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Abstracts

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Po7.03(392) PRELIMINARY STUDIES ON RAPDs TECHNIQUE AS AN AID FOR THE IDENTIFICATION AND CHARACTERIZATION OF *DICROCOELIUM DENDRITICUM*

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The liver fluke *Dicrocoelium dendriticum* is a little known parasite responsible for the disease dicroceliosis in ruminants in various parts of the world. For several years the Parasitology team of the CSIC has been carrying out a wider research programme on the life-cycle of *D. dendriticum* in the intermediate (molluscs, ants) and definitive hosts (sheep and cattle) naturally and experimentally infected.
Nevertheless, the knowledge of its Molecular Biology is very scant. Due to this, for this paper we tried to obtain a specific primer of DNA from *D. dendriticum* in order to develop a useful and discriminating method of diagnosis in infected molluscs, ants and ruminants and study any possible genetic variation. The approach has been the use of the RAPDs (Random Amplified Polymorphism DNA) technique. The optimal conditions were: initial denaturation at 94°C for 1 min following a temperature denaturation at 94°C for 1 min, 36°C for annealing step for 1 min and 72°C for extension for 2 min in 45 cycles. The concentration of DNA template was 100-200 pg. Using genomic DNA from 20 single parasite coming from several infections, we tested different 10-mer oligos obtaining discrete patterns for all except one of them. Most discernible patterns were obtained with primer 5 AGGGTCTTG 3 able to amplify common 1 kb fragments as well as different size fragments. From one of the samples we cloned in PGEM 5 (+) 1, 0,5 and 0,2 Kb fragments that are being sequenced. Comparing in the Genome Database (Fasta Program, EMBL) using a partial 254 bp sequence we have evidence we cloned a fragment gene involved in ATP binding function. A fragment of this gene as well as other cloned fragments will be used to develop specific primers able to discriminate the presence of the parasite by PCR in mixtures of final and/or intermediate host DNAs.

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Po7.04(758) SMALL SUBUNIT (18S) RIBOSOMAL RNA GENE DIVERGENCE IN EUROPEAN SPECIES OF THE GENUS *LYMNAEA* (MOLLUSCA: BASOMMATOPHORA: LYMNAEIDAE)

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Many species of freshwater pulmonate snails have an important role as intermediate hosts for trematode parasites causing several medical and veterinary diseases around the world. Several species of the genus *Lymnaea* act as intermediate hosts of *Fasciola hepatica* with variable grades of epidemiological relevance. The European species *L. truncatula* is its major and original intermediate host of this distomatosis which has spread affecting human populations in various countries adapting to a diversity of ecological conditions. Traditionally, among the Lymnaeacean pulmonates, the assessment of the phylogenetic relationships between families has been based on a small number of studies on reproductive tract characteristics or in outgroup analysis and component analysis. The parameters used, however, do not always correlate, leaving considerable doubt on the present taxonomic classifications. Moreover, in the case of the genus *Lymnaea*, molecular data on genes that are used as currents tools in molecular phylogeny studies (18S and 28S rRNA, ribosomal gene spacers, mitochondrial DNA) are not available today. In order to establish the phylogenetic relationships among European *Lymnaea* spp., the entire 18S rRNA gene sequence from five European species of the genus *Lymnaea* [*L. stagnalis* (Linnaeus, 1758), *L. palustris* (Müller, 1774), *L. peregra* (Müller, 1774), *L. glabra* (Müller, 1774), and *L. truncatula* (Müller, 1774)] have been determined by direct cycling sequencing and silver staining methods. Analysis of these regions by both maximum parsimony and distance methods, using the previously published sequence of *Limicolaria kambeul* (Mollusca: Stylommatophora) 18S rRNA gene as outgroup, showed that these nucleic acid sequence data can be used to recognize three different European *Lymnaea* phylogenetic groups: *L. peregra*, *L. truncatula* and *L.palustris-L.stagnalis-L.glabra*, respectively. In this case, the results indicate that 18S rRNA sequence becomes satisfactory for resolving lower levels of phylogeny (i.e. at the subgenus level), and not only at family or genus level, as previously proposed by several authors.

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Po7.05(757) GENETIC STRAIN VARIABILITY AMONG THE SPECIES *FASCIOLA HEPATICA* DETERMINED USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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The usefulness of random amplified polymorphic DNA (RAPD) markers, as a discriminating tool which allows distinguishing among species, strains and individuals, has been already demonstrated for several helminth species (i.e. of the genera *Schistosoma*, *Echinococcus* and *Trichinella*). Fasciolosis is a disease of mammals (mainly domestic ruminants, but also Man) caused by digenetic trematodes of the genus *Fasciola*, of which *F. hepatica* is the most common. In *Fasciola hepatica*, worth mentioning is the unusual fact of the absence of detection, up to the present, of polymorphic loci by means of isoenzymology studies, as well as restriction fragment lengh polymorphism (RFLP) or sequencing methods. However, the RAPD marker technique permitted us to detect diversity over the whole genome of *F. hepatica*. This technique offers several advantages over the other techniques previously cited. Twenty arbitrary decamer oligonucleotides were used as primers to amplify total DNA by the polymerase chain reaction (PCR) method. Random amplified polymorphic markers were assayed on 20 adult specimens of *F. hepatica* from four isolates naturally obtained in the Bolivian Altiplano human focus of fasciolosis (the liver fluke cycle for cattle, sheep, pig and human isolates is currently maintained in our laboratory using Bolivian *Lymnaea* strains as intermediate host and laboratory Norvergian white rat as definitive host) and on 6 *F. hepatica* adults obtained from the bile ducts of naturally infected cattle in a local slaughter-house at Valencia (Spain). The use of the RAPD tool extended considerably the previous information available from studies on morphology, isozymes, limited restriction fragment length polymorphism and sequence data, and facilitate the genetic analysis at intra- and interpopulational levels, allowing a rapid obtention of molecular data which show to be useful for accurate identification at individual and populational levels. The images of agarose gels stained with ethidium bromide showed that RAPD patterns often demonstrate differences for the absence or presence of a single product. The primers tested revealed polymorphic DNA fragments. This approach must be extended to *F. hepatica* egg DNA in the way to allow epidemiologists to distinguish different parasite populations and disentangle their genetic and biogeographical origins.

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Po7.06(729) CLONING OF SEQUENCES ENCODING P-GLYCOPROTEIN HOMOLOGUES OF *FASCIOLA HEPATICA*

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P-glycoproteins are proposed to function as energy-dependent membrane transport proteins in a wide variety of species. Members of the P-glycoprotein superfamily have been shown to possess a broad substrate specificity and are able to transport anions, peptides, amino acids and cytotoxic drugs. Overexpression of, and/or mutations within, P-glycoprotein gene sequences have been shown to be associated with resistance to a wide range of drugs in a number of mammalian and parasite systems. These gene sequences have historically been termed "Multidrug Resistance" (MDR) genes to reflect the cross-resistance phenotype exhibited by cells with altered P-glycoprotein expression.

We are studying the role of P-glycoproteins in the biology of *Fasciola hepatica*. Using a PCR-based approach we have identified and cloned a 600bp genomic DNA sequence (Fhmdr) from *F.hepatica* that encodes a putative P-glycoprotein nucleotide-binding domain. The predicted coding sequence reveals 56% homology at the amino acid level with the corresponding region of a mammalian P-glycoprotein (MDR1). Northern blot analysis has failed to reveal the presence of P-glycoprotein specific mRNA's within adult *F.hepatica*, whilst two distinct transcript classes are present within 5 week old parasites suggesting that the expression of P-glycoproteins are regulated in a stage-specific manner.

Partial "MDR-like" cDNA sequences have been cloned from a *F.hepatica* 5 week old library and are virtually identical except for their 5' termini. We are currently raising antisera to recombinantly expressed portions of the molecule and are attempting to isolate genomic MDR sequences in order to further characterize this putative transport system within *F.hepatica*.