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Diagnosis of parasitic disease

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Summary: Diagnosis of parasitic diseases requires highly sensitive and specific tests. In many cases the identification of parasites concerns their epidemiology and it is important to distinguish between species and subspecies. Conventional techniques including serology and microscopy do not always meet these requirements. The principle of nucleic acid probes is that a specific sequence of the parasite's DNA is isolated and used in a hybridisation assay to identify homologous parasite DNA from infected material. Since DNA normally remains the same during every stage of the parasite's life cycle this technique has many applications. The use of DNA diagnostics in the identification and differentiation of certain animal parasites is discussed.

KEYWORDS: Diagnosis - DNA hybridisation - Nucleic acid probes - Repetitive sequences - Serology.

INTRODUCTION

It has been estimated that one hundred years from now the population of the earth will exceed ten billion people, about twice what it is today (23). The question that arises is whether or not our species will be able to feed itself and if so, how? Parasites are a major cause of disease of man and domestic animals. More than half the human population live in misery and pain and suffer vast economic losses due to parasites. A possible solution to these problems lies in the development of new agricultural technologies for expanding food production. One of the ways in which livestock production can be increased is by reduction of losses due to disease. Estimates suggest that only a 6% reduction in disease could provide food for an additional 250 million people (97). The situation is most serious in developing countries where 75% of the world's population resides. According to the Food and Agriculture Organisation, it is estimated that up to 70% of the world's livestock resources exist in these regions, yet they account for only 30% of the world's meat output (97).

The effective control and treatment of parasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic protocol. The usual immunological tests do not always meet these requirements and are not able reliably to distinguish between closely related species. In fact, diagnostic serology is in many cases not specific (131). Molecular biology has provided new methods for identifying parasites and their

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vectors; methods that reach the genomic organisation of the organism and often replace traditional approaches because of their rapidity, sensitivity, ease of use and accuracy. Yet, perhaps more importantly, identification using DNA probes is rapidly moving beyond the laboratory environment and becoming a technique readily available for field epidemiologists and controlling bodies. The subject of this review is the application of nucleic acid diagnostics to the detection and classification of economically important rickettsial, protozoan and helminthic parasites of domestic animals and wild ruminants.

PRESENTLY USED DIAGNOSTIC TECHNIQUES

Epidemics among animals are still one of the main obstacles to the increase in production of animal protein and contact with vector-borne diseases often results in considerable losses (104). The most reliable way to diagnose a parasitic infection is by detection and identification of the infecting organism. Laboratory diagnosis is a basic step in the evaluation of the disease process, at times confirming a presumptive diagnosis or providing evidence of an unsuspected agent of disease. However, failure to demonstrate or recover a parasite does not exclude the possibility of infection. Many of these parasites, especially the protozoa, can be identified only by microscopic examination. This requires considerable skill and apart from being time-consuming and labour-intensive the method has limitations. This also applies to most of the commonly used serological techniques.

Direct identification

Parasitic infections are usually diagnosed from samples of faeces, urine, blood and tissue.

Faeces: Evidence of intestinal parasitism, apart from the general clinical signs, is obtained from faecal or post-mortem examination. There is no "general" technique, nor is there an "ideal" technique for the microscopic examination of faeces. In fact, a reliable diagnosis can usually be made only by using a combination of several techniques (14), such as:

– *Direct saline smear:* This procedure provides only an indication of the parasites present and cannot be used quantitatively. To prepare a direct faecal smear a drop of saline is placed in the centre of a microscope slide and a 2 mg faecal sample is suspended in this drop without spreading it. This is then covered with a coverslip and examined (13).

– *Stained smears:* This type of smear is essential for accurate diagnostic detail and is also suitable for long-term storage for record purposes. The two stains generally used are haematoxylin and trichrome (13, 60).

– *Parasite concentration in faeces by flotation:* This is used for the identification of oocysts of coccidia (14, 105) and helminth eggs (39, 115). One drawback of this technique is that there is not always a direct relationship between the number of eggs in faeces and the number of parasites present.

Urine: Examination of urine sediment is used mainly for the identification of *Encephalitozoon cuniculi* (116) and *Schistosoma* eggs (83).

Blood: Testing is used to identify the various stages of blood parasites and is routinely applied to diagnose malaria, theileriosis, babesiosis, anaplasmosis, ehrlichiosis, trypanosomiasis and most types of filariasis (14). Trypanosoma can also be diagnosed with wet smears. Depending on the application and purpose, two types of blood films are used.

Thin blood films (13) are useful for studying morphological changes of blood cells and blood parasites. The main disadvantage is that sample volume is small, making the detection of low parasitaemia and carrier animals difficult.

Thick blood films (35, 70) contain 6 to 20 times as much blood per unit area as thin films. The thick film is suited for rapid diagnosis of parasitaemia that is too low to be detected with thin films. This method is not suitable for detailed morphological studies of parasites.

Tissue: Recovery of protozoa or helminths from biopsy material is often an important aid to diagnosis. Lymph node, spleen, liver, lung, bone marrow or spinal fluid biopsies are frequently used to diagnose a variety of diseases (13).

Post-mortem: Post-mortem examination is currently the most effective way to accurately diagnose helminth infection (7, 92, 119). Brain-cortex smears are examined for babesiosis (*Babesia bovis*), turning sickness (cerebral theileriosis) and cowdriosis.

Indirect identification

All methods for the direct identification of parasites fail if the parasite density in the specimen is below the sensitivity of the method employed, or if the parasite cannot be directly demonstrated due to the life cycle in the host (e.g. toxoplasmosis, echinococcosis and cysticercosis). In such cases indirect methods must be used.

Ideally, serology should allow differentiation between recent and latent infections and should be able to demonstrate whether an animal is a carrier as well as the elimination of the parasite after therapeutic measures have been applied. In practice, serology seldom achieves this. There is a lack of dependable tests for the serodiagnosis of parasitic disease in animals. Commercially available tests present problems in reliability and interpretation of results. Costly, specialised apparatus is often needed to perform the tests (31). With the majority of tests, specificity is not satisfactory and cross-reaction seldom allows distinction between closely related organisms. However, monoclonal antibodies may overcome this problem and enable the identification of highly specific antigen sites.

Tests commonly in use include the complement fixation test (CFT), immunodiffusion (ID), indirect haemagglutination (IHA), indirect immunofluorescent antibody test (IFA), enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) (137) (Table I). Less frequently used tests include latex agglutination, capillary agglutination and card agglutination (137). Most of these tests are based on the reaction of antibodies with antigenic parasite components (whole or soluble) resulting in antigen-antibody complexes. These complexes are detected by the addition of antiglobulins coupled to fluorescein and rhodamine dyes, radioisotopes or enzymes.

Because antibodies can persist for a long time after elimination of the parasites, another drawback of serology is that the demonstration of a specific antibody does not indicate the present parasitological status of the host. The results of a serological

test are therefore retrospective. Serodiagnosis of helminth infections is even more difficult because cross-reactivity is more the rule than the exception. Only highly purified, defined antigens allow serodiagnosis to the genus level; species-specific serodiagnosis is unusual (137). Antigen-capturing ELISA can be used for demonstrating infection with trypanosomes and *Cowdria* for instance.

Antigen

The application and reliability of serology is in many cases dependent on the availability of sufficient amounts of high quality antigen. The inability to culture many parasites *in vitro* and the lack of suitable animal models often hamper antigen preparation.

TABLE I

Commonly used serological assays

Assay	Applied to	Reference *
Complement fixation test (CFT)	Anaplasmosis	91
	Babesiosis	71, 34, 102
	Helminthiasis	53
	Toxoplasmosis	100, 134
	Trypanosomiasis	41
Indirect fluorescent antibody test (IFA)	Anaplasmosis	79
	Babesiosis	50, 68, 98
	Besnoitiosis	32
	Cowdriosis	26
	Helminthiasis	52
	Malaria	113
	Theileriosis	61, 103
	Toxoplasmosis	146, 19
	Trypanosomiasis	122, 147, 143
Radioimmunoassay (RIA)	Babesiosis	54
ELISA	Anaplasmosis	12
	Babesiosis	90
	Besnoitiosis	107
	Cowdriosis	126
	Helminthiasis	89, 42, 43, 52
	Toxoplasmosis	129
	Trypanosomiasis	130, 63
Rapid card agglutination test (CAT)	Anaplasmosis	4
	Babesiosis	120, 5
	Trypanosomiasis	69

* The following review articles could also be consulted (121, 22, 137, 109, 145).

NUCLEIC ACID-BASED DIAGNOSTICS

The use of nucleic acid probes in the diagnosis of parasitic infections is based on the premise that every organism carries unique DNA sequences which differentiate

it from other organisms. A diagnostic probe is developed by identifying and isolating these sequences. Two issues are of major importance in nucleic acid diagnostics: the specificity and sensitivity of the probe, i.e. can the probe differentiate between species of parasite and what is the lowest level of infection that can be detected?

Sensitivity: Today's technology makes it possible to detect even a single base change in complex genomes (117). Sensitivity of detection depends on the abundance of unique sequences in the parasite genome. The most sensitive DNA probe is one originating from total genomic DNA since all the nucleic acid sequences of the organisms would be present in such a genomic probe. Genomic probes for *Plasmodium falciparum* can detect as little as 40 parasites per microlitre of blood (10). However, since the host cell genome is on average 1,000 times larger than the parasite genome, the major problem associated with genomic probes is that of host cell DNA contamination. It is therefore important to isolate uncontaminated parasite DNA as a prerequisite to the development of this type of probe. This is not always easy to achieve. Yet another problem is that genomic probes may hybridise to DNA from closely related species. For example, *Leishmania major* kinetoplast DNA (kDNA) cross-hybridises with virtually every other *Leishmania* spp. kDNA (9).

With few exceptions, biological functions have not been determined for the highly repetitive DNA sequences that constitute a large percentage of parasitic genomes (1). Such repetitive DNA, however, is excellent for DNA-based diagnostics because of its abundance and rapid evolution that allows sensitive and specific detection (67). These sequences should be able to detect parasite DNA at high sensitivity. Highly repeated DNA can be detected by standard hybridisation techniques with a sensitivity of only one order of magnitude less than genomic DNA probes (10).

Specificity: The specificity of nucleic acid probes is due to the ability of DNA to denature under certain conditions and to renature in a highly specific manner. DNA can easily be denatured by heat or by increasing the pH. This leads to a reversible separation of the two complementary strands which will reassociate under the appropriate conditions to reform a duplex molecule. Two separated polynucleotide chains will reassociate only if they are complementary. This reaction is termed nucleic acid hybridisation and occurs both with DNA and RNA.

Since the requirement for complementarity is not absolute, non-identical but related nucleic acids can participate in the hybridisation reaction. However, these hybrids are less stable when exposed to low ionic strength buffers or heat allowing the distinction between precisely and imprecisely matched duplexes. The specificity of a nucleic acid probe therefore depends on its base sequence. By using the Southern transfer technique (110) it is possible to detect a single-copy gene with a probe of less than 20 nucleotides long (84).

Hybridisation in the detection of parasite DNA

It is a fundamental requirement for DNA diagnostics that techniques exist which allow the detection of parasite sequences in individual animals. This task is complicated by the location of the infecting parasite during different stages of the life cycle.

Detection of membrane-immobilised sequences: This is by far the most common way to detect hybrid formation. In the Southern blot technique (110) DNA is digested with restriction endonucleases that specifically cleave at their individual recognition sequences. These fragments are separated according to their length by electrophoresis

to yield bands in an agarose gel. The DNA molecules are then transferred upwards out of the gel onto a solid support such as nitrocellulose or nylon. The position of the DNA bands transferred to the filter corresponds to their original position in the gel. Bound DNA can then be probed with a suitably labelled radioactive probe and the binding of that probe can be visualised by autoradiography. A similar technique termed Northern blotting (140) has been developed for RNA/DNA hybridisation.

The dot-blot or slot-blot technique was developed specifically for the rapid screening of large numbers of samples (51). The specimen of interest is immobilised on nitrocellulose or nylon filters by direct application. One advantage of this technique is that a known amount of sample DNA is applied to the filter making quantitation possible. Because many samples can be immobilised very quickly the technique can be used to screen large numbers of samples. A major disadvantage, compared to Southern transfer, is that total DNA is analysed rather than DNA fragments generated by restriction endonuclease digestion. There is therefore no simple way of distinguishing between cross-hybridisation of the probe to other nucleic acids present in the sample. Also, hybridisation backgrounds will be higher than in Southern blots (75). The first practical application of this method was for the detection of Epstein-Barr virus in lymphoblastoid cells (17). Since then there have been many applications of dot-blotting in different organisms such as viruses (111, 112), bacteria (46, 78, 122), protozoa (10, 18, 32, 81, 144) and helminths (55, 65, 96, 132).

A modification of dot-hybridisation, so-called sandwich hybridisation, has also been described (8). This technique uses two probes that hybridise to adjacent sections of the target DNA. One probe is bound to the solid support so that when the target DNA hybridises to it, it is also bound to the support. The second probe is labelled and will not hybridise to the first probe. In the absence of target DNA, the labelled probe will not bind to the support. If the target is present then both probes are bound to the support.

Detection of target sequences in solution: Several rapid techniques that do not require nucleic acid purification or immobilisation have been developed. Probe/target hybrids may be selectively isolated on a solid matrix such as hydroxyapatite (57). Probe nucleic acids may be immobilised on a solid support and used to bind target sequences from solution. The target sequence can be selected with a second, labelled probe that is displaced from the support by the target in a competition-type assay (26).

Labelling nucleic acid probes

In order to apply probe-based diagnostics it is necessary to have a sensitive means of detecting the probe. This can presently be achieved by radioactive as well as non-radioactive labelling. Radioisotopes offer the possibility of incorporating many labels per probe, increasing the sensitivity of detection and thus resulting in very sensitive probes. This is usually the method of choice.

Radioactive labelling: The most common radioisotopes used in nucleic acid labelling are ^{32}P and ^{35}S (Table II). Because of its high energy, short scintillation counting times and short autoradiographic exposures, ^{32}P is the isotope of choice for nucleic acid hybridisation in solution and on filters. Low backgrounds and low energy made ^{3}H the isotope of choice for *in situ* hybridisation. However, a drawback of this low energy is the very long autoradiographic exposure times of slides. Because of this, ^{125}I is now often used instead.

TABLE II

Radioisotopes for probe labelling and major labelling methods

Isotope	1/2 life	Type of decay	Energy	Labelling methods
^{32}P	14.3 days	ß ß	High	Nick translation (94) Random priming (30)
^{3}H	12.3 years	ß	Low	Phage polymerase (27, 77)
^{125}I	60.0 days	γ	Med.	Transcription (44)
^{35}S	87.4 days	ß	Med.	End labelling (106, 44)

Isotope	Enzymes required	Template	Method of detection
^{32}P	DNase/Polymerase I	ds DNA	Autoradiography, Scintillation counting, Gamma ray spectroscopy
	Klenow polymerase	ss DNA	
	Reverse transcriptase	ss DNA	
^{3}H	SP6/T7/T3/T5	ds DNA	
^{125}I	RNA polymerase	ds DNA	
^{35}S	T4 polymerase Kinase Klenow polymerase T4 DNA polymerase	ds DNA	

Non-radioactive labels: The hazards, expense and instability of radioactive labels have led to a search for alternative non-radioactive DNA labelling and detection methods. Considerable effort has been devoted to the development of hybridisation methods using non-radioactive probes (44, 58, 99). The main objective was to simplify and shorten the protocol while increasing the sensitivity of detection and resolution compared to conventional radioactive procedures. Methods for non-radioactive labelling of DNA include the incorporation of reporter molecules such as biotin (80), acetylaminofluorenzyl modified guanosine (118) and sulphonated cytidine (88). Detection of these molecules is with an appropriate antibody, or in the case of biotin, with avidin or strepavidin coupled to a colorimetric, fluorimetric or chemiluminescent signal. Direct cross-linking of probes to enzymes which act as signal generators has also been described (49, 93).

Compared to radioactively-labelled nucleic acids, biotinylated DNA or RNA probes have the following advantages: they can be stored for long periods without loss of sensitivity; they require no special safety or disposal precautions; the detection procedure is rapid and they are compatible with several different detection systems. However, the major disadvantage of biotin, and of other non-radioactive labels, is their low sensitivity compared to radioactive labels. Depending on the skills of the user, detection varying between 5 and 50 pg is usually obtained. This would not be sufficient for reliable detection of, for example, single copy genes in mammalian genomes where a detectability of 3 pg is required (6). A 7 kb insert from Epstein-Barr viral DNA has been detected by immunofluorescence in human metaphase nuclei (59) and two copies of a 5 kb fragment of the same viral DNA were detected in

interphase nuclei (59). Using non-radioactive labelling Garson *et al.* (36) reported the detection of a 7 kb nerve factor gene in the pBR322 plasmid by *in situ* hybridisation. Although non-radioactive labelling has been used successfully in different laboratories for dot-blot, Southern and *in situ* hybridisation (9, 63, 125) it is still not the method of choice for parasite detection. Lopez *et al.* (62) reported the detection of low numbers of *Leishmania* with non-radioactive probes and recently new non-radioactive detection systems have been described which claimed detection of better than 0.5 pg DNA (16). Whether these systems will be suitable for routine use remains to be seen.

Probes for the detection of Rickettsia-like organisms

Anaplasmosis, caused by the rickettsial parasites *Anaplasma marginale* and *Anaplasma centrale* (87), is the most widely distributed of the tick-borne haemoparasitic diseases. This disease occurs worldwide and is enzootic in tropical and subtropical regions. It has been estimated that over one billion cattle are at risk (38, 45). Anaplasmosis in sheep is caused by a third species of *Anaplasma, Anaplasma ovis*. Although not as economically important as bovine anaplasmosis, losses have been reported from time to time.

Anaplasmosis is characterised by severe anaemia, weight loss, decreased milk production and, in a number of cases, death. Surviving animals become lifelong carriers of the parasites and are a constant reservoir of infection (86). A rapid and sensitive diagnostic test is essential to monitor the disease and to understand its epidemiology.

Probes specific for *A. marginale* (38, 128) and *A. centrale* (128) have been isolated. The *A. marginale* probe described by Goff *et al.* (38) could detect 0.01 ng DNA, which is equivalent to a parasitaemia of 0.000025% (28). This probe could also detect the presence of the parasite in the infected tick vector. The probes described by Visser and Ambrosio (128) could detect 127 ng *A. centrale* DNA and 8 ng *A. marginale* DNA. These probes could be used on blood from field samples, to follow experimentally induced parasitaemia in cattle. The carrier state of anaplasmosis is presently poorly understood and a key epidemiological question is what the prevalence is among carriers in enzootic regions. Recently a DNA probe that can detect *A. ovis* has been isolated (Visser and Ambrosio, manuscript in preparation). This probe can detect 7.5 ng *A. ovis* DNA in infected ovine blood and when used together with the *A. centrale* and *A. marginale* probes should be very useful in epidemiological studies of these parasites.

Heartwater (cowdriosis) is a tick-borne disease of ruminants caused by the rickettsia-like parasite *Cowdria ruminantium*. This disease is endemic in Africa and the Caribbean islands and poses a serious threat to livestock in the United States where potential vectors are present, but not the disease (11, 123). As with anaplasmosis, the existing diagnostic tests for cowdriosis are unreliable. Blood smears are of no value and the disease cannot be diagnosed with certainty (123, 124).

The isolation of nucleic acid probes for *C. ruminantium* has been hampered by low yields of organisms from infected animals. *C. ruminantium* has recently been successfully cultured in a bovine endothelial cell line (15). This has allowed the construction of genomic libraries of this parasite and *Cowdria*-specific DNA sequences have been isolated (3, 139).

Recently Welburn and Gibson (138) reported the isolation of repetitive DNA probes from a rickettsia-like organism symbiotic in tsetse flies (*Glossina* spp.) that is reportedly associated with the presence of *Trypanosoma* in the fly midgut. This probe identified as few as twenty organisms in the fly host and therefore may provide a rapid method of assessing the potential susceptibility of a tsetse population to *Trypanosoma brucei* and *Trypanosoma congolense* infections. Probes have not been isolated for other Rickettsial organisms that infect animals, such as *Ehrlichia*, *Cytoecetes*, *Rickettsia conori* and *Coxiella burnettii*.

Probes for the detection of protozoan parasites

The diagnosis of most protozoan diseases is performed by a variety of serological tests and by microscopic examination of stained blood smears. The sensitivity of the latter method varies widely and it is not suited for routine applications because of its time-consuming nature.

The development of a DNA probe for the diagnosis of *P. falciparum* has been described in detail by Barker *et al.* (10). This probe could detect 10 pg *P. falciparum* DNA, representing 40 parasites per microlitre of blood (10). Plasmids containing cloned repetitive DNA have been used to detect as little as 25 pg of *P. falciparum* DNA (33). These hybridisation probes contain additional plasmid and parasite nucleotide sequences which may lessen the specificity of the hybridisation reaction. For this reason, a synthetic DNA probe was developed and used to detect *P. falciparum* (64). This probe achieved sensitivities comparable to those reported for repetitive DNA probes and could detect 50 pg DNA (33).

Repetitive DNA fragments from *P. falciparum* have been evaluated as species-specific probes for clinical use (24, 33, 82, 114, 135) and repetitive DNA probes for the identification of *P. falciparum*-infected mosquitoes have been described (24). Probes have also been used to differentiate between rodent malaria species and strains (127) as well as to analyse the dynamics of infection based on banding patterns specific to different parasite lines (108).

Babesiosis is a haemoparasitic disease common in ruminants, horses, dogs and, occasionally, humans and is caused by *Babesia bovis*, *Babesia bigemina*, *Babesia divergens*, *Babesia ovis*, *Babesia motasi*, *Babesia equi*, *Babesia caballi*, *Babesia canis*, *Babesia microti* and other species. DNA probes have been isolated for the detection of *B. bovis* (65), *B. equi* (85) and *B. caballi* (Posnett and Ambrosio, manuscript in preparation) and a synthetic probe for *B. bovis* is being developed (65). Probes for the other *Babesia* species have not been reported. A synthetic probe for *B. equi* based on a highly repetitive sequence is currently being evaluated for routine use (Posnett and Ambrosio, unpublished data).

The *Theileria parva* complex of tick-transmitted intra-cellular parasites are the causative agents of East Coast fever, Corridor disease and Rhodesian malignant theileriosis. Approximately fifteen million cattle over large areas of Africa are at risk (47). The complex has been divided into subspecies but the phylogenetic significance of this is not clear. Serological characterisation of these parasites is difficult and the need exists for rapid and sensitive tests that can differentiate subspecies. DNA probes have been isolated which distinguish between *T. parva* strains (47). Probes have also been used to distinguish *T. parva* strains in buffalo and to detect *T. parva* in the tick vector (2, 47).

Trypanosomiasis is a serious threat to human and animal health in most of Africa. The disease affects 37 countries covering approximately 10 million km^2 of sub-Saharan Africa placing over 45 million people and a much greater number of animals at risk (74). Epidemiological studies of African trypanosomiasis are hampered by lack of methods which can be used to rapidly identify and characterise trypanosome species. As with many other protozoan diseases, the methods presently used to distinguish between trypanosomes are laborious and can be expensive (74).

It is difficult, if not impossible, to identify trypanosomes occurring in the host and vector and to distinguish different species on the basis of purely morphological criteria (37). This situation is complicated by the variability in morphological forms (74). A further factor complicating the use of serology in trypanosome identification is the antigenic variation of the organisms (73, 74, 140). The first nucleic acid-based detection and characterisation of trypanosomes was done by using the genes coding for trypanosome variable surface glycoprotein (VSG's) (73, 74, 141). These probes did not cross-hybridise to other trypanosome species and even distinguished groups within the species *Trypanosoma congolense* (72). Nucleic acid probes have thus been developed that can distinguish between closely-related trypanosome species (25, 48, 74, 76) and that can detect the parasite in the tsetse fly vector (37, 56). These probes are all based on repetitive DNA sequences and can detect as few as 100 trypanosomes by dot-blot hybridisation (37).

A variety of probes are available for the detection and differentiation of *Leishmania* species. Recently, double probes which simultaneously identify *Leishmania major* within its squash-blotted sandfly vector *Phlebotomus papatsi* have been reported (9). Touch-blots of biopsy material from infected tissue probed with *Leishmania* kinetoplast DNA showed that it was possible to detect parasite DNA in the presence of large excesses of human or animal DNA (9).

Probes for the detection of helminths

There are two approaches to the use of DNA probes in species identification. One is to generate probes specific for the DNA of a given species that will not hybridise to DNA from others. The other approach is to generate probes that hybridise to DNA of all the parasites, but that can distinguish between DNA of different parasites on the basis of restriction fragment length polymorphisms (RFLP's). This approach is useful because it can yield information about the degree of genetic similarity between different species. In the case of the diagnosis of helminth infections the emphasis is not so much on the rapid diagnosis of low numbers of parasites, but rather on the taxonomy of the infecting worm. DNA probes have been isolated that identify RFLP's in *Trichinella* (21), *Brugia malayi* (55, 142), *Wuchereria bancrofti* (55) and several species of cestodes including *Taenia ovis* and the pig tapeworm, *Taenia solium* (40). *T. solium* and the beef tapeworm *Taenia saginata* can be differentiated with DNA probes (40). This should make a major contribution to epidemiological studies of these cestodes.

Cloned DNA probes have been used to differentiate *Schistosoma mansoni* from *Schistosoma rodhaini* (133). Recently, highly repetitive probes have been isolated that are female-specific and their use in a cercarial sex assay has been described (20, 132, 136). These probes hybridise to a repetitive sequence in the schistosome that occurs 75 times in the genome of the female worm. A method has been developed to apply this sex-specific probe to the rapid determination of the sex of cercariae without the

need for DNA isolation or Southern blotting. Simple assays based on these probes are currently being developed for species-specific detection of these parasites in their hosts (132). A DNA fragment specific to *Echinococcus granulosus*, a hydatid disease organism that infects horses, sheep, pigs and dogs, has been used as a probe to assess the extent of genetic variability within *E. granulosus* (66). DNA probes have also been used to distinguish between horse and sheep strains of *E. granulosus* that also suggest a very close affinity between isolates from cattle and sheep (95).

FUTURE PROSPECTS

There are several advantages of nucleic acid detection of parasites which make this approach suitable for diagnostic tests. Firstly, these tests can be used to diagnose any specific organism and can differentiate between closely-related species. Secondly, they are sensitive and highly suited to detect carrier animals, making probes ideal for epidemiological studies. Thirdly, these tests are versatile and can be used to detect micro-organisms which cannot replicate *in vitro* or that lack protein components.

Immunoassays preferentially detect surface proteins or antigens which are synthesised during replication of the parasite. Serological tests cannot always distinguish between closely-related organisms and may be unable to detect the low parasitaemia, or low levels of antibodies associated with carrier animals. Perhaps the most serious disadvantage of immunoassays is that the diagnosis is retrospective because of the presence of antibodies for varying periods of time after infection.

However, it is unlikely that nucleic acid probes will ever replace serology entirely unless the techniques associated with probe technology are improved. Dependence on radioactive labelling and on short-lived isotopes are major drawbacks to the routine application of probes in the field. The hybridisation protocol itself might be improved to a method that does not depend on filter-immobilised DNA.

The polymerase chain reaction (PCR) (29, 101) allows the amplification of DNA fragments of up to 6,000 base pairs and can be exploited to yield highly sensitive, labelled probes. PCR has made the amplification of single-copy genes in individual parasites possible, yielding sufficient DNA for identification. This technique may also be applied to the amplification of parasite sequences in infected blood resulting in increased sensitivity of detection. This is especially important for the identification of carrier animals.

A number of instruments are available to simplify routine DNA diagnostics. The DNA extractor can prepare DNA suitable for blotting in three hours. DNA synthesisers produce oligonucleotides of up to 200 nucleotides in length in short times. A variety of routine manipulations have been automated including DNA sequencing and several instruments are available that size-separate large DNA molecules (55, 57). Software for computers with specialised co-processors is being specially designed for the task of searching for sequence similarities and sequence patterns in data bases (57).

With the large number of laboratories developing nucleic acid probes it is only a matter of time before solutions are found to the present probe-associated problems. Nucleic acid-based diagnostics will then become a matter of routine in the diagnosis of parasitic disease in the host and the parasite vector in epidemiological studies.

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DIAGNOSTIC DES PARASITOSES. – R.E. Ambrosio et D.T. de Waal.

Résumé: *Le diagnostic des maladies parasitaires requiert des épreuves extrêmement sensibles et spécifiques. L'identification des parasites est importante, dans bien des cas, du point de vue épidémiologique, et il est essentiel de reconnaître l'espèce et la sous-espèce. Les techniques traditionnelles, y compris les épreuves sérologiques et l'examen microscopique, ne le permettent pas toujours. Le principe des sondes d'acides nucléiques est le suivant : une séquence spécifique de l'ADN du parasite est isolée et utilisée lors d'essais d'hybridation pour identifier l'ADN du parasite présent dans le prélèvement examiné. Comme l'ADN ne subit pas de modifications aux différents stades du cycle évolutif du parasite, cette technique trouve de nombreuses applications. L'utilisation de l'analyse de l'ADN pour identifier et différencier certains parasites animaux fait l'objet de la discussion.*

MOTS-CLÉS : Diagnostic - Hybridation de l'ADN - Séquences répétitives - Sérologie - Sondes d'acides nucléiques.

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DIAGNÓSTICO DE PARASITOSIS. – R.E. Ambrosio y D.T. de Waal.

Resumen: *El diagnóstico de las enfermedades parasitarias requiere pruebas sumamente sensibles y específicas. En muchos casos, la identificación de los parásitos es importante para su epidemiología, siendo importante también distinguir especies y subespecies, lo que no siempre es posible con las técnicas convencionales, incluidas las serológicas y microscópicas. El principio de las sondas de ácidos nucleicos es el siguiente: se aísla una secuencia específica del ADN del parásito y se usa en pruebas de hibridación para identificar el ADN del parásito presente en la muestra examinada. Como el ADN no sufre ninguna modificación en los diferentes estadios del ciclo de vida del parásito, esta técnica ofrece muchas posibilidades de aplicación. El artículo trata del uso del análisis del ADN para identificar y diferenciar ciertos parásitos animales.*

PALABRAS CLAVE: Diagnóstico - Hibridación del ADN - Secuencias repetitivas - Serología - Sondas de ácidos nucleicos.

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