

Molecular analysis reveals two lineages of *Fasciola hepatica* in an endemic region of Brazil

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Fasciola hepatica is trematode parasites that cause infection in mammals during their adult stage. Within the liver host's, the fluke cause a severe disease called fasciolosis. This parasite is cosmopolitan with high prevalence in lowlands where the intermediate host snail is present. In southern Brazil, *f. hepatica* infections have large economic importance because livestock are commonly infected in the field. The aim of this study was to evaluate the genetic diversity of *f. hepatica* in areas of parasite high prevalence in southern Brazil (Rio Grande do Sul - RS) to understand the fluke natural history in this region. Adult *f. hepatica* were collected in slaughterhouses in three regions from an endemic area in the state of Rio Grande do Sul, Brazil: southern region (n=19), western region (n=5) and northern region (n=4). DNA was isolated using phenol-chloroform protocol from single worms and a partial segment of the cytochrome oxidase, subunit i (*coi*) mitochondrial gene was amplified using specific primers for subsequent sequencing. Sequence quality and contigs was evaluated using staden package. For additional comparisons, we downloaded 28 *coi* sequences from genbank: Australia (1), Egypt (6), Iran (6), Peru (6), Poland (7), United Kingdom (1) and Uruguay (1). Sequence diversity parameters such as haplotype number, haplotype and nucleotide diversity were computed in dnasp v.5 and a network tree was built in network 5.0 software, using medium joining parameter. To build a neighbor joining phylogenetic tree, using all sequences available, we used mega v.7 and to access the composition of molecular variance and fixation index (*fst*), we used arlequin v.3.5.2.2. We found 6 haplotypes in the region of study, only 1 haplotype was shared among the three regions (h_1 n=21), 4 haplotypes were exclusive from southern region (h_2 n= 1, h_4 n=1, h_5 n=1, h_6 n=2) and 1 from western region (h_3 n=2). Total haplotypic and nucleotide diversity was 0.439 and 0.00231, respectively. When we partitioned the molecular variance, we found out that 97.29% of the variation was within populations and only 2.71% among populations and the *fst* calculated was very low 0.02711. The topology of network tree shows a star like pattern, in which the shared haplotype (h_1) was the most abundant and central. Neighbor joining tree using sequences from all around the world resulted in two well defined clades. Both clades grouped samples from Asia, Africa, Europe, Oceania and South America. Our samples were divided between them, and the h_6 haplotype grouped separately from other southern Brazilian samples. Our results suggest that at least two lineages of *fasciola hepatica* took part in the colonization of Brazil: a low nucleotide diversity in contrast with relatively high haplotype diversity are in agreement with the recent colonization in the historic times while the low *fst* found may be due to the high animal traffic within the study region.