1740

STUDY ON THE INTRAORGANIC LOCATION OF PARAMPHISTOMIDAE FLUKES INFECTING ADULT CATTLE IN GALICIA (SPAIN) AND THE SPECIES IDENTIFICATION

M. GONZÁLEZ-WARLETA[1], M. MARTÍNEZ-VALLADARES[2], A. M. MARTÍNEZ-IBEAS[2], J. A. CASTRO-HERMIDA[1], B. MIÑAMBRES[2], C. GONZÁLEZ-LANZA[2], M. MEZO[1], M. Y. MANGA-GONZÁLEZ[2]

[1]Laboratorio de Parasitología, Centro de Investigaciones Agrarias de Mabegondo, Xunta de Galicia, Mabegondo, CORUÑA, Spain
[2]Departamento de Sanidad Animal, Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ganadería d, Grulleros, LEÓN, Spain

The forestomachs of 722 cows, over 2 years old, from 628 farms in the 4 provinces of Galicia (Coruña, Lugo, Ourense and Pontevedra), NW Spain, were collected from slaughterhouses. All the rumens and reticula were examined to detect and count the Paramphistomidae. To determine the parasite intraorganic distribution, 31 animals were chosen randomly and the parasites found in the different anatomical areas of the rumen (ruminal atrium, rumenoreticular sulcus, ventral sac, dorsal sac) and in the reticulum were counted and measured. Fifty parasites were collected from each infected animal. The specific identification was done morphologically and by ITS2 (Internal Transcribed Spacer 2) sequencing.

The infection prevalence was 17.3% and the parasite loads varied between 1- 21200 (Mean = 1293 ± 2780 SD). Significant differences were observed between the infection prevalences in the four provinces, the highest being in Pontevedra (27.6%). Most parasites were located in the rumen (94.3%), with the percentages found in the atrium (58.2%) and in the rumenoreticular sulcus (26.5%) significantly higher than those found in the dorsal and ventral sacs. Statistically significant differences were also recorded in parasite size.

In order to study the genetic variability of *Calicophoron daubneyi* a 401 bp fragment of the ribosomal RNA internal transcribed spacer 2 (ITS2) was sequenced. Firstly, we analysed the intraindividual variability after sequencing a total of 10 *C. daubneyi* recovered from two cows. No difference was shown in the ITS2 sequence between the adults taken from different parts of the rumen (ruminal atrium, rumenoreticular sulcus, ventral sac, dorsal sac) and reticulum , in the same cow. Secondly, the interindividual variability was also tested in 21 samples obtained in the four provinces of Galicia (Spain). All sequences were also similar between *C. daubneyi* adults from different origins.

Study supported by Spanish INIA, Project FAU2006-00021-C03-00.

1741

PCR DIAGNOSIS OF *DICROCOELIUM DENDRITICUM* INFECTION IN MOLLUSC AND ANT INTERMEDIATE HOSTS

M. MARTÍNEZ-VALLADARES, A. M. MARTÍNEZ-IBEAS, B. MIÑAMBRES, C. GONZÁLEZ-LANZA, M. Y. MANGA-GONZÁLEZ

Departamento de Sanidad Animal, Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ganadería d, Grulleros, LEÓN, Spain

Dicrocoeliosis, caused by *Dicrocoelium dendriticum*, is an important hepatic trematodosis which affects a wide range of mammals, mainly ruminants, in Spain and many other countries. To apply successful control programmes against dicroceliosis, prior study of its epidemiology is needed. This requires specific and early diagnosis in mammals, as well as in molluscs and ants, the first and second intermediate hosts, respectively. The aim of this study was to develop an analytical method based on PCR (Polymerase chain reaction) to detect the infection by *D. dendriticum* in their intermediate hosts. A PCR based on the diversity of nucleotide sequences in mitochondrial DNA among species was carried out. Since no mitochondrial sequence of *D. dendriticum* was available, firstly we identified conserved regions of different parasite species with phylogenetic similarity to design general primers which anneal with *D. dendriticum* DNA. So ten primers,

and several combinations, which flanked the large and small rRNA subunits , the NADH dehydrogenase gen and different cytochrome C oxidase subunits were tested. The pair of general primers, which partly flanked the cytochrome C oxidase I and the large rRNA subunits, amplified a band from adult *D. dendriticum* samples. Once this band was sequenced, internal specific primers were designed to detect the infection in the intermediate hosts. Using PCR the primers amplified DNA obtained from: *D. dendriticum* adults, different species of land molluscs experimentally and naturally infected with *D. dendriticum* and infected ants collected in tetania (in León province, Spain). The PCR products, observed in agarose gel, permitted the detection of *D. dentricum* sporocysts in mollusc hepatopancreas as well as metacercariae and brainworm in ant abdomen and head, respectively. The specificity of the PCR was also established after testing the primers with other different parasites such as *Fasciola hepatica, Calicophoron daubneyi* and Brachylaimidae.

Study supported by Spanish CICYT, Project AGL2007-62824.

1742

JUST WHAT PFCRT NEEDED: REQUIREMENTS FOR THE HETEROLOGOUS EXPRESSION OF PFCRT

R. V. Marchetti[1], **S. Bröer**[1], **K. Kirk**[1], **R. E. Martin**[1,2]

[1]*Research School of Biology, The Australian National University, Canberra, ACT, Australia*
[2]*School of Botany, The University of Melbourne, Parkville, VIC, Australia*

Mutations in the chloroquine resistance transporter (PfCRT) are the primary cause of chloroquine resistance in the malaria parasite. PfCRT has proven difficult to express in heterologous systems and consequently the function of this protein, and the mechanism by which it confers resistance, have only recently been resolved (1). PfCRT is located on the membrane of the parasite's internal digestive vacuole and it is possible that the elements responsible for targeting PfCRT to this organelle hinder its study in heterologous systems by directing the protein to analogous organelles (rather than the plasma membrane) in the host cell. Bioinformatic analysis has identified multiple putative organellar targeting motifs in both termini of the PfCRT protein sequence. When a modified version of PfCRT in which these residues were mutated was expressed in *Xenopus laevis* oocytes, the resistance-conferring form of the protein ($PfCRT^{CQR}$) mediated the transport of chloroquine across the oocyte plasma membrane, whereas the chloroquine-sensitive form ($PfCRT^{CQS}$) did not (1). Localization of the motif-free version of PfCRT to the oocyte plasma membrane was confirmed by immunofluorescence microscopy assays as well as surface biotinylation and Western blot analysis. By contrast, oocytes injected with cRNA encoding native (motif-replete) $PfCRT^{CQR}$ did not show increased chloroquine uptake, nor was the protein present at significant levels in the plasma membrane. The sequence modifications therefore facilitated the functional expression of PfCRT. When reinstated in isolation, a number of the putative trafficking motifs resulted in the complete loss of $PfCRT^{CQR}$-mediated chloroquine uptake. Insights into the roles these motifs play in the localization and functional expression of PfCRT will be presented.

(1) Martin et al. Science 2009; 325: 1680-82