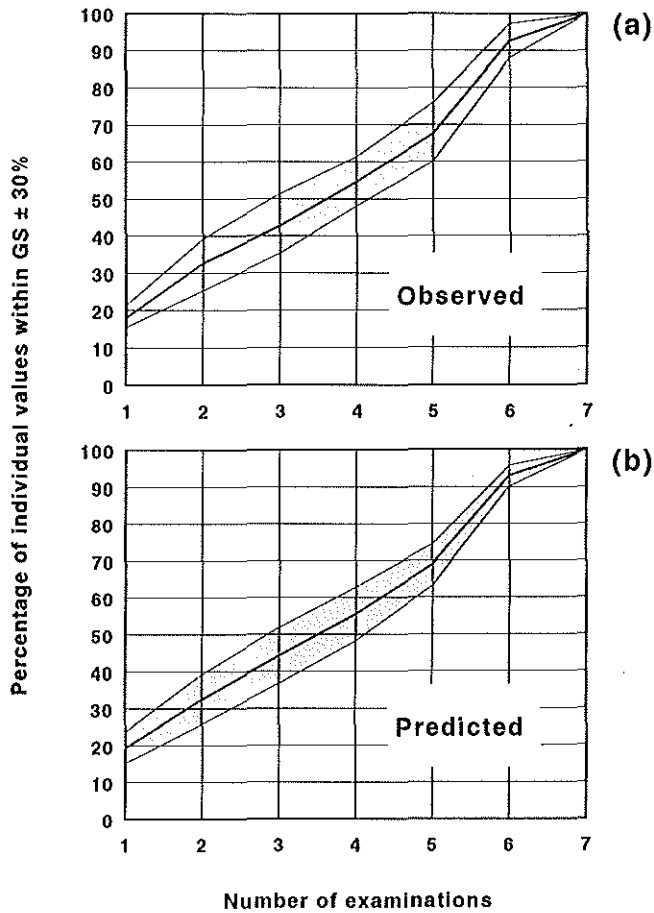

Figure 9.2 Comparison of (a) empirically observed and (b) model-predicted percentages of individual cumulative values lying within GS ± 30% after up to 7 repeated examinations with duplicate 25 mg Kato-Katz slides on different days in a group of 150 people infected with *S. mansoni*. The GS used in this graph was the overall mean egg count for each individual in the duplicate slides examined on days 1-3-5-8-10-32-37. Bold lines indicate mean values; grey areas show minimum-maximum ranges.

repeated measurements). From the fifth examination onwards, the line is also being pulled to the 100% level, but in a way that is much more marked than in Figure 9.1b.

The good agreement between observed and simulated data sets suggests that the assumption concerning the distribution of individual egg counts after repeated measurements is adequate. The assumed negative binomial distribution with aggregation parameter $r = 1.23$ can thus be used to predict the distribution of mean egg counts after more repeated examinations. As done empirically, these egg counts were compared with

the 'true' individual gold standard (being the expected mean egg count, i.e. the mean = *h**WP of the used binomial distribution) which is directly related to the assumed individual worm pair load. The chart was constructed for 1 up to 25 repeated examinations and for individuals with 100 WP (which corresponds to a mean egg count per slide of 5.0) and 1000 WP (or a mean egg count per slide of 50.0).

Figure 9.3 shows the model estimations of the individual egg load accuracy for day-to-day sampling with duplicate 25 mg slides. The estimated values for WP loads of 100 and 1000 were very close. Up to 3-4 repeated examinations, the predicted figures in this graph matched the empirical observations (Figures 9.1b and 9.2a) fairly well. Thereafter they deviated because of the use of a theoretically 'true' gold standard, which eliminated the 'pulling-up' bias. According to the model, it would take about 4-5 repeated measurements before 50% of the individual values would lie within GS $\pm$ 30%. Even after 25 measurements, still 10% of the individual values would not yet meet this level of precision. On the other hand, nearly 60% of the individual values would lie already within GS $\pm$ 50% after 2 repeated measurements. But still between 5-6 measurements would be necessary before 80% of the individual values reach this level of precision, and 9-10 measurements before 90% of the values do so. For more intensely infected individuals (e.g. 10,000 WP), the results were almost identical to the 1000 WP figures. For more lightly infected people (not shown), the percentage of individual values lying within the specified precision ranges fluctuated considerably. For low WP numbers, this type of graph is indeed less relevant because the corresponding slide egg counts are numerically too low to use such precision ranges (see Appendix 9.1).

9.5 Discussion

The findings presented here demonstrate the limits of estimating individual *S. mansoni* egg loads by (even repeated) stool examinations. Yet, precise estimates are required for certain types of research activities, e.g. the investigation of the relation between individual pathology and egg output, the evaluation of new (quantitative) immunologic diagnostic methods (Deelder *et al.* 1994) and immune mechanisms, the quantitative assessment of treatment or the investigation of factors determining immunity and transmission dynamics (Gryseels, 1996). In order to obtain such accurate estimates, a high number of examinations has to be performed. It is preferable to take samples from different stools collected on different days, than to examine multiple slides from one specimen.

We have used an existing mathematical model to extrapolate the empirical findings beyond 7-10 repeated examinations. In addition to previous validations (De Vlas, Van Oortmarssen & Gryseels, 1992; De Vlas *et al.* 1992, 1997) the agreement between observed and predicted figures constitutes a further step in the confirmation of the

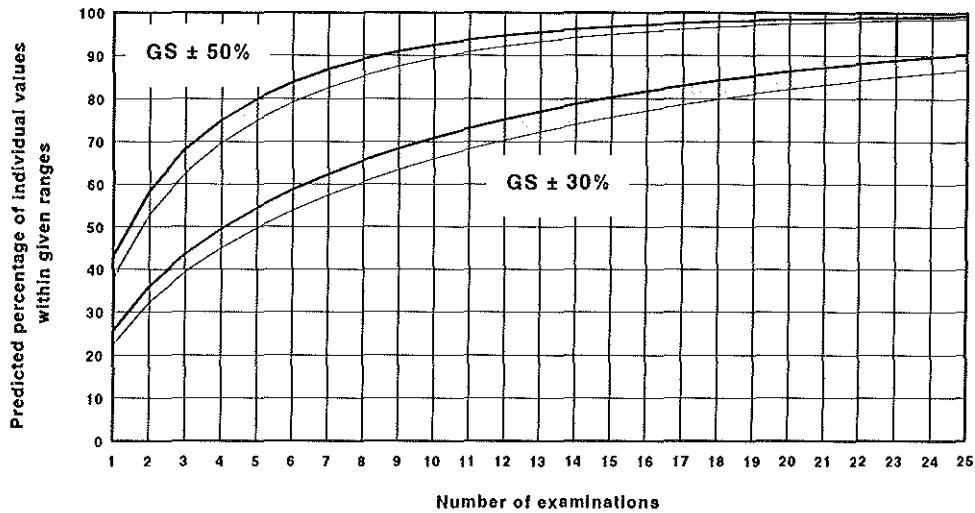

Figure 9.3 Model estimations of individual egg load accuracies after the examination of up to 25 repeated duplicate 25 mg slides on different days. The GS used here is the model-expected "true" individual intensity of infection. Two levels of precision are considered: GS ± 30% and GS ± 50%. The grey areas indicate the ranges of mean values for individuals harbouring between 100 worm pairs (lower lines) and 1000 worm pairs (upper lines). Given the estimated parameter value h = 0.05 eggs/worm-pair/sample, this corresponds to individuals where, respectively, 5 and 50 eggs are expected to be detected in 50 mg of stool.

abilities of the model to describe (now also quantitatively) *S. mansoni* egg counts. The aim of these extrapolations was to give more insight in the ability of coprological diagnosis to supply individual *S. mansoni* egg load estimations close to the real infection status. The multitude of different operational approaches used to obtain precise estimates indeed indicate a lack of rational basis for decision making in this field. Awaiting the operational application of alternative diagnostic techniques (which would probably also have to be validated in comparison with egg counts), we have constructed a chart from which the expected accuracy of individual *S. mansoni* egg load estimations can be read in function of the number of repeated stool examinations (Figure 9.3). The accuracy of these estimations appears not to differ much between moderately and heavily infected individuals. This would mean that, unlike the estimations of prevalences (De Vlas *et al.* 1993a), the accuracy of estimations of individual intensities of infection does hardly depend on the endemic level. Due to inherent numerical restrictions, the methodology used to construct the chart is not relevant in the case of light infections. For them, one can assume that even more repeated examinations are necessary in order to attain the same precision levels.

Acceptable accuracy appears to require an unrealistically high number of repeated examinations. The data set used for our analysis may seem a proof that, for specific research purposes, quite a number of consecutive stools can be obtained from a fairly large group of people without a substantial loss of compliance. However, this requires considerable persistence of a field team and a compliant population. Yet, the degree of accuracy which is 'desirable' has also to be put in perspective. A deviation of 30% of the gold standard which, intuitively, might seem a reasonable standard, appears to be an almost unachievable goal according to the chart. However, such a percentage of difference could easily exist between individuals or between several measurements on the same individual because of simple biological factors such as individual variations in size and consistency of stools. If we assume that on average 150 g of stool is produced every day, a variation from 115-215 g could already produce these 30% deviations in egg count. Scott & Headlee (1937) have shown that such variations in mean weight of stool do even occur at group level. Hence, it may be more reasonable to aim for a deviation of less than 50% from the gold standard. This would require the examination of 5-6 duplicate slides on different days to get about 80% of the individual values within this range.

Up till now, very few studies have used such extensive series of measurements to define individual intensities of infection. The contents of this chapter might therefore throw new light on a number of existing controversies such as the relationship between morbidity and infection status, the presence or nature of immunity and resistance to infection, cure rates after treatment and the performance of immunodiagnostic techniques. Some conclusions might have to be reconsidered in view of the here presented findings.

Acknowledgments

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Appendix 9.1

Irregularity of the model for low worm pair numbers

In our model, an individual with, for example, 10 WP has a corresponding mean egg count, or GS, of 0.5 eggs per slide. After 1 stool examination, all possible empirically observed egg counts (0, 1, 2, ...) will necessarily fall outside the range of, for example, a GS $\pm$ 30% accuracy interval (i.e. from 0.35 to 0.65). After 2 examinations, the expected

sum of both egg counts will be 1. Empirically observed individual cumulative egg counts of (0, 2, 3, ...) will then fall outside that level of precision, which now corresponds to the range 0.7-1.3, but a substantial percentage of people will have a cumulative egg count of 1, which falls within that range. After 3 examinations, however, the expected sum will be 1.5 and a GS $\pm$ 30% then corresponds to the range of 1.05-1.95, so that again all possible individual cumulative egg counts will fall outside this range.

PART IV

Implementation into SCHISTOSIM

10

THE MICROSIMULATION APPROACH TO EPIDEMIOLOGICAL MODELLING OF HELMINTHIC INFECTIONS, WITH SPECIAL REFERENCE TO SCHISTOSOMIASIS[1]

Summary

The microsimulation technique has been used since 1985 as a tool for epidemiological modelling of helminthic infections. This technique is characterized by mimicking individual life histories, which makes it possible to include several relevant processes and mechanisms that have not so far been considered in applied modelling. Biological, epidemiological, and social processes can be simulated in detail, which allows realistic prediction of the impact of control strategies. It is clear that careful quantification and validation of the many processes and parameters in the model requires close collaboration with experts working on control projects. In the development and application of a microsimulation model, we distinguish eight steps, ranging from the identification of questions the model will be designed to address, to the completion of a model that can be used as a routine decision-making tool in a control programme.

10.1 Introduction

Efforts to model infectious diseases have so far been based on systems comprising a small number of mathematical equations (Anderson & May, 1991a). These models, therefore, tend to oversimplify the processes underlying the dynamics of infection and transmission. In his review of mathematical models in schistosomiasis, Woolhouse (1991, 1992a) concluded that these fail to take into consideration a number of important aspects. Although conventional modelling has contributed much to the understanding of schistosome epidemiology, its use in the practice of schistosomiasis control is restricted. To our

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[1] Habbema JDF, De Vlas SJ, Plaisier AP & Van Oortmarssen GJ (1996). The microsimulation approach to epidemiological modeling of helminthic infections, with special reference to schistosomiasis. *American Journal of Tropical Medicine and Hygiene* **55**, Schistosomiasis Supplement (in press). Re-used with permission of The American Society of Tropical Medicine and Hygiene.

knowledge, only one schistosomiasis model exists which has been verified on the basis of data from a control project (Rosenfield, Smith & Wolman, 1977), and models for evaluation and planning of schistosomiasis control have so far not been applied.

The increased capacity of computers facilitates the building of large models and permits simulation of dynamic and stochastic processes in detail. We have used stochastic microsimulation for helminthic infections since 1985, starting with the development of the ONCHOSIM computer program for modelling onchocerciasis (Remme, De Sole & Van Oortmarssen, 1990; Plaisier *et al.* 1990). Recently, similar modelling efforts have been initiated for schistosomiasis and lymphatic filariasis (Habbema *et al.* 1992).

The microsimulation approach is characterized by considering individuals (and possibly parasites) as modelling units. Life histories of hypothetical persons are simulated by means of a computer program. The simulated individuals are fictitious, unlike participants in a field study whose characteristics are recorded in a database. Comparison with field data is undertaken in aggregate by combining the status of simulated individual life histories.

In the following sections, we explain the basic principles of microsimulation and discuss the different steps involved in developing such a model to the stage that it can be a practical tool in field situations. Most examples are related to the microsimulation model for schistosomiasis: SCHISTOSIM. The accompanying paper by De Vlas *et al.* (1996) describes all assumptions in the current version of SCHISTOSIM and presents some preliminary results.

10.2 How is microsimulation done?

The backbone of a microsimulation computer program is a long list of hypothetical individuals whose life histories are being recorded. Together they constitute a community that is followed over time. Running the computer program simply represents the book-keeping of all relevant characteristics of these individuals. During the simulation run, new persons may join the list due to birth or because they have immigrated into the community from areas that are or are not endemic. Individuals are only removed from the list after they have died or emigrated. At each step in the simulation, the computer program checks, for each individual, whether his or her characteristics should be updated following a particular event. Some of these events are rather trivial; with every timestep in the simulation (typically one month) the individual gets older and the age-dependent characteristics, such as exposure, will change. Others concern specific epidemiological events, such as an increase in new infections or the administration of an anthelminthic drug during a chemotherapy campaign. In this section, we will illustrate phenomena which can be incorporated into a microsimulation model for helminthic infections. Several

of these phenomena are already part of the current version of SCHISTOSIM (De Vlas *et al.* 1996).

Suppose that at a given moment (say 1950, month 6), the simulated community consists of 1000 inhabitants, of which 50 are women between 20 and 30 years. According to the model input, the fertility rate in this age group is 0.027 per month, and therefore the expected number of newborns among these women is 1.35. After drawing random numbers, it appears that one baby joins the community. A number of characteristics are assigned to this new individual. First, the sex is determined by the computer by a simple heads or tails process (result: male), followed by drawing randomly from the life table the age (48 years and two months old) at which he will die. The resulting time of death (1998, month 8) may be moved forward during the simulation due to life-shortening effects of heavy chronic schistosome infection. Figure 10.1 represents the life history of this individual and some fellow villagers.

The newborn will further be assigned several epidemiological and social features that remain unchanged during the course of the simulation. The most important epidemiological characteristic is the individual's relative risk to gain new worms. Differences in infection rates can be explained by differences in susceptibility and exposure to infection. It is well known that exposure varies with age and sex, but in addition to these variables, we assign to each individual a personal exposure index. The assigned exposure index of this baby is 2.0, indicating that the expected number of infections later in his life will be twice the mean number of infections for men of the same age. Similarly, the ability to build up an efficient immune system and the tendency to develop pathology can be modelled as individual characteristics, if the user of the simulation program so wishes. Social characteristics may, for example, concern a person's willingness to participate in certain control efforts. Characteristics that may change during the simulation, like infection, immune and disease status, have the initial value zero for babies.

Now we will move on to an arbitrary step in the simulation. During month 3 of 1961, several events may have happened. New persons may have entered the community, while people that have reached their age of death have left the simulation. Our focal individual, who has now reached the age of 10 years and nine months, may have gained new infections or lost some dead worms. If we assume that (after drawing some random numbers) the net gain of worms is positive, his or her intensity of infection will increase, leading to a higher mean egg output later in the simulation. Furthermore, some female worms may start to produce eggs early in their development, or may have an increased productivity when they are still younger than six months. An additional result may be that our individual now has a more serious disease status, but he will probably also have a more efficient protection against new infections due to an improved immune status. Contamination of the water and consequently release of miracidia, infection of snails, and shedding of cercariae are considered on the community level; the force of infection at the

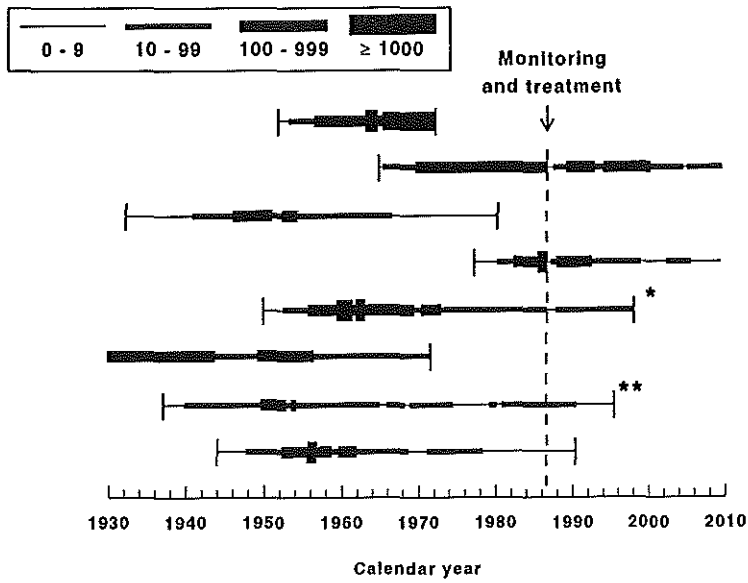

Figure 10.1 Example of hypothetical individuals as they may exist within the microsimulation computer program SCHISTOSIM. The life histories of eight individuals infected with schistosomes are indicated between the period 1930 and 2010. The thickness of the lines represent their respective worm burdens: the high infection rates at age 10-15 are typical for schistosomiasis. In 1986 month 7 (dashed line), a selective treatment has been performed, followed, however, by rapid reinfection. The asterisk indicates the person (born in 1950 and died in 1998) described in the text. The double asterisk shows an individual whose worm load did not change during the treatment campaign; this person could be one of the non-attenders, or his egg count was a false-negative result.

next simulation step is assumed to be a function of the combined egg output of all individuals. This function can be defined in as much detail as one wishes, and may include miracidial and cercarial population dynamics and vector dynamics, even by micro-simulating life histories of individual snails.

Let us now consider the situation during a selective treatment campaign. In 1986 month 7 (the focal individual has reached adulthood), screening is undertaken followed by administration of praziquantel to all positive individuals. The overall attendance in this round of screening is quite high (71%), and after drawing a random number, it appears that this individual will be examined. In proportion to the number of mated female worms, his egg count is determined, taking the variation in egg count measurement into account (De Vlas *et al.* 1992). The resulting draw of five eggs in the stool sample designates him as candidate for treatment. Subsequently, a binomial random number with

98% probability indicates how many of his 50 worms will be killed. During the period after treatment, his worm load will increase again due to reinfection.

In each simulation run, the number of persons to be simulated has to be specified. Output, like prevalences of infection, to study the effects of control, or detailed egg counts, to test the model with observed field data, is obtained by aggregating the characteristics of all individuals.

10.3 The development of a microsimulation model

Table 10.1 shows the phases that can be distinguished in the development of a microsimulation model for a helminthic infection. Some of them have already been considered in the construction of SCHISTOSIM; others are still to be incorporated. The different steps are described below. It must be emphasized that in reality there will be many loops, as illustrated in Figure 10.2. In addition, since modelling is an ongoing process, work will often be undertaken on several phases at the same time; e.g., while the current version of the model is being used for predictions in the context of a control programme, a new version is being developed because important new data have been published.

Identification of questions to be addressed

In epidemiological modelling of infections, the interest often lies in the evaluation of measures that are already being applied in control programmes. The aim is not primarily to prove the effectiveness of control measures, but rather to determine the magnitude or size of the effects as influenced by factors such as intensity, duration, and coverage. For example, one of the questions in the Onchocerciasis Control Programme (Plaisier *et al.* 1991) was how long should vector control by larviciding be continued to make the risk of recrudescence acceptably small?

Setting clear priorities in the questions to be answered is crucial for determining the essential processes and aspects to be included in the model and, above all, for making the modelling exercise relevant. New or modified questions will undoubtedly arise during the project.

Investigation of existing knowledge

A useful way of synthesizing existing knowledge, from the viewpoint of model development, is to review knowledge in the literature (such as laboratory results, experiences of existing models, and data from control programmes). This review should be quantitatively oriented and organized according to the main components of transmission, epidemiology, natural history of disease, and control options. Once the review has been discussed with experts from different disciplines, it could then form the basis for structuring and

quantifying the model. New applications (e.g., effects of future schistosomiasis vaccines) may bring about the need for additional targeted review.

Model design

A first step in the actual construction of the model is to make a list of all relevant factors to be included. These factors are grouped into a number of modules of closely related parameters and their interactions, such as human demography, vector dynamics, worm characteristics, development of disease, vector control, and chemotherapy. For example, the worm characteristics module deals with the lifetime distribution of adult worms, the male-to-female ratio, the pattern of egg production, etc. Relationships within modules and between modules can be described by means of flow charts (see De Vlas *et al.* 1996).

Microsimulation allows for a step-by-step development of the model. Some modules can be omitted initially (for example morbidity and control), and one can start with highly simplified modules that will gradually be refined and extended. Parts of the transmission cycle may first be considered as 'black boxes' and be described by simple mathematical functions, without explicitly taking details into account, for example the dynamics of the larval stages or the population dynamics of the vector hosts.

A choice has also to be made regarding the 'degree' of microsimulation. As a minimum, human subjects and their personal characteristics are to be simulated individually. Inhabitant worms may be simulated individually when their numbers are not too high, as is the case in the ONCHOSIM model. In schistosomiasis, worm burdens of several hundreds have been counted and estimated (Cheever, 1968; Gryseels & De Vlas, 1996), and a *macro*simulation approach in which only different worm age classes, with corresponding transition rates are considered, is more efficient.

Most decisions about the design should be made in close collaboration with field epidemiologists and experts in the relevant research disciplines.

In the early phases, the computer program will already include data structures for the microsimulation units (humans, worms, etc.) with corresponding procedures (fertility, mortality, treatment, etc.), and input/output routines. Input should be conveniently and clearly arranged to facilitate discussion about the assumptions of the model. The output should provide intermediate and final results of the model, which should be formatted in a way suitable for comparison with actual data to test and validate the model.

Model quantification

In parallel with the programming of the model, functions and distributions have to be specified and parameters quantified. Right from the start this requires the extensive and continuous collaboration with experts both within and outside the control programs involved. Several quantifications, especially those related to the biology of disease and to transmission (egg production and the lifespan of worms and vectors), should ideally be

Table 10.1 Steps in the development and use of a microsimulation model.

	Step	Major aspects
1)	Identification of questions to be addressed	• Questions to be addressed with the model
2)	Investigation of existing knowledge	• Literature • Existing models • Expert opinion, expert workshops
3)	Model design	• Listing of relevant aspects to be included in the model. • Organizing aspects into separate modules • Implementation in computer program
4)	Model quantification	• In each module: description of processes and quantification of parameters from existing knowledge, expert opinion and field observations • Estimation of parameters by data analysis and additional modelling • Identification of missing information
5)	Model validation	• Validation against data from control programme • Comparison with findings of mathematical-analytical models
6)	Prediction and optimization	• Prediction of trends and public health effects under different control strategies. • Comparison on basis of cost-effectiveness • Sensitivity analyses
7)	Decision making	• Present and discuss the results of prediction and optimization • Recommendations about control policies • Recommendations about research priorities
8)	Transfer of simulation program	• Working model, to be used as routine tool in control project, or for educational purposes • Transfer to other control programme or other infectious diseases

based on existing knowledge. Other parameters represent typical characteristics of the control programme under study and should be determined or estimated on the basis of locally available data. Examples are operational data (e.g., coverage, compliance, treatment protocol), demography (lifetable, fertility rates and migration patterns), and social factors (e.g., exposure and contamination indices).

At this stage, the main problem is often lack of reliable and consistent data from both the literature and the control programme. Sometimes data analysis, e.g., by statistical modelling, allows the quantification of specific parameters. For example, our analysis of repeated egg count data resulted in estimates for parameters on egg production and the variation in repeated surveys (De Vlas *et al.* 1992). In the case of very limited direct or indirect evidence on important model parameters, research for filling these gaps in knowledge can be proposed. For instance, during the development of ONCHOSIM, it became obvious that the relationship between vector infectivity and skin microfilarial load was a crucial parameter and field experiments were undertaken to quantify it (Plaisier *et al.* 1991).

To characterize a region where a control programme is carried out, specific observations are usually needed. For example, in schistosomiasis, the age- and sex-related exposure to contaminated water is an important part of the dynamics of infection and should be estimated by water contact studies. Since social conditions may differ greatly between communities, these values cannot simply be applied to other situations.

The timestep to be used in the simulation program also has to be chosen. For example, it could be equal to the prepatent period or the time between infection of the vector and the release of cercariae or third stage (L_3) larvae. In SCHISTOSIM, a timestep of one month seems adequate because this is both the minimum time between infection with cercariae and the subsequent excretion of eggs and the average time between the infection of snails and the shedding of cercariae.

Model validation

A fully quantified model will not necessarily be a valid and reliable model. The model quantifications can be validated by checking the predictions of the full model (or major parts of model) against independent empirical data sets. Ideally, one would like to use comprehensive data sets covering all aspects of transmission and disease, including results of the control measures, e.g., observed endemicity levels, disease prevalences, and intensities. A statistical check on these data (by goodness-of-fit) will reveal whether or not the model may be considered adequate for making predictions about the effects of alternative control strategies.

Another way to validate the model is to study its behaviour for different values of parameters (within plausible ranges). Results of the model should be biologically and epidemiologically reasonable. Some errors or inconsistencies in the model can be detected by checking the outcome against those of mathematical-analytical models for simplified input assumptions. A less formal, but crucial type of validation is to expose the model to criticism from various experts in the field of schistosomiasis control.

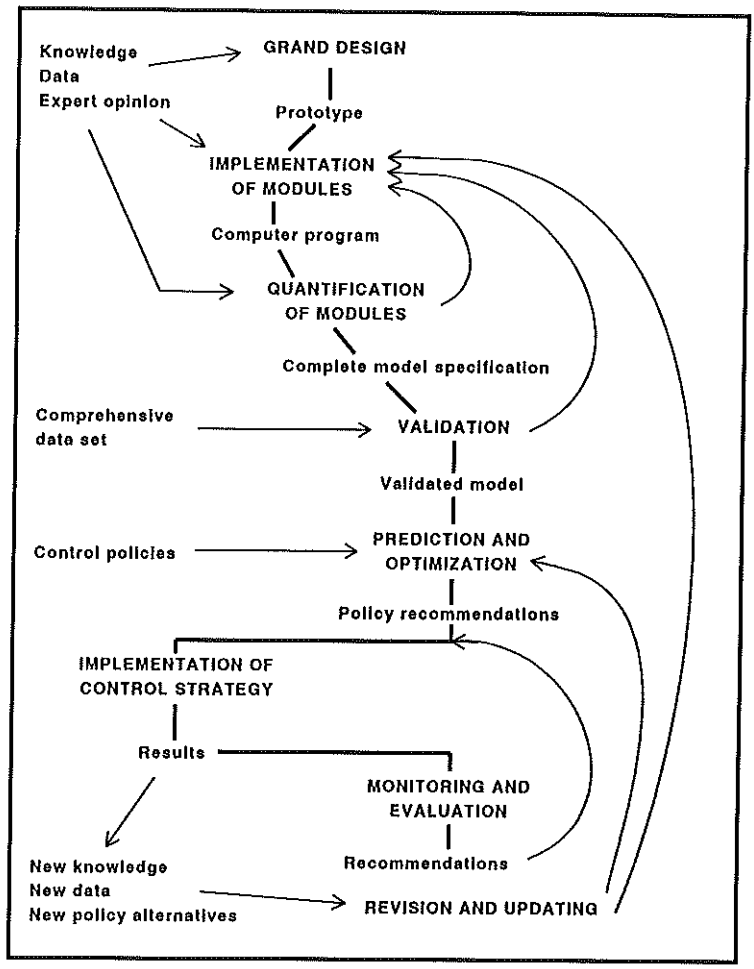

Figure 10.2 Schematic representation of the development and use of a microsimulation model. The relationships and transitions between the different phases of Table 10.1 are indicated.

Prediction and optimization

Although preliminary evaluations of available control options will have been undertaken during the early stages of model development, a more definite assessment and choice of control strategies can only safely be made after the model has been thoroughly validated. Model predictions of the effects of control include the reduction in incidence, morbidity, and (if relevant) mortality. When relevant, the resources required for executing the control strategy should also be taken into account. For example, in a selective chemotherapy campaign, one could consider the costs of microscopic examination, as well as

drugs and their distribution. Optimization methods can then be applied to find the most effective control strategy for a given amount of resources, to minimize the resources needed to obtain a given effect, or for optimizing the balance between resource use and effects.

Predictions depend critically on the assumptions made in the model. Unfortunately, some of the assumptions may only be 'guestimated'. Sensitivity analyses, by running the model for different plausible parameter values, can be used to assess the implications of such uncertainty. In case the ordering of different control strategies remains (virtually) unchanged, a conclusion on what control strategy is optimal can be made with more confidence.

Decision making

To arrive at predictions that are actually used in decision making, they should be understandable to field workers and managers; a purely technical-mathematical description will not be sufficient. Discussion between modellers and users is needed to find the most appropriate representation of model outcomes regarding the effects of control strategies. Of special importance in this respect is the embedding of the model conclusions in a broader organizational and societal perspective, including unintended, adverse, or beneficial effects. For example, schistosomiasis control using molluscicides could lead to deterioration of fishing yields due to environmental pollution, and when schistosomiasis is controlled by sanitation or safe water supply, the improved health and the implied economic profit resulting from healthier workers may be due in part to prevention of other parasite infections that are also transmitted by surface water, such as amoebiasis and Guinea worm infection.

Policy decision will not be taken by the modellers but by control project managers, health care authorities, or other government bodies. Funders, other scientists, and external advisors may also be consulted. All parties involved will have to form an opinion about the conclusions of the modelling project.

Recommendations on the basis of modelling may not only concern control strategies, but also the needs for additional research to fill the gaps in knowledge that have been identified during model development, and which limit the predictive possibilities of the model.

Transfer of simulation program

When fundamental changes in model design and quantification are not to be expected in the near future, the model can be transferred to the management of cooperating control projects for routine use in further planning and evaluation of control options. To facilitate this technology transfer, it is necessary to properly document the computer program, to

prepare user manuals, and to provide a user interface for modifying and inspecting model parameters and displaying intermediate and final results of simulation runs.

When possible, the efforts in developing and documenting the microsimulation model should also be used to the advantage of schistosomiasis control programmes in other areas. Transfer to other control programmes requires a critical review of all basic assumptions, including the control options, to find out whether or not they have to be modified to fit the new setting.

Additionally, the model may have value as an educational tool. Students can get an insight into the processes and mechanisms in the dynamics of infection and disease by 'tuning' the parameter values and studying the resulting changes in outcome of the model.

Finally, we would like to stress the importance of continuously updating models for helminthic infections: what is a complete reflection of the state of knowledge today will be obsolete tomorrow. Criticism of the present model and new achievements in research and control bring about the need for periodic revision and adaptation. Updated versions of the computer program should be made available to all users.

10.4 Conclusions

The development, application, and updating of microsimulation computer models is a resource-intensive enterprise. It should not be embarked upon lightly. For example, many years after its initiation, the ONCHOSIM model is still being adjusted and extended. The modular structure of a microsimulation model allows one to change or extend specific parts of the model without changing its basic structure. New insights or new control options in the field of the disease under study can be integrated into the model quite easily. Adaptation of the model for other regions and control programmes is relatively straightforward.

The microsimulation approach to epidemiological modelling has a legitimate place in the control of helminthic infections, and forms a useful addition to, rather than replacement of, the established modelling approaches (*Science*, 1994).

11

SCHISTOSIM: A MICROSIMULATION MODEL FOR THE EPIDEMIOLOGY AND CONTROL OF SCHISTOSOMIASIS[1]

Summary

A computer simulation model, SCHISTOSIM, has been developed for the epidemiology and control of schistosomiasis, based on the stochastic microsimulation technique. The eventual aim is to evaluate and predict the effects of different control strategies. In the current state of the model, human-, worm-, and infection-related aspects have been included. However, many others, including most transmission and transmission-related mechanisms, have yet to be modelled. By simulating a series of surveys and treatments in Burundi, short-term effects of this programme were satisfactorily explained by the model. However, long-term predictions did not match the observed data. Possible extensions of the model to properly describe these effects are identified. The potential of SCHISTOSIM as a tool for the prediction of the outcome of alternative control strategies is illustrated and discussed.

11.1 Introduction

This chapter describes current progress in the development of a model for the dynamics of infection, transmission, and morbidity of human schistosomiasis, called SCHISTOSIM. The eventual aim is to evaluate and predict the outcome of different control strategies. Such an ambitious goal requires a comprehensive model that incorporates a multitude of interrelating processes. Therefore, input from several areas of schistosomiasis research and control is essential. Following the experience with the ONCHOSIM model for onchocerciasis (Plaisier *et al.* 1990; Habbema *et al.* 1992), the basic technique we apply is stochastic microsimulation, as discussed elsewhere in this supplement (Habbema *et al.* 1996).

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[1] De Vlas SJ, Van Oortmarssen GJ, Gryseels B, Polderman AM, Plaisier AP & Habbema JDF (1996). SCHISTOSIM: a microsimulation model for the epidemiology and control of schistosomiasis. *American Journal of Tropical Medicine and Hygiene* **55**, Schistosomiasis Supplement (in press). Re-used with permission of The American Society of Tropical Medicine and Hygiene.

Microsimulation allows one to start with a basic global model structure, which is then gradually extended and refined. In this chapter, we illustrate which questions can already be addressed and studied with the current, incomplete, version of SCHISTOSIM. The mechanisms incorporated in the model are indicated in Figure 11.1. The SCHISTOSIM model is presently designed for *Schistosoma mansoni*, but can later be adjusted for *S. haematobium* and *S. japonicum*.

The current version of the model has been used to simulate a series of surveys and selective treatment campaigns in the village Gihungwe, Burundi between 1986 and 1989 (Gryseels, Nkulikyinka & Engels, 1991, 1994). Field observations and model predictions are compared. For further illustration of its possibilities, we use SCHISTOSIM to project the short-term effects of alternative control measures, with emphasis on the comparison of selective and mass treatment.

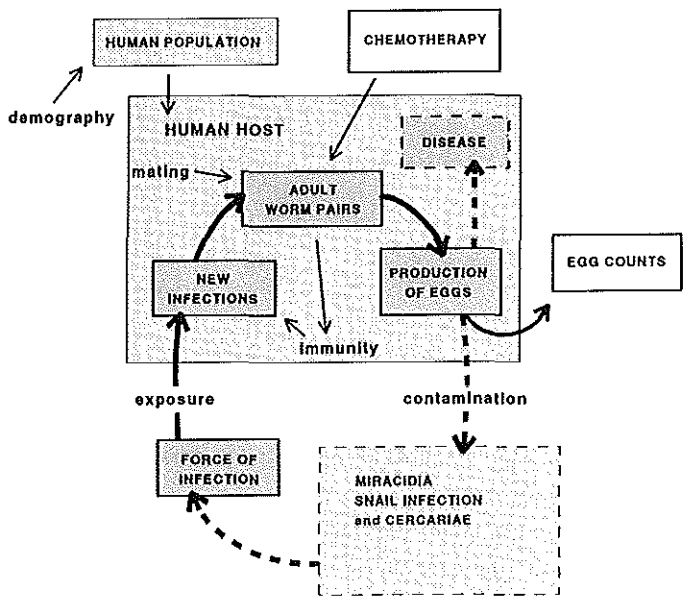

Figure 11.1 Schematic representation of the transmission cycle. Components included in SCHISTOSIM are indicated with solid lines (see the text for details). Major components to be defined in the near future are represented by dashed lines.

11.2 Model structure and specification

In the current state of the model (see Figure 11.1), all included factors and their relationships can be grouped into four modules: humans, parasites, infection and control.

SCHISTOSIM mimics life histories of hypothetical individuals by simulating, step by step and based on these modules, all events in the population relevant for the dynamics of infection. Output of the model and comparison with field data occurs by aggregating life histories. This technique is called microsimulation; for further details, see the paper by Habbema *et al.* (1996) in this supplement.

Most parameter values in the model are based on existing knowledge or are specified according to empirical data from the village under consideration. Some other quantifications are merely tentative and need further support by research.

11.2.1 Human population

The dynamics of the human population are determined by its lifetable (Figure 11.2a) and birthrates (Figure 11.2b), which have been based on data from Burundi. The lifetable has been constructed to correspond with the available age-pyramid for the Bubanza region, where Gihungwe is situated. The mean life expectancy is about 45 years. The population size doubles every 25 years; during the field project it was about 1000.

Individual exposure to *Schistosoma* infection depends on age and sex. The values in Figure 11.2c are based on extensive water contact studies in Gihungwe (Gryseels, 1991; Gryseels, Nkulikyinka & Engels, 1994). These relative exposures were calculated as multiplications of duration, frequency, and type (area of body exposed) of water contact, and scaled to a population average of 1.0.

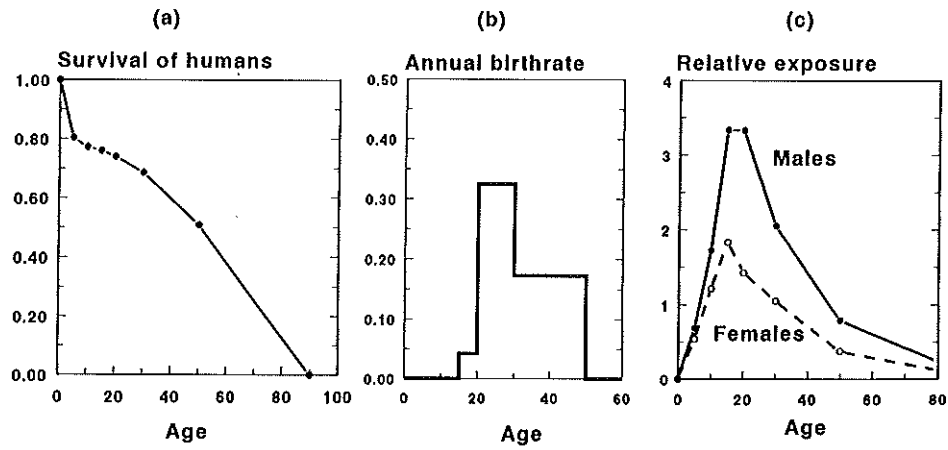

Figure 11.2 Characteristics of human demography and relative exposures in Gihungwe, Burundi. The lifetable (a) and annual birthrates (b), i.e., number of births per female per year, have been obtained from official government census figures (Bujumbura, 1980) and are according to the area (Bubanza) where Gihungwe is situated. Relative exposures (c) were based on water contact studies in Gihungwe (Gryseels, 1991; Gryseels, Nkulikyinka & Engels, 1994) Age is in year.

11.2.2 Parasite population

This part of the model comprises several aspects of worm dynamics and egg production. The average lifespan of the worms is assumed to be 40 months (Goddard & Jordan, 1980; Anderson & May, 1991a) and survival follows a Weibull distribution (Figure 11.3a), which implies that the risk of dying depends on the age of the worm (Law & Kelton, 1982). The sex of worms is taken into account, assuming a ratio of 1:1 of male:female worms and monogamous mating.

We assume that female worms start production of eggs at two months of age, with an increase to a maximum value of 150 eggs per mated female worm per day at the age of six months, and a stable production thereafter (Figure 11.3b). These assumptions have been based partly on an extensive analysis of inter-individual and intra-individual variation in egg counts (De Vlas *et al.* 1992). In addition, we took into account that 10% of the eggs are excreted with a delay, 5% leaving the body the next month and 5% a month later. This means that if all worms were removed from a person and no reinfection took place, eggs could still be found in that person two months after treatment.

11.2.3 Infection

The transmission cycle is not closed in the model: the aspects of the transmission cycle involving water (miracidia, vector snails, and cercariae) have not yet been incorporated (Figure 11.1). The force of infection (*FOI*) is therefore assumed to be constant. The *FOI* is here defined as the expected number of new successful infections per month, for the average exposed individual without acquired immunity; successful infections are those that ultimately lead to an adult worm. In later versions, the *FOI* may vary dependent on a range of transmission-related factors.

Acquisition of infections is considered a Poisson process and is simulated according to the equation: expected number of new infections for person i during month t = *FOI* × individual exposure × individual immune protection =

$$FOI \times \{ Exp(a,s) \times Exp_i \} \times \left\{ 1 - \alpha \times Imm_i \times \sum_{\tau=0}^{t} M(\tau).\beta^{t-\tau} \right\}$$

in which exposure variation has an age- and sex-dependent component $Exp(a,s)$ (Figure 11.2c) and an individual component Exp_i, α represents the effect of the immunological response on the acquisition of new infections, Imm_i is the individual's ability to develop an immune response, $M(\tau)$ is the worm burden at time τ (τ = 0, 1, ..., current time t), representing the infection history, and ß is the immunological memory. The outcome of the simulation is highly dependent on the values of these parameters. The *FOI* and

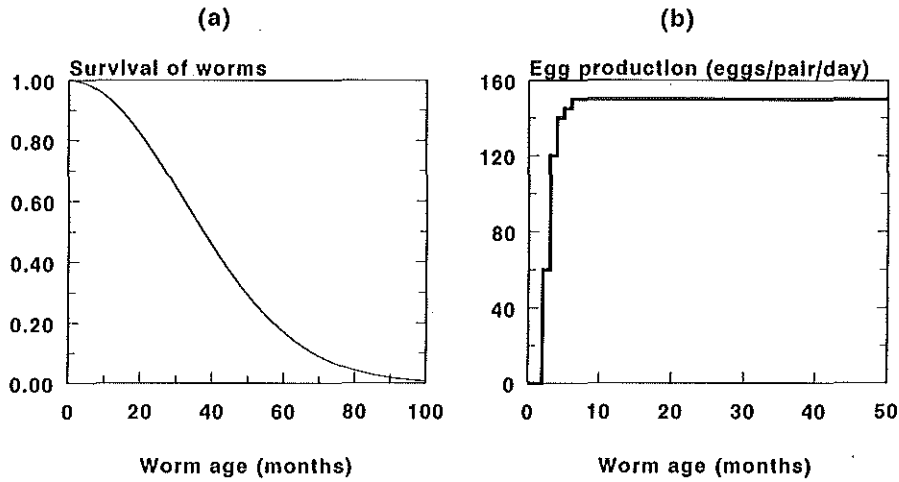

Figure 11.3 Assumed worm characteristics in SCHISTOSIM: lifespan of worms (a) in months (follows a Weibull distribution with a mean of 40 and a shape parameter of 2.0) and the egg production (b) in number of eggs passed into the intestine per worm pair per day.

exposure variation are specific for the simulated endemic situation, while the others can be considered biological constants. In the rest of this section we will explain the choice of the parameter values used to describe the field data from Gihungwe.

The value for the FOI = 40, which means that the mean number of worms in adults would be $FOI \times MLS$ (mean worm lifespan of 40 months; Figure 11.3a) = 1600 if there were no protective immunity (Anderson & May, 1991a). The individual exposure index Exp_i is assumed to be gamma-distributed (a mean 1.0 and a shape of 0.3). The resulting distribution of worms among the individuals of the population would then follow a negative binomial distribution with aggregation parameter k = 0.3. Without protective immunity, this would lead to very high worm numbers. The way in which infection history impacts on immunity is derived from a model proposed by Anderson and May (1985b). The immunological memory ß = 0.9715 corresponds with a half-life time of the immune effect of 24 months (0.9715^{24} = 0.5): i.e., every two years after a person's worms are completely removed by treatment, with no reinfection taking place, the immune protection would be reduced by half.

It can be calculated that the mean worm burden, taking the immune process into account will approximate:

$$\frac{FOI \times MLS}{\alpha \times FOI \times MLS / (1 - \beta) + 1}$$

The value for the immunological effect (α=0.00001779) is chosen so that the mean worm burden = 800, which is half the value if there were no immune protection. If other values for *FOI* had been assumed, the effect would be different for the same α, ß, and *MLS*: a mean worm burden of 6400 (*FOI* = 160) would be decreased by immunity to 1280 (80% reduction), and a mean worm burden of 400 (*FOI* = 10) would be decreased to 320 (20% reduction). These numbers are higher than those observed in autopsy series (Cheever, 1968), but are consistent with the results of our egg count model (Gryseels & De Vlas, 1996).

A consequence of immunity is that the variation between individuals due to differences in exposure is much reduced. The index of immunity Imm_i (gamma-distributed with a mean of 1.0 and a shape of 0.5) can be considered as an individual's ability to build up an effective immune response. The variability means that persons have different threshold levels at which the acquisition of infections is completely interrupted.

11.2.4 Control

Parameters concerning control can, to a large extent, be based on the specific characteristics of the Gihungwe field project (Gryseels, Nkulikyinka & Engels, 1991, 1994). Nine surveys (with attendance between 63% and 75%) were performed; four annual surveys were followed by selective treatment of all detected positive individuals with praziquantel (40 mg/kg) (Table 11.1). In the model, the attendance pattern is assumed to be partly non-random; i.e., the previous attenders have a higher chance of participating again than the previous non-attenders. The parameter indicating the attendance pattern, which can range from 0.0 (totally random) to 1.0 (totally non-random), = 0.6.

Furthermore, we assume that on average 98% of the worms in an individual are killed by treatment with praziquantel, according to a binomial distribution. The intra-individual variation in egg counts is accounted for in this part of the model. Egg counts for a given number of mated female worms are based on the examination of 50 mg of stools and are assumed to follow a negative binomial distribution with aggregation parameter 0.865 (De Vlas *et al.* 1992).

11.3 Fitting the model to the data

The observations of the Gihungwe control project are represented in Figure 11.4a. The initial prevalence of about 60% decreased to 30% three months after the first treatment, followed by a rapid reinfection. The prevalences decrease further after repeated treatment. The results have been described in more detail elsewhere (Gryseels, Nkulikyinka & Engels, 1991, 1994). Figure 11.4b shows the outcome of a simulation run of SCHISTOSIM, with assumptions and specifications as explained above. The average number of

Table 11.1 Characteristics of surveys and chemotherapy carried out in Gihungwe 1986-1989 (Gryseels, Nkulikyinka & Engels, 1991, 1994).

Year	Month	Treatment	Attendance (%)
1986	January	No	75
1986	April	No	69
1986	July	Yes	71
1986	October	No	70
1987	January	No	69
1987	April	No	69
1987	July	Yes	68
1988	July	Yes	65
1989	July	Yes	63

simulated individuals was 1000, the size of Gihungwe. Only two parameters have been varied to find the best fit with the observed data: the *FOI* and the individual variation in exposure Exp_i. Both have been based on the egg count distribution in the three pre-treatment surveys. As seen in Figure 11.4, observations and predictions of prevalences and intensities agree very well before and after the first treatment, but differ markedly after repeated treatment.

There may be several reasons for the poor agreement after repeated treatment. First, the transmission cycle has not been closed in the model; the *FOI* is considered constant and is not affected by seasonal or yearly variations, or by these population treatments. Heterogeneity in time and space can lead to highly variable rates of transmission (and reinfection) between successive seasons and years (Gryseels, 1991; Woolhouse, Watts & Chandiwana, 1992). The (repeated) selective treatment may have had an impact on the *FOI*. However, in the actual field situation, this would be surprising because of the incomplete coverage of the population, and the contribution to transmission of false-negative individuals and of infected individuals from neighbouring non-treated areas; Gihungwe is in fact part of a larger community (Gryseels, Nkulikyinka & Engels, 1991). To account for varying cercarial densities, SCHISTOSIM has to be extended by closing the transmission cycle and including the vector and larval stages of transmission. Second, social aspects could have changed in the course of the control project: the (decreasing) attendance might be biased towards low-risk groups or might become less random, leading to relatively better results in this group of attenders. More research and analysis is needed here. Third, there may be age-related cohort effects: the surveys were in fact not carried out cross-sectionally but based on a cohort. Therefore, the surveyed

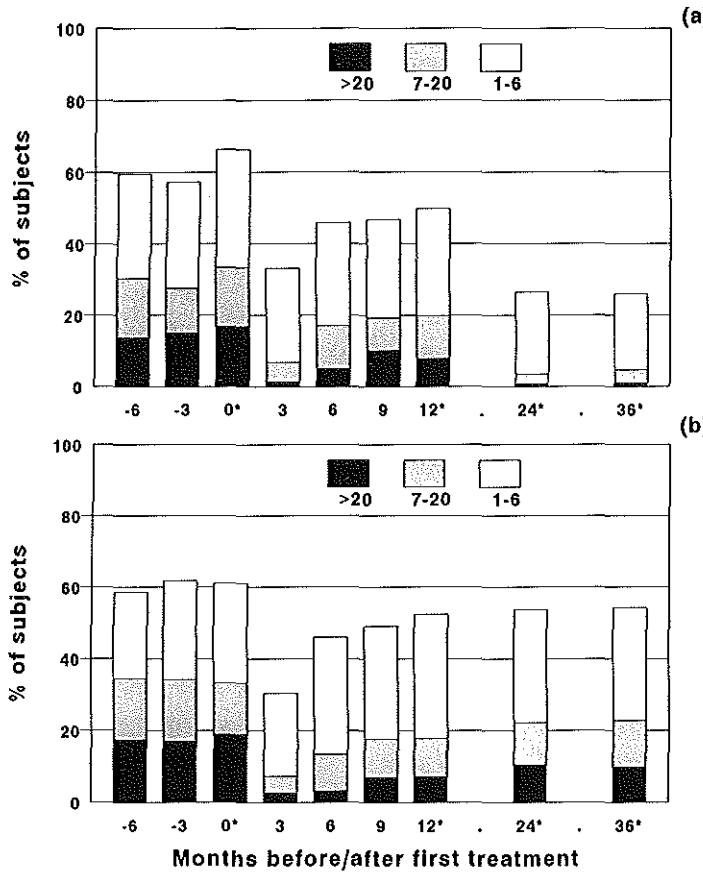

Figure 11.4 Comparison of observed and simulated egg count results: (a) observations of the selective chemotherapy campaigns in Gihungwe 1986-1989, and (b) results of a simulation run using SCHISTOSIM with assumptions and specifications as explained in the text. Indicated are the prevalences of low (1-6), medium (7-20) and high (>20) egg counts in samples of 50 mg of stools. Surveys followed by treatment are marked with an asterisk.

population became older and the model does not yet account for this factor. Finally, in the model the level of immunity is assumed to be quantitatively dependent on infection history (Anderson & May, 1985b). A reduction of worm burden by treatment may then result in reduced immune protection and thus increased reinfection rates. Such an impact of chemotherapy on immune resistance is not confirmed, and the immune sub-module may need further refinement.

The above discussion illustrates that many complex, interrelated aspects must be taken into account to make SCHISTOSIM a proper model for describing a specific set of field data. The dissimilarities between observations and predictions focus the modeller on

priorities in further development of the model, and the field worker on relevant topics for further research.

We are aware that models with too many free parameters might lack robustness, and therefore have tried to base as many parameters as possible on additional research data and other modelling experiences. It should, however, be noted that many of the parameters in SCHISTOSIM are actually used to describe the characteristics of a specific environment and control programme. Ultimately, we hope to develop a single broad model that can easily be adapted to make predictions for the effects of interventions in any endemic situation. To that end, a user-friendly input program, SCHISTO-IN, accompanies the SCHISTOSIM package. It can be used as an aid in specifying, modifying, and inspecting quantifications of all parameters, relationships, and distributions.

11.4 Evaluation of control

By comparing worm burdens and the corresponding egg counts of simulated individuals, the underlying mechanisms of this selective chemotherapy campaign and the subsequent reinfection are further clarified. Figure 11.5 shows in more detail the proportion of positive and negative individuals before and three months after the first treatment (Figure 11.4b).

The model indicates that before treatment, only one-third of all negative individuals would have no worms or single sex infections, and may therefore be considered true negatives. The other supposed negative individuals have at least one worm pair, but have negative stool examination results due to the low sensitivity of the Kato thick-smear technique for light infections, and thus receive no treatment in selective chemotherapy campaigns.

The administration of praziquantel to the detected positive individuals reduced the prevalence from 60% before treatment to 30% three months after treatment. According to the model, however, the proportion of true negative results did not change. Due to the high average worm burden, all positive individuals, including those of whom egg counts became negative, would have kept some worm pairs, in spite of the assumed 98% killing rate of worms, or they obtained new infections during the three months after treatment. However, one must beware of using true prevalences for expressing the effect of treatment. To evaluate the impact of intervention on morbidity, the reduction of the intensity of infection is much more important than removing the last few worms from each infected individual (WHO, 1993). SCHISTOSIM predicts that the mean worm burden of the survey population would have decreased markedly from 851 to 207, and even one year after treatment, the mean worm burden is still 390. These numbers also include the previous non-attenders and false-negative individuals.

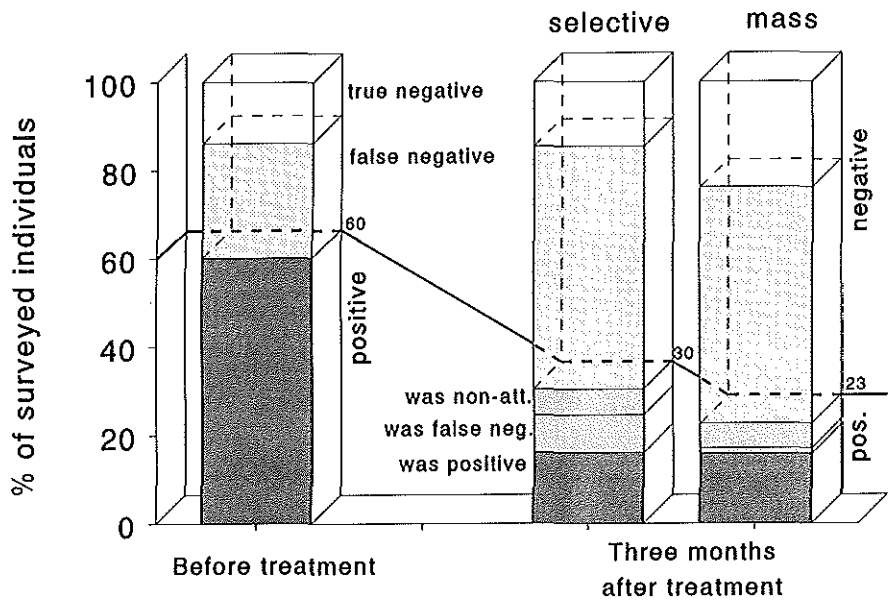

Figure 11.5 Composition of diagnosed positive (pos.) and negative (neg.) individuals in the surveys before and three months after the first treatment (assuming selective or mass treatment). Material above the bold line represents negative individuals: dotted area = false-negative individuals; open area = true negative individuals. Positive individuals are indicated below the bold line and have been distinguished into three categories according to their performance before treatment: non-att. = non-attenders; See text for additional information.

Besides incomplete cure and reinfection, two other categories contribute to the observed positive individuals after treatment. First, some false-negative individuals before treatment show up as positive in the next survey due to the intra-individual variation in egg counts (De Vlas & Gryseels, 1992). Second, a small category, constituted by non-attenders to the previous screening (and treatment) round, now show up with worm burdens high enough to be detected. Both categories cloud and complicate studies of cure and reinfection rates.

11.5 Prediction of alternative control strategies

From the above discussion, it appears that due to the low sensitivity of the screening technique, many infected individuals are not identified and therefore not treated. Health planners might be interested to project, without having to embark upon further field studies, what would happen if indiscriminate mass treatment were applied instead of selective treatment. Using SCHISTOSIM with the same assumptions as above, but introducing treatment of all individuals that attend the surveys, results in a prediction of

almost the same result as in Figure 11.4b: the observed prevalence after the first treatment would decrease slightly to 23% (Figure 11.5), but after one year of reinfection, almost the same levels as with selective treatment would be reached. However, the category of true negative individuals after three months would now double, due to the treatment of false-negative individuals. On the other hand, the proportion of positive egg counts after treatment is only affected by those previous false-negative individuals that, in the case of selective treatment, would show up as positive. This explains the limited reduction of the observed prevalence. The mean worm burden would be reduced to 153.

Table 11.2 shows the predicted results of other alternative control options compared with selective treatment. As a first example, attendance may be improved through health education. Assuming that half of the non-attenders would now participate in selective treatment, SCHISTOSIM predicts that the result expressed as further reduction of the observed prevalence is rather poor; only some of the previous non-attenders are added to the negative category after treatment. However, one should consider not only proportional results: by increasing attendance many more individuals would now be covered by the control project. The result expressed in proportion of individuals with high infections (worm burdens higher than 100) in the whole population is therefore comparable with the result after mass treatment (Table 11.2).

Table 11.2 Predicted results three months after different chemotherapy control measures.[a]

Alternative control measure	Prevalence (%) of positive counts in the survey population	Percentage with worm burdens > 100 in the whole population
Selective (baseline)[b]	32	34
Mass treatment	23	30
Higher attendance[c]	29	30
Greater efficacy of the drug[d]	25	32
All combined[e]	12	21

[a] Each result represents the average of 10 different simulation runs of approximately 1000 persons.

[b] Baseline characteristics of the project in Gihungwe: selective treatment of all persons showing a positive egg count (attendance to the survey and treatment is 71%) with praziquantel, 40 mg/kg, assuming a 98% killing rate of worms.

[c] Attendance at treatment improved to 85.5%.

[d] Rate of killing of worms by drug improved to 99.99%.

[e] Mass treatment, with an attendance of 85.5% and a killing rate of 99.99%.

Another alternative is the use of a 'perfect' drug with a 99.99% killing rate. This prevents cases of incomplete cure, but due to reinfection, the effect on prevalences is still rather moderate. Expressed as the proportion of intense infections in the whole population, the predicted effect of this theoretical example is even less than those for mass treatment or improving the attendance. Combining all three modifications, finally, results in considerably lower prevalences.

The simulated effects of these alternative control policies obviously depend on the assumptions of the model. SCHISTOSIM is still under development and has limitations and uncertainties that may influence the outcome of the model. It must be stressed that the described predictions merely illustrate the potential of SCHISTOSIM; furthermore, the long-term impact of control is much more important than the short-term effects described in this example. The exercise shows, however, that if the model could be further corrected and refined, SCHISTOSIM may become a powerful tool to predict and evaluate the effects of different control strategies, without having to go through costly and lengthy comparative trials in the field.

11.6 Conclusions

This chapter shows that SCHISTOSIM can already satisfactorily describe the short-term effects of chemotherapy, and plausibly predict effects of alternative strategies. To predict long-term results of control after (repeated) chemotherapy, the model needs further extension and refining, including, among others, closing the transmission cycle by including the dynamics of the intermediate host and larval stages. Equally important, SCHISTOSIM should also include the dynamics of morbidity, the main objective of control, and should further refine the mechanisms of immunity. In the hope that SCHISTOSIM will eventually contribute to the planning and evaluation of control programmes, efforts to further develop the model will be continued. To that end, input and expertise from all fields of schistosomiasis research, including other modelling efforts, are essential.

PART V

General discussion

12

MODELLING HUMAN *SCHISTOSOMA MANSONI* INFECTION: THE ART OF COUNTING EGGS IN FAECES

12.1 Introduction

The overview in Chapter 1 leaves little doubt about the significance of faecal egg counts in schistosomiasis research and control. The model, as graphically illustrated in Figure 1.4, turned out to be a great help in understanding the variability in egg counts. Several independent data sets were described in detail, leading to the general conclusion that standard Kato-Katz surveys miss many infections. The pocket chart has proven to be a practical and statistically valid tool to predict true prevalences from observed population egg counts. The chart has already been applied in practice (Van Lieshout *et al.* 1995a; Barakat *et al.* 1995; Engels, Sinzinkayo & Gryseels, 1996). Finally, the general mechanisms of egg count variation as derived from this modelling exercise have been implemented into SCHISTOSIM and could be compared with other phenomena that affect the outcome of a chemotherapy campaign.

In this final chapter, I will try to integrate the findings of the different chapters. Since the individual studies in this thesis have been discussed extensively at the end of each chapter, this general discussion will especially focus on recent ideas and future perspectives. In order to keep the studies accessible for a non-mathematical audience, we have avoided technical details underlying the general model and the construction of the pocket chart in the preceding chapters. This discussion will therefore start in section 12.2 with a more in depth analysis of important topics like goodness-of-fit, bias and behaviour of parameters in the model. In section 12.3, the phenomenon of aggregation in repeated individual measurements is studied in more detail by comparing the contribution of day-to-day and within-stool variation to the value of the aggregation parameter r. Section 12.4 follows with more practical considerations for use of the pocket chart to estimate true prevalences. Here, for example, expressions for confidence intervals and charts for other standards than 50 mg Kato-Katz faecal samples are provided. In section 12.5, the actual usefulness of the pocket chart for current control considerations is discussed, followed by a description of how the model could be used to provide an answer to the important control question of how many repeated surveys suffice for adequate screening. Section 12.6 concludes by discussing other aspects that make modelling for schistosomiasis

control relevant, and this section could be considered as the guide for further development of SCHISTOSIM.

12.2 Technical aspects of the model

The model to be addressed deals with how to make inferences on the distribution of worm pair burdens in the human population from observed (repeated) egg counts. The probability of finding y eggs in the stool of someone harbouring a given number x of worm pairs can be expressed as $P(y|x)$, and thus the probability of observing y eggs in the population is given by

$$\sum_{x=0}^{\infty} P(y|x) \, . \, P(x)$$

where $P(x)$ denotes the probability of harbouring x worm pairs. In our model $P(y|x)$ is the negative binomial with $E(y|x)$ being a *linear* function of x, $E(y|x) = h.x$, and aggregation parameter r. Thus, h represents the average egg count for an individual with 1 worm pair. The (unobserved) worm pair distribution in our model was derived (cf. Chapters 2 and 3) from the assumption of a negative binomial distribution (with mean M and aggregation parameter k) of the number of worms, and monogamous mating of male and female worms into worm pairs.

12.2.1 Goodness-of-fit

Using the model in practice can only be justified after successfully checking model output against data, addressing features of model and data most likely to be perturbed by misspecification. Such features are individual variation in egg counts and variation in mean egg counts between individuals. Application of the model to data sets with both single and three repeated individual measurements (Chapter 3) showed good fit. Also the relationship between the proportion of individuals with identified infection after one and three surveys (Chapter 4) could be predicted adequately. Application of the model (via the pocket chart) to predict 'true' prevalences (Chapters 6 and 8) and 'true' individual intensity of infection (Chapter 9) after many measurements further supports the validity of the model.

In Chapter 3, we tested the model using the commonly used chi-square goodness-of-fit criterion. For this the data sets were divided into n categories (indexed by i), and

$$\sum_{i=1}^{n} \frac{(Obs_i - Exp_i)^2}{Exp_i}$$

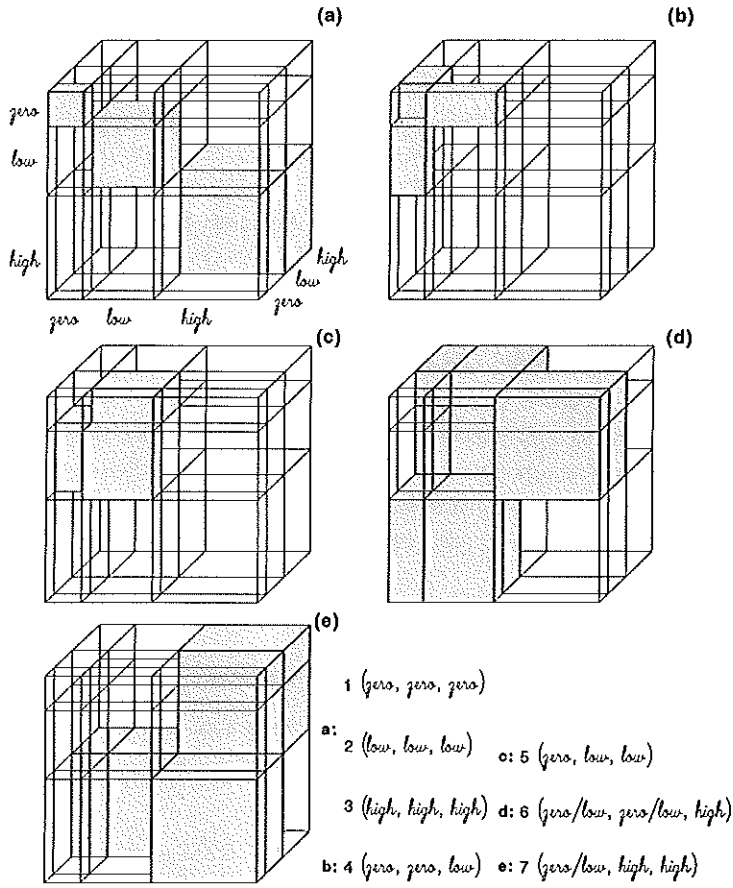

Figure 12.1 Combination of the 27 egg output cells for 3 repeated measurements and 3 possible egg count categories: *zero*, *low* and *high*. Reduction of the number of cells was obtained by combining permutations in the order of individual counts. For instance, (c) shows the combination of (*zero*, *low*, *low*), (*low*, *zero*, *low*) and (*low*, *low*, *zero*). Furthermore, (e) shows the combination of relatively rare counts (*zero*, *high*, *high*) and (*low*, *high*, *high*). The resulting 7 cells usually allow chi-square testing of the goodness-of-fit for data sets with about 50 to 100 individuals. This approach was used for the Gihungwe and Kizina data sets in Table 3.3.

compared to the χ^2 distribution with DF $= n-1$ minus the number of fitted parameters. For this test to be reasonably accurate, the expected number of individuals in each cell Exp_i must be at least 5 (Glantz, 1989). As shown in Chapter 3, this way of testing the goodness-of-fit already becomes rather complex in case of 3 repeated egg counts per individual. For example, 3 egg count categories (zero, low and high) leads to $3^3 = 27$ different cells. Thus, the number of individuals must be rather high to have enough entries in each cell. By systematically combining groups of categories, the number of

cells can be reduced to 7 (Figure 12.1). However, this reduction may lead to a considerable loss of power.

This way of testing the goodness-of-fit of the model in case of the extensive Gihungwe data set with 7 repeated measurements (Chapters 8 and 9) cannot reasonably be done, as the number of cells would be too large. In Figure 12.2, we have therefore compared observations and model predictions for only a few essential egg output categories. The agreement between observed and expected number of individuals in categories $(0)^1$, 1−, 30−, 100−, 300−, 1000− and 3000− for example shows that the model accurately describes the distribution of single egg counts. The good fit for the series $(0)^1$, $(0)^3$ and $(0)^7$ furthermore confirms that prevalences after 1 to 7 repeated measurements can be predicted adequately, implying that reliable prediction of prevalences after more repeated measurements might be possible. However, the small difference between observed and expected number of individuals in categories $(0)^3$, $(0)^7$ and $(\geq 300)^2$ does not provide enough evidence that repeated individual egg counts can be described properly with this model. An overall chi-square test cannot be applied to Figure 12.2 as categories partly overlap. Thus, despite the good fit of Figure 12.2 additional aspects need to be considered.

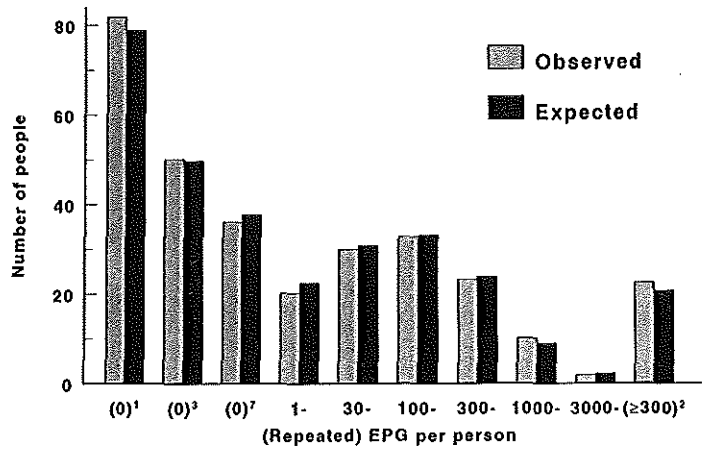

Figure 12.2 Goodness-of-fit for the Gihungwe data set with 7 repeated measurements from 200 individuals (17 missing values) by comparing the observed and predicted number of individuals in 10 different egg output categories, with $(0)^w$ representing the individuals still showing zero counts after w repeated measurements, 100− those with egg counts from 100 to 299 EPG, and $(\geq 300)^2$ the persons that show egg counts ≥300 EPG twice. Calculation of observations are based on the average number in all permutations of the 7 repeated surveys. For example, the value for 100− is the mean number of individuals in this category in the 1st, 2nd, ..., 7th survey, and the value for $(\geq 300)^2$ is the mean number of individuals in this category in the 1st and 2nd, 1st and 3rd, ..., 6th and 7th surveys. Predictions are based on the model with parameter h fixed at 0.05 eggs per 50 mg sample per worm pair and free parameters (with 95% CI): r = 1.23 ± 0.16, k = 0.37 ± 0.07, and M = 537 ± 253, 939 ± 439, 260 ± 144 and 131 ± 60 for the age groups 5-8, 9-16, 18-33 and 34+, respectively. This parameter set provides the highest likelihood with the least number of significant parameters (i.e. the lowest AIC, see Chapter 3).

The Kolmogorov-Smirnov one sample test is another commonly applied approach to testing goodness-of-fit. It uses the maximum absolute difference

$$D = \max \mid F(X) - S_N(X) \mid$$

between the theoretical and the observed cumulative frequency distribution functions $F(X)$ and $S_N(X)$ of a sample of N observations, for all possible counts X. In Figure 12.3, we show the observed and predicted cumulative frequency distributions for 5 specific characteristics of the data and the model: single counts, minimum, maximum, sum and standard deviation of the 7 measurements from each individual. Although an overall goodness-of-fit criterion cannot be calculated, each aspect can be tested separately. According to the Kolmogorov-Smirnov one-sample criterion (Siegel & Castellan, 1988), the observed and predicted distributions agree very well for all 5 characteristics. The finding that only 6 parameters (see legend Figure 12.2) suffice to describe this extensive data set with 1383 measurements further corroborates the egg count model.

12.2.2 Monte Carlo simulations

In the previous subsection we have shown that the model can adequately fit real data. Although a necessary condition, a good fit is not sufficient to conclude that model parameters can be estimated, even if the propounded model were correct. To show that reliable estimates can be obtained, we resort to Monte Carlo simulations. For given parameter values, we generate (100) data sets and try to re-estimate the parameter values (of r, M and k) from these data sets (Table 12.1). It appears that the bias in the estimates is small compared to standard errors.

Table 12.1. Summary statistics of sampling distributions for maximum likelihood estimates of M, k and r for 100 Monte Carlo simulations of populations with size 100 or 1000, and 7 repeated measurements per individual. Parameters have been set at values corresponding with an average endemic situation: M = 100.0, k = 0.40 and r = 1.0. Parameter h is fixed at 0.05 for simulation and estimation. Figure 12.4 illustrates the difficulty in re-estimating h.

	M	k	r	M	k	r
	SIZE 100			SIZE 1000		
Mean	97.5	0.401	1.018	99.8	0.401	1.000
S.d.	15.8	0.061	0.124	5.4	0.022	0.033
Median	95.1	0.396	1.028	100.3	0.401	0.999
Lower 25%	86.9	0.355	0.930	96.3	0.388	0.982
Upper 25%	108.3	0.435	1.098	103.5	0.416	1.028

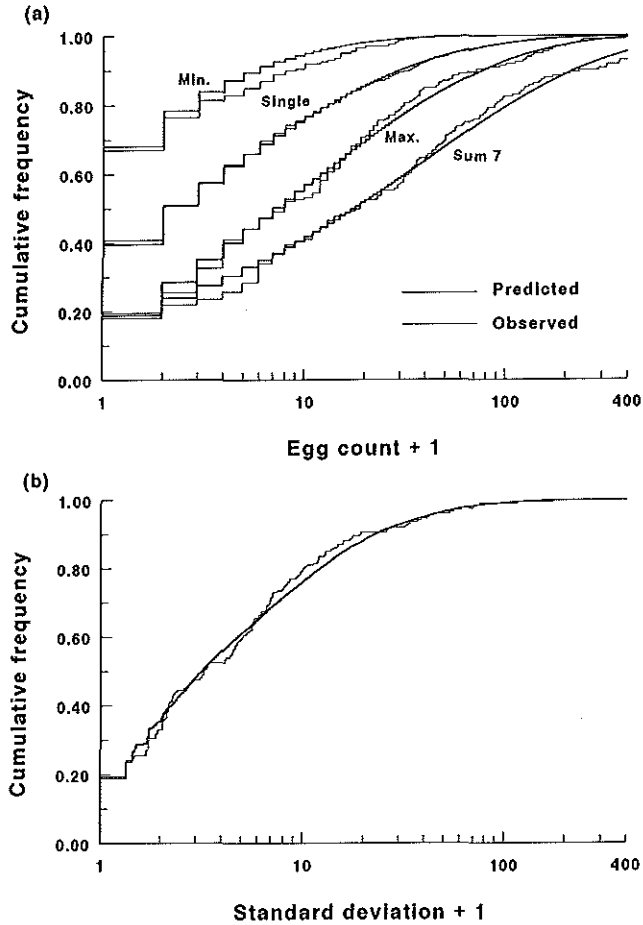

Figure 12.3 Goodness-of-fit for the Gihungwe data set with 7 repeated measurements from 200 individuals (17 missing values) by comparing the observed and predicted cumulative egg output distributions for 5 characteristics of the data: (a) single measurements (i.e. the combination of all 7 measurements of the 183 individuals with complete follow up, and 6 for the 17 with a missing value), the minimum, maximum and sum of the 7 (or 6) repeated measurements, and (b) the standard deviation of the 7 (or 6) repeated measurements. Predictions have been obtained by Monte Carlo simulation for 100 000 individuals using the model with the best fitting parameter values (see Figure 12.2). Differences in observed and predicted cumulative distribution functions can be tested by means of the Kolmogorov-Smirnov one-sample test. Test statistic D equals 0.046, 0.012, 0.041, 0.047 and 0.041 for *Min.*, *Single*, *Max.*, *Sum 7* and *Standard deviation*, respectively, implying no significant differences ($P > 0.20$ for each aspect).

However, parameter h was not considered in Table 12.1. For one of the 100 Monte Carlo data sets Figure 12.4a shows the profile likelihood as a function of h. Figures 12.4b and 12.4c show the corresponding maximum likelihood estimates of r, k and M. The continuous lines denote the result for the general model, while the dotted

lines are the result for an alternative (simpler) model that ignores worm pair formation and assumes worm pair burdens to be negative binomially distributed (with mean M' and aggregation parameter k'). It is clear from Figure 12.4a that the profile likelihood poorly distinguishes values for $h < 0.05$. However, values > 0.10 can be rejected on the basis of the data. This typical 'saxophone curve' was already visible in Figure 3.2 for a data set with 5 repeated measurements. Further simulations show that confidence intervals of h narrow by increasing the number of repeated observations. In practice, however, obtaining more than 7 separate stool specimens per individual in population surveys is extremely cumbersome. This limits the feasibility of estimating (lower bounds of) h from such data. Therefore, other sources of data (human autopsy studies, animal experiments) have to be (and were!) used to arrive at acceptable estimates of h (Chapter 5).

Parameters r, k and M are better identifiable because they represent specific distinct aspects of the data. Aggregation parameter r parameterizes the variation in repeated measurements within individuals, and its value is therefore hardly affected by changes in h. Also the shape of the assumed distribution for worm pair burdens does not influence its value, as shown by the overlap of the continuous (general model) and dotted (alternative model) lines for r in Figure 12.4b. For a given h, parameter M is largely determined by the mean egg count, which is approximated[1] by the expression $M.h/2$. For high values of h, and thus small numbers of worms, the effect of mating becomes more important as smaller proportions of worms are paired. This leads to relatively higher values of M as illustrated by an increasing value of $M.h/2$ in Figure 12.4c. In the alternative model, no mating is taken into account and the mean egg count is about equal to $M'.h$ for all values of h. Parameter k accounts for between-individual variation, and depends largely on the probability of harbouring zero worms. As h increases and thus M becomes smaller, the chance of zero worm burdens $((k/(M+k))^k$ would increase for fixed k. This is compensated by a higher value of k (Figure 12.4b), i.e. by assuming less variation in the worm burden distribution. Similarly, the intrinsically higher chance of zero worm pair burdens for a model with mating, compared to the alternative model without mating, explains why k is larger than k'.

---

[1] Assuming worm burdens negative binomially distributed with mean M and index of aggregation k, monogamous mating between male and female worms, and an average production of h eggs per worm pair per sample, the mean egg count equals:

$$\frac{M.h}{2}\left\{1-\sum_{x=1}^{\infty}\frac{\Gamma(k+2x)}{\Gamma(x+1)\,\Gamma(x)\,\Gamma(k+1)}\cdot\left[\frac{M}{2(M+k)}\right]^{2x-1}\cdot\left[\frac{k}{M+k}\right]^{k}\cdot\left[\frac{1+2x.(M+k)}{M(k+2x-1)}\right]\right\}$$

Further note that for inference about the parameters the alternative (simpler) model appears to be just as good as the general model. For small values of h, when the effect of mating vanishes, the continuous (general model) and dotted lines (alternative model without mating) converge (Figure 12.4).

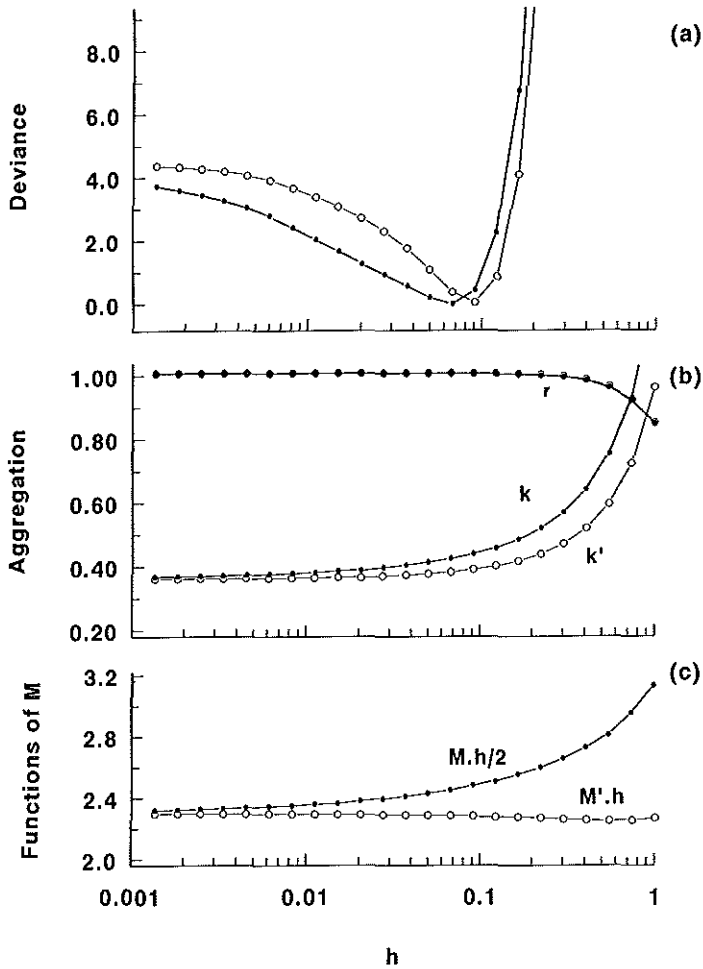

Figure 12.4 Maximum likelihood estimation of the parameters from a Monte Carlo simulated data set with 1000 individuals, examined 7 times, as obtained from the general model assuming M = 100, k = 0.40, h = 0.05 and r = 1.0. Parameters were re-estimated using the same model (•), and an alternative model (○) that does not take into account the process of mating between male and female worms but assumes a single negative binomial (M', k') distribution for *worm pair* burdens. Indicated are (a) the profile likelihood (deviance) as a function of the productivity parameter h, and (b) the corresponding values of aggregation parameters r in repeated measurements and k in worm burdens (or k' in worm pair burdens for the alternative model), and (c) the expression $M.h/2$ (or $M'.h$ for the alternative model). The mean egg count in this data set equals 2.28 per 50 mg of stools. See the text for further explanation.

12.2.3 Extensions of the model

The assumptions underlying our model could be relaxed in several ways. The assumption of a negative binomial distribution of worms can be relaxed, for example by introducing a mixture model of two distributions, a negative binomial plus a group of 'true negatives'. In every population there may be groups, such as recent immigrants from non-endemic areas or recently dewormed individuals, who do not 'belong' to the population at large. The proportion of individuals without worms will then be higher than predicted by the model. However, in most endemic areas these groups could be identified and - if necessary - analyzed separately.

On the other hand, false positive measurements may lead to less individuals without worms than predicted by the model. The chances of misdiagnosing uninfected individuals are probably minimal: one will not easily confuse foreign material in stool samples with eggs, and detection of residual eggs in faeces after death of the last schistosomes (as suggested by Goddard, 1977) is also not very likely, given the fact that infected persons usually harbour many more than one worm pair (Chapter 5) and the long life-span of schistosomes (Wilkins, 1987). False positive diagnoses may sometimes also result from mislabelling stool samples, especially among relatives (mother and child).

An additional extension of the model is provided by allowing parameter h to depend on worm load (density dependence) and/or allow it to vary between individuals. Chapter 5 explores the issue of density dependence. It appears that for schistosome infections there is no clear evidence that density dependence plays a role. The issue of between-individual variations in h is difficult to address. We expect that making h stochastic will have little effect on predicted average worm burden, but that it may reduce variations in worm pair burden. As a result, e.g. the 'tails' of worm pair burden distribution could be less pronounced than in Figure 5.1, and 'true prevalences' could even be higher than predicted thus far. Continuing research on relating individual egg counts to another (better?) indicator of worm (pair) burdens, such as circulating antigen concentrations in serum and urine samples (Van Lieshout *et al.* 1995b), might help us to understand density dependence and stochasticity in h.

It should be noted that our models are intended to apply to equilibrium situations only. Dynamic processes, such as occur after the implementation of a control program, are far more complex and depend on the details of the particular intervention. For example, selective treatment based on screening will disproportionately affect high worm burdens and thus vitiate the arguments leading to a negative binomial distribution. Understanding of dynamic processes requires a different approach. Currently, the most appropriate approach seems to be microsimulation, in which individual life histories are simulated (Chapter 10). In Chapter 11, we discuss a successful application of this technique. The microsimulation model SCHISTOSIM includes our general assumption

that repeated individual egg counts are negatively binomially distributed with mean h.WP and index of aggregation r (WP denotes number of worm pairs). The successful fit to a series of egg count distributions (up to one year) after control in Figure 11.4 therefore suggests that this assumption remains valid for situations after control.

12.3 Aggregation in repeated individual egg counts

The negative binomial distribution for repeated individual egg counts is central to our modelling exercise. The negative binomial assumes 'heterogeneity' in the variation of repeated egg counts, so that the variance exceeds the mean (i.e. 'overdispersion'). The aggregation parameter (or index of aggregation) r represents the extent of heterogeneity. The lower the value of r, the more the eggs are aggregated (or concentrated) in a few of the repeated samples. The Poisson distribution is an extreme case of the negative binomial in which $r \to \infty$. Then the variance is equal to the mean, and the variation in repeated counts is called 'homogeneous' or random.

12.3.1 Testing the negative binomial

Table 3.2 showed that r did not differ significantly between age groups in the Buhandagaza data set (respectively 0.67, 0.91, 0.80, 0.99). For convenience, the overall value $r = 0.87$ was treated as a biological constant and has been used throughout this thesis. In the previous section, we have estimated r from the more complete Gihungwe data set with 7 measurements per individual, and the best fitting value appeared to be 1.23 (legend Figure 12.2). Also here, r did not differ significantly between age groups (respectively 1.16, 1.32, 1.15 and 1.20), but the higher values imply less aggregation in repeated egg counts than assumed thus far.

The appropriateness of the negative binomial to describe repeated individual egg counts has only been tested as part of validating the complete model (see previous section). In theory, the negative binomial could be tested independently if we had enough repeated measurements from some individuals. Probably the most valuable data in this respect have been published by Scott (1937). He collected total faecal outputs of three Egyptian boys for 30 consecutive days and published the number of eggs counted in stool samples from each specimen. Egg counts were obtained by the dilution technique of Stoll (1923) and effectively concern examination of about 30 mg of faecal material. We use egg counts from the largest daily stool samples to determine the distribution of day-to-day egg count variation. Figure 12.5 demonstrates that the negative binomial nicely fits the observed distributions, and in all three cases superior to the Poisson distribution. The maximum likelihood estimates of r (6.1, 7.6 and 10.6) are however much higher than we found in our data (see later for a possible explanation).

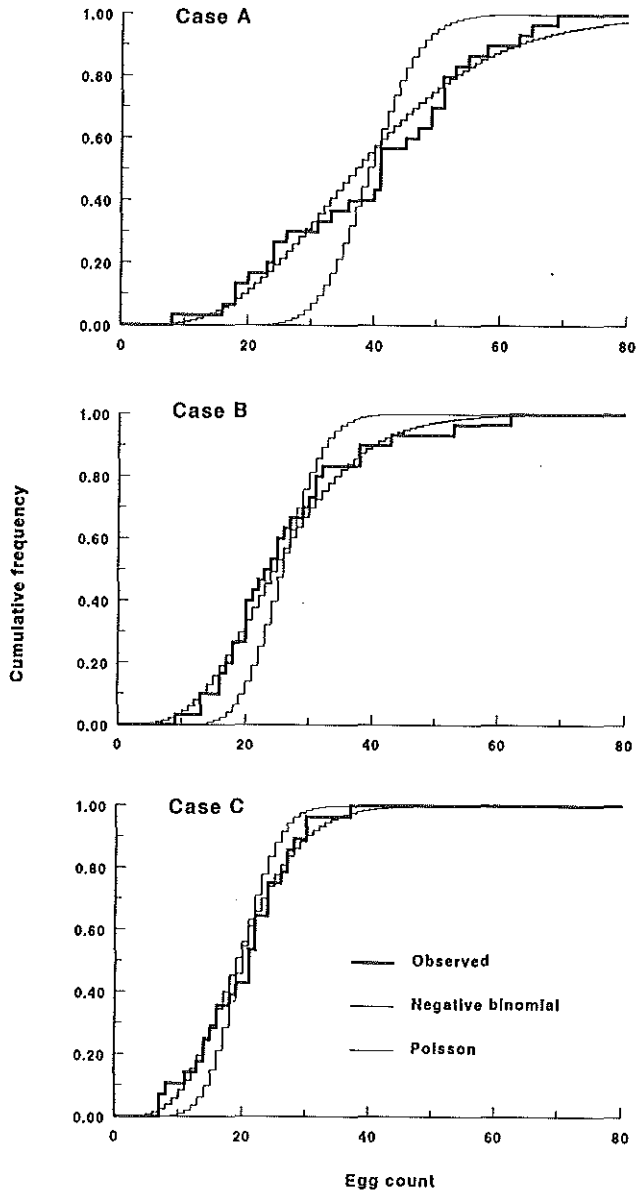

Figure 12.5 Cumulative frequency distributions of 30 consecutive *S. mansoni* egg counts compared with the best fitting negative binomial and Poisson distributions. The data have been derived from the study by Scott (1937) where for three persons all stool specimens were collected during one month and tested for infection (see text). Mean egg counts are 40, 26 and 20 for case A, B and C, respectively. Maximum likelihood estimates of the index of aggregation r are 6.1, 7.6 and 10.6.

12.3.2 Day-to-day variation

Thus, the negative binomial really seems to be adequate for describing individual variation in egg counts, but the value of r differs between situations. Theoretically, one could consider the negative binomial distribution the result of gamma distributed mean egg counts on different days, and Poisson variation in (repeated) samples from daily stool specimens. Mean individual egg counts could vary from day to day due to e.g. changes in stool size (and consistency), fluctuation in schistosome oviposition, or dynamical patterns in the total number of worms harboured. Evidently, these mechanisms become more important (i.e. r becomes smaller) as the interval between examinations increases. This may in part explain why the value of r is higher for Gihungwe (intervals of 2 days to 2 weeks, $r = 1.23$) than for Buhandagaza (3 months intervals, $r = 0.87$). Analysis of a data set with individual examinations on 7 consecutive days even resulted in $r = 1.56$ (Chapter 3).

We use the above data sets for a more detailed analysis of parameter r. Figure 12.6 shows the maximum likelihood estimates of $1/r$ for each individual in the three data sets. Piegorsch (1990) showed that maximizing the likelihood with respect to $1/r$, instead of r, allows one to take into account situations with $r < 0$ (i.e. 'underdispersion'). The estimates of $1/r$ show a very wide range due to the relatively small number of measurements per individual (5 to 7). Estimates of aggregation are sensitive to zero counts, such that the variation in $1/r$ declines as the mean egg count increases and the probability of zeros becomes small. This typical relationship of $1/r$ with mean egg count was also found in Monte Carlo simulated data sets (not shown).

The individual points from the three data sets confirm that r decreases as the duration between subsequent measurements becomes larger (Figure 12.6). In all three situations the combined estimate of $1/r$ (continuous line) is smaller than the value of $1/r$ obtained after applying our egg count model (dotted lines). This is due to the tendency of the maximum likelihood to overestimate the aggregation parameter, especially if the number of measurements is small (Taylor, Woiwod & Perry, 1979; Piegorsch, 1990). Furthermore, the egg count model explicitly takes into account the possibility that series of only zero counts represent persons with (a low number of) worm pairs. A series of zero counts provides no information about an individual estimate of $1/r$, and these persons are therefore not considered in Figure 12.6. The figure further shows that in none of the three cases a linear relationship between $1/r$ and mean egg count provides a significantly better fit than simply using a fixed value. Thus, a constant r for all individuals in a population seems to be a plausible assumption.

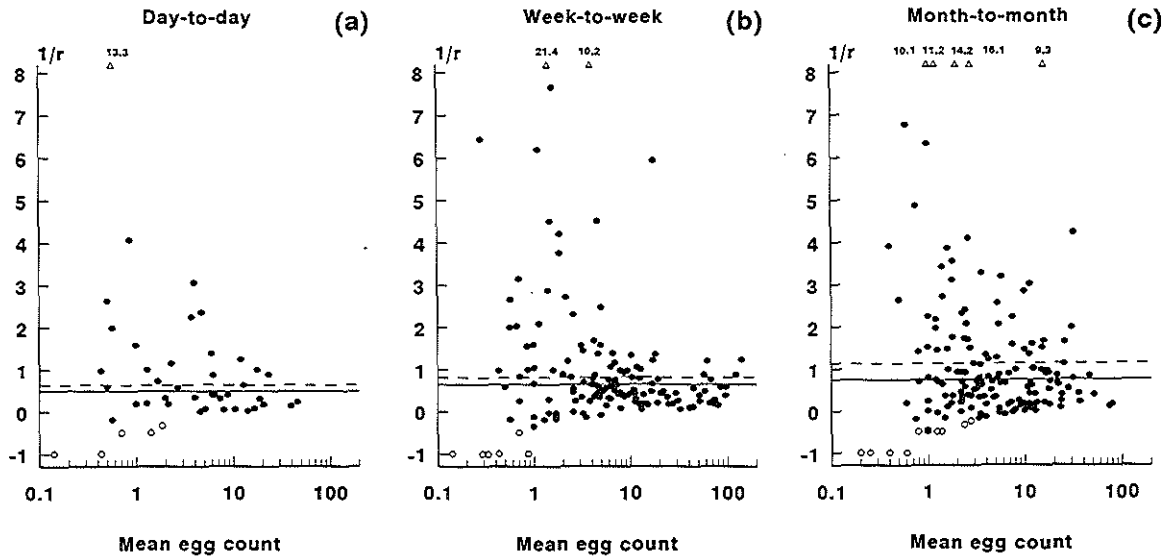

Figure 12.6 Scattergrams of individual estimates of $1/r$ as a function of the mean egg count. Data come from three population studies on variation in *S. mansoni* egg counts with different duration between subsequent surveys. Day-to-day variation (a) was measured by 7 consecutive 25 mg Kato-Katz samples from 73 inhabitants of a village in Burundi (unpublished data, with courtesy of Dr. B. Gryseels). The week-to-week variation (b) concerns the data from Gihungwe where 200 individuals have been measured 7 times with intervals of 2 days to 2 weeks (Engels, Sinzinkayo & Gryseels, 1996). The month-to-month (c) variation concerns the study in Buhandagaza where 435 individuals have been examined 5 times with about three months between subsequent surveys (Gryseels, Nkulikyinka & Engels, 1991). The Gihungwe and Buhandagaza studies have been based on duplicate 25 mg Kato-Katz slides. For all individuals that showed at least one positive slide (n = 47, 161 and 238, respectively), estimates of $1/r$ have been obtained by maximizing the likelihood of the negative binomial using the method described by Piegorsch (1990). Values of $1/r$ above 0 indicate overdispersion or aggregation, and values below 0 imply underdispersion. The triangles indicate the values that are located outside the chart. The open dots represent values that show maximum underdispersion. In that case, $1/r$ is limited to $-1/y_{max}$, where y_{max} is the largest observed value from the individual (Piegorsch, 1990). The solid lines indicate the overall estimate of $1/r$, respectively 0.49, 0.65 and 0.74. The dotted lines indicate the estimate of $1/r$ using our egg count model on the three complete data sets: 0.64 (r = 1.56), 0.81 (r = 1.23) and 1.16 (r = 0.865).

Note that for inferences on the underlying worm pair distributions from single survey egg counts (as in the pocket chart), a value of r has to be chosen. With a too short interval between successive surveys (e.g. day-to-day), the corresponding value of r may not account for all possible variation in individual egg counts. Very long intervals may violate the assumption that individual worm burdens do not change over time. This is especially important for young children who just started with accumulating the infection. Still, given the long lifespan of schistosomes, we believe that (for persons $\geq$ 5 yrs) the Buhandagaza study (month-to-month variation) provides the most adequate estimate of r.

12.3.3 Within-specimen variation

The above reasoning that the negative binomial for repeated egg counts arises from mean egg counts being gamma distributed and repeated counts from the same stool specimen being Poisson distributed, would imply that the value of r does not depend on the amount of stool examined. The sum of Poisson distributed random variables is again Poisson (Mood, Graybill & Boes, 1988), so that the heterogeneity introduced by the gamma distribution is not affected. We can test this assumption by applying our model to only one of both duplicate 25 mg egg counts in Buhandagaza and Gihungwe. For Buhandagaza, the estimate of r is lower than when using all data (r = 0.81, instead of 0.87). For Gihungwe, this reduction is even more pronounced (r = 0.96 instead of 1.23). Apparently, eggs are not homogenously mixed in the faeces and heterogeneity (or clustering) of eggs within stools contributes to the observed aggregation in repeated samples.

The distribution of schistosome eggs in faeces has been the subject of long debate. Khalil & Salah El Din (1930) reported that smears prepared from the surface of stools (especially at parts containing mucus or blood) show many more eggs than smears from the centre. Such clustering of eggs was however not found by Martin & Beaver (1968) and Ratard *et al.* (1990), who demonstrated that samples from different locations in the stool (beginning compared to end, surface compared to centre) did not have different mean egg counts. However, clustering of eggs could still exist on the micro-level. Woodstock *et al.* (1971) showed for 10 out of 11 individuals that the variance in repeated egg counts from the same stool specimen exceeded the mean. This overdispersion was significant in 3 cases.

Recently, Engels *et al.* (1996) studied intra-specimen egg count variation by preparing 10 Kato-Katz slides from each of 59 stool specimens. Plotting variances against means of each series of 10 measurements revealed a typical pattern with overdispersion becoming more pronounced for high mean egg counts. This pattern is highly suggestive of the negative binomial, where the variance V and mean m are related by $V = m + m^2/r$ (Chapter 2). Note that possible clustering of eggs is not likely to be observed directly in the Kato-Katz slides because the faecal samples are sieved before screening. In direct

smears without sieving, clumps of eggs can sometimes be observed (Dr. A.M. Polderman, personal communication).

12.3.4 Integrating within- and between-specimen variation

Within-specimen variation could be included in the model by assuming a negative binomial instead of the Poisson for repeated samples from the same stool specimen, and the mean egg counts between specimens could still be considered gamma distributed. This means that now two components contribute to the observed aggregation in repeated samples: between-specimen and within-specimen aggregation. The index of between-specimen aggregation (henceforth denoted by r_1) depends on the duration between successive measurements (see above), and the index of within-specimen aggregation (denoted by r_2) depends on the amount of stool examined. The sum of two negative binomial variables with equal mean and aggregation is again negative binomially distributed with twice the values for mean and aggregation (Grafen & Woolhouse, 1993). Thus, if r_2 denotes the index of within-specimen aggregation for egg counts in 25 mg stool samples, the index of aggregation in duplicate 25 mg samples will equal $2r_2$. When adding many more samples from the same stool specimen, the value of $r_2 \rightarrow \infty$, and within-stool variation will approximate the Poisson distribution.

Unfortunately, mathematical analysis shows that the resulting distribution of repeated individual egg counts is no longer negative binomial (which would leave the general structure of our model unaltered). However, Monte Carlo simulated data, generated from an extended model which includes the proposed mechanisms of between-specimen and within-specimen aggregation, can be well described by the original model (not shown). Thus, within the context of our egg count model, the negative binomial seems to remain adequate to describe repeated individual egg counts, even when accounting for two components of heterogeneity. The simulations further suggest $r^{-1} = r_1^{-1} + r_2^{-1}$ as a convenient expression for the overall aggregation parameter r (Table 12.2).

In order to estimate r_1 and r_2, we can use this expression for the values of r as estimated from the Gihungwe and Buhandagaza study (see above). In Gihungwe, egg counts in single 25 mg samples resulted in $r = 0.96$ (so, $r_1^{-1} + r_2^{-1} \approx 0.96^{-1}$), whereas duplicate 25 mg counts resulted in $r = 1.23$ (so, $r_1^{-1} + 0.5r_2^{-1} \approx 1.23^{-1}$). Thus $r_1 \approx 1.68$ and $r_2 \approx 2.24$. In fact, the value of r_2 should be comparable for Gihungwe and Buhandagaza, as the diagnostic methods in both studies were similar. Using $r_2 = 2.24$ for the single 25 mg egg counts in Buhandagaza ($r = 0.81$) results in $r_1^{-1} \approx 0.81^{-1} - 2.24^{-1} = 1.27^{-1}$. However, the expected $r = 0.99$ for duplicate samples ($1.27^{-1} + 4.48^{-1} = 0.99^{-1}$) is considerably higher than estimated from the data ($r = 0.87$).

The study in Gihungwe was specifically designed with the objective to understand variation in repeated individual egg counts, and much care was taken to prevent missing

Table 12.2 The value of aggregation parameter r as estimated by simulation compared with the value as expected from pre-set between-specimen aggregation r_1 and within-specimen aggregation r_2 using the presumed expression $r^{-1} = r_1^{-1} + r_2^{-1}$. Estimations are obtained from fitting the model to 5 Monte Carlo simulated data sets with 1000 individuals, examined 7 times, using the model for $M = 100$, $k = 0.40$, $h = 0.05$ and for the indicated values of r_1 and r_2. See the text for further explanation.

r_1	r_2	$r_{expected}$	$r_{estimated}$
0.25	0.5	0.17	0.13
0.25	1.0	0.20	0.18
0.40	1.0	0.29	0.25
0.40	2.0	0.33	0.32
1.25	5.0	1.00	0.98
1.25	10.0	1.11	1.12
2.0	2.0	1.00	0.96
2.0	4.0	1.33	1.32
6.0	12.0	4.00	3.92
6.0	24.0	4.80	4.59

values and other disturbing effects (Engels, Sinzinkayo & Gryseels, 1996). The five repeated surveys in Buhandagaza were part of a longer population-based study which aimed at understanding the dynamics of *S. mansoni* infection before and after control (Gryseels, Nkulikyinka & Engels, 1991). In the latter study, missing values were more common. We therefore consider the index $r_2 = 2.24$ (in 25 mg samples) from Gihungwe as most realistic for aggregation in within-specimen variation. As explained above, the index of aggregation from Buhandagaza is considered more relevant for variation in mean egg counts over time. Using this $r_1 = 1.27$ for between-specimen aggregation, the overall value for aggregation in repeated individual duplicate 25 mg samples equals $r = 0.99$, which we will use henceforth.

Figure 12.7 demonstrates how extra aggregation (lower values of r) in duplicate 25 mg samples could result from actually using single 25 mg counts which had been multiplied by 2. The simulations show that if this had happened for 15% of the individuals in the Buhandagaza study, the discrepancy between the expected and estimated value of r could fully be explained. For some cases only one slide is available either because the amount of faeces provided was little or because the other slide was broken or simply missing. Generally, such counts are multiplied by 2 to make them comparable to

the other observations. A more important phenomenon might be the fact that two microscopists who each screen one of every pair of slides sometimes tend to compare both counts to prevent large differences. Perhaps, even only one slide is incidentally examined to save time. Obviously, these phenomena are not very likely to occur for 15% of the cases, but it could certainly have happened (Dr. B. Gryseels, personal communication).

Finally, it also seems contradictory that the estimated aggregation in daily egg counts (r = 1.56 in Figure 12.6a) is much more intense than when directly estimated from Scott's data (r = 6.1, 7.6 and 10.6 in Figure 12.5), although both studies were based on about the same amount of stool examined per individual (25 and 30 mg). However, one should note that Scott's egg counts were obtained after dilution and homogenizing large amounts of faeces (3 grams) in a solution from which a few samples were examined under a microscope (Stoll, 1923). This means that in fact r_2 should express a Poisson distribution ($r_2^{-1} \approx 0$), so that r_1 from the data in Figure 12.6a ($r_1^{-1} \approx 1.56^{-1} - 2.24^{-1} = 5.1^{-1}$) should be compared with r from Scott's data.

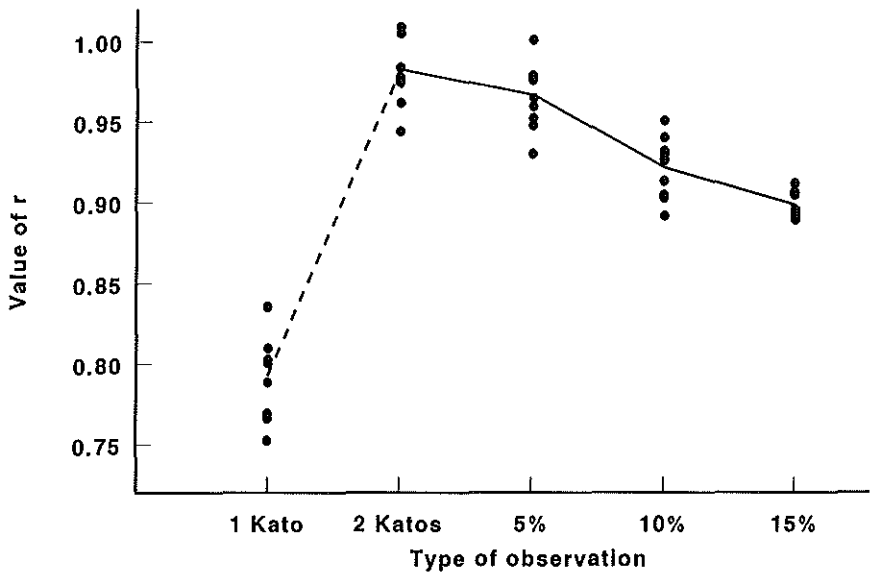

Figure 12.7 Aggregation parameter r as estimated from simulated data sets using different combinations of 25 mg Kato-Katz samples: single counts (1 Kato), duplicate counts (2 Katos) and three mixtures of duplicate Katos and 5%, 10% or 15% of the individuals with only a single count, which was multiplied by 2. Parameter values have been set at M = 100, k = 0.40, r_1 = 1.27, r_2 = 2.24 (for 25 mg samples) and h = 0.025 (for 1 Kato) or h = 0.05 (for all other cases). In each situation, 10 replicated Monte Carlo simulated data sets have been considered.

12.4 Estimating true prevalences from the pocket chart

Our approach to model variation in *S. mansoni* egg counts allows one to predict the proportion of individuals with at least one worm pair from the observed distribution of egg counts. The value for the sensitivity of Kato-Katz slides to detect *S. mansoni* infection that is derived by simply comparing this 'true prevalence' with the observed prevalence of positive counts is specific for the situation considered, and cannot be used in other situations. As has been discussed and demonstrated in Chapters 2 and 6, sensitivity depends on the intensity (and also the prevalence) of infection in the population, so that for every community (and age category) the model should be applied again. In Chapter 7, it has been explained how the actual model can be circumvented by plotting two standard epidemiological measurements, single survey prevalence and geometric mean of detected positive individuals, in the proposed 'pocket chart'. Construction of this chart was based on the assumption that the parameters for egg production, $h = 0.05$ eggs/worm pair/sample, and aggregation in repeated individual measurements, $r = 0.87$, are constants. Only the two parameters of the underlying distribution of worm burdens, mean M and aggregation parameter k, remain to reflect any combination of prevalence and geometric mean of positive counts. The true prevalence can subsequently be calculated from M and k. For 12 tested data sets, pocket chart predictions appeared to agree rather well with prevalences measured with much more sensitive techniques (Chapter 8). Below, we will further discuss some technical and practical considerations for use of the pocket chart to estimate true prevalences.

12.4.1 Application of the pocket chart

In the previous section, it was demonstrated that the degree of aggregation in repeated measurements has been overestimated, and a value $r = 0.99$ seems to be more adequate. The Kato-Katz technique is more sensitive than originally assumed, and the pocket chart (Figure 7.1) therefore could overestimate true prevalence. Figure 12.8, which gives the adapted version of the chart for $r = 0.99$, however shows that predictions are only slightly lower than by the original chart. Moreover, Figure 8.4 demonstrates that the suggested alternative value of r also leads to a successful validation. The value for the productivity parameter h could also differ from the one initially assumed. In Chapter 5, it was concluded that all available empirical and theoretical evidence (however limited) indicates that $h = 0.05$ is a plausible value, but this may differ from the true value by a factor two or more. Figures 8.3 and 8.4, however, show that pocket chart predictions are very robust to such changes in the value of parameter h. Thus, it is unlikely that alternative assumptions on the relationship between worm burden and corresponding egg counts, including density dependent egg production, will seriously affect the chart.

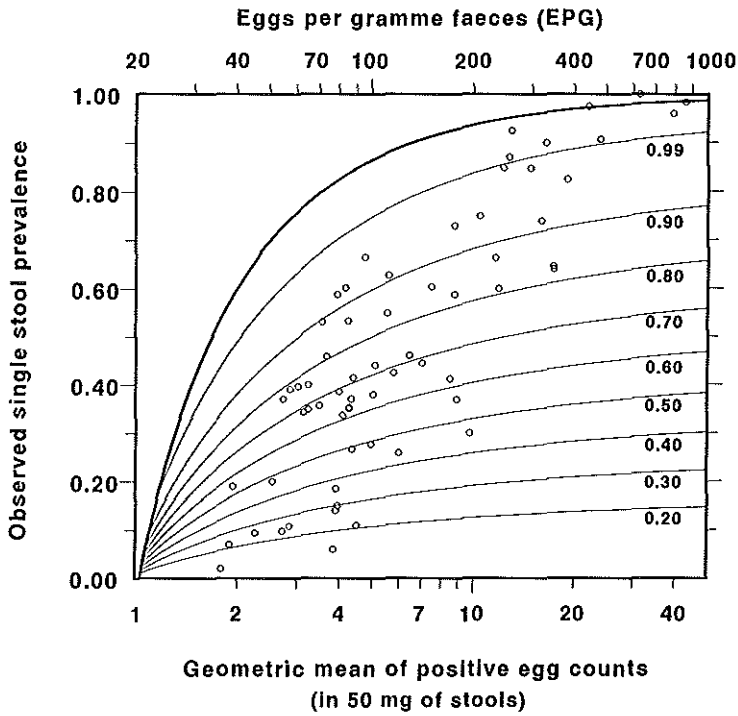

Figure 12.8 Pocket chart to estimate true *Schistosoma mansoni* prevalences. For each combination of observed single stool prevalence and geometric mean of positive egg counts, the estimated proportion of individuals that harbour at least one worm pair can be read from the contour lines. The predictions are based on an underlying stochastic model which only holds for pre-control situations and which is only defined below the bold line. This chart is specific for egg counts obtained in 50 mg stools. The bottom x-axis shows the geometric means from the actual counts, the top x-axis the geometric means after transformation to eggs per gramme faeces (EPG). The points represent various pre-control situations (when possible divided into age groups) recently observed by colleagues who have collaborated in this study. The data come from 5 different countries: Brazil (see Chapter 8 of this study), Burundi (Gryseels, 1988; Gryseels & Nkulikyinka, 1988, 1989, 1990; Gryseels, Nkulikyinka & Engels, 1991; Engels, Sinzinkayo & Gryseels, 1996), Senegal (Stelma *et al.* 1993), Surinam (Polderman *et al.* 1994), and Zaire (Polderman *et al.* 1985; Gryseels & Polderman, 1987; Polderman & De Caluwé, 1989). This chart is different from Figure 7.1 in its use of a different, but more accurate, value for aggregation in repeated measurements ($r = 0.99$).

Despite small differences with the original chart, we will henceforth use Figure 12.8 as *the* pocket chart. In this figure, we have further plotted the observed prevalences and geometric means for several endemic situations (and different age groups). The resulting points are scattered within a broad band diagonally across the chart. It can be seen that a single stool prevalence over 80% implies that approximately all individuals are infected. Predicted true prevalence for lower observed prevalences depend largely on the

corresponding geometric mean EPG. In combination with a relatively high geometric mean of 100 EPG, an observed prevalence of 20% indicates extreme aggregation of infection (k is small): some are heavily infected but most persons harbour no or a few worms. Thus, zero counts have a high chance to really represent uninfected individuals, so that the true prevalence is predicted to be only 40%. By contrast, a geometric mean of 40 EPG suggests less aggregation and a true prevalence of almost 60%. Analysis for each point in the chart further reveals that aggregation or overdispersion tends to decrease with geometric mean egg counts, reflected by a positive correlation between mean M and aggregation parameter k of the assumed underlying negative binomial worm burden distribution. The two points located on the bold line indicate minimal aggregation ($k \rightarrow \infty$) and thus a Poisson distribution. The single point above the bold line suggests that the underlying worm burden distribution is underdispersed (i.e. the opposite of aggregation), and therefore does not fit the negative binomial. Random fluctuation due to the small size of each data set, usually less than 100 persons, may account for such exceptions.

In most helminthic infections such as hookworm and *Ascaris* simple relationships between prevalence and intensity of infection were found (Guyatt *et al.* 1990; Guyatt & Bundy, 1991; Lwambo, Bundy & Medley, 1992). However, in *S. mansoni* infection, in spite of the overall positive correlation between M and k, the width of the band of points in Figure 12.8 proves that one parameter does not suffice to describe a particular endemic setting. This explains why Guyatt *et al.* (1994), pursuing the same approach as for other helminths, failed in their attempt to predict *S. mansoni* egg count distributions from prevalences only. Apparently, the spread of schistosome infections within populations differs substantially between populations. Acquisition of schistosome infections is primarily a matter of water contact behaviour, with exposure patterns in a population highly dependent on the distance to and use of infective water. This is e.g. illustrated by our finding that aggregation of infection in an artificial miners' village in Maniema (Zaire) and in a neighbouring traditional (but also highly endemic) community, differed substantially (Table 3.3).

12.4.2 Reliability of the pocket chart

The pocket chart assumes that the observed prevalence and geometric mean of positive egg counts suffice to determine the values of M and k. The 'loss of information' from using only these two field measures instead of fitting the model to all data may, however, lead to less accurate predictions. We therefore compared the distributions of M and k and the resulting true prevalence as estimated by maximum likelihood from 100 simulated complete data sets with the values of M and k corresponding to the prevalence and geometric mean from the same data. Table 12.3 reports the results for two endemic situations and two population sizes. Notably, in all four cases the mean values of M and k

are in almost perfect agreement with the pre-set values, and also the corresponding true prevalences agree very well with the expected value. Thus, bias seems negligible (Fulford, 1994). Complete data sets give more accurate results, but this improvement is insignificant relative to effects of population size. Thus, the prevalence and geometric mean of positives not only yield 'unbiased' estimates of M and k, the predicted true prevalences are also almost as accurate as those obtained from fitting complete data.

Table 12.3 further shows that the mean of each simulated distribution of the true prevalence approximates to the median and lies symmetrically between both quartiles, so that we will apply the normal distribution for descriptive purposes. Intervals around predicted true prevalences depend crucially on population size. In Table 8.2, e.g. we saw that 50 individuals is too little for reliable estimates. By means of Monte Carlo simulation we attempted to gain more insight into variability in the true prevalence. Figure 12.9 shows confidence intervals for the predicted true prevalence for a range of population sizes. Predictions are based on an average endemic situation with single stool prevalence equal to 0.40 and a geometric mean among positive individuals of 100 EPG (i.e. pre-set values of $M = 217$ and $k = 0.195$). Again, estimations are almost symmetrically distributed around the theoretically predicted true prevalence of 0.67, and the confidence intervals are unacceptably wide for population sizes under 100. Binomial variation in the single survey prevalence largely accounts for this (Figure 12.9). Evidently, according to the central-limit theorem, the latter approximates the normal distribution. Indeed, e.g. the 90% confidence intervals (CI) of the single stool prevalence p obtained from populations with size n agree with the theoretically expected $p \pm 1.645 \times \sqrt{\{p(1-p)/n\}}$.

Another factor which negatively affects reliability of estimates of the true prevalence is the additional inaccuracy in measurement of the geometric mean of positive egg counts. Figure 12.9 suggests that the relationship between true prevalence variation and population size n resembles the relationship between single survey prevalence and n. In fact, the 90% CI around the predicted true prevalence P can conveniently be expressed by $P \pm 1.645.C \times \sqrt{\{P(1-P)/n\}}$, with C a fixed value > 1. In Figure 12.9, the value of C only ranges from 1.57 to 1.61. Further simulations show that this expression also applies for other situations, with C only depending on the geometric mean of positive counts. C decreases with geometric mean egg counts (e.g. $C \approx 2.3$ for 40 EPG, and $C \approx 1.4$ for 200 EPG). As a 'rule of thumb' (and provided that the underlying egg count model and its quantifications are correct), the 90% confidence intervals for true prevalences predicted by the pocket chart, can be derived from the expression:

$$P \pm 1.645.C \times \sqrt{\{P(1-P)/n\}}$$

with n representing the population size, and $C \approx 1 + 0.4 \times \{\mathrm{Log}_{10}(geom)\}^{-1}$, where *geom* denotes the geometric mean of actual positive counts (in 50 mg of stools), or equivalently $C \approx 1 + 0.4 \times \{\mathrm{Log}_{10}(\mathrm{EPG}) - 1.3\}^{-1}$.

Table 12.3 Summary statistics of sampling distributions for distributions of M and k and the resulting predicted true prevalence (%) for 100 Monte Carlo simulations of populations with size 100 or 1000, and a single measurement per individual. Parameters have been set at (a) $M = 100$ and $k = 0.40$, theoretically corresponding with a single survey prevalence of 43.1%, geometric mean of positive counts of 60.2 EPG and a true prevalence of 82.1%, and (b) $M = 200$ and $k = 0.10$, corresponding with 27.3%, 115 EPG and 46.5%, respectively. The values of M and k are obtained directly from the complete data sets by maximum likelihood estimated, or indirectly by calculating the maximum agreement to the prevalence and geometric mean of positive counts from the same 100 data sets. True prevalences are calculated from the values of M and k as has been described in Chapter 7. S.d. means standard deviation.

	(a) Assuming $M = 100$ and $k = 0.40$						(b) Assuming $M = 200$ and $k = 0.10$					
	M	k	True prevalence	M	k	True prevalence	M	k	True prevalence	M	k	True prevalence
	SIZE 100			SIZE 1000			SIZE 100			SIZE 1000		
Directly from complete data set:												
Mean	101.1	0.442	82.5	100.5	0.399	81.8	213.2	0.107	47.2	198.5	0.101	46.6
S.d.	22.2	0.141	6.7	8.0	0.039	2.4	88.5	0.032	8.3	22.5	0.009	2.4
Median	100.0	0.402	82.4	99.7	0.399	82.1	203.5	0.099	46.5	198.8	0.100	46.5
Lower 25%	83.9	0.340	78.2	94.3	0.376	80.6	141.2	0.081	41.5	183.2	0.094	44.9
Upper 25%	113.8	0.506	86.4	106.3	0.427	83.4	247.3	0.130	52.5	211.0	0.107	48.0
Indirectly, by using only prevalence and geometric mean:												
Mean	103.2	0.438	82.2	100.6	0.400	81.8	224.2	0.105	47.0	200.2	0.101	46.6
S.d.	26.5	0.154	6.9	8.6	0.044	2.6	91.9	0.034	8.7	26.8	0.009	2.4
Median	99.4	0.389	82.4	99.8	0.401	82.4	211.8	0.100	46.5	198.7	0.099	46.3
Lower 25%	83.8	0.334	77.0	93.6	0.370	80.2	162.4	0.082	41.7	180.9	0.095	44.8
Upper 25%	119.7	0.495	86.3	106.7	0.426	83.7	269.4	0.126	53.1	214.5	0.105	48.0

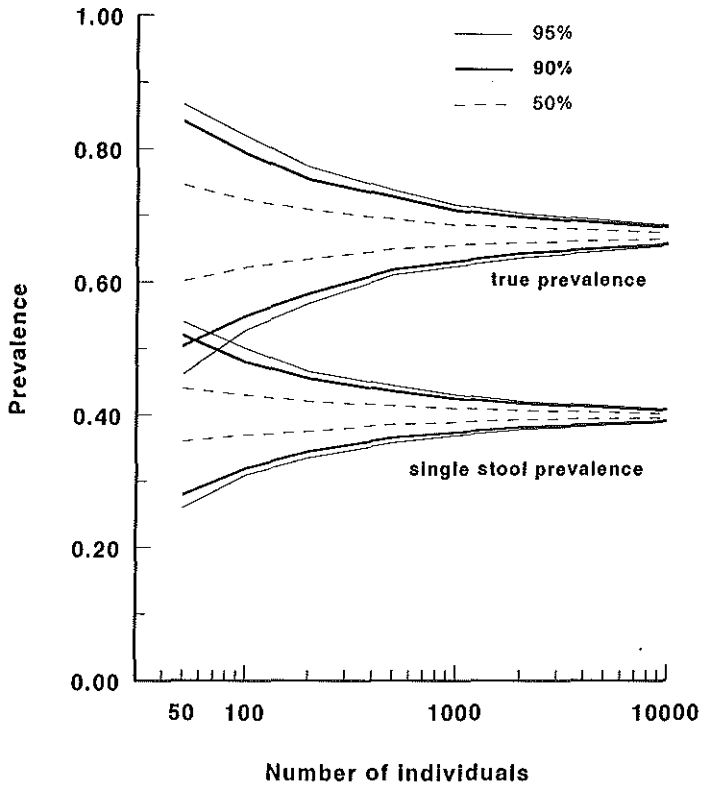

Figure 12.9 Confidence intervals of predicted true prevalence and single survey prevalence for a given number of individuals examined in an average endemic situation (prevalence 40% and geometric mean of positive counts of 100 EPG). The ranges are obtained by Monte Carlo simulation of 1000 different subsets using the model for variations in *S. mansoni* egg counts. For example, an interval of 90% means that 900 simulations resulted in a prediction within these ranges.

Previous experiences with fitting population egg counts suggest that models should be fitted for different age categories separately. Using one value of M (and k) for all ages leads to a poor fit (e.g. Table 3.2). Thus, we recommended applying the pocket chart for different age groups separately (Chapter 7). However, for prediction of true prevalences only, this restriction is not really needed. Table 12.4 shows the results of using the pocket chart for five situations, defined in terms of their position in the chart. For example, a hypothetical village consisting of equal numbers of individuals from 'low' and 'high' endemicity would show a prevalence of $0.5 \times (20+70) = 45\%$ and a geometric mean among positives of $60^{2/9} \times 200^{7/9} = 153.1$ EPG. Plotting both values in the chart would

Table 12.4 Use of the pocket chart, illustrated by 5 selected combinations of single stool prevalences and geometric means of positive egg counts in 50 mg Kato-Katz slides and the predicted true prevalences as can be read from Figure 12.8. The name of each endemic situation corresponds with its location in the chart. In addition, pocket chart predictions for fifty-fifty mixtures of two endemic situations are compared with the exact value from both single components.

Endemic situation	Geometric mean EPG of positives	Single stool prevalence	True prevalence as predicted from the pocket chart	Predicted true prevalence from single components
LOW	60	20	43.7	–
MIDDLE	100	40	67.2	–
HIGH	200	70	91.4	–
LEFT	80	60	93.4	–
RIGHT	200	30	45.9	–
LOW+MIDDLE	84.3	30	55.1	55.4
LOW+HIGH	153.1	45	68.3	67.6
MIDDLE+HIGH	155.5	55	79.8	79.3
LEFT+RIGHT	108.6	45	72.6	69.7

result in a true prevalence of 68.3%, almost equal to the (better) value of averaging the two constituents, viz. $0.5 \times (43.7+91.4) = 67.6\%$. Other combinations in Table 12.4 also indicate that the chart is adequate for heterogeneous data sets, e.g. comprising both children and adults or even different communities. Moreover, the higher overall number of individuals may further reduce confidence intervals. Thus, use of the pocket chart to obtain an approximation of the true prevalence of *S. mansoni* in a large geographical area, as Barakat *et al.* (1995) did for the northern Nile delta, seems to be justified.

In conclusion, empirical and theoretical evidence support the view that our pocket chart for predicting true *S. mansoni* prevalences from simple field observations is a reliable tool in epidemiological research. However, use of the chart is restricted to studies that are based on standard 50 mg faecal examinations (duplicate 25 mg Kato-Katz faecal smears). Provided the general model structure is valid, comparable pocket charts can in theory be constructed for any standard in *S. mansoni* coprological diagnosis, if the alternative values of h and r are known. The value of h can simply be adapted according to the amount of faeces examined using the norm of 1 EPG per worm pair (Chapter 5). The general expression for the relationship between aggregation and the number of repeated 25 mg Kato-Katz faecal examinations, as derived in the previous section, can be applied for alternative values of r. Figure 12.10 contains pocket charts to estimate true prevalences from surveys based on single 25 mg examinations ($h = 0.025$ and $r = 0.811$) and 41.5 mg Kato-Katz slides ($h = 0.0415$ and $r = 0.947$). The first standard

represents the 'quick Kato' as currently often used in subsaharan Africa (Polderman *et al.* 1985), the latter is roughly the traditional Kato-Katz smear as presented by Katz, Chaves & Pellegrino (1972) and the standard in most Egyptian and Brazilian research and control programmes. Because both alternatives are less sensitive in detecting infection, predicted true prevalence are higher than in Figure 12.8. For example, an average situation of prevalence 40% and geometric mean 100 EPG obtained from 50 mg faecal samples, implies about 67% really infected (Figure 12.8), whereas the same combination from 41.5 or 25 mg samples would result in true prevalences equal to 72% and even 90%, respectively (Figure 12.10). Although neither alternative chart has been explicitly validated, we believe that using them is preferable to adjusting predictions from the original chart (Figure 12.8).

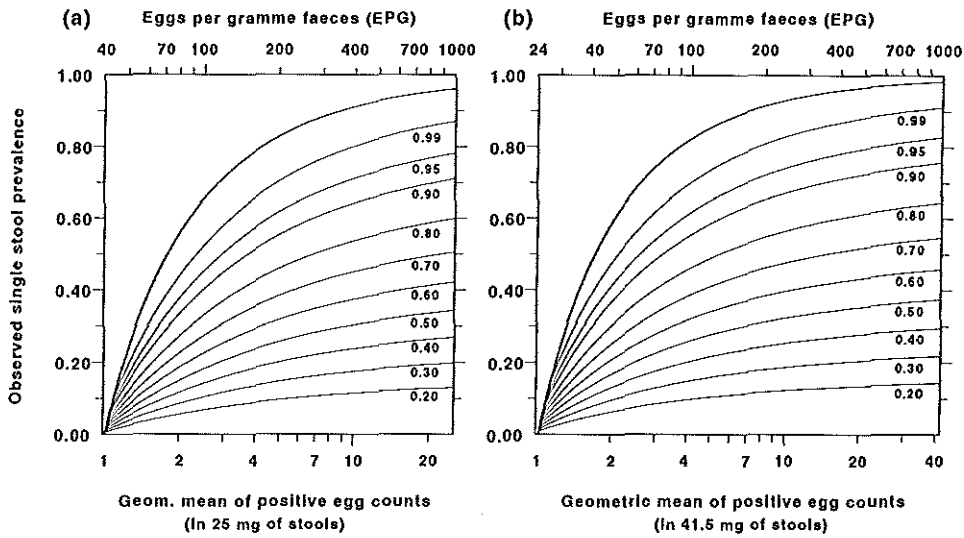

Figure 12.10 Alternative charts to predict true *Schistosoma mansoni* prevalences from observed single stool prevalence and geometric mean of positive egg counts in case measurements are based on examination of 25 mg (a) or 41.5 mg (b) Kato-Katz stool samples. For use of the charts see Figure 12.8.

12.5 Implications for control

Pocket charts to predict true *S. mansoni* prevalences are a useful tool in schistosomiasis research (Chapter 7). Knowledge about how many infected people have been missed by screening can help to understand the results of selective chemotherapy campaigns, and a statistical estimate of the real number of uninfected people certainly is a better gold standard for the evaluation of sensitivity and specificity of new diagnostic tests than the number of negatives in one or a few faecal examinations. However, the fact that pocket chart predictions only provide information at the population level is a serious restriction

for further practical use. Obviously, one cannot use the chart to identify the individuals that do harbour the infection but showed no eggs in a faecal examination.

12.5.1 Number of successive examinations for adequate screening

Use of the pocket chart for control oriented decisions is especially relevant to those situations where public health authorities are confronted with the choice between indiscriminate mass treatment and selective treatment based on screening. In current medical practice it is actually 'not-done' to treat a person without conclusive evidence of the presence of infection. The high cost of medication (praziquantel) further supports this viewpoint. However, our model predictions could suggest that e.g. instead of an observed 70% in reality 95% people in a population are infected, so that simply providing treatment to all individuals might be a strategy worth considering. The extra medical costs could easily be outweighed by the labour and material cost of a tedious series of surveys. Moreover, praziquantel is safe enough not to expose the few persons that unwarrantedly received treatment to irresponsible risks. Still, this application of our modelling work for control questions should be applied carefully and selectively.

Of much more importance for the management and planning of control is the following question: How many repeated examinations suffice for adequate screening? After presenting our model for egg count variation and the general conclusion that a single Kato-Katz examination is certainly not reliable enough to detect most *S. mansoni* infections, this question has often been posed to us. In Chapter 9, we have shown how many examinations would be necessary to arrive at reliable estimates of individual infection status. Charts like Figure 9.3 are useful for purposes such as the development of (quantitative) diagnostic techniques, but application for control is limited for the simple reason that the decision whether or not to treat a person only depends on the presence of infection (Chapter 9). Thus for control purposes, the sufficient number of faecal examinations must be considered merely in terms of detecting at least a certain percentage of all infected individuals. Earlier, the difficulty was that model-predicted answers could hardly be tested without a reliable gold standard of infection. However, since the successful validation of the pocket chart against empirical estimates of the 'true prevalence' (Chapter 8), we believe that our model can safely be applied for answering this question as well. Below, we indicate how an estimate of the optimal number of individual examinations (or screening rounds) can be derived, although firm recommendations will need more elaboration.

As explained in Chapter 1, current *S. mansoni* control programmes mostly aim at screening to detect as many infected persons as possible, followed by treatment of positive individuals to prevent development of (irreversible) morbidity, which is a function of worm load. Figure 12.11 illustrates such a campaign for three endemic

situations. By means of our egg count model, we have predicted the probability that individuals with each possible worm pair burden WP are detected after 1 to 7 successive stool examinations. Assuming a negative binomial with mean h.WP and aggregation r for his/her egg counts, the chance to be detected after w repeated examinations can be expressed by (see e.g. Chapter 2)

$$1 - \left(\frac{r}{h.WP + r} \right)^{w.r}$$

Again, we only consider 50 mg faecal samples and assume $h = 0.05$ and $r = 0.99$.

In the situation with mild endemicity (Figure 12.11a), it appears that even after 7 examinations a considerable number of infected persons remain undetected. But most likely they do not constitute a serious public health problem as their infections are very mild. On the other hand, in the situation with very intense endemicity (Figure 12.11c) most people harbour so many worms that sensitivity of faecal examination will almost be perfect. This means that even after a single screening almost all infected persons will be detected. However, one can wonder whether the screening was necessary in the first place, because all persons appear to be infected anyway (see also Figure 12.8). Evidently, the most interesting considerations exist for communities with moderate endemicity (Figure 12.11b).

First, we have to define a practically useful criterion of what we would like to accomplish with one or more screening rounds. To make different endemic situations comparable, it is best to focus on the result after screening, e.g., by the condition of 'no more than 5% of the population should still harbour undetected (and thus untreated) dangerous infection'. An alternative criterion of, say 80%, of all infections detected is not very practical. This would require unrealistically intensive screening efforts in low endemicity areas, whereas in situations with intense endemicity too many individuals with hazardous infections might remain untreated. We further take into account a specific cut-off point below which infections are not considered dangerous enough to imply treatment. Because in reality all detected infections are treated, we have for the sake of simplicity selected a cut-off point of 10 WP. Given the value of $h = 0.05$ eggs/WP/sample, this corresponds with on average 0.5 eggs per sample, just on the edge of detection.

Thus, our aim is to predict the required number of repeated examinations so as to leave only 5% of the population with more than 10 WP undetected. The limit of 5% usually is small compared to what will already be missed due to non-participation and poor compliance. In order to construct a general applicable chart for different endemic situations, we again use the fact that measurements of single survey prevalence and geometric mean egg count for positives are accurate statistics to determine M and k of the underlying egg count model (see previous section). For any combination of both

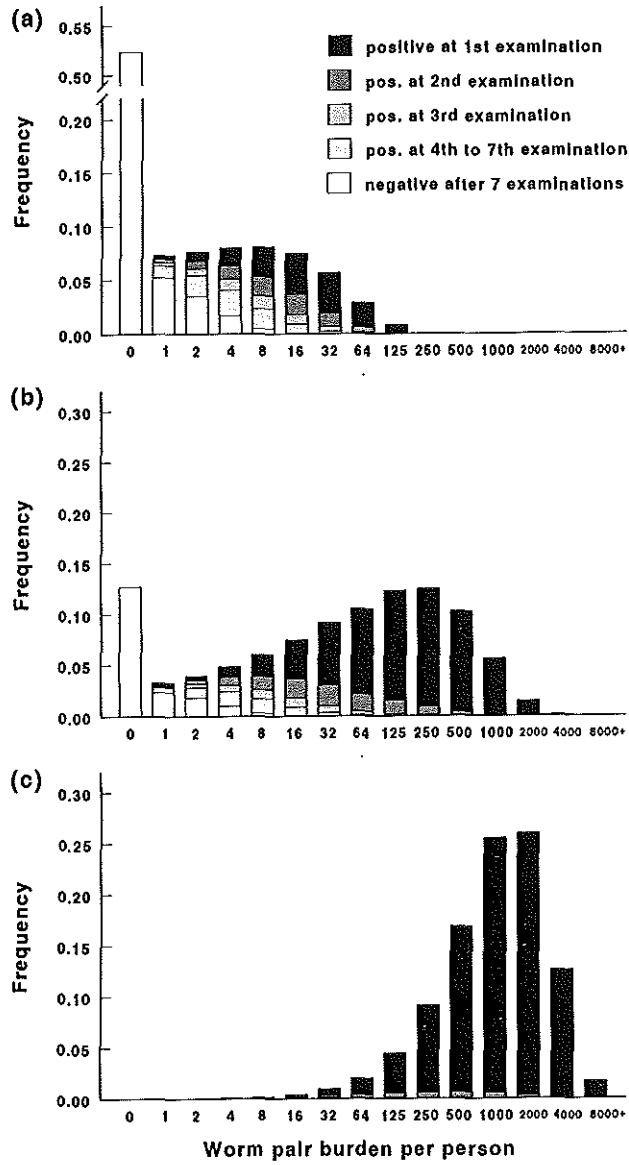

Figure 12.11 Predicted distribution of *Schistosoma mansoni* worm pairs among individuals from three different endemic localities: (a) a hypothetical situation with low endemicity (prevalence 16%, geometric mean 40 EPG); (b) Gihungwe, moderate endemicity (61%, 149 EPG); and (c) Makundju, high endemicity (96%, 791 EPG). On the x-axis, the lower limits of different worm pair burden intervals are indicated. By means of different grey-colours (see legend) the proportion of individuals in each group that is found positive after 1, 2, 3, 4-7 or even 8+ repeated examinations is shown (see text for mathematical expression). Figure 5.1 shows the parameter values for Gihungwe (b) and Makundju (c). In hypothetical situation (a), the estimated parameter values of the negative binomial distribution are $M = 21$ and $k = 0.19$.

epidemiological measurements we can calculate the corresponding values of M and k, and then predict the proportion of infected individuals detected after successive surveys and compare the result with the above criterion.

Figure 12.12 shows that the resulting chart contains typical more or less elliptical rings. In the upper-right corner, situations like Makundju (Figure 12.11c) are represented, where only one survey suffices to detect most of the (very intense) infections. In the lower-left corner, one (or even no) screening rounds suffices because there hardly are individuals with more than 10 WP. In the middle-left part of the chart, next to the bold line, the corresponding distribution of worm pairs shows very little aggregation ($k \rightarrow \infty$). Here, almost all people harbour more than 10 WP but their burdens are not intense enough to guarantee easy detection, so that up to five repeated examination would be necessary to meet our objective. However, given the distribution of the points for several observed prevalences and geometric means (Figure 12.12), such situation are rather unrealistic. Generally, it seems that only two repeated examinations suffice to leave not more than 5% of all persons in the population with 10 WP undetected. In some cases, three surveys would be better.

Use of the chart can, e.g., be based on a pilot study in part the population to obtain estimates of prevalences and geometric means. But it is also possible to decide after the first screening round whether a second one would be necessary. Of course, only previously negative individuals need to be invited for repeated screening. In practice, a second or even third examination is something one will not too lightly embark upon. The control team has to return to the population, which will significantly increase labour and transport cost. Moreover, willingness of the population to give up another working day for handing-over a stool specimen and waiting for the result of the faecal examination will definitely decline. An interesting practical alternative might be to improve the sensitivity of the diagnostic test by increasing the number of faecal samples prepared from one specimen (Engels *et al.* 1996).

12.5.2 Multiple sampling of one stool specimen as alternative

Starting from the same negative binomial in repeated examinations as above, the probability of an individual with a burden of WP worm pairs to be detected from w 50 mg samples from the same stool specimen would equal

$$1 - \left(\frac{r}{w.h.WP + r} \right)^r$$

For all $w > 1$, the probability of detection is lower than based on examination of w examinations from different stool specimens. However, the above expression assumes a

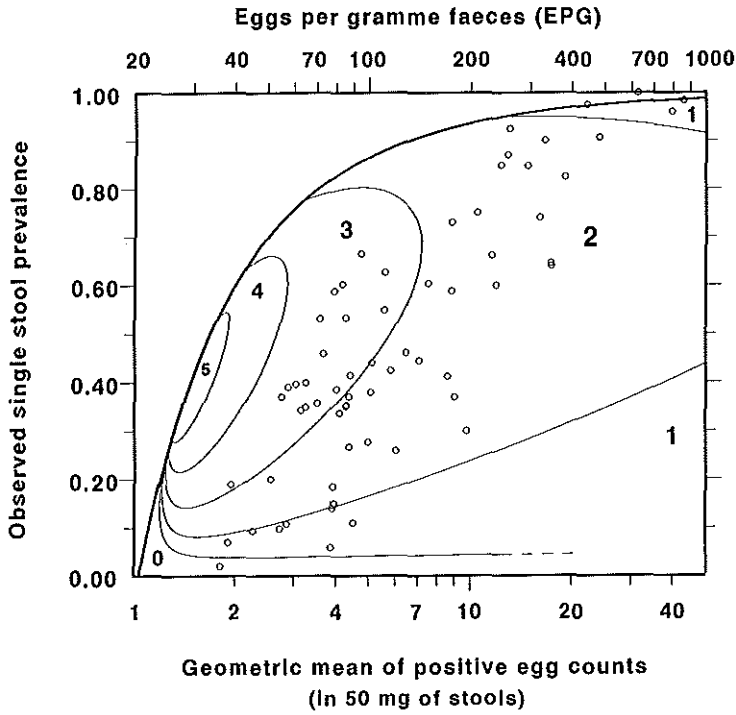

Figure 12.12 Chart to predict the necessary number of repeated measurements (each based on 50 mg Kato-Katz faecal smears from different stool specimens) to leave only 5% of the population with more than 10 *S. mansoni* worm pairs unidentified. Predictions can be obtained from plotting the observed single stool prevalence and geometric mean of positives as in the pocket chart for true prevalences (Figure 12.8). The points represent various endemic situations (see Figure 12.8).

random (homogeneous) distribution of eggs in the stool. Earlier we saw (section 12.3) that both between-specimen ($r_1 = 1.27$) and within-specimen aggregation ($r_2 = 4.48$, i.e. for 50 mg faecal samples) contribute to the overall aggregation ($r = 0.99$) in the negative binomially distributed individual egg counts, with $r^{-1} \approx r_1^{-1} + r_2^{-1}$ being a convenient expression. From the additive features of the negative binomial (Grafen & Woolhouse, 1993), it can now be calculated that using w 50 mg samples from the same stool specimen will lead to $r^{-1} \approx r_1^{-1} + (w.r_2)^{-1}$. The higher value of r (approximating r_1) as w increases means that aggregation decreases, so that the investigation of more faecal material implies some additional improvement of sensitivity.

Figure 12.13 shows that now up to 5 or 6 times 50 mg samples (thus 10 standard 25 mg Kato-Katz microscopic slides) from the same specimen appear to be sufficient to leave only 5% with more than 10 WP undetected. Obviously, the lines for no and one examination are equal to those in Figure 12.12. Again, use of the chart can be based on the estimated prevalence and geometric mean from part of the population. Another

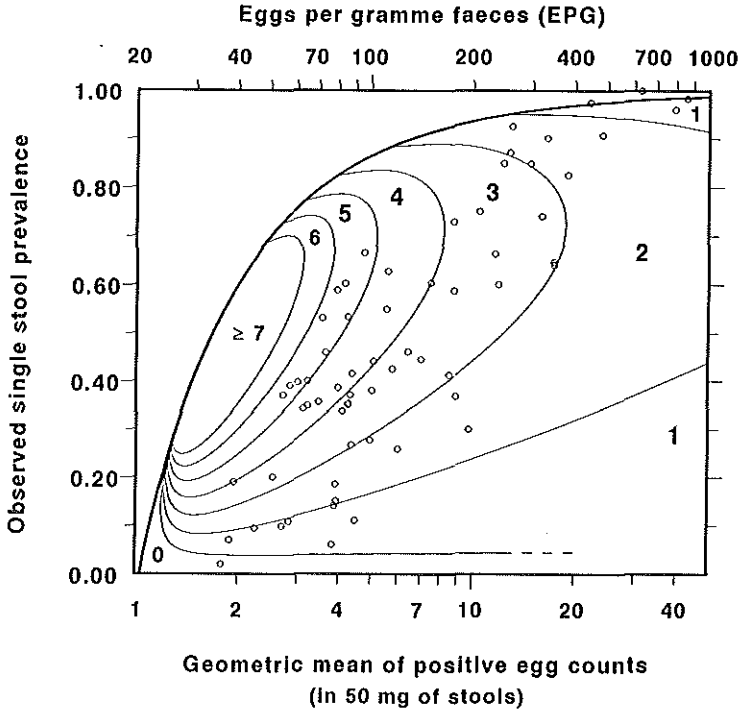

Figure 12.13 Chart to predict the necessary number of repeated measurements (each based on 50 mg Kato-Katz faecal smears from the same stool specimen) to leave only 5% of the population with more than 10 *S. mansoni* worm pairs unidentified. See Figure 12.12 for further explanation.

possibility is to save each stool specimen after preparation of 50 mg samples, and then decide whether or not more slides should be made. In this case adequate storage facilities are needed. One can also decide to prepare many more preparations from each specimen than initially needed, and determine after examination of 50 mg samples from each individual whether microscopic investigation of the other preparations would be needed. In general, the time to make an extra preparation (< 1 minute) is short compared to the time it takes to microscopically screen the slide for eggs (up to 5 minutes). Also, cost for possible extra equipment (slides, templates, etc.) are low in comparison with the extra labour cost.

12.5.3 An optimal screening approach?

Starting from the objective to leave in the population only a small proportion (say, 5%) with high *S. mansoni* burdens (say, >10 WP) undetected, and carefully taking into account all cost considerations (labour, transport, equipment, medicine), and assuming that the only adverse effect of providing too much praziquantel is its extra cost, it is

possible to devise an optimal chemotherapy campaign based on screening for a control programme in any endemic situation (or specific group in the population, e.g. school-children). The choice will be between (a) indiscriminate mass treatment, (b) selective treatment based on screening of a certain number of Kato-Katz slides from one stool specimen, (c) selective treatment of previously found negative individuals screened for a second or third time, or (d) any combination of (b) and (c). Using charts as Figures 12.8, 12.12 and 12.13 to this end is no more arbitrary than the grounds for selecting the approaches in many existing vertical programmes. The general recommendation from Figure 12.12 to do two or three repeated stool examinations can also be relevant for integrated control approaches. When suspecting *S. mansoni* infection for a person with (vague) abdominal complaints, he/she can be asked to come back to the health centre for a second or third test if a standard 50 mg faecal examination turns out to be negative. Alternatively, examination of 250 mg of stool (10 'quick Katos') from a single stool specimen will in most cases also be conclusive (Figure 12.13).

The '5% criterion' and tools for its easy implementation such as the charts in Figures 12.12 and 12.13 are meant to be illustrative for the potential of our egg count model to be a tool for decision making in *S. mansoni* control. When aiming at cost-effectiveness considerations more refinement is needed. For example, we lack knowledge about the individual relationship between worm pair burdens and the risk of morbidity development. The rather arbitrary cut-off point of 10 WP should be elaborated by assigning certain weights to each burden, so that missing a person with e.g. 1000 WP is considered worse than leaving someone with only 10 WP undetected. Furthermore, for use of Figure 12.12 one needs to take into account how many individuals remain negative after successive screenings and are the candidates for another round. In low endemicity areas their presence will be abundant, so that the extra costs of another screening round are much higher compared to more intensely endemic situations. Obviously, 1 minus the observed single stool prevalence can be used for the number of candidates in a second survey. Another important phenomenon is the time needed for microscopical screening of slides. The lower the number of eggs per slide the more time it takes to find the first egg to define a person infected. Again this will be most profitable in high endemicity areas. Assuming that screening of a slide without eggs takes T minutes, the average time to find the first egg in a sample with y regularly distributed eggs equals about $T/(y+1)$ minutes. In case of a heterogeneous distribution of eggs within a sample, this relationship becomes more complex.

Eventually, it is our aim to expand charts like Figures 12.8, 12.12 and 12.13 into a computer program that, using specific cost and timing assumptions as input, provides the optimal screening strategy for any endemic situation with given epidemiological characteristics (prevalence and geometric mean). It should be noted that 'optimal' does not necessarily refer to the long term objectives of screen-and-treat campaigns: preventing

development of chronic morbidity. Such predictions require a much more extensive modelling effort, such as is aimed for by the SCHISTOSIM model (see next section).

12.6 Comprehensive applied modelling for schistosomiasis control

In this thesis, it has been explained how an exercise to understand variations in *S. mansoni* egg counts has resulted in a practically applicable model which can help to decide between different screen-and-treat strategies. Still, this commonly applied approach to screen an endemic population for schistosome infection, followed by treatment of positive individuals, does not mean that it is always a good one. The recommendation from our model that most infections are detected after two or three screening rounds should only be considered relevant given the appropriateness of this prevention strategy. In section 1.2, we explained that the screen-and-treat approach emanated from the presumption that killing the worms prevents or at least significantly delays the development of morbidity. However, is this really the case? In our opinion, it is here where we can find the most challenging objectives for further applied modelling for schistosomiasis.

To our knowledge, there are no mathematical models in schistosomiasis research that explicitly take into account prevention or reduction of the development of morbidity, whereas this is/should be the main objective of control. Most existing models aim to describe the transmission cycle, and then predict outcome of control in terms of eradication of transmission ($R_0 < 1$). All field research on schistosomiasis has cogently proved that this is much too optimistic a starting point. Alternatively, model-predicted results of interventions are expressed as the reduction in (high) infection levels (Chan *et al.*, 1995, and e.g. our Chapter 11), but this again only indicates the 'risk of morbidity'. Pathology development from schistosome infection is the result of continuous accumulation of parasite eggs in the tissues and subsequent granulomatous inflammatory responses around dead eggs. Although the human body can remove such granulomata, at certain levels this is not possible any more and (supposedly irreversible) fibrotic lesions appear. The balance between accumulation and removal of parasite eggs as a function of experience of infection is a typical phenomenon to be studied by mathematical modelling. It can then be analyzed how the period after treatment, when supply of new eggs is reduced until the re-establishment of worms, allows the human body to reverse or interrupt progress of pathology.

Microsimulation, as in SCHISTOSIM (Chapter 10), is a very convenient modelling technique to describe the complex dynamical processes of morbidity development. Differences in the degree of infection and the resulting accumulation of eggs, both between individuals and within individuals over time, can realistically be mimicked taking into account specific immunity and water contact patterns (Chapter 11). Our experience in

relating worm burdens to egg counts can further help to compare simulated trends in infection levels against data. The association between morbidity development and experience of infection is more difficult to test, especially for *S. mansoni*, given the problem of recognizing consequences of schistosome infection among the many other infections in endemic areas (section 1.2). However, from the current use of ultrasonography in large schistosomiasis trials, a new type of information is rapidly becoming available (Wiest, 1996). Individual trends in grades of schistosome related pathology before chemotherapy and during the gradual build-up of infection thereafter can be compared with model predictions. *S. haematobium*-related morbidity is easier to identify. However, one of the most serious consequences, hydronephrosis, can be the result of a single granuloma in the ureter-wall, and is therefore difficult to predict by modelling. Experiments of animal models may further help to identify the more general structure of accumulation and regression of schistosome eggs in the host, which can then be translated to the human situation. In this respect, recent population based biological studies on Danish pigs as hosts for *S. japonicum* seem very promising (Willingham & Hurst, 1996).

If a valid model for morbidity development has been derived, the long term effects of temporary absence of worms due to chemotherapy could be predicted for different endemic situations. In that case it might turn out that, for example, provision of chemotherapy a limited number of times in an individual's life is effective enough to prevent most morbidity. Such an approach would then be a good alternative to a possible vaccine, especially when attendance is high due to the selection of an efficient moment for provision of praziquantel, such as the first and last year at primary school, as suggested by Gryseels (1988). Knowing that so much money is currently spent on vaccine development, modelling efforts to find out what is possible with the already existing tools might be rewarding. Another promising consideration to be tested is the long term effect of integrated approaches. Supporting basic health care facilities, so that at least persons who report themselves with mild schistosomiasis always receive proper treatment, might also prevent most of the chronic morbidity cases. This would even make the costly vertical screening programmes redundant.

In order to model morbidity development on a population level, other phenomena should be taken into account as well. Individual experience of infection and thus the chance to develop pathology hinges on the history of water contact, something which is still inadequately understood. Current exposure is a poor determinant of current infection, since establishment of infection is a matter of several years during which individual exposure might have changed considerably. A better way to study this topic is to relate current exposure to the speed of reinfection after treatment. Of special interest is trying to find out to what extent reinfection is due to the number of water contacts or to the total duration, possibly in combination with the area of body exposed (swimming or fording). The derived 'exposure index' could be an important parameter to explain much of the

variation in morbidity between members of a population. Immunity, acquired over experience of infection, will obviously affect this variation. However, differences in immunity levels are especially important in their protective effect against new infections after treatment. It is very likely that individuals with a complete immune response (i.c. adults) receive relatively more protection from chemotherapy, because reinfection is delayed. Thus, high worm burdens may on the one hand lead to more morbidity, but also to more acquired immunity and more protective effect from treatment. On the population level, reinfection might be delayed by other interventions at the same time, or by the fact that the chemotherapy intervention can have an effect on transmission itself. In Chapter 11, we have shown that the rapid return of infection after one intervention compared with the slow reinfection after a second or third intervention, could not readily be explained without taking into account a reduction in the force of infection. In any case, a proper schistosomiasis model should include a specific (genetic ?) factor which accounts for the considerable differences in the infection-morbidity relationship between different geographical localities (Gryseels & Polderman, 1991). Ideally, the existing level of morbidity in comparison with infection can be used to estimate this factor, just as the prevalence and geometric means suffice to explain the distribution of infection in a population (this thesis).

In conclusion, applied modelling in schistosomiasis urgently needs to emphasize more the aspects of morbidity and its reduction after chemotherapy. Much research effort and resources are currently spent on the development of new tools, particularly vaccines. Obviously, it would be worthwhile to invest at least as much in the proper evaluation and planning of already available control options. We believe that SCHISTOSIM will be a valuable tool to that end.

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SUMMARY

Identification and counting of eggs in faecal samples, by the Kato-Katz thick-smear technique, is the standard procedure to detect and quantify human infection with *Schistosoma mansoni*, a common fluke parasite in many tropical countries. Subsequent treatment of detected infections is advocated to prevent development of schistosomiasis related morbidity. Quantitative faecal egg counts are also central to epidemiological research on schistosomiasis. Several studies have demonstrated that, due to the low density of eggs in faeces and its variation in repeated stool samples, a large proportion of infections remain undetected. The probability of detecting parasite eggs in the stools depends on the intensity of an individual's infection, so that sensitivity of the test cannot be considered a fixed characteristic.

In this thesis, a stochastic model is presented which distinguishes (1) variation in egg counts between individuals due to differences in the number of schistosome worms harboured and (2) variation in egg counts within individuals due to the variability of egg excretion and its measurement. Data from several endemic communities in Burundi, Zaire and St. Lucia are satisfactorily described by this model. The structure of the model allows to make inferences about the underlying distribution of worm burdens in the human host population. This has lead to the insight that schistosome worms in the human host easily number hundreds to several thousands, instead of the few to dozens indicated by autopsy studies.

The model further cogently demonstrates that many more individuals with (mild) infections are missed at screening than generally assumed. Existing autopsy studies have shown that such individuals can still show severe pathology, and missed cases may be at the origin of the persistence of transmission after population based chemotherapy of all positive cases.

From the model, a practically applicable 'pocket chart' has been developed to predict the true prevalence for any given combination of observed single stool prevalence and mean egg count. The chart has successfully been validated, using independent data sets, by comparing predicted true prevalences with prevalences obtained with more sensitive methods (repeated measurements and filtration techniques). Such more accurate estimates can help to decide between indiscriminate mass treatment or selective treatment based on screening.

Further application of the egg count model shows that examination of 2 or 3 stool specimens collected on different days usually suffices to detect most moderate to heavy infections, but only part of the light ones. Multiple sampling of a single specimen could be an alternative, but arriving at the same sensitivity would require examination of up to 5 or 6 samples. Another chart has been developed to assess the number of different stool specimens required for obtaining a reliable quantitative estimate of an individual's intensity of infection, as necessary e.g. in immuno-epidemiological research. For such quantitative purposes, however, multiple sampling of one specimen does not provide a good alternative.

The egg count model with its quantifications has subsequently been implemented as a module into SCHISTOSIM, a comprehensive microsimulation model which aims at the evaluation and prediction of the outcome of alternative strategies for the control of schistosomiasis. The microsimulation technique stands for mimicking the life histories of hypothetical individuals, thereby allowing great detail and transparency in model assumptions and output. The version of SCHISTOSIM described in this thesis also includes an existing immunity submodel and certain plausible assumptions on human factors (demography and attendance behaviour), pharmacological factors (efficacy of the drug) and operational factors (timing and coverage of interventions).

This version of SCHISTOSIM adequately explains short-term results of selective treatment after screening for a particular setting in Burundi. It appears, among others, that one third of the individuals with positive egg counts at the follow-up three months after the intervention, were in fact missed at the screening and therefore remained untreated. Other positive cases at the follow-up were the result of reinfection, incomplete cure or non-attendance.

To become a fully applicable public health tool, SCHISTOSIM needs to be further refined and extended to include also transmission, morbidity and economic factors.

GEARFETTING

Yn in soad tropyske lannen is de minske unfektearre mei *Schistosoma mansoni*, in algemien foarkommend plat wjirmke. Om út te finen oft, en yn hokker mjitte dit oan'e oarder is, wurdt de Kato-Katz technyk tapast: Yn in nochal tsjok meunster fan húskeguod siket en telt men de aaien fan dat wjirmke. Men is fan miening dat mei de behanneling fan ûntdutsen ynfeksjes it ûntwikkeljen fan de sykte schistosomiasis foar te kommen is. It ûndersyk nei fersprieding fan schistosomiasis is ek basearre op sok tellen. Ut ferskate stúdzjes docht bliken dat nochal wat ynfeksjes net ûntdutsen wurde. Dit komt fan de lege tichtens fan op dizze manier telde aaikes en de fariaasje dêryn as men it ûndersyk wer docht op oare dagen by deselde persoan. De kâns aaien fan de parasyt yn'e poepmeunsters te finen, hinget ôf fan de earnst fan de ynfeksje by in minske, sadat de gefoelichheit fan de test net beskôge wurde kin as in fêst sifer.

Yn dizze dissertaasje wurdt in stochastyk model presentearre dat op twa manieren fariaasje ûnderskiedt. Oan de iene kant fariaasje yn aaitellingen oangeande ferskillende persoanen as gefolch fan it oantal wjirms dat yn harren húzet, oan de oare kant fariaasje yn aaitellingen by ien persoan op ferskillende dagen. De beskriuwing dy't dit model jout, strykt op befredigjende wize mei observaasjesifers út ferskate doarpkes yn Burundi, Zaïre en St. Lucia, dêr't de sykte foarkomt. De struktuer fan it model makket it mooglik konkluzjes te lûken oer de ferdieling dy't past by de wjirmoantallen fan de gasthearpopulaasje. Sa komt men ta it ynsjoch dat it by dizze wjirms earder giet om hûnderten of tûzenen dy't yn de gasthear húzje, as om in stikmannich dy't fûn wurde by autopsy.

It model lit fierder oertsjûgjend sjen dat der folle mear (licht) ynfektearre persoanen by ûndersyk oer de kop sjoen wurde as oer it algemien oannommen is. Ut autopsy-stúdzjes docht bliken dat der yn soksoarte gefallen dochs sprake wêze kin fan slimme syktеferskynsels. Miste ynfektearden kinne ek noch de earste skeakel wêze fan fierdergeande fersprieding fan de ynfeksje yn de bevolking at allinne de ûntdutsenen genêskundige behanneling krije.

Fia it model is der in praktysk brûkber, lyts figuerke beskikber kommen, dêr't men, by eltse kombinaasje fan obserfearre foarkommen en aaitellingsgemiddelde, mei útfine kin wat it wiere foarkommen is. It figuerke is mei sukses falidearre oan de hân fan ûnôfhinkelike data-samlingen, troch it foarseine, wiere foarkommen te fergelykjen mei foarkommens-útkomsten dy't beskikber kamen mei gefoeliger metoaden (werhelle

mjittings en filtraasje techniken). Sokke krektere skattingen kinne helpe om te kiezen tusken behanneling fan eltsenien sûnder ûnderskied en selektive behanneling op basis fan ûndersyk.

Fierdere tapassing fan it aaitellings-model lit sjen dat ûndersyk fan twa of trije - op ferskillende dagen opheinde - trochgongen fakentiids genôch is om de measte al of net swiere ynfeksjes by befolkingsgroepen oan it ljocht te bringen. Men kin ek hieltyd wer in meunster fan deselde trochgong ûndersykje. Om in gelikense gefoelichheid te krijen moatte der dan wol 5 of 6 meunsters besjoen wurde. Om fêst te stellen hoefolle ferskillende trochgongen ûndersocht wurde moatte foar in betroubere kwantitative skatting fan de earnst fan de ynfeksje by in persoan, sa't dat bygelyks foar in soad laboratorium- en fjildûndersiken nedich is, is in oar figuerke beskikber kommen. Foar sokke kwantitative doelen is it lykwols net in goed alternatyf om mear meunsters fan ien en deselde trochgong te ûndersykjen.

It aaitellings-model mei syn waarden is dêrnei modulair boud yn SCHISTOSIM, in wiidfiemjend simulaasje-model doeljend op evaluearjen en foarsizzen fan it resultaat fan bestridings-alternativen foar it yn'e hân hâlden fan schistosomiasis. SCHISTOSIM is basearre op de mikrosimulaasjetechnyk, wat ynhâldt dat libbensskiednissen fan hypotetyske minsken sa neispile binne dat der by de oannames en de útkomsten fan it model romte jûn wurdt foar in protte details en foar trochsichtichheid. By de yn dizze dissertaasje beskreaune ferzy fan SCHISTOSIM binne in submodel foar ymmuniteit en oannimlike útgongspunten oangeande minsklike (demografy, belutsenheid), farmacologyske (medikaasje-effect) en operasjonele (berik en tiid fan de bestridingskampagne) faktoaren ynbegrepen.

Dizze ferzy fan SCHISTOSIM ferklearret adekwaat koarte termyn resultaten fan selektive behanneling nei ûndersyk foar in spesjale tapassing yn Burundi. It docht trije moannen nei dy bestridingskampagne bliken dat it tredde part fan de minsken dy't dan in positive aaitellingsútslach hawwe, earder oer de kop sjoen binne en dus ek gjin behanneling krigen hawwe. Yn de oare gefallen dêr't aaien fûn waarden by kontrole, giet it om werynfeksje, in net folsleine behanneling of men hie earder net meidien. SCHISTOSIM kin pas in goed tapasber stik ark wêze foar folkssounens, as it fierder útplúze en útwreide wurdt om ek noch faktoaren te omfiemjen, sa as it oerbringen, de syktetastân en de ekonomy.

SAMENVATTING

Het identificeren en tellen van eieren in faeces monsters, met de Kato-Katz 'dikke smeer' techniek, is de standaard manier om infectie met *Schistosoma mansoni* te detecteren en te kwantificeren. *S. mansoni* is een algemeen voorkomende platworm in veel tropische landen. Naar wordt aangenomen, voorkomt behandeling van gedetecteerde infecties de ontwikkeling van ziekteverschijnselen (schistosomiasis) bij die mensen. De analyse van eitellingen in faeces vormt ook de basis voor epidemiologisch onderzoek naar schistosomiasis. Verscheidene studies hebben aangetoond dat een groot deel van de infecties ongedetecteerd blijft, hetgeen te wijten is aan de geringe aantallen eieren in de faeces en de variatie in uitkomsten van herhaalde metingen aan een persoon. De kans op het aantonen van parasieteieren in de ontlasting van een persoon hangt af van de intensiteit van zijn infectie, zodat de gevoeligheid van de test niet door één getal kan worden weergegeven.

In dit proefschrift wordt een stochastisch model gepresenteerd dat een onderscheid maakt in (1) variatie in eitellingen tussen personen veroorzaakt door verschillen in het aantal wormen dat men herbergt, en (2) variatie in eitellingen binnen een persoon veroorzaakt door de variabiliteit in de uitscheiding van eieren en de gebruikte meetmethode. Gegevens uit verschillende lokaties in Burundi, Zaïre en St. Lucia waar schistosomiasis voorkomt, kunnen goed worden beschreven met dit model. Met het model kunnen gevolgtrekkingen worden gemaakt over de onderliggende verdeling van wormlasten in de menselijke gastheerpopulatie. Dit heeft geleid tot het inzicht dat het aantal wormen in de mens gemakkelijk honderden tot vele duizenden kan bedragen, in tegenstelling tot de enkele tientallen die blijken uit autopsie onderzoek.

Het model laat verder overtuigend zien dat veel meer personen met (lichte) infecties bij screening worden gemist dan algemeen wordt aangenomen. Autopsie studies hebben eerder al laten zien dat bij deze mensen toch ernstige ziekteverschijnselen kunnen voorkomen, en de gemiste geïnfecteerden zouden de bron kunnen zijn van de voortzetting van de verspreiding van schistosomiasis na behandeling van alle positieven in een populatie.

Uitgaande van dit model is een simpele grafiek ontwikkeld die door onderzoekers in het veld gebruikt kan worden om een voorspelling te maken van het werkelijke aantal geïnfecteerde personen op basis van de combinatie van het gemeten aantal geïnfecteerde

personen na één faecesonderzoek en het gemiddelde aantal getelde eieren. De figuur is met succes getest op onafhankelijke gegevens, door de voorspelde ware aantallen geïnfecteerden te vergelijken met de aantallen die werden vastgesteld na gebruik van gevoeliger methoden (herhaalde metingen en filtratietechnieken). De via de grafiek verkregen preciezere schattingen kunnen helpen bij de afweging tussen massabehandeling en selectieve behandeling op basis van screening.

Verdere toepassing van het model laat zien dat onderzoek van 2 tot 3 stoelgangen, verzameld op verschillende dagen, gewoonlijk volstaat om de meeste gemiddelde tot zware infecties aan te tonen. Herhaalde bemonstering van één stoelgang kan een alternatief zijn, maar dan zijn 5 tot 6 monsters nodig voor hetzelfde resultaat. Er is tevens een grafiek ontwikkeld om vast te kunnen stellen hoeveel verschillende stoelgangen getest zouden moeten worden voor een betrouwbare schatting van de intensiteit van infectie in een individu, zoals bijvoorbeeld benodigd voor immuno-epidemiologisch onderzoek. Echter, voor zulke kwantitatieve doeleinden is herhaalde bemonstering van één stoelgang nooit betrouwbaar genoeg.

Het eitellingen model en de gevonden kwantificaties zijn vervolgens als een module ingebouwd in SCHISTOSIM. Dit is een veelomvattend microsimulatiemodel dat ontwikkeld is voor het evalueren en voorspellen van de uitkomsten van verschillende strategieën ter bestrijding van schistosomiasis. De microsimulatietechniek houdt in dat levensgeschiedenissen van hypothetische personen kunnen worden nagebootst, zodat een grote mate van detail en een grote doorzichtigheid mogelijk zijn met betrekking tot de modelaannames en uitkomsten. De versie van SCHISTOSIM beschreven in dit proefschrift bevat tevens een eerder ontwikkeld submodel voor immuniteit en bepaalde redelijke aannames over menselijke factoren (demografie en participatie gedrag), farmacologische factoren (effect van het geneesmiddel) en operationele factoren (duur en dekking van interventies).

Deze versie van SCHISTOSIM kan korte termijn effecten van selectieve behandeling na screening voor een bepaalde situatie in Burundi goed beschrijven. Het blijkt ondermeer dat één derde van de positieve eitellingen tijdens de follow-up drie maanden na de interventie, in feite afkomstig is van gemiste en onbehandeld gebleven personen tijdens de screening. De andere positieve gevallen tijdens de follow-up waren het gevolg van herinfectie, incomplete behandeling en het eerder niet deelnemen aan de screening.

Om uiteindelijk tot een gereedschap te komen dat voor volksgezondheidsvragen volledig toepasbaar is, moet SCHISTOSIM verder worden verfijnd en uitgebreid met aspecten die de overdracht van infectie, de morbiditeit en de kosten betreffen.

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Sake de Vlas

CURRICULUM VITAE

Sake de Vlas werd op 26 juli 1965 in Harlingen geboren. Hij groeide op in Achlum en behaalde in 1983 aan de Rijksscholengemeenschap *Simon Vestdijk* te Harlingen het Atheneum-B diploma. In datzelfde jaar begon hij aan de Rijksuniversiteit Groningen met de studie biologie, waarbij de theoretische en oecologische vakken zijn grootste interesse hadden. Zijn eerste doctoraalonderwerp betrof een studie op Schiermonnikoog naar het foerageergedrag van de scholekster in relatie tot het getijde. In 1988 behaalde hij de propaedeuse wiskunde, waarna hij een doctoraal onderzoek deed bij het Instituut voor Theoretische Biologie (ITB) van de Rijksuniversiteit te Leiden. Hier heeft hij gewerkt aan een model dat de beslissingsregels van de sluipwesp beschrijft om een gebied met gastheer-larven optimaal te exploiteren. In november 1989 studeerde hij in Groningen *cum laude* af als Theoretisch Bioloog. Aansluitend begon hij als Assistent in Opleiding (AIO) aan het Instituut voor Maatschappelijke Gezondheidszorg (*i*MGZ), Erasmus Universiteit Rotterdam. Zijn onderzoek betrof het modelleren van de bestrijding van schistosomiasis, met nadruk op de rol van de diagnostiek. Deze studie werd vanaf 1994 voortgezet binnen het kader van de onderzoeksschool *The Netherlands Institute for Health Sciences* (NIHES). Een deel van de werkzaamheden vond plaats bij het Instituut voor Parasitologie (Rijksuniversiteit Leiden). In de zomer van 1993 werkte hij gedurende drie maanden als *Young Scientist* aan het ontwikkelen van optimaliseringsmethoden voor microsimulatiemodellen op het *International Institute for Applied Systems Analysis* (IIASA) te Laxenburg, Oostenrijk. Hij bezocht verder enkele malen veldstudies voor schistosomiasis in Kenya. Naast het schistosomiasisonderzoek is hij op het *i*MGZ betrokken bij andere projecten waar modellen voor infectieziekten centraal staan (slaapziekte, rivierblindheid en seksueel overdraagbare aandoeningen).

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