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The molecular epidemiology of parasites

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Summary. The explosion of new techniques, made available by the rapid advance in molecular biology, has provided a battery of novel approaches and technology which can be applied to more practical issues such as the epidemiology of parasites. In this review, we discuss the ways in which this new field of molecular epidemiology has contributed to and corroborated our existing knowledge of parasite epidemiology. Similar epidemiological questions can be asked about many different types of parasites and, using detailed examples such as the African trypanosomes and the *Leishmania* parasites, we discuss the techniques and the methodologies that have been or could be employed to solve many of these epidemiological problems.
Key words. Molecular epidemiology; epidemiology; parasites; DNA probes; trypanosomes; *Leishmania*; parasitic nematodes.

1 Introduction

One of the central areas of investigation considered in the study of parasites and the diseases they cause concerns the dynamics of interaction of the parasite, its host(s) and the environment. The study of such interactions – epidemiology – seeks to describe, both qualitatively and quantitatively, the rules which govern the ways in which a given parasite is transmitted from one host to other hosts, and how this influences the spread of disease within and between populations of hosts. Such studies on transmission must encompass a wide range of factors in order to document fully the epidemiology of a given parasitic disease.

One of the primary problems in parasite epidemiology is the correlation of a given disease with its causative organism. In some cases such an association can be clearly made. For example, in disease due to the bovine lungworm, *Dictyocaulus viviparus*, the clinical signs of bronchitis and pneumonia can be readily associated with the presence of adult worms in the trachea and bronchi. In contrast, the association between parasite and disease may be much less obvious, as is the case with the morphologically identical trypanosomes of the *Trypanosoma brucei* complex, the causative agents of African sleeping sickness. Here, apparently identical parasites have different specificities for the host and therefore different ranges.

Having established what is the causative agent of a disease, the epidemiologist must then consider the ways in which transmission occurs. Parasites can utilise very straightforward transmission routes; for example, *Trypanosoma equiperdum* is transferred from one host to the next by direct venereal transmission. Other parasites may require more complex life cycles, like the malaria parasite, *Plasmodium*, which uses the mosquito as a vector for transmission, or the blood fluke, *Schistosoma*, which requires an intermediate host to complete its life cycle. Mechanisms of transmission are almost as diverse as the parasites that use them.

The rate of transmission between hosts is another domain of the epidemiologist. The study of the components which influence transmission rates involves consideration of such diverse factors as parasite density, host density, intermediate host density, vector density, parasite virulence, animal reservoirs and host resistance. In addition to modes and rates of transmission, environmental and socio-political factors contribute to the overall distribution (geographical and host range) of the parasite.

The ultimate objective of the epidemiologist is to be able to describe all such parameters completely, perhaps using mathematical modelling, in such a way that predictive statements can be made in the evaluation of different control measures. A considerable wealth of information has been built up on the epidemiology of most parasitic diseases using classical parasitological, medical and veterinary studies. More recently, contributions to these studies have been made using biochemical and molecular approaches, which provide useful tools for the investigation of some epidemiological questions. In this review we will consider the contributions of DNA technology to our understanding of epidemiological processes, the approaches used, and the limitations of the technique. In considering the application of molecular probes to the study of the epidemiology of parasitic diseases, it is necessary to look at four main areas. Firstly, the nature and type of epidemiological question being asked must be identified; secondly, the molecular techniques and reagents which are available must be identified and the information that has been gained from their use examined; thirdly, it is important to consider what these reagents and probes are detecting and their level of sensitivity; finally, it is necessary to consider the limitations and problems of the existing technology and how these could be overcome in the future. It is clear from a brief survey of the literature that, despite intensive research effort, we are only just reaching the point where such studies can be undertaken in some parasite species.

Considering first the nature and type of epidemiological questions that need to be asked, it is clearly impossible in a review of this type to cover all parasite systems in detail. However, many parasite systems raise very similar epidemiological questions, and therefore it is appropriate to define these in general terms before going on to examine what tools are available and how they have been applied. In this review we will consider how molecular probes have been used to begin to examine the following questions:

1) Can the species or strain causing a particular clinical disease state be defined? This is particularly relevant where several morphologically similar parasite species co-exist and infect a range of mammalian hosts.
2) What vectors or intermediate hosts carry a particular parasite which causes a particular disease?
3) What other mammalian hosts carry the parasites causing a particular disease (reservoir hosts)?
4) Are particular strains/variants associated with particular disease states, outbreaks of a disease or epidemics?

These questions can be broadly considered as qualitative, i.e. aimed at defining the disease-causing organism and how it is distributed. Once this is established, quantitative studies concerned with disease transmission, the relative importance of different hosts and vectors, and the overall dynamics of a particular disease can be undertaken. An outline of the qualitative questions and their relationship to each other is provided in the flow chart (fig. 1). The specific questions associated with a particular disease will of course differ, depending on the disease concerned, and so will the relative importance of the factors outlined in the flow chart.

While the present discussion is totally focused on the parasite, it is also of importance to remember that molecular probes also have a role in defining host and vector parameters which may be determinants of the incidence, geographical distribution or severity of the disease. An example of such a parameter is the presence of rickettsia-like organisms found in tsetse flies which, at least under laboratory conditions, have a major effect on the level of mature infection by trypanosomes [5,6] and therefore potentially on trypanosome transmission rates. Other examples include the differential susceptibility of different

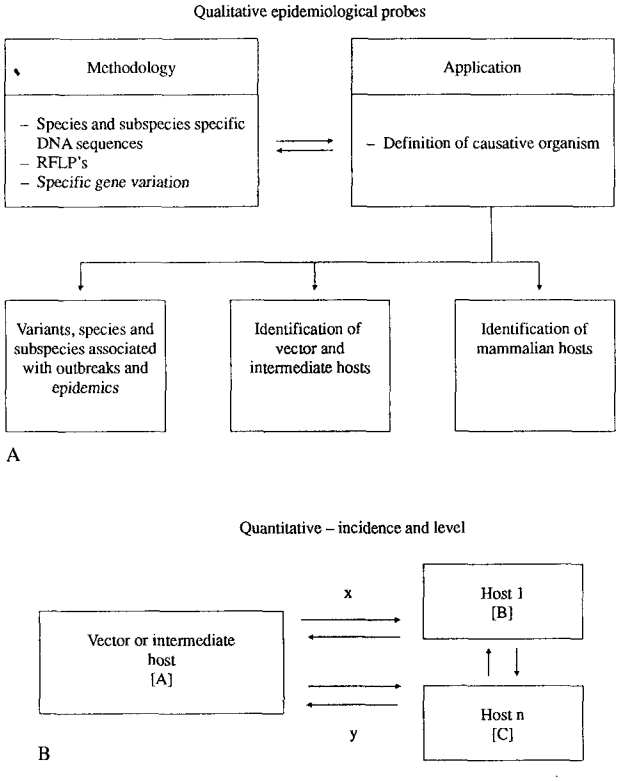

Figure 1. *A* Outline of the types of epidemiological DNA probes and their applications. *B* Outline of the quantitative measurements that are required for defining the epidemiology of a disease. X, Y and Z are rates whereas [A], [B] and [C] are levels of a particular parasite. In many instances other parasites, either causing other diseases or of a non-pathogenic nature, are present in both vectors and hosts.

breeds of sheep and even of individuals to the nematode *Haemonchus contortus*, and variation in the levels of parasitaemia and the ability of *P. falciparum* to invade red blood cells of individuals with different genotypes[51] – the most classical example here is the effect of sickle cell anaemia. In addition when the host is considered, there is the whole research area of immune responsiveness and disease resistance which has primarily been studied in laboratory animal models e.g. *Leishmania* and mice[9]. From these examples alone it is clear that there are a large number of genes and genotypes, in both host and vector, which are determinants of disease incidence, transmission and severity of symptoms; clearly these need to be identified in order to understand the epidemiology of a disease. If such genes and genotypes are identified, molecular probes allowing their identification within populations of the host or vector could be of immense value. This is clearly an area for future research investigation and evaluation, but discussion of these questions is beyond the scope of this review.

2 *Methods and approaches*

Classical epidemiological methods and procedures have provided answers to many of the major epidemiological questions about parasitic diseases. However, the advent of recombinant DNA has provided a battery of new tools and approaches that have been or could be used to complement and extend our knowledge of the epidemiology of many parasites. Much research effort in epidemiological studies is put into the identification of parasites and the study of the variation within and between groups of parasites so that epidemiological questions can be answered. For these purposes, molecular methods have a number of technical advantages. Basically, two levels of distinction can be made: an absolute distinction between two clearly defined species or groups of parasites, or a finer distinction that examines differences between individuals within a group. In molecular epidemiology this distinction can be made relatively simply. First, absolute distinctions can be made by examining the DNA of given groups for sequences which distinguish the groups on the basis of presence or absence. Second, finer discrimination can be made by examining DNA sequences which show variation between individuals or groups of individuals. A number of approaches are available for the study of variation at the DNA level.

2.1 *Hybridisation of gene probes*

2.1.1 *Dot blot analysis*

An absolute distinction between groups of parasites can be made using a dot blot analysis. This technique is based on the isolation of a DNA sequence which is unique to one of the parasite groups being compared. DNA samples from 'unknown' parasites are immobilised on nitrocellulose or nylon filters and hybridised to the radiolabelled group-specific probe. The technical advantages of this type of system are that parasites belonging to a particular species, subspecies or group can be identified specifically with high sensitivity, and that many samples can be processed simultaneously. Such a procedure could form the basis of a diagnostic test, as quantities of DNA down to 10–100 pg can be detected. This would be the equivalent of detecting 10^2–10^3 protozoal parasites such as trypanosomes or $^1/_{10}$ of the DNA of a filarial worm such as *Onchocerca*.

2.1.2 *In situ hybridisation*

The technique of in situ hybridisation[43, 71] has potential as a technique for the identification of parasites. The technique is essentially similar to the dot blot technique except that individual parasites can be immobilised and the probe hybridised to them in situ. Thus, individual parasites within a mixture can be assayed for the ability to hybridise to a specific probe. The results from such a technique could be of considerable interest to epidemiologists who seek to design mathematical models of epidemics and require information about the frequencies of particular parasite groups within hosts, vectors and reservoir hosts. However, this technique is considerably less sensitive than the dot blot assay, and it is technically more difficult to use.

2.2 *Detection of DNA polymorphism*

2.2.1 *Restriction fragment length polymorphisms*

Finer distinctions between parasite strains can be made by examining the variation in DNA sequence in specific genes; such variation can be detected using a series of restriction enzymes which cut the DNA at specific sequences. If there is polymorphism within the specific sequence recognised by each restriction enzyme, this will be detected as the presence or absence of a particular site. Typically, such an approach consists of assessing variation by examining electrophoretically separated banding patterns of parasite DNA digested with restriction enzymes after transfer onto nitrocellulose using the Southern blot technique[85] followed by hybridisation on the filter with a radiolabelled DNA probe.

The DNA sequences used as probes can be cloned fragments of any region of DNA which shows differences between the groups or individuals being examined. The probes used are single-copy housekeeping genes, multicopy gene families (e.g. tubulin, histone or ribosomal genes) or highly repetitive DNA sequences of unknown function. The banding patterns observed for these types of probes range from 1–4 bands with a single copy gene probe to 30–40 bands produced by a highly repetitive probe. Figure 2 shows an example of a Southern blot showing variation between trypanosome stocks using a ribosomal RNA gene probe and a housekeeping gene probe (phosphoglycerate kinase).

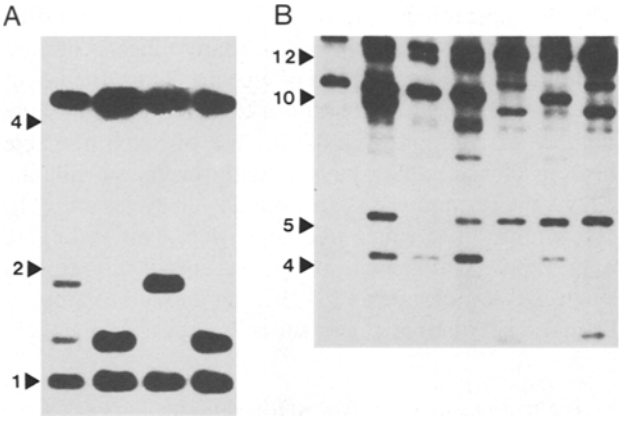

Figure 2. Examples of Southern blots of trypanosome DNA probed with (A) a low copy number probe (phosphoglycerate kinase - PGK) and (B) a high copy number probe (ribosomal RNA gene non-transcribed spacer). Molecular sizes are indicated in kilobases.

DNA polymorphism and variation can be detected by observing differences in banding patterns when comparing total DNA isolated from different parasite stocks. The greatest degree of variation can be detected by using repetitive probes since more DNA sequences can be examined simultaneously; however, the complexity of the results is increased and the degree of interpretability decreased unless mathematical analyses, such as cluster analysis techniques[22], are invoked to assess levels of similarity. The level of variation observed varies between genes. In some cases it is sufficient to distinguish species, whereas in other cases it can be used to identify individual strains. For example, some genes are highly conserved, such as the ribosomal RNA gene coding region or specialised housekeeping genes, while others are much more variable such as the ribosomal RNA gene non-transcribed spacer, or non-functional DNA sequences which are under low selective pressure. The choice of probe, therefore, depends on the level of discrimination required. Molecular 'fingerprinting' using particular genes can result in each individual having a different banding pattern. As yet, no parasite gene sequences with such properties have been identified, but in humans a hypervariable minisatellite probe can be used, and with this it has been shown that each individual has a unique banding pattern[39,40]. In general, when examining restriction enzyme patterns from parasite stocks within a species the assumption can be made that differences between stocks or groups of stocks arose by divergent evolution, therefore comparisons of similarities in banding patterns between groups within a species has the potential to allow the epidemiologist to make statements about the ancestry and origins of groups of stocks, and to make deductions about the spread of particular parasite genotypes over a time-span.

2.2.2 DNA sequencing

DNA sequencing is the ultimate method of assaying polymorphism between given parasite strains. However, such an approach is extremely laborious, and not very useful to the molecular epidemiologist. There is one approach, which has been used in determining phylogenies of different eukaryotic and prokaryotic organisms, which may have potential for the epidemiologist. This approach involves a rapid DNA sequencing analysis of a specific region of the ribosomal RNA genes. The technique involves the use of specific primers to a region of the ribosomal RNA genes which is conserved throughout the eukaryotic and prokaryotic species. This primer can be used for sequencing in a specific region of the rRNA genes and then sequence differences can be analysed by computer and relationships between different organisms defined[42]. Such an approach could be used to analyse variation within and between parasite species and perhaps be used to deduce information about origins or dynamics of distribution of the given parasites. The technique can be carried out in a period of about 1 week and can be undertaken using a whole parasite lysate or isolated DNA. Relatively little parasite material is required.

2.3 Analysis or molecular karyotypes

2.3.1 Nuclear karyotype

In many parasite species, in particular protozoan parasites, the chromosomes are not condensed and consequently it is not possible to determine the karyotype. This situation led to the development of electrophoretic techniques capable of separating chromosome-sized molecules. A number of these techniques exist: Pulsed Field Gel Electrophoresis (PFGE)[79,93], Orthogonal Field Alternating Gel Electrophoresis (OFAGE)[14], Contour-clamped Homogeneous Electric Fields (CHEF)[16] and Field Inversion Gel Electrophoresis (FIGE)[15]. These techniques work on the principle of applying changing electric fields to preparations of total cell DNA, prepared without any shearing, in such a way that separation of large chromosomes occurs. The differences in the techniques are in general due to different electrode arrangements and the resulting differences in the shape and strength of the electric field generated. Analysis of a number of parasites by this technique has demonstrated the presence of different chromosome patterns within and between related parasite groups, thus potentially allowing stock identification. The technique is relatively time-consuming and requires large quantities of parasite material, which reduces its usefulness for epidemiological studies. For example, gel runs lasting several days each with only 8–10 samples per gel are required.

2.3.2 Extranuclear DNA

All parasites have been found to have extranuclear DNA species, in particular mitochondrial DNA; in addition, many groups of parasites have other forms of extrachromosomal DNA. Such DNA species may include simple episomes, such as the episomes found in *Theileria*[33] and *Leishmania*[7], and the larger complex kinetoplast DNA networks of the Kinetoplastida. The application of re-

striction endonuclease mapping, Southern blotting and DNA sequencing to such extranuclear DNA species can also be used in the identification of parasite groups.

2.4 Polymerase chain reaction

The polymerase chain reaction is a very powerful technique which has been recently developed for the analysis of DNA[77,78]. The principle behind the technique is that specific pieces of DNA can be amplified many millions of times from a mixture of DNA (e.g. a sample of total genomic parasite DNA) using the thermostable DNA polymerase from *Thermophilus aqueus* (Taq polymerase) and a pair of oligonucleotide primers homologous to different regions of the gene of interest. The basic outline of the technique (illustrated in figure 3) is as follows: two oligonucleotides are synthesised on the basis of the previously determined sequence of the piece of DNA to be amplified (target DNA). These oligonucleotide primers are designed to be some 500–1000 bp apart on the piece of target DNA and on opposite DNA strands. The target DNA is then subjected to a 3-phase temperature cycle. The DNA is denatured at a high temperature (e.g. 94 °C) for 1–2 min, then the temperature is rapidly shifted to a much lower level (e.g. 37 °C) for 2 min to allow hybridisation of the primers to the target DNA. Finally, the temperature is raised (e.g. to 72 °C) so that the Taq polymerase can utilise the annealed oligonucleotide as a primer to synthesise a second strand of DNA on the single-stranded template (target) DNA. Thus, during this cycle the number of copies of the target DNA has doubled.

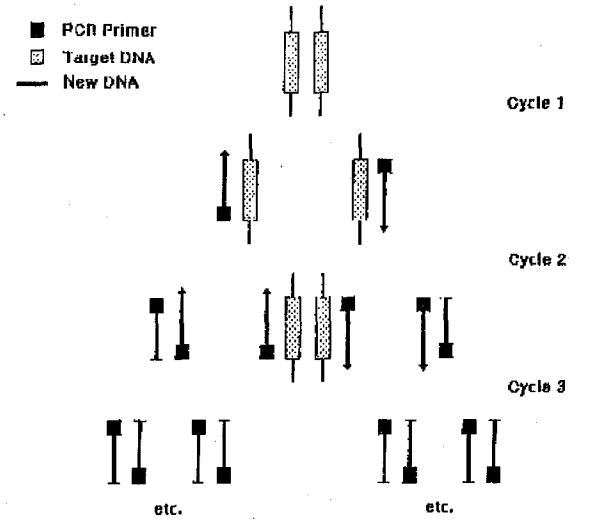

Figure 3. Illustration of the basic polymerase chain reaction technique. During the first cycle, the target DNA is denatured, the specific primers are allowed to anneal to the single-standed target DNA and the strand is copied by the DNA polymerase. Similarly, in the second cycle, the DNA is once again denatured, the primers allowed to anneal and the DNA is copied by the DNA polymerase. The third cycle consists of the same procedures and at this stage a population of DNA molecules, which are flanked by the specific primers, is produced. During the fourth and subsequent cycles these molecules are further amplified and eventually become the predominant DNA species within the mixture.

The thermostable nature of the Taq polymerase means that this cycle can be repeated as many times as desired without the further addition of enzyme. Thus, the target DNA sequence can be amplified 2^n times (where n = the number of reaction cycles). The use of programmable heating blocks, which have recently become available, allows the researcher to carry out 25–40 cycles on up to 100 samples in a single overnight step. This technique may be powerful enough to amplify, specifically, from a single DNA molecule and is efficient enough to amplify from a small number of parasite cells or even a single cell.

3 Applications of molecular epidemiology

3.1 African trypanosomes

As a detailed example of the applications of molecular methods to epidemiology, the study of African trypanosomiasis is useful in highlighting practical approaches and limitations as well as discussing future developments of molecular epidemiology. The *Trypanosoma* species which are mainly responsible for causing trypanosomiasis in Africa are classified into 3 subgenera; *Duttonella* (represented by *T. vivax*), *Nannomonas* (represented by *T. congolense* and *T. simiae*) and *Trypanozoon* (represented by *T. brucei* ssp., *T. evansi* and *T. equiperdum*). The main epidemiological problems in African trypanosomiasis can be summarised as follows:

a) Identification of the parasite causing the disease. Although trypanosomes from each subgenus can be distinguished by microscopy, many of the species and subspecies cannot. For example, members of the *Trypanozoon* subgenus are all morphologically identical but have different host ranges, geographical distribution and transmission mechanisms. Similarly, *T. congolense* and *T. simiae* can only be distinguished on the basis of their different disease-causing capabilities in cattle and pigs. Recent evidence suggests, especially in the *Nannomonas* and *Trypanozoon* subgenera, that our classification of the African trypanosomes is not yet complete and that there are groups of parasites, currently included within existing taxa, which may need to be defined as epidemiologically distinct from the group in which they are currently placed.

b) Classification and frequency of trypanosomes in the vector. The distribution and transmission rate of African trypanosomiasis appear to be controlled closely by the distribution and behaviour of the tsetse fly vector. In determining the significance of vector involvement in different types of disease, in different areas and different hosts, the epidemiologist must know what types of trypanosomes can be found in any one area and at what frequency particular types are found.

c) The involvement of alternative hosts. A number of studies, classical, biochemical and molecular, have identified human infective trypanosomes (*T.b.rhodesiense* and *T.b.gambiense*) in both wild game[27,35] and domestic animals[29,61,68,69,80] and have thus defined reservoir

hosts. At the present time the significance of these reservoirs as a source of human infection remains to be evaluated, as does the involvement of wild game in relation to the animal trypanosomes. If animal reservoirs are a major source of infection, then disease control measures will need to be directed at these reservoirs.

3.1.1 Approaches to the molecular epidemiology of African trypanosomes

Three basic approaches have been taken to investigate these problems. First, the isolation of DNA probes which uniquely recognise particular trypanosome groups. Second, the identification of related trypanosomes or individual trypanosome stocks by comparison of restriction enzyme patterns. Third, the study of differences in molecular karyotype (either nuclear or extranuclear, e.g. kDNA) as a means of identifying groups of trypanosomes.

3.1.1.1 Species and sub-species specific DNA probes. A considerable amount of effort has been invested recently in obtaining 'epidemiologically useful' probes. In the *Trypanozoon* subgenus, much effort has been concentrated on distinguishing the three subspecies of *T. brucei*. Classically, *T.b.gambiense* infects man and is found in West Africa, *T.b.rhodesiense* infects man in East Africa and *T.b.brucei* infects animals throughout Africa. As will be seen, these classical distinctions have turned out to be an oversimplification of the profile of *Trypanozoon* groups involved in disease in Africa.

A considerable amount of effort has been put into obtaining DNA probes which can be used to discriminate between different African trypanosomes. These probes discriminate at different taxonomic levels. These levels of discrimination are summarised in figure 4 for all of the DNA probes currently available.

T. b. gambiense can be distinguished from *T. b. brucei* and *T. b. rhodesiense* by *T. b. gambiense*-specific antigen gene probes[6, 69, 70, 72], while other antigen gene probes can distinguish both *T. b. rhodesiense* and *T. b. brucei* from *T. b. gambiense*[55]. As yet no published data is available on probes which distinguish *T. b. brucei* and *T. b. rhodesiense* and current thinking is that these two subspecies are indistinguishable[29, 30, 36, 37, 69, 70, 91]. However, recently an RNA transcript has been found whose presence can be correlated with the presence of human serum resistance in *T. brucei*. This observation may lead to a useful method for distinguishing *T. b. rhodesiense* from *T. b. brucei*[19].

The distinction between *T. evansi, T. equiperdum* and *T. brucei* is less well studied at the molecular level. Some antigen gene probes will distinguish *T. brucei* from *T. evansi*[55] and the kDNA maxicircle, absent from *T. evansi*[12], could also be used as a probe. Other probes, for example, the ribosomal RNA gene non-transcribed spacer[36, 37] and the 177 bp satellite DNA[32], recognise all of the *Trypanozoon* species, thus confirming their relatedness, but not providing a means of defining these species.

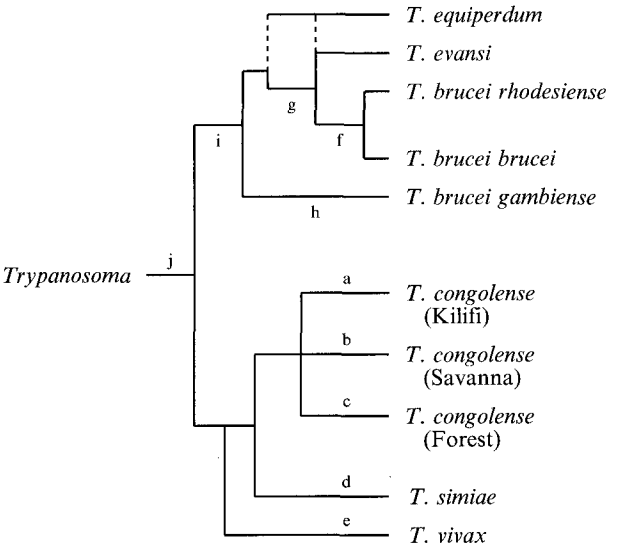

Figure 4. Diagram illustrating the levels of discrimination, using DNA probes, of the various African trypanosome species, subspecies and groups. The letters refer to available probes which discriminate at the level indicated: a) 400 Alu probe[32], pgNIK 450[46, 53]; b) 369 satellite probe[32], pgNRE372[46, 53]; c) 350 cfoI probe[32]; d) 200 CfoI[32], pgNS600[45]; e) 400 bp probe[60], lambda gDIL-10[46]; f) Probes, A_1, A_2, A_3 and B[55], kDNA maxicircle[12]; g) antat 1.1 probe[69, 72]; h) X1 probe[69], L2, U1, P1 probes[6]; i) ribosomal non-transcribed spacer[37], pBE2[37], Antat 1.8, Antat 1.13[69], probe X2[69], 177 bp satellite[32], pgDR1[46]; j) ribosomal coding region[37].

A number of probes are available which distinguish the different species of trypanosomes in Africa. Most of these are repetitive DNA sequences and thus increase the sensitivity of detection of trypanosomes in the vector. These probes include *T. vivax* specific sequences[32, 53], *T. simiae* specific sequences[32, 52] and sequences which distinguish between the *Trypanozoon* species from the other subgenera[32, 36, 37, 46].

It is clear from the summary of probes (fig. 4) that some types of sequences can be used for finer discrimination than others. Thus, it may be important to consider the types of probes used in the study of the African trypanosomes and the taxonomic level at which they discriminate in terms of choosing probes for other systems. Those probes which discriminate at the lowest taxonomic level (i.e. subspecific level) tend to be the highly variable antigen gene probes. Satellite DNAs and repetitive probes tend to discriminate at the specific level (or subgenus level in *Trypanozoon*) and highly conserved probes, like the ribosomal RNA gene coding region, appear to hybridise to all species of African trypanosome. The ribosomal RNA genes show different levels of homology in different adjacent regions of the genes[36, 37]. While the coding region hybridises to all of the African species, the non-transcribed spacer from *T. b. brucei* hybridises only with the *Trypanozoon* species. Thus certain ground rules have emerged which begin to define the type of sequence which should be selected when attempting to isolate a

DNA sequence for discriminating at a particular level of relatedness between groups of organisms.

The use of probes as epidemiological tools has led to a number of changes of groupings within the existing taxa of African trypanosomes. The species *T. congolense* is now thought to consist of a number of groups. Studies using repetitive DNA probes have demonstrated the presence of two distinct types of *T. congolense*, the 'savanna' type and the 'coastal' (or Kilifi) type[32,46,53]. Gibson et al.[32] describe a probe which defines a third group, the 'forest' type of *T. congolense*. These observations with molecular probes confirm groupings based on isoenzyme studies[26,45,103]. It is unclear, at present, whether these groups represent different species, subspecies or geographical variants and whether the same epidemiological parameters are involved in their life cycles.

Many of the approaches taken in the development of these probes have been orientated towards the identification of trypanosomes in the vector. Kukla et al.[46] developed a dot blotting method for detecting the presence of different species of trypanosomes using their repetitive probes in different parts of the vector. Their 'abdominal touch blotting' method, which involved rubbing the abdomen of a single tsetse fly sequentially onto filters and probing each filter with a different specific probe, allowed them to assay which species were present in a given tsetse fly. Similarly, they could assay the trypanosome species present in the proboscis and salivary glands of the tsetse. Thus, it would be possible to compare incoming trypanosomes from a feed (midgut assay) with those which would be transmitted to another host (proboscis salivary gland assay) and thereby identify any selective transmission in the vector. As few as 10–100 trypanosomes per vector could be identified in this way.

Gibson et al.[32] developed a different approach to detection in the tsetse fly. They used an in situ hybridisation assay, with species-specific probes to identify individual trypanosomes (in a smear on a microscope slide) which hybridised to each probe. Thus, using this system, it would be possible not only to identify which species of trypanosome was present in parts of the tsetse fly, but also to determine the frequency of each species. Such an approach has considerable implications for those seeking to develop mathematical models for the epidemiology of African trypanosomiasis. If, in addition, tools were available (for example antibodies) to identify the host species by virtue of the blood present in the gut of the tsetse, it would also be possible to identify the host species on which the fly had been feeding. The dot blot assay using such probes can also be applied to the identification of the trypanosome species within a sample of host blood either by direct lysis of the blood on filters or by preliminary concentration of trypanosomes from larger volumes of blood.

Thus, in principle, it is now possible to take samples from tsetse flies and all mammalian hosts in a particular area and then identify the species of trypanosome present, their relative numbers, the hosts carrying particular species, the species ingested by the tsetse and the species of host on which the tsetse has most recently fed. Most of the probes have been developed with a view to simple, rapid collection procedures (e.g. on filters etc.) such that field samples could be readily collected. Although some of the probes and techniques are not fully developed for field use, we are very nearly in a position to collect very relevant epidemiological data using these reagents.

3.1.1.2 Restriction enzyme patterns. Restriction enzyme pattern differences or Restriction Fragment Length Polymorphisms (RFLPs) have been used to make fine distinctions between closely related trypanosomes. This type of approach identifies, very sensitively, differences between closely related trypanosome stocks but suffers from the limitations that it is quite laborious and requires a considerable amount of parasite material. Thus, it is not very suitable for large-scale analyses or analyses of small amounts of material such as that obtained from the tsetse fly vector, and cannot be used for the identification of single trypanosomes.

This type of approach has been used in the *Trypanozoon* species in which the relationships between different, but morphologically identical, parasites are complex. The approach has been used in two ways. First, to assign a 'molecular fingerprint' to any given strain of trypanosome such that, by sampling trypanosome isolates from hosts and vectors from a given area, epidemiological questions can be answered regarding the spread of that trypanosome strain. As yet, this type of approach has not been used extensively. One example of its use is in the analysis of *T. b. rhodesiense* stocks, taken from a 1980 sleeping sickness epidemic in a single Zambian village[21]. In this case it was shown, using ribosomal RNA and repetitive gene probes[36,37,104], that a single trypanosome strain was responsible for the epidemic.

The second approach is based on the assumption that different patterns are generated by the gradual accumulation of divergent changes during the evolution of the parasite. Thus, a hierarchical grouping analysis technique can be used which constructs groupings of related trypanosomes based on similarities in their restriction enzyme patterns. If the initial assumption is correct, then the groups reflect common ancestry and, therefore, common origins. Thus, by combining information obtained from this grouping approach with other epidemiological data (e.g. place of isolation, host, vector etc.) it allows the epidemiologist to ask questions about the origins and relatedness of populations of trypanosomes. A study using this type of approach was carried out, using restriction enzyme patterns generated by antigen gene probes[69,70], and ribosomal RNA gene and repetitive DNA probes[36,37,104], to characterise a set of West African *T. brucei* stocks infective for humans, from the Daloa Focus in the Ivory Coast.

Classically, the West African human trypanosomes are classified as *T. b. gambiense* and cause chronic sleeping sickness, but analysis of collections of such isolates has revealed that the stocks from the Daloa Focus can be clustered into 3 distinct groups. The first group comprises the 'classical' *T. b. gambiense* stocks because within this group are stocks previously characterised as *T. b. gambiense* by isoenzyme studies [29, 61, 90]. The second group consists of isolates which are indistinguishable from animal isolates in West Africa. In East Africa *T. b. rhodesiense* (human infection) is considered to be indistinguishable from *T. b. brucei* [10, 91]. It is thought, therefore, that this group of West African human-infective stocks represents the West African equivalent of *T. b. rhodesiense* stocks from East Africa. The third group of stocks is, at present, represented by a single stock [36, 37]. This stock is indistinguishable from East African *T. b. rhodesiense*. As all of the Daloa isolates were obtained from the same region, it must be concluded that all three groups co-exist in the same area and may contribute to the epidemiology of sleeping sickness in this area. *T. b. rhodesiense* is thought to cause a more virulent form of the disease in humans than *T. b. gambiense*, thus the presence of *T. b. rhodesiense* may significantly affect transmission rates and, therefore, the epidemiology of sleeping sickness in this area.

3.1.1.3 Molecular karyotype and extranuclear DNA. In addition to nuclear DNA, the African trypanosomes also possess extranuclear DNA, the kinetoplast DNA, which exists as a large network of two classes of intercalated circular molecules – maxicircles and minicircles. A number of approaches have been used to investigate the relationships between stocks using markers based on restriction enzyme variation in the kDNA. Variation in the nuclear karyotype can be assayed by PFGE; however, the techniques involved are so laborious that, as yet, they have not seriously been applied to epidemiological questions. However, when examining the molecular karyotype of various species of African trypanosomes a number of laboratories have described differences both within and between species in the numbers and sizes of chromosomes [31, 53, 54, 93]. Such an approach could therefore be used to complement other methods in the identification of the parasitic disease agent.

The analysis of kDNA has been more extensively applied and structural studies on the kDNA of the various species of African trypanosomes has revealed considerable differences between and within species. *T. vivax* was found to have much larger maxicircles and much smaller minicircles than the other species [11] while little sequence homology was found between the kDNA of *T. vivax, T. congolense* and *T. brucei*. The kDNA of *T. equiperdum* and *T. evansi* was found to be even more strikingly different, in that no maxicircles occur at all; thus, these species can be completely distinguished from the others [12]. Within the *T. brucei* subspecies a number of observations were made which could be applied more directly to epidemiological questions. When examining restriction site polymorphisms in the kDNA maxicircle, Gibson et al.[30] found that there was only a small degree of variation between different *T. brucei* stocks except for one particular region of the genome, the variable region, which was variable both in length and in the presence/absence of particular restriction enzyme sites. A correlation could be made between the length of this variable region in a given stock and the geographical location from which the stock was isolated. Stocks from West and Central Africa were found to have a shorter variable length region than those of East Africa. Thus, this observation could form the basis of a marker to identify the geographical origins of trypanosome stocks, particularly in groups of animals or humans that migrate or move over large distances. By this means an assessment of the flow, if any, of parasites between East and West Africa could be made.

3.1.2 Limitations of molecular approaches

Molecular approaches to the epidemiology of African trypanosomiasis have been very useful in a number of areas. First, the techniques have produced methods of identifying the species and subspecies involved as disease causing agents, and this has led to potential diagnostic reagents. Second, the study of variation, at the molecular level, has identified subgroups within previous groupings which may differ in their specificity for host, disease virulence, vector preference and geographical distribution. Finally, the probes have been used (or have the potential to be used) to identify qualitatively the animal reservoirs, the distribution in vector populations and the distribution in host populations.

The limitations of these approaches become apparent when considering the contributions made to our understanding of the quantitative dynamics of African trypanosomiasis. Many of the trypanosome isolates, be they from hosts, vectors or animal reservoirs, contain mixtures of parasites, and as many of the procedures require large quantities of trypanosomes there is an inevitable selection for single lines either by cloning or by growth-rate differences in rodent hosts. Thus, although the original isolate may start out as a mixture of trypanosomes, many of these will be lost during the analysis stage and therefore their contribution to the epidemiological profile will be lost. Such problems also exist with other characterisation techniques such as isoenzyme analysis. In addition, most of the techniques are not suitable for examining large numbers of isolates. Thus, these methods are not very useful for large-scale studies on the frequencies of trypanosomes in hosts, vectors and reservoirs which is the sort of information required for mathematical modelling.

3.1.3 Future trends

One of the most promising molecular techniques available for the epidemiology of African trypanosomiasis is the Polymerase Chain Reaction (PCR) technique. In

principle, if the right conditions are used, this technique should be able to amplify DNA from a single trypanosome. This would allow the epidemiologist to identify the particular types of trypanosomes from within a mixed population and quantitate the relative importance of different types within the mixture. Another advantage of the PCR technique is that it works by amplifying DNA directly from the parasite and, therefore, there is no requirement for growing the parasites in culture or in rodents and thereby risking the possibility of selection occurring during growth of the parasite. Finally, the PCR technique is sufficiently convenient to be applicable to large-scale analysis of trypanosome samples. At present there has only been a limited application of the PCR technique to the identification of trypanosomes[65], but it is anticipated that such an approach will be used extensively in the near future.

3.2 Leishmaniasis

The protozoan parasites of the genus *Leishmania* are a group of morphologically very similar organisms which cause a range of different diseases in man, are transmitted by a range of vector species, and also infect different groups of wild and domestic animals. Thus, at the epidemiological level there are numerous questions to be answered in relation to the diseases states caused by different parasite species and the role and significance of the various reservoir hosts. Epidemiological studies carried out over the last few decades show that leishmaniasis is a zoonotic disease, and that of the numerous species found in mammals, a subset of at least 15 species and subspecies are infective for man. The classification of these species and subspecies used here is as described elsewhere[5]. Human-infective *Leishmania* species are distributed right across the tropical and subtropical regions of the world; the diseases they cause can be broadly divided into three types: simple cutaneous, mucocutaneous and visceral. While all forms of the disease are found in many areas of the world, the disease is caused by different species in different geographical areas of the world, as summarised in figure 5. The need for accurate identification of the Leishmanias arises from the need to prescribe the highly toxic drugs only to patients infected with the more pathogenic species, such as those causing the visceral forms of the disease.

3.2.1 Application of molecular methods to Leishmania epidemiology

As with the African trypanosomes, molecular epidemiological approaches to the study of the Leishmanias have concentrated on defining and differentiating between the different species of *Leishmania*. However, a considerable amount of effort has also been invested in using molecular probes to identify animal reservoirs and also to distinguish the organisms causing the different clinical manifestations of the disease.

Karyotypic analysis of different *Leishmania* species has been used as an approach to the differentiation and identification of the Leishmanias[8,18,28,86]. It was found that the members of different species could be readily distinguished[28], and the chromosome patterns could be used to identify unknown *Leishmania* isolates by comparison of banding patterns. However, it was shown that all *Leishmania* stocks examined had a different karyotype[86], suggesting that this approach may not be of

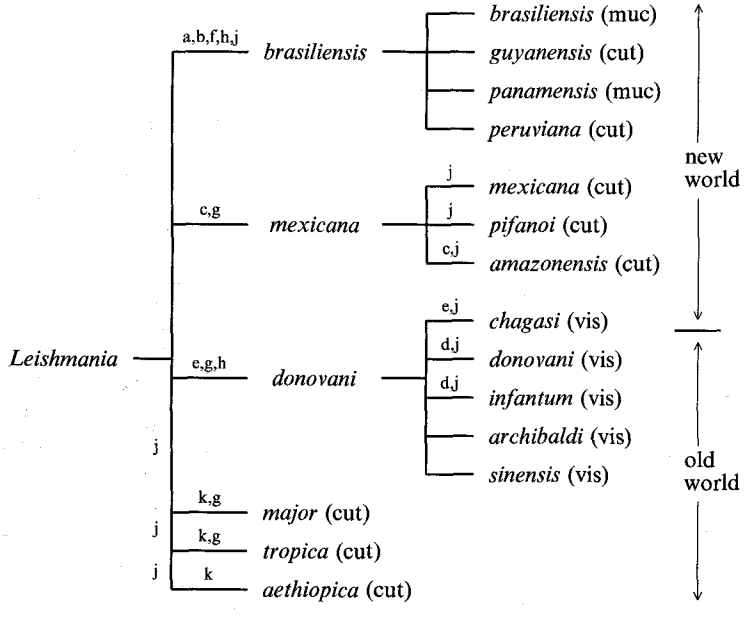

Figure 5. Diagram of the levels of discrimination of the *Leishmania* species and subspecies using kDNA probes. Letters refer to references describing the discriminating probes. a) ref. 100, b) ref. 101, c) ref. 75, d) ref. 48, e) ref. 50, f) ref. 49, g) ref. 97, h) ref. 38, j) refs 2, 3, 5, k) ref. 44. The diagram also indicates some of the epidemiological features of the *Leishmania* subspecies. The abbreviations indicated represent the following disease states: muc, mucocutaneous; cut, cutaneous; vis, visceral.

great value because the plasticity in the genome can make it difficult to assess relationships.

Analysis of *Leishmania* species using nuclear DNA probes has also been used as an approach to investigating the epidemiology of leishmaniasis. The basis of this approach is that it is desirable to have available DNA probes which show low intra-species diversity but high interspecies diversity, in such a way that similarities and relationships can be deduced. On this basis probes have been isolated which distinguish, on the basis of restriction enzyme polymorphisms, the species causing visceral leishmaniasis from those causing the other pathological manifestations[92], and also probes which identify each of the major lineages of *Leishmania* spp.[7].

The greater part of the work carried out using molecular probes has been undertaken using probes derived from kDNA sequences. Species-specific DNA probes have been obtained for a number of the *Leishmania* species: *L. donovani*[4,38,50], *L. major*[44,87], *L. tropica* and *L. aethiopica*[38,44], *L. brasiliensis*[2,4] and *L. mexicana*[2,75].

As yet, few studies have made use of these probes to answer specific epidemiological questions. However, some work has begun in this area. Barker et al.[4] have developed an in situ hybridisation approach which allows them to identify different *Leishmania* species within vectors. Similarly Jackson et al.[38] developed a touch blot analysis using infected hamster organs and blood which could be further developed to examine wild animal species as potential secondary or reservoir hosts. In other work, kDNA probes have been used in a study of infected sand-flies to distinguish those carrying the pathogenic *Leishmania* parasites from those carrying the non-pathogenic *Endotrypanum* species[76].

3.2.2 Future approaches

Epidemiological studies on leishmaniasis over the last 20 years have been carried out using classical approaches. Such approaches have gone a long way towards qualitatively identifying and describing epidemiological parameters, such as human pathogenic species, vectors, alternative hosts and transmission modes[47]. While it could be said that the contribution of molecular approaches to our overall understanding of leishmaniasis is not large, it is clear that the basic ground-work of producing reagents (DNA probes) and developing methods has now been carried out. In the future this will allow the epidemiologist to use such reagents for large-scale analysis in the field. It is likely that future studies will be designed with a view to making quantitative statements on parasite loadings in different hosts, vectors and transmission rates.

4 Other parasite systems

In the previous sections we have reviewed the application of molecular methods to two specific examples of epidemiological systems, the African trypanosomes and *Leishmania*. It is not possible, in a review of this size and nature, to discuss the contributions of molecular approaches in all parasite systems. However, we will highlight some of the applications in systems where different epidemiological questions arise.

4.1 Parasitic protozoa

In general, the approaches and techniques that have been applied to studies of the other parasitic protozoa are similar to those used for the African trypanosome and *Leishmania* systems. DNA probes have been developed in a number of organisms for diagnostic and identification purposes, for example in *Babesia*[73] and *Plasmodium*[3,20,58,66,94]. Restriction endonuclease polymorphism has also been extensively used for strain characterisation in *Plasmodium*[25] and *Naegleria*[17]. In *Trypanosoma cruzi*, the causative agent of Chagas' disease in South America, considerable efforts have been made to characterise strains by examining restriction endonuclease polymorphism in the kinetoplast DNA[63,64].

The use of the polymerase chain reaction as a tool for characterising parasites has, as yet, only been reported in a few instances although it is clear that in the near future there will be a wealth of published material on this subject. One such study concerns the characterisation of different isolates of the African cattle parasite, *Theileria parva*, which causes East Coast Fever. In this study, oligonucleotide probes were made for a variable region of the *T. parva* genome and this region amplified, using the PCR technique, in order to define differences between isolates of different origins[1].

At present, many of the molecular studies carried out in protozoa parasites have been aimed at producing diagnostic reagents and have not, as yet, been applied seriously to epidemiological questions. In the future, it is envisaged that these diagnostic probes will be used, in conjunction with techniques such as the polymerase chain reaction, to assess parasite distributions and movement between vectors and hosts.

4.2 Parasitic helminths

The term 'parasitic helminths' loosely encompasses a multitude of organisms of medical and veterinary importance, and a large number of animal parasites for which man has only a passing curiosity. The degree to which the epidemiology of each of these organisms is understood largely reflects the economic importance of the individual organism. This situation is reflected in the application of molecular epidemiology to these organisms. In a large proportion of the parasites in this group, molecular approaches to epidemiology have never been carried out and probably never will be. In a handful of systems, such studies are just beginning and in very few cases, such as that of the schistosomes, considerable effort has been made in applying molecular techniques.

Another factor influencing the extent to which molecular methods have been employed is their importance for a particular system. In many cases, the classical approaches to defining life cycles, hosts, vectors and transmission have been perfectly adequate for the epidemiologist. The fact that helminths are multicellular means that the basis for morphological definitions is more clear-cut; in addition their resulting greater mass (compared to the parasitic protozoa) mitigates, to some extent, the need for high sensitivity in the detection of the parasite. Thus molecular identification is more important for the protozoa, which are often morphologically similar, than for epidemiological systems involving the parasitic helminths. Another difference between the parasitic helminths and the parasitic protozoa involves sexual processes. While it is known that sexual processes occur in some protozoan parasites[41,88,89,95], it is not known to what extent such processes contribute to the epidemiology of these parasites. However, in all parasitic helminths, the existence of mating, sexual dimorphism, parthenogenicity and hermaphroditism is clear, and these processes almost certainly contribute significantly to the epidemiology of these parasites. Thus, the epidemiologist may need to take account of the relative proportions of different sexes and mating frequencies as contributory factors in the transmission, distribution and variability of the parasite.

4.2.1 Schistosomes

Schistosomes are flatworms belonging to the trematode group (flukes), which cause a chronic debilitating disease in humans. This group of flukes has a life cycle which involves two hosts: the mammalian host and a snail species. Some 18 species of *Schistosoma* exist, 5 of which are important human pathogens, and each species differs from the others on numerous criteria including host specificity, infectivity, pathogenicity, growth rates, and egg production. In many areas and within many hosts these different species co-exist, and thus it becomes important to the epidemiologist to understand the interrelations of the species and the overall contribution of each species to the disease pattern found in a given area.

As with many of the other parasite systems, identification of the parasites responsible for causing the disease is the primary objective for the application of molecular techniques. In general, the identity of the human-infective species can be determined when sufficient information is available on a number of characters such as egg shape, geographical location, intermediate host and others. Difficulties arise, however, when the epidemiologist wants to turn the question around and examine specific epidemiological factors associated with a given species – for example, examining which snail species act as hosts to the given parasite species or collecting information on differences in pathogenicity, growth rate, transmission rates and modes, reservoir hosts and other characters associated with a given species. The other major difficulty arises when only the cercarial stage (the stage which enters the snail) is available for analysis and the species and the sex of the cercariae need to be determined. Thus, much effort has been made by molecular parasitologists to devise molecular methods to solve these problems.

As with the trypanosomes and *Leishmania*, DNA hybridisation has been used as an aprroach to the identification of the *Schistosoma*. Simpson et al.[83], using cloned DNA sequences derived from the ribosomal RNA genes of *Schistosoma mansoni*, showed that intraspecific variation could be observed, and furthermore, it was shown that this variation could be used to discriminate between three Puerto Rican isolates[84]. These isolates differed from each other with respect to their abilities to infect the snail host. These studies demonstrated another potential epidemiological use for this system. McCutchan et al.[57] extended this analysis to distinguish strains of *S. mansoni* from different continents, to discriminate between the species, *S. mansoni, S. haematobium* and *S. japonicum*, and finally to distinguish different sexes.

Further development of the ribosomal RNA gene system was carried out to provide discriminating markers for the identification of six species of *Schistosoma* related to *S. haematobium*[96]. The potential of this approach for the epidemiologist can be illustrated by considering this group of schistosomes. For example, *S. mattheei*, a schistosome species normally found as a cattle parasite, can sometimes be found in man in association with the human parasites *S. mansoni* and *S. haematobium*. Both *S. mattheei* and *S. haematobium* use the same species of snail as an intermediate host, thus it is of considerable interest to be able to distinguish the morphologically identical cercarial stages in the intermediate host in order to determine the contribution of that host to human disease in a given area. Using the ribosomal RNA genes this discrimination is possible, and the technique has been extended so that discrimination between a number of other species of schistosomes and the determination of their sex is now possible at the cercarial stage[97,98]. This procedure requires the use of some 10^3 cercariae, which means that the technique could be used on cercariae from a single snail host.

4.2.2 Filarial worms

The epidemiological questions pertinent to the filarial worms are, broadly, similar to those illustrated previously in other parasites. The filarial worms comprise a large number of species and genera which infect both man and animals. As would be expected, the main epidemiological focus has been concentrated on those worms which infect man. The main human parasites are *Wuchereria bancrofti* and *Brugia malayi* which are responsible for elephantiasis, *Onchocerca volvulus* which causes 'river blindness' and *Loa loa* the 'eye worm' which is responsible for swelling in the eyes. In general, these helminths are clearly distinguishable from each other and from other species for whom man is not a host. However, in some cases

closely related animal-infective species can be found in the same vectors as the human-infective species, and in many cases these parasite stages are indistinguishable. This is the case in *Onchocerca* spp. where a number of related species use *Simulium damnosum* as a vector. The procedure for the daily evaluation of insecticide programs, carried out to combat onchocerciasis, measures the annual transmission potential. This figure is based on the total numbers of infective larvae capable of being transmitted to man per year. Such figures are dependent on vector numbers and also on the parasite load carried by the vector. This latter figure will be grossly overestimated if the vector contains species of animal infective *Onchocerca* larvae. A number of groups have developed DNA probes[62,67,81] or synthetic oligonucleotide probes[34] for the identification of the species of the larvae.

Similar approaches have been used in the identification of the vector stages of *Brugia malayi* and other human and animal filarial worms[82,99]. Specific probes have also been used as an approach to the identification of variants of the same species. For example, in the case of *Onchocerca* two strains exist which have different epidemiological attributes; the forest and savanna forms. Erttmann et al.[24] identified a DNA sequence which could be used to discriminate between these two forms. In addition, restriction fragment length polymorphisms have also been employed to distinguish between strains and species of filarial worms. For example, Cameron et al.[13] compared restriction enzyme banding patterns between various human and animal species of filarial worms and showed that they could be identified on the basis of their banding patterns.

4.2.3 Cestode worms

Very little in the way of molecular epidemiology has been applied to these parasites, with the exception of the development of DNA probes to distinguish species and strains. The parasites to which this approach has been most extensively applied are *Taenia* spp.[74] and *Echinococcus* spp.[59,102].

5 Future perspectives

As has been described, there has been a boom in the development of molecular biological probes for the study of parasite epidemiology, and many of these are now available to be added to the battery of techniques available to the epidemiologist. At present, the field of molecular epidemiology has reached the stage of applying technologies and acquiring an understanding of the ways in which molecular biological techniques could be used to answer epidemiological questions. For example, for many organisms there has been a search for diagnostic DNA probes which can be used to distinguish epidemiologically distinct groups of parasites. Also the analysis of variation in parasite DNA, at a number of levels, has been used to investigate relationships between given parasites. Furthermore, technologies have been developed to make such investigations more rapid, convenient, cost-effective and sensitive and thus more suitable for use in the field.

Much of the work carried out so far has concentrated on the characterisation of small numbers of parasite strains with very little reference to the overall distribution of the given parasite. Thus, as yet, molecular epidemiology has contributed rather little 'real epidemiological data' compared with classical epidemiological approaches. However, it is now clear that the current investment in molecular epidemiological research has produced a number of useful reagents or tools which will allow the epidemiologist to apply these technologies on a very large scale in the field, and this could make a valuable contribution to the epidemiology of given parasite. In particular, the molecular approaches based on the Polymerase Chain Reaction technique, with its high sensitivity and specificity, promise to launch the field of molecular epidemiology into a new era of data gathering and analysis. Finally, this phase will merge into a third phase where the data obtained from molecular approaches can be used by mathematical epidemiologists, clinicians and veterinarians, public health authorities and governments in the development of strategies for effective control measures for parasitic diseases, and allow the evaluation of the effectiveness of such measures when they are applied.

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Molecular diagnosis of parasites

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Summary. New developments in molecular biology have generated exciting possibilities for improved diagnosis of parasitic diseases. Through gene cloning and expression and peptide synthesis, defined parasite antigens can be produced in vitro for use in serodiagnosis, while nuclear hybridization techniques offer a vastly improved approach to identification of parasites in the tissue specimens of infected hosts as a means of diagnosis. Furthermore, the advent of the polymerase chain reaction technique has made it possible to increase the sensitivity of nuclear hybridization techniques, through amplification of target DNA sequences of the parasites in test material, by in situ synthesis of these sequences prior to hybridization with the diagnostic probe. Finally, through the use of monoclonal antibody technology, it is possible to design highly specific and sensitive serological assays, as well as assays for parasite antigen detection in tissue fluids and in the excreta of infected hosts, as a means of diagnosis.
Key words. Parasites; DNA; oligonucleotides; probes; monoclonal antibodies; diagnosis.

Introduction

Diagnosis is an essential element in the management of disease, both at the level of individual patient care and at the level of disease-control in populations. The diagnostic tests used should be simple, rapid, specific and highly sensitive, and ideally they should differentiate between closely related parasites if the disease syndromes they cause require different management approaches. They should be suitable for field application, and the cost for the tests should be within the means of the communities affected by the disease. With most parasitic diseases, the tests currently in use do not meet these criteria.

Recent developments in molecular biology, however, have opened new avenues for a vast improvement in parasite detection. Firstly, recombinant DNA technology offers a means for cloning and expression of genes encoding specific parasite antigens needed for use in designing highly specific and sensitive diagnostic tests. Secondly, nuclear hybridization techniques have shown a high potential for use in parasite diagnosis with increased specificity and sensitivity. Thirdly, monoclonal antibody technology has also given rise to highly sensitive and specific assays for detection of parasite antigens in the tissues of the infected host.

The aim of this review is to highlight the potential for these new technologies in parasite diagnosis. The emphasis will be placed on disease diagnosis rather than parasite characterization per se, which will be reviewed in a separate article in this journal issue[9].

DNA diagnostics

Parasite DNA is made of nucleotide sequences which can be divided into two broad classes, namely, those sequences which exist in single copy and those which are in multiple copies within the parasite genome. The repeated DNA sequences, known as 'satellite' DNAs, have no known cellular function and typically they contain a simple concensus sequence that is repeated thousands and often millions of times. In *Trypanosoma brucei* and *T. cruzi*, for instance, these elements constitute 12% and 9% of the genome, respectively[3, 4, 11, 23].

Parasite species-specific differences can be found in either class of DNA sequences, but it is among the repetitive DNA sequences that the most convenient ones for diagnostic purposes are to be found. The repetitive sequences give good sensitivity when used as hybridization probes for detection of complementary sequences in small amounts of test DNA extracted from parasites or in whole organisms.

The principle of DNA hybridization is that a single-stranded DNA fragment containing the specific DNA sequences, such as parasite species-specific sequences, is identified and, preferably, purified. It is then labelled