

## Review

# The Interplay of Molecular Biology and Veterinary Parasitology: A Need of the Time

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## ABSTRACT

The tools of molecular biology are increasingly relevant to veterinary parasitology. The techniques used with eukaryotic cells are generally applicable to the study of parasites and their hosts. The sequencing of the complete genomes of helminths and protozoa is allowing great advances in studying the biology, and improving diagnosis and control of parasites. Unique DNA sequences provide very high levels of specificity for the diagnosis and identification of parasite species and strains, and PCR allows extremely high levels of sensitivity. The polymerase chain reaction is particularly important for identification and diagnosis of parasites, as well as for many other applications. The accumulation of more information on the DNA sequences of parasites will reveal many more unique sequences which can be used for identification, diagnosis, molecular epidemiology, vaccine development and for studying the evolutionary biology and the physiology of parasites and the host-parasite relationship. The study of the molecular biology of antiparasitic drug receptors, potential targets for chemotherapy, and the molecular genetics of drug resistance is allowing molecular screens to be used with combinatorial chemistry in the search for new antiparasitic drugs, improvements to existing chemotherapeutic families and better diagnosis and monitoring of drug resistance. Molecular biology and the benefits from its application are relevant for veterinary parasitologists in developing countries as well as developed countries and we should introduce aspects of molecular biology to the teaching and training of veterinary parasitologists.

## INTRODUCTION

During the last 20 years, the diagnosis of agents of infectious disease has begun to include the use of nucleic acid-based technologies. Diagnosis of parasitic organisms is the last field of clinical Microbiology to incorporate these techniques, due in part to the expense of new technology as well as a scarcity of these parasites in countries where this research is ongoing. Despite the slow start on developing these assays, the further progress and utilization of nucleic acid-based assays to detect parasitic pathogens has started to play a role in the epidemiology, prevention, and treatment of parasitic diseases. The nucleic acid-based methods are much better than conventional techniques which are being used in the diagnoses of various parasites (Table I).

Molecular biology has brought a revolution in every walk of life and it has provided many areas of the veterinary sciences with powerful new tools which can be used to advance our basic understanding of biological systems and improve health. Understanding the molecular biology of the host-parasite relationship is particularly relevant to the development of veterinary parasitology. Parasites are often small and are frequently difficult to obtain and work with without sacrificing their host. Using molecular biology in veterinary parasitology can help us further our field without

the frequent need to sacrifice animals and can provide unparalleled opportunities to study the regulation of the parasitic way of life with great precision and specificity (Prichard, 1997).

The advancement of veterinary parasitology, the objective of the World Association for the Advancement of Veterinary Parasitology (WAAVP), is intimately dependent on the engagement of veterinary parasitologists with molecular biology (Prichard, 1997). The genetic codes of most parasites and the parasites' hosts, reside in the DNA of each species which can be transcribed into mRNA and then translated into protein. Specific applications of molecular biology in veterinary parasitology include the classification of parasites, diagnosis of infection, understanding parasite pathophysiology, the development of antiparasite vaccines, the development of new antiparasitic drugs, the mechanisms of resistance to antiparasitic drugs and the selection of hosts with genetic resistance. Molecular approaches can be applied to all host-parasite systems of interest to veterinary parasitologists. While much of the development of molecular biology in veterinary parasitology is taking place in developed countries, the applications of molecular biology are valid for both developed and developing countries. Furthermore, many of the techniques are becoming simpler and relatively less expensive, allowing

**Table I. Comparison of methods to diagnose parasite infection**

S. #	Method	Advantages	Disadvantages
1.	DNA probe and PCR	<ul style="list-style-type: none"> <li>a. Fast</li> <li>b. Sensitive and specific (PCR)</li> <li>c. Direct detection of parasite</li> <li>d. Can detect and differentiate variants</li> <li>e. Independent of immunocompetence or previous clinical history</li> <li>f. Parasites do not need to be viable</li> <li>g. Automatable</li> </ul>	<ul style="list-style-type: none"> <li>a. Poor sensitivity and specificity (DNA probe)</li> <li>b. Expensive</li> <li>c. Multistep</li> <li>d. Detects dead organisms</li> <li>e. Possible false-negatives from PCR inhibitors</li> <li>f. Possible false-positives from carryover contamination (PCR)</li> </ul>
2.	In vitro culture and mouse inoculation	<ul style="list-style-type: none"> <li>a. Measure of virulence and infectivity</li> <li>b. Only viable parasites detected</li> </ul>	<ul style="list-style-type: none"> <li>a. Expensive</li> <li>b. Slow</li> <li>c. Interstrain variation</li> <li>d. Need to maintain parasite viability in specimen</li> <li>e. Uses animals</li> </ul>
3.	Microscopic examination	<ul style="list-style-type: none"> <li>a. Simple</li> <li>b. Direct detection of parasite</li> <li>c. Can differentiate morphologically distinct organisms</li> </ul>	<ul style="list-style-type: none"> <li>a. Slow</li> <li>b. Laborious and tedious</li> <li>c. Requires high levels of parasite for good sensitivity</li> <li>d. Cannot discriminate between morphologically similar organisms</li> <li>e. Requires experienced microscopist</li> </ul>
4.	Serologic detection of antibodies	<ul style="list-style-type: none"> <li>a. Simple</li> <li>b. Fast</li> <li>c. Automatable</li> <li>d. Appropriate for screening large numbers of samples</li> </ul>	<ul style="list-style-type: none"> <li>a. Poor specificity</li> <li>b. Does not distinguish active from prior or latent</li> <li>c. Requires standardized reagents</li> </ul>
5.	Xenodiagnosis	<ul style="list-style-type: none"> <li>a. Measure of parasitemia and infectivity</li> </ul>	<ul style="list-style-type: none"> <li>a. Slow</li> <li>b. Laborious</li> <li>c. Unpleasant</li> <li>d. Poor sensitivity</li> </ul>

**Source:** (Weiss, 1995)

some uses of molecular biology to have universal utility (Prichard & Tait, 2001).

This review focuses on developments and some examples of the application of molecular biology to veterinary Parasitology.

**1. Basic molecular tools used in parasitology.** With a world-wide increase in morbidity and mortality due to some parasitic infections, a drastic increase in (multi-) drug resistance of some major human pathogenic parasites, a paucity in newly developed drugs, and with many parasites causing opportunistic infections in AIDS patients, quick and accurate tests for the early detection and identification of these parasites are urgently needed. The introduction of the polymerase chain reaction (PCR) has stimulated the development of new epidemiological and diagnostic tools. With the PCR tiny amounts of DNA fragments corresponding to a few or even one parasite can be detected with high specificity in almost every type of specimen such as tissue, sputum, cerebrospinal fluid (CSF), blood, urine or stool. The value of this potential gain in sensitivity and specificity, though, should be critically evaluated against the clinical observations and compared with that of other available tests such that a

sound selection of appropriate detection methods can be made.

One of the very special and useful features of nucleic acids is that single strands of DNA will readily pair up, under simple conditions, with identical or similar strands of DNA or the transcription equivalent strands of RNA. This means that DNA can be extracted from cells, or synthesized in the laboratory, the bonds forming the double helix broken and then the single strands used to rebind to transcription similar sequences of RNA or to other single strands of DNA of similar sequence. The breaking of the bonds between the double strands of DNA is called denaturation and is usually accomplished by heating. Following the mixing of single-stranded DNA at elevated temperature to another single strand of DNA, or to RNA, double-stranded DNA or DNA/RNA can be formed under lower temperatures. This reformation of double strands is known as hybridization. Some variant of this process is used in most techniques of molecular biology (Hyde, 1993; Braun & Shirley, 1995).

**2. Identification and Diagnosis of Parasites.** The use of PCR for the identification of parasites and for diagnosis has become well established in veterinary parasitology in recent

years. It is predicted that it will become the tool of choice in the near future when specificity and sensitivity are important. However, PCR can fail because of contamination (Longo *et al.*, 1990) producing false positives and inhibition of amplification producing false negatives. The use of negative and positive controls is important. Often a positive control is employed by the co-amplification of a specific fragment unrelated to the fragment of interest. Another restriction on the more widespread use of PCR for identification and diagnosis has been its cost. Costs are decreasing and kits are making the assays simple. A remaining deficiency is the ability to use PCR quantitatively. Semi-quantitative PCR methods are being developed. Routine implementation of PCR for diagnosis requires, at present, transportation of samples to a central facility for processing, and personnel trained in the use of this technology. Because of the cost and need for sophisticated facilities and well-trained personnel, PCR is unlikely to replace conventional tests for parasites, especially in developing countries. Nevertheless, its sensitivity, specificity and speed make it imperative that veterinary parasitologists work to develop routine tests based on PCR technology.

Different species of parasites can be detected and distinguished by a variety of molecular biological techniques. Because different species of parasites and their hosts have unique DNA sequences, it is possible, if such sequences are known, to diagnose and identify the species of parasite(s) involved with exquisite specificity and very high sensitivity. The most common techniques are based on hybridization patterns after restriction enzyme digestions and electrophoresis, or on PCR.

PCR is rapidly becoming the technique of choice because it allows selected sequences of DNA or RNA to be greatly amplified, increasing sensitivity enormously. Following amplification, the PCR products are directly stained with ethidium bromide or cut with a restriction enzyme and then stained with ethidium bromide or transferred to a membrane for hybridization with a radioactive or biotin-labelled probe.

Three types of sequences are used for PCR for diagnosis: (1) Ribosomal DNA or RNA which contain highly repeated sequences, permitting high sensitivity but specificity may be reduced because repeat sequences tend to be conserved between closely related species. (2) Specific sequences of genomic DNA which are highly specific for the species of interest. While specificity is high, sensitivity may be less than with highly repeated sequences. However, PCR can overcome a lack of sensitivity by greatly amplifying the sequence of interest. (3) Sequences obtained after random primer amplification (RAPD-PCR). RAPDs produce numerous fragments, some of which may vary between different species. While RAPDs may produce high sensitivity, specificity is usually less than when specific sequences are used for diagnosis and identification.

The use of molecular tools for diagnosis of parasites of importance in veterinary medicine has been reviewed by Mathis *et al.* (1996), Felleisen *et al.* (1996) and Comes *et al.* (1996).

**Diagnosis of protozoans.** Ellis and Bumstead (1990) showed that different species of avian *Eimeria* could be distinguished on the basis of Southern blot hybridization patterns using ribosomal RNA gene probes. The disadvantage of this type of approach is that it requires relatively large amounts (micrograms) of high molecular weight DNA. The identification of *Eimeria* species has also been achieved by PCR to amplify a 560 bp intergenic sequence between 5S ribosomal RNA genes (Stucki *et al.*, 1993) and required as little as 1 pg DNA or < 10 oocysts.

*Babesia bovis* has been diagnosed in chronically infected cattle by using PCR for a 60 kDa merozoite surface protein (Figueroa *et al.*, 1994). High sensitivity was achieved with a limit of detection of  $1 \times 10^{-6}$  % parasitaemia. RAPD has been used to reveal markers of species and isolates of *B. bovis* and *B. bigemina* (Carson *et al.*, 1994). Fragments characterizing species or isolates of *Trypanosoma* from ruminants and pigs have been found using RAPD-PCR (Dirie *et al.*, 1993; Waitumbi and Murphy, 1993). In these studies it was necessary to purify the parasites from host blood before amplification.

Tanaka *et al.* (1993) utilized PCR primers and a probe derived from a gene encoding a 32 kDa intra-erythrocytic piroplasm surface protein of *Theileria sergenti* to detect this parasite with a sensitivity of 4 parasites  $\mu\text{l}^{-1}$  blood using a 10  $\mu\text{l}$  sample. D'Oliveira *et al.* (1995) used PCR primers encoding a portion of a 30 kDa major merozoite surface antigen of *Theileria annulata* to detect parasites in cattle blood with a sensitivity of 3 parasites  $\mu\text{l}^{-1}$  blood. Allsopp *et al.* (1993) were able to differentiate between six different species of *Theileria* in cattle by using PCR with primers for small subunit ribosomal RNA, while Bishop *et al.* (1993) achieved high specificity in identifying *Theileria* species using RAPD.

**Diagnosis of helminthes.** There are also numerous reports of the use of molecular biology to detect and identify helminth infections. Identification of egg and larval stages of Trichostrongyles of ruminants is usually carried out by culturing the early stages to third-stage larvae. This takes about a week. Beh *et al.* (1989) demonstrated that it was possible to use repeat sequences to identify different species without using PCR. Roos and Grant (1993) developed a PCR assay, based on parts of the DNA sequences of  $\beta$ -tubulin in these nematodes, which can discriminate *Haemonchus (H.) contortus* DNA from *Trichostrongylus colubriformis* DNA and from several other sheep nematode parasites using all stages, including eggs. Zarlenga *et al.* (1994b) used genomic DNA from adult *H. contortus*, *H. placei*, and *H. similis* to clone and map complete ribosomal RNA gene repeats from each species. Sequence analysis demonstrated 100% conservation of a 1758 bp fragment from the small subunit ribosomal DNAs from each species.

Nevertheless, amplification by PCR, using primers complementary and proximal to the 3'-end of the large subunit and the 5'-end of the small subunit ribosomal DNAs enabled a rapid differentiation of individual worms of *H. contortus* from *H. placei* by using size variability within this region of the repeat sequence.

Campbell *et al.* (1995), Chilton *et al.* (1995), Gasser *et al.* (1994), Hoste *et al.* (1995) and Stevenson *et al.* (1995) have all shown the utility of ribosomal DNA or RNA for strongyle identification, with sensitivities as low as a single egg. Christensen *et al.* (1994) utilized repeat sequences to distinguish between four genera of Trichostrongyles with the ability to detect as few as 25 eggs. Humbert and Cabaret (1995) and Jacquet *et al.* (1995) used RAPD to find specific marker probes to identify three species of *Haemonchus* adults or larvae.

Bretagne *et al.* (1993) developed a PCR assay for detection of *Echinococcus (E.) multilocularis* eggs in fox faeces with sensitivity down to 1 egg per 4 g faeces. The assay used primers for the non-coding flanking sequence of the U1 sRNA gene which is repeated at least 50 times in the *E. multilocularis* genome. The primers were reported to be specific for *E. multilocularis* and no product was amplified for other cestode species including *E. granulosus*. Mathis *et al.* (1996) found that many faecal samples inhibited PCR amplification and developed an improved method involving concentration of the cestode eggs before PCR.

The larval stages of *Fasciola (F.) hepatica* have been identified within the snail intermediate host using a ribosomal RNA probe (Shubkin *et al.*, 1992), while Adlard *et al.* (1993) were able to identify three species of Fasciolidae (*F. hepatica*, *F. gigantica* and *F. magna*) using ribosomal RNA from adult flukes.

**3. Molecular epidemiology.** All of the techniques for molecular diagnosis and classification can be employed in molecular epidemiology. However, some molecular tools have been specifically designed for epidemiological studies. The use of mini-satellite variant repeat analysis (MVR-PCR) by PCR, for epidemiological studies of parasites, was reviewed by Arnot *et al.* (1994). MVR-PCR exploits the hypervariable mini-satellite tandem repeats.

Oligonucleotide primers can be designed specifically to hybridize to each mini-satellite variant of a parasite. Combining such variant repeat specific primers and a primer which hybridizes to a conserved site in the DNA flanking the repeats, it is possible to generate PCR products extending from the flanking primer to each variable repeat. These variable length products can be separated by electrophoresis and visualized by autoradiograph or staining. The product is analogous to a supermarket barcode which can be converted into a type-specific digital code for computer analysis (Prichard, 1997).

**4. Evolutionary biology of parasites.** The study of the evolutionary biology of parasites has received new impetus with the development of molecular phylogenetics. The sequences of gene classes can be used to provide data on

phylogenetic relationships. Nuclear ribosomal RNA genes, which encode structural RNA of ribosome, usually occur in eukaryotes as series of tandemly repeated units, each containing three coding regions (5.8S, ~ 150 bp; 18S, ~ 2000 bp; 28S, ~ 4500 bp) separated by spacers. The rate of evolution has differed both among and within these genes and spacers. The spacers between the 18S and 28S genes and separated by the 5.8S gene are the least conserved regions whilst the 18S gene is the most conserved. These ribosomal RNA genes and their spacers tend to be conserved between different members of the same species, thus intraspecific variation is very low compared with interspecific variation.

Another common source of phylogenetic information is the mitochondrial genome. This clonally/maternally inherited circular molecule typically encodes 37 genes and is less than 20000 bp in animals (Avisé, 1994). Mitochondrial genes tend to accumulate changes at a higher rate than nuclear genes, and are thus useful for analysing recent divergences. Each homologous fragment of DNA from one species is aligned according to the nucleotide sequence to that of the other species of interest. This is best done by computer. Various programs exist for comparing alignments and producing phylogenetic trees.

The evolutionary biology of the platyhelminths has been analysed using both conventional approaches and molecular phylogeny (Blair *et al.*, 1996). In another study, Zarlenga *et al.* (1994a) amplified, by PCR, a 1758 bp cloned fragment of small subunit ribosomal DNA from *Nematodirus battus* and compared the sequence with that from *H. contortus* and *Caenorhabditis (C.) elegans*. These workers also compared the divergence in this fragment seen between different species of *Haemonchus* and were able to conclude that the divergence between these subfamilies was limited and suggested that this molecule alone may be inappropriate for assessing phylogenetic relatedness within the superfamily Trichostrongyloidea. In addition to identification, diagnosis and understanding the genetic relationship between different species, strains and types, molecular biology is increasingly being used in efforts to improve the control of veterinary parasites.

**5. Host resistance.** Molecular biology is being used to understand the mechanisms and map the genes responsible for resistance to infection. Most advanced has been the study of resistance to such intracellular parasites as *Mycobacterium bovis*, *Leishmania donovani* and *Salmonella typhimurium* where the gene known as *Nrampl* (synonyms: *Bcg*, *Lsh* and *lty*) controls the capacity of mature tissue macrophages to restrict the proliferation of ingested parasites in the reticuloendothelial organs (Ivanyi, 1994; Vidal *et al.*, 1995a; Vidal *et al.*, 1995b). The *Nrampl* gene has been cloned and sequenced and found to code for a macrophage-specific polytrophic protein with twelve predicted transmembrane domains and a consensus transport motif. Furthermore, it was found on sequence analysis that susceptibility to infection was associated with a

single non-conservative Gly to Asp substitution at position 169 within predicted transmembrane domain 4 of the Nramp protein. Vidal *et al.* (1995a) produced *Nrampl* gene knockout mice and analysed their phenotype in relation to susceptibility to infection with intracellular parasites. The *Nrampl-deficient* mice were more susceptible to infection and it was shown that Nramp functions by a cytotoxic/cytostatic mechanism at the early stage of the macrophage-parasite interaction.

Beh and Maddox (1996) have reviewed the prospects for developing genetic markers for resistance to gastrointestinal parasite infection in sheep. They concluded that while many phenotypic markers were available such as haemoglobin type, anaemia, MHC type, circulating eosinophil counts, serum enzymology, faecal egg counts and others, none of these markers predicted an individual's resistance status satisfactorily and that genetic markers were the best way to incorporate parasite resistance into selection programs. However, resistance to metazoan parasites is polygenic, quite complex and modulated by environmental factors. Linker analysis is used to test for the co-inheritance of marker alleles and the trait of interest.

The advent of PCR technology has provided the means to investigate a virtually limitless supply of DNA markers for a characteristic (genetic resistance) of interest. Currently, efforts are being expended to systematically scan the entire genome of sheep and to genotype sheep families using a number of hypervariable DNA markers to tag all chromosomes (Beh & Maddox, 1996). The presence of a gene with a significant effect on genetic resistance can then be detected using least-squares analysis.

Another approach is to look at differential expression of mRNA (Callard *et al.*, 1994) and RNA fingerprinting (Murphy & Pelle, 1994). Eventually specific genes which make a significant contribution to resistance against parasite infections will be identified. In this respect, Gullard *et al.* (1993) found significant association between the adenosine deaminase (*Ada*) genotype and mortality due to parasites in sheep, and between *Ada* genotype and nematode burden. As genome sequencing projects are completed there should be a rapid acceleration in our ability to select for genetically resistant livestock.

**6. Vaccine development.** A great deal of effort has been made to develop antiparasite vaccines by conventional approaches and, until recently, the only vaccines to be successfully developed against helminth parasites in animals were irradiated larval vaccines against *Dictyocaulus viviparus* (Urquhart, 1985), *D. filaria* (Sharma *et al.*, 1981) and *Ancylostoma caninum* (Miller, 1978). Other means of attenuating live vaccines, such as repeated passage *Babesia* (Callow, 1979; Wright, 1990) and *Theileria hirci* (Hawa *et al.*, 1981), and the development of 'precocious' lines of *Eimeria* (Jeffers, 1975; Shirley, 1992), have been employed against protozoa with limited success. The state of vaccines against veterinary parasites has been reviewed by Barriga (1994), Eckert and Deplazes (1996) and Emery (1996).

In recent years, molecular biology has been employed to develop new vaccines of interest to veterinary parasitologists. The approach that is beginning to prove successful is to define a parasite antigen which confers resistance and to produce it by recombinant technology either as a polypeptide, by infecting the host with a benign expression vector, or by vaccinating with a nucleic acid. A subunit vaccine against *Boophilus microplus* has been developed in Australia (Willadsen *et al.*, 1995) and is commercially available in a number of countries under the name of TickGARD®. A tick antigen called Bm86 is produced by recombinant technology. *E. coli* and baculovirus have been successfully employed as expression vectors producing levels of immunity comparable to that against the native antigen (Tellam *et al.*, 1992). It is a 89 kDa glycoprotein with an extracellular location on the digestive cells of the tick gut (Gough & Kemp, 1993).

Attempts are being made to develop recombinant vaccines against a number of tick-borne haemoparasitic diseases of livestock such as anaplasmosis (Barbet, 1995; Palmer & McElwain, 1995), cowdriosis (Mahan, 1995), theileriosis and babesiosis (Brown *et al.*, 1993; Palmer & McElwain, 1995). The development of a recombinant 45W vaccine against *Taenia ovis* (Johnson *et al.*, 1989) followed many years of research that showed that sheep could be vaccinated against *T. ovis* with antigens from oncospheres (Rickard & Bell, 1971). A similar approach has been taken to develop recombinant vaccines against other parasites (Lightowers, 1996). Liebau *et al.* (1996) have cloned, using PCR, and expressed a recombinant glutathione S-transferase (rGST) from *E. muhilocularis* and on the basis of the activity of rGSTs as vaccines against some trematodes such as *Schistosoma mansoni* (Boulanger *et al.*, 1991) and *Fasciola hepatica* (Sexton *et al.*, 1994) propose to test the IGST as a vaccine against *Echinococcus*. The major advantages of using a defined recombinant antigen over impure and often less well-characterized natural antigen extracts or whole organisms are that a consistent vaccine product can be produced cheaply and with predictable effects against a given parasite strain.

## 7. Chemotherapy

**Development of molecular screens for antiparasitic drug discovery.** Economic pressures have led to virtually all major pharmaceutical companies starting the search for new classes of drugs with mechanism-based screening. In the case of anthelmintics, the role of mechanism-based screening has been reviewed by Geary *et al.* (1999b). Mechanism-based screening depends on the identification of a biochemical process in the parasite that can be used for high throughput screening in the laboratory. Typically, the gene for a particular receptor or enzyme is expressed in a suitable vector, usually a eucaryotic cell (e.g. yeast). The assay may be run with whole transfected cells or sub-cellular preparations. Typically, the gene of interest may be expressed with a reporter gene (Bronstein *et al.*, 1994) which allows for rapid automated monitoring of responses

to chemical libraries, by fluorescence or chemiluminescence detection in multiwell plates. Isolation of receptors and enzymes from parasites is not feasible for high throughput screening, so the whole screening process has become dependent on the expression of parasite genes (or model organism genes, such as *C. elegans*). This process tends to produce many 'hits' and second stage screening, e.g. in whole *C. elegans in vitro* is used prior to *in vivo* assays in small animal model host/parasite systems.

Most of the initial 'hits' do not survive through the initial *in vivo* screens due to toxicity or lack of sufficient efficacy. Candidate drugs that have progressed through these different screens from the molecular screens to the model *in vivo* screens may then proceed to initial target animal screening. As can be seen the molecular, mechanism-based screens are now the foundation of the anthelmintic discovery process. As has been emphasized by Geary *et al.* (1999a), our ability to discover new anthelmintics depends on our understanding of the basic biology of parasites.

**Mode of action and development of resistance in anthelmintics.** The antiparasitic activity of the avermectins was discovered by empirical screening. However, the mechanism of action was determined by cloning the putative receptor, a glutamate-gated chloride channel into *Xenopus* oocytes. *Xenopus* oocytes have been used extensively as an expression system for the characterization of neurotransmitter receptors. The avermectin receptor was expressed after micro-injection of mRNA from *C. elegans* into the oocytes where it is translated into protein with appropriate post-translational modifications, assembly, sorting and other processing and retains the properties and pharmacology of the donor tissue. It was possible to use expression cloning in *Xenopus* oocytes to clone and sequence the receptor gene from *C. elegans* and to study the current/voltage relationships after exposure of the transformed oocytes to ivermectin, glutamate and other ligands active on chloride channels and glutamate receptors (Arenas, 1994).

The action of the benzimidazole anthelmintics has been shown to be binding to tubulin and differences in this binding were found to correlate with benzimidazole resistance (Sangster *et al.*, 1985). However, it was not known whether the benzimidazoles bound to  $\alpha$ -tubulin or  $\beta$ -tubulin or the  $\alpha\beta$ -heterodimer, or what region of the tubulin protein was responsible for the benzimidazole receptor. Parasitic nematode tubulin genes have been cloned for *Brugia pahangi* (Guenette *et al.*, 1991), *H. contortus* (Geary *et al.*, 1992) and *Teladorsagia circumcincta* (Elard *et al.*, 1996). By conducting mebendazole binding studies on *in vitro* expressed recombinant *H. contortus*  $\beta$ -tubulin obtained from Geary *et al.* (1992), Lubega *et al.* (1993) demonstrated that the benzimidazoles could bind to nematode  $\beta$ -tubulin alone. Kwa *et al.* (1994, 1995) showed that the mutation of phenylalanine to tyrosine in *H. contortus*  $\beta$ -tubulin was correlated with benzimidazole resistance and produced benzimidazole resistance phenotype in *C. elegans*

transformed with *H. contortus*  $\beta$ -tubulin. Lubega *et al.* (1994) showed by restriction fragment length polymorphism analysis of genomic DNA from individual *H. contortus* that two  $\beta$ -tubulin genes were involved in benzimidazole resistance. This was confirmed in a genetic analysis using PCR with  $\beta$ -tubulin primers (Beech *et al.*, 1994) and the genetic restriction involved in benzimidazole resistance was also quantified in this study. Finally, Nare *et al.* (1996), using sequence analysis following the binding of a photoactive benzimidazole ligand to recombinant *H. contortus*  $\beta$ -tubulin, expressed in *E. coli*, showed that benzimidazole binding took place in the N-terminal 63-103 sequence. From this series of research, using the tools of molecular biology, a very full understanding of the mechanism of action and the mechanism of resistance to benzimidazoles has been obtained and the prospects are good that simple and highly sensitive nucleic acid probes will be available for the practical determination of benzimidazole sensitivity of parasitic nematodes.

Levamisole resistant mutants of *C. elegans* lack a normal complement of physiologically functioning acetylcholine receptors (Lewis *et al.*, 1980). The cloning and expression of the levamisole resistance genes, *lev-1*, *unc-29* and *unc-38* has revealed that they encode subunits of acetylcholine-gated cation ( $\text{Na}^+$  &  $\text{K}^+$ ) channels in *C. elegans* including a levamisole/morantel binding site (Fleming *et al.*, 1997). The *lev-1* appears to be a structural subunit of a five subunit acetylcholine-gated channel. A mutation, Glu237Lys in the sequence encoding a transmembrane region of *lev-1* changes the ion channel from cationic to anionic and renders *C. elegans* insensitive to levamisole. In *H. contortus*, there appear to be high and low affinity levamisole binding sites on cholinergic receptor subunits, with differences between resistant and susceptible worms consistent with the low affinity receptors in resistant worms readily becoming unresponsive to levamisole (Sangster *et al.*, 1998a,b). These studies suggest that several genes may be involved with the effects of levamisole and possibly with levamisole resistance and in one isolate of *H. contortus* in which this question was addressed, levamisole resistance appeared to be multigenic (Sangster *et al.*, 1998a,b).

A novel chemotherapeutic approach to parasite control is the use of antisense nucleic acid. The use of antisense oligonucleotides for therapy generally relies on an antisense oligonucleotide being synthesized and delivered to the target, in our case, the parasite's nucleic acids. Hybridization of antisense to sense sequences may block the expression of the targeted protein. Numerous problems confront this theoretical approach to chemotherapy. The antisense oligonucleotide has to be synthesized, delivered into the parasite cells, not be degraded too rapidly, and must hybridize with the parasite's nucleic acids to prevent protein expression essential for the survival of the parasite and do so at a high enough level to compromise the parasite. Furthermore, the antisense sequence must be very specific

for the parasite nucleic acids so that it will bind strongly to it and not bind to host nucleic acid and cause toxicity, an adequate *in vivo* delivery system remains a fundamental problem in the practical application of antisense therapeutics to parasites (Miller & Vile, 1994).

## CONCLUSION

Applications of molecular parasitology are advancing particularly rapidly in the development of diagnostic probes, in the study of parasite antigens and in vaccine development. In addition, the completion of various genome sequencing projects should provide a significant boost to efforts to select genetically resistant livestock. The development of vaccines and resistant livestock should eventually have a significant effect on sustainable animal production in developing as well as developed countries.

The basic techniques of molecular biology are not complicated and offer enormous advantages over conventional techniques because DNA is far more stable than protein, nucleic acids have the special ability to hybridize to other single strands of similar nucleic acid offering a huge range of technical advantages, PCR has opened up the possibility to amplify enormously DNA and RNA sequences, and the genetic code of a species, type or individual is a unique template which allows exquisite specificity in mapping biology. Veterinary parasitologists must embrace molecular knowledge and use the tools of molecular biology in our research; teaching and clinical work if the field is to advance, be respected, funded and is given an appropriate place in the curriculum in our universities.

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