Transmission dynamics

Several characteristics of human infections by intestinal amoebae and flagellates create problems for the epidemiologist who wishes to measure the parameters that determine the dynamics of these infections. (1) Incidence rates are generally low, a notable exception being the waterborne outbreaks of Giardia, so that direct measurement of incidence by longitudinal parasitological data is not normally feasible. (2) A high proportion of new infections is asymptomatic or oligosymptomatic so that surveillance based upon clinical reporting will greatly underestimate incidence. (3) Infections by the pathogenic species induce conversion in only a limited proportion of infections and in these titres fall relatively rapidly; thus seropositivity is an inefficient indicator for estimating incidence of infection. (4) The sensitivity of current parasitological methods is relatively low (this problem will be discussed separately), creating further problems in the direct estimation of the rates of gain and loss of infection.

Analysis of age prevalence curves, however, does enable inferences to be made about infection dynamics. The long duration of many of these infections generates relatively high levels of prevalence despite low incidence. In this context data concerning nonpathogenic species are also valid because of the similarity of their transmission dynamics; observations upon related organisms in their natural animal hosts are also relevant. There are two important determinants of the validity of community based age prevalence data. (1) The width of the age classes used in the data analysis; in general age classes should be narrower in pre-adults since their prevalence rates differ more between adjacent age classes. To achieve precision each age class should have a sufficient sample size; age stratified samples may be more efficient than random samples of whole populations, although the latter must be documented demographically. (2) The diagnostic sensitivity should be known so that observed prevalence can be adjusted.

Among the wealth of published data on community based prevalence some of the most useful for analysis (Knight, 1975) derive from the Southern States of USA between 1930 and 1955. In general the findings of these studies are similar to those made in most developing countries, both currently and in the past. The various data sets are qualitatively very similar, but show important quantitative differences both secular and...
between populations. With the notable exception of *Giardia*, the curve pattern is one of a steady rise from early childhood that continues with diminishing slope into a more or less steady plateau in adults. The order of prevalence in adults of the different amoebic species is highly consistent: *Entamoeba coli* being the highest followed by *Endolimax nana*, *E. histolytica*, *E. hartmanni* (often referred to in early studies as 'small race' *E. histolytica*) and lastly *Iodamoeba buetschlii*; in pre-adults, the respective curves for the different species do not cross. The striking similarity in form of these curves to the reversible catalytic models of Muench (1959), suggests that a plausible hypothesis for their generation is one of a constant 'force of infection' $\lambda$, that is age independent, balanced by a constant rate of loss of infection $\gamma$. These rate parameters can be estimated from the data by graphical curve fitting procedures or by maximum likelihood or other statistical procedures. In general the fit of data to curves determined by the two estimated parameters is good and supports the use of this model as a working hypothesis.

Three other consequences of the model are that the age of first infection is given by $1/\lambda$; loss of infection is random with a mean duration of infection of $1/\gamma$; and the limiting stable prevalence in adults is $\lambda/(\lambda+\gamma)$. Survey data for *E. histolytica* commonly show an 'adult' prevalences of 10–45% with the plateau being reached between the ages of 15 and 20 years; estimates of $\gamma$ predict a mean duration of infection of 2 years which is consistent with clinical experience and also the data from 23 experimentally infected persons (Beaver et al., 1955), estimates of $\lambda$ predict mean ages of first infection between 4 and 12 years (predicted age for first infection will not show a simple relationship to observed prevalence at that age because the size and prevalence of each age class determines its contribution to $\lambda$). The fit of the model for *E. histolytica* in rural, semirural and poor urban areas in developing countries suggests that in these contexts the force of infection $\lambda$ is principally age independent and therefore that the main routes of transmission are age independent also: namely waterborne (communal or domestic); food contaminated by food handlers or flies; vegetables grown with 'night soil', contaminated by indiscriminate defaecation or sewage contaminated irrigation water; or 'freshened' in the market place or washed at home with infected water. Clearly all infected persons can contribute to $\lambda$ and they must inevitably do so unequally; nevertheless since most infectives are older persons, infections in children are likely to be derived 'horizontally' from adults rather than 'vertically' between children of similar ages. Exceptions to this age prevalence pattern are not uncommon in certain countries and suggest that other mechanisms may be operating. Thus in parts of Nigeria (Nnochiri, 1965) and South Africa (Scragg, 1975) prevalence and morbidity is unusually high in older infants and young children. That this is due to the practice of hand feeding is strongly suggested by the finding that the mothers of many infected infants were infected carriers (Nnochiri, 1965). The alternative explanation that this is due to more-or-less direct faecal-oral transmission between young children is perhaps less likely because, although many other enteric pathogens are transmitted in this way, high *E. histolytica* infection rates in children are not a widespread phenomenon in natural communities even when total prevalence is high.

Unlike $\lambda$, the value of the rate of loss of infection $\gamma$ may vary much less in different contexts and indeed for a non-pathogen, like *E. coli*, it may be constant for a host-parasite species pair. The observation that the curves for *E. histolytica* and *E. coli* are so similar, at least in contexts where the former is not commonly invasive, suggests that $\gamma$ is relatively constant for *E. histolytica* also. That immunological mechanisms can lead to expulsion from the gut is however suggested by the common lack of commensal gut infections in liver abscess patients and also perhaps by the relative infrequency of parasitological relapse in patients with invasive disease treated with drugs with low efficacy against luminal parasites. Community studies of infection rates among untreated seropositive persons could provide good evidence of immunological rejection but the effect, if it occurs, may be a small one; moreover the prevalence of significant seropositivity is low in many communities even when prevalence is high.
The age prevalence curves for Giardia in the studies referred to, and indeed the great majority of such data from poor or developing countries are very different to that of the amoebic species. Infection appears in infancy and rises to a modal peak between the ages of 2 and 8 years followed by a decline to a steady 'adult' level well below the childhood peak, the ascending limb of this mode is much steeper than that seen in amoebic species. In general the higher the level of total population prevalence, the earlier in childhood the modal peak occurs. Two non-exclusive hypotheses can be proposed. Firstly, in a context of an age independent force of infection, loss of infection y may differ in different age groups as a result of immunological or non-immunological mechanisms. In normal adults, not previously exposed to Giardia, infection rarely lasts more than 3 months, giving, for a mean duration of 2 months, a y value per year of 6 compared with 0.25 for E. histolytica. Thus using the catalytic model for an adult limiting prevalence \((\lambda/(\lambda+\gamma))\) of 10%, the value of \(\lambda\) would be 0.66 and the mean age of first infection, 1.5 years; this would also be the mean interval between new infections and adults. Impaired immune competence at any age can prolong Giardia infections and the high prevalence in children could be due to their low mean y value caused by an immune responsiveness depressed by malnutrition or intercurrent infection; or alternatively by conditions within the small bowel including its bacterial and mycotic flora, an altered motility of achlorhydria - these being themselves determined by recurrent bacterial gut infections in the context of poor nutrition. Another factor affecting y would be specific immune responses, thus primary infections in children might be longer than secondary infections in adults. While it is almost certainly true that both G. muris infections in mice and G. intestinalis infection in man are normally terminated by immunological mechanisms, it is uncertain, for example, whether adults reinfected after an 18-month interval have shorter infections than those with primary infections.

The second hypothesis is that while y does not vary with age, the force of infection \(\lambda\) does. Current information (Knight, 1980) suggests that for Giardia as compared with E. histolytica; the daily cyst output is higher, the infective dose of cysts is lower and cyst viability in the environment is greater; the latter including resistance to chlorine treated water and survival in pit latrines and sewage, and beneath fingernails. Thus, in terms of transmissibility, Giardia is a more efficient parasite; when opportunities exist for more or less direct transmission between children with high \(\lambda\) values, Giardia will be much more readily transmitted than E. histolytica. Clearly, these hypotheses can be combined so that longer infections in children due to lower y, produce high prevalence in an age group whose behaviour in the presence of efficient parasite transmission, facilitates 'horizontal' infection.

It is relevant to consider these parasites in the context of newer concepts of infectious disease dynamics (Anderson & May, 1982). Thus the force of infection \(\lambda\) can be considered to be the product of the rate of effective contact between individuals (the transmission coefficient) \(B\) and the number of infectives. For the most directly transmitted infections \(B\) is itself a function of the population density \(N\) so that it is greater in dense urban populations; the functional relation between mean \(B\) and \(N\) is a non-linear one with a general form \(BN^c\) where \(c\) takes values between 1 and 0; the former indicating a linear relation between \(B\) and population density while 0 indicates no relationship so that \(B\) depends only upon prevalence of infection. For most human gut protozoa, population density does not appear to be a major determinant of transmission and high prevalence can occur in dispersed rural communities. As acquired immunity is often of little or no significance, parasites can persist, perhaps indefinitely, in small isolated host populations such as hunter-gatherer groups. The basic reproductive rate \(R_0\), the mean total number of new infections generated by one infective during their period of infectivity, in an uninfected non-immune population cannot be estimated directly but provided the major modes of transmission are indirect, it will be density independent (this will be less true for Giardia) and will approximate to \(B/y\) (\(B\) can be estimated from the catalytic model at equilibrium population prevalence). Values of \(R_0\) will be greater in growing populations but even in developing countries are unlikely to exceed two.
In general the incidence of these infections will be directly proportional to the \( P \) values, which are considerably greater in *Giardia* than in *E. histolytica*. As \( P \) declines with improvements in socio-economic conditions, personal and environmental hygiene, so will incidence rates fall. For *E. histolytica*, prevalence will fall progressively and the age distribution will not change, eventually virtual extinction is likely, as currently in UK, apart from population subgroups where \( P \) remains high, as in a few institutions. For giardiasis, the modal peak will be progressively delayed and may disappear perhaps because the determinants of long infections in children are less evident under improved nutritional and environmental conditions. Because of higher \( P \) values, extinction will occur later than for amoebiasis; the infection is endemic still in UK cities and transmission will occur readily in institutions and in the households of persons infected in the tropics. Lastly, the threat of waterborne epidemics, which will affect all water-drinkers independent of age, will remain a continuing threat.

As prevalence falls, the significance of individual heterogeneities in \( P \) values, will become important and extinction is not inevitable as mean \( R_0 \) values fall below 1; the same applies to other faecal-oral infective agents when the modes of transmission are largely indirect. It is likely that the good fits obtained with catalytic models in developing countries, are due to \( \lambda \) being determined by the mean \( P \) value of many infectives; also in such countries, individuals are less likely to become 'superspreaders' by contaminating large scale communal water and food sources, nevertheless, the current proliferation of communal water supplies must increase the likelihood of *Giardia* outbreaks.

Epidemiological consequences of pathogenic and non-pathogenic *E. histolytica* zymodeses

The new substantial evidence (Sargeaunt et al., 1984) that *E. histolytica* isolates from invasive disease represent a distinct set of total known specific phenotypes (zymodeses - characterised by isoenzyme mobility patterns), considerably alters concepts of amoebic infection and disease.

1. Provided further data continue to maintain the clear distinction of pathogenic and non-pathogenic zymodeses (PZ and NPZ respectively) then seropositivity will be wholly attributable to PZ, whose relative frequency in different communities can be compared by the ratio of seropositivity to parasite prevalence. Since seropositivity, like infection, can be described by a catalytic model with two parameters; it may be possible to compare the forces of infection and seropositivity in different contexts and to relate this to the ratio of PZ to PNZ in stool isolates.

2. These genetic markers will enable the course of mixed infections to be documented in man, experimental animals and laboratory cultures. Thus the possible outcome of random or non-random exclusion can be studied and also the effect of mixed infections upon the total duration of infection. It is assumed in the simple amoebic model that superinfection does not affect duration of infection as it is the total trophozoite population that is determined by fluctuating variables controlling parasite growth and multiplication. It is exceptional for any gut amoeba to exceed a prevalence of 50%; were superinfection to extend duration significantly, then for high levels, prevalence might soon reach 100%. Thus current infection acts like presumptive against new infection and is, in effect, the only limiting factor on prevalence when \( \lambda \) is high. If competitive exclusion does occur, then persons with NPZ might have some protection against PZ and this would be a contra-indication to treating NPZ.

3. Since morbidity and relative prevalence of PZ show quite a marked geographic variation, selective factors may operate in different environments. The nutritional needs and selected aspects of parasite metabolism should be compared in PZ and NPZ in *vitro* as it might be possible to discriminate against PZ by dietary or other means. An example might be the known iron dependence of certain strains; iron supplements enhance pathogenicity in hamsters and it is a general impression that amoebic pathogenicity is low where iron deficiency is common, as in the Gambia.
(4) The P2/NP2 distinction must not, however, lead to host factors being ignored since the former are clearly not always invasive as evidenced by classical volunteer experiments of Walker and Sellards (1913) and the association of tissue invasion with the presence of Schistosoma mansoni and Trichuris, both in mice and in man (Knight & Wright, 1978; Knight, R. and Draper, C., 1972 unpublished observations). However, it appears that NP2 have joined the ranks of 'atypical' E. histolytica (Laredo strains etc.) and E. hartmanni as organisms that can no longer be regarded as potential pathogens.

Diagnostic problems relevant to epidemiology

Stool microscopy. For all human gut protozoa, it is probably true that faecal parasite output, per day or per gramme of stool, shows heterogeneity between persons and varies in an unpredictable way, in time, within individuals. Parasite detectability is affected by parasite numbers and by the physicochemical characteristics of the stool. In the past, both parasitologists and mathematicians (Lancaster, 1950; Mantel, 1951) gave attention to this problem (usually only cysts were considered) in an effort to estimate detectability (4) by repeated examinations and to study its frequency distribution among persons; both non-exclusive and exclusive methods were used; in the latter positives were not re-examined. The similar problems of estimating d for low level malaria parasitaemia has recently been discussed by Aron (1982), who favours, as did former mathematicians concerned with amoebae, the beta distribution which is itself characterized by two parameters. This subject should be further studied for gut parasites. When prevalence surveys are used for critical epidemiological analysis, the sensitivity of the technique must be measured or at least estimated. d refers to one examination but sensitivity will depend upon the number per individual. It is evident that d is even more critical when gain and loss of infection is being considered, since even with concentration and other 'sophisticated' techniques values for d are within the range of 0.6 to 0.9. Of at least equal importance is the need to measure the level of interobserver agreement. It is recognised that motivation and practice greatly improve accuracy in faecal microscopy, even amongst the most experienced. An appropriate statistic must be used when diagnostic reliability is being measured since the prevalence of positives will determine the amount of agreement that occurs by chance alone; for pairs of observers the Kappa statistic (Koran, 1975) is perhaps the best measure of agreement. Paradoxically, it is in the clinical context, when the detection of haematophagous trophozoites in fresh material is of critical importance, that diagnostic reliability can be least easily measured. Furthermore, d is likely to vary considerably among bowel disease patients depending upon the extent and site of the lesions.

Serology. For amoebiasis, serodiagnosis is very successful in the clinical context, especially for liver abscess. However, despite good specificity, problems remain in the use of amoebic serology as an epidemiological tool. Thus, the frequency distributions of antibody level (a discrete variable for many tests but continuous for RIA and ELIZA) obtained in population surveys are difficult to interpret since clear bimodality is unusual and the distinction between positivity and 'background noise' is never clear-cut. Thus seropositivity rates quoted for symptomless carriers tend to be rather arbitrary. However, provided a demarcation point is defined the rates of gain and loss of seropositivity (and hence its mean duration) can be calculated from population data using a catalytic model. Whether newer diagnostic tests will resolve this problem is uncertain. For giardiasis serology is currently less useful in a clinical context since in immunocompetent persons, morbidity is usually self-limited, although they remain seropositive. Possibly newer tests will be able to differentiate patients with current morbidity. In contrast, serologic methods could become more useful in population studies provided that seroconversion rates are higher in those with, than without, morbidity attributable to Giardia. This is because faecal microscopy can show trophozoites, cysts or both in either symptomatic or asymptomatic patients, although trophozoites are more prevalent in the former.
Immunological detection of parasite antigen. Detection in serum of amoebic protein by RIA or ELIZA, if properly validated, could become a valuable tool but it would presumably be a rare finding in survey specimens. Methods for detecting both E. histolytica and Giardia antigen in faeces are being developed and raise the possibility, apparently welcomed by those who regard faecal microscopy as obsolescent, that microscopy will eventually be dispensed with, at least in surveys. It is still unclear to what extent these tests detect soluble or particulate antigen in faeces, or antigens from parasites disrupted when faeces are passed; and also their ability to differentiate symptomatic from asymptomatic infections although presumably the total amount of antigen, whatever its source, will normally be greater in the former. Commensal amoebae, whether E. coli or E. histolytica, within the bowel lumen, may be 'immortal' in the sense that all faecal antigen is in the form of intact parasites but possibly secretory antigens are also present; in contrast, when host immune mechanisms destroy parasites, 'free' faecal antigen may be plentiful. The use of faecal antigen detection in prevalence surveys is likely to face the problem of heterogeneity of output between, and within, individuals. Further, since the tests are immunological, there is no predetermined demarcation line between positive and negative. Thus antigen 'positivity' will have to be calibrated against microscopic positivity and the relation between the two is not likely to be a simple one, nor uniform in different contexts.

Deployment of diagnostic facilities and chemotherapy in the primary care context.

The current availability of safe and potent drugs, particularly the nitroimidazoles, for both amoebiasis and giardiasis, emphasizes the need for them to be used at the community level wherever these diseases cause significant morbidity. The frequency of amoebic seropositivity in population surveys indicates that self-limited invasive disease, of variable duration, is not uncommon. Documented mortality rates (Adams et al., 1977) for uncomplicated amoebic dysentery and uncomplicated liver abscess are both about 1% when they are treated adequately but when complications arise, this rate is increased at least tenfold for both conditions. Diagnostic, and thus therapeutic, delay is the major factor leading to complications. The extent to which Giardia infection precipitates, prolongs or exacerbates protein energy malnutrition (PEM) merits further urgent study since wasting and stunting are such important risk factors for infant and child death due to many causes. Nevertheless, the high prevalence of Giardia in PEM, its known severity and long duration in immunocompromised hosts, and its documented association with weight loss, inappetence and malnutrition, even in many normal persons on first exposure, implies that a much more aggressive attitude must be taken towards the parasite.

As part of national diarrhoeal disease control programmes (which should also include diseases due to gut protozoa) there is a need to establish, perhaps at the district level, relatively sophisticated laboratories, using modern techniques, to act as sentinel surveillance units. These should (1) monitor the relative frequency of the causal agents of diarrhoea among patients attending the health facility to which they are attached, (2) conduct field surveys and (3) investigate epidemics of diarrhoeal disease.

However, it will normally neither be feasible nor cost-effective to deploy specialized technicians in the peripheral health centres. There is an urgent need for well-designed case control studies using discriminant or multiple logistic regression analysis to define a set of predictor variables that can be used in the form of a clinical algorithm to decide who should receive chemotherapy. The variables would include demographic ones, and also symptoms, signs and macroscopic stool characteristics. It is likely that such diagnoses would have greater sensitivity and specificity for clinically significant infections than those achieved by the isolated microscopist with his attendant problems of detectability, reproducibility, etc. The situation would be similar to that currently used for the selective treatment of malaria, acute respiratory infections and Schistosoma haematobium; for the latter, a history of haematuria are visible haematuria are valuable predictors, in addition to urine dipstick tests for blood and protein (Nott et al., 1985). Efforts should be made to develop simple 'side room' tests for faecal fat and specific gravity in symptomatic giardiasis.
REFERENCES


Walker, E.L. & Sellards, A.W. (1913) Philippine Journal of Science and Tropical Medicine, 8: 253-330