A Study of *Cryptosporidium parvum* Genotypes and Population Structure

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Genetic evidence for the occurrence of two Cryptosporidium parvum *subgroups is presented. This evidence is based on restriction fragment length polymorphism analysis of several independent loci. Sequence analysis of the b -tubulin intron revealed additional polymorphism. The stability of the genetic profiles following passage of* C. parvum *isolates between different hosts was investigated.*

Key words: *Cryptosporidium parvum* - restriction fragment length polymorphism - tubulin

Cryptosporidium parvum is an enteric protozoan parasite which commonly infects immunosuppressed individuals. Ruminants, in particular calves, are important reservoirs. Recent genotypic analyses of *C. parvum* from human cases of cryptosporidiosis have identified two groups of genotypically distinct parasites. One of these genotypes, designated genotype C, infects animals and humans, whereas the other, known as genotype H, is only found in humans. Differences in infectivity between H and C isolates were found in animal models. These observations have led to the hypothesis that *C. parvum* is transmitted via different transmission routes, each transmitting parasites of one genotype. An alternative view is that both genotypes circulate among different host species, and that genotypically different populations can arise from mixed infections through selection in different host environments.

MATERIALS AND METHODS

DNA purification - PCR amplification was performed either on DNA isolated directly from stool or extracted from purified oocysts. For stool DNA extraction, 100 to 200 ml of stool was incubated overnight in 0.2% SDS and 200 mg/ml proteinase K, extracted with phenol/chloroform and ethanol precipitation. Alternatively, oocysts were purified from stool and DNA recovered by proteinaseK/SDS treatment.

Restriction fragment length polymorphism - Multilocus RFLP was performed using four unlinked RFLP markers; polyT (Carraway et al. 1997), COWP (Spano et al. 1997), TRAP-C1 (Spano et al. 1998) and RNR (Widmer et al. 1998). A sequence-specific PCR assay aimed at the ribosomal internal transcribed spacer 1 (Carraway et al. 1996) was also used.

Isopycnic fractionation of oocysts- Semi-purified oocysts were sedimented on a 15-30% (w/v) Nycodenz (Sigma) for 1 hr at 55,000xg. Fractions of approximately 1 ml were recovered and oocysts concentrated by centrifugation.

RESULTS AND DISCUSSION

In order to investigate the epidemiology of *C. parvum*, we have developed PCR and PCR-RFLP markers. Several coding and a non-coding region were examined for sequence polymorphism. Using a combination of polymorphic markers developed in our laboratories, *C. parvum* isolates originating from different host species and different geographical locations were subject to a multilocus genotypic analysis. Isolates were found to segregate into H (41%), C (52%) and mixed (7%) type isolates. Significantly, in a sample of 29 isolates no recombinants were identified, suggesting reproductive isolation between H and C parasites.

RFLP and sequence analysis of a non-coding region (the b-tubulin intron) identified a high degree of polymorphism (Fig.). A multiple sequence alignment of cloned PCR products spanning the b-tubulin intron and part of exon 2, revealed four groups of sequences and additional polymorphism within groups. Sequences indicative of interallelic recombination were found in two isolates.

The population structure of isolates serially transmitted through calves or passaged from calves to mice, human to mice or calves to humans was examined. Several infections showing changes in RFLP profiles following serial transmission were observed. Using isopycnic fractionation of oocysts, it was possible to separate, in the calf-propagated isolate GCH1, two populations of oocysts bearing different genotypes.

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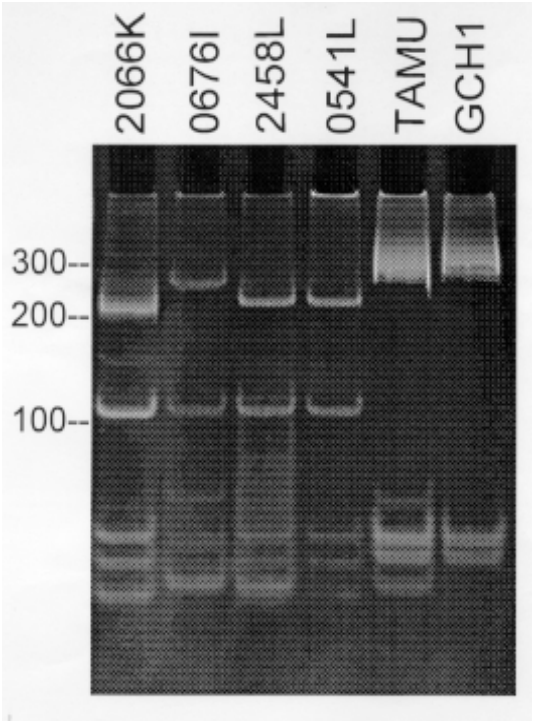

Restriction site polymorphism in the beta-tubulin gene of *Cryptosporidium parvum.* PCR products amplified from the intron and adjacent exon 2 were digested with restriction enzyme Tsp5091. Three restriction profiles were detected among these samples; one in the bovine isolate GCH1 and TAMU, one in human isolate 06761 and one in human isolates 2066K, 2458L and 0541L.

These observations indicate that the epidemiology of *C. parvum* is complex and that individual hosts can excrete heterogeneous populations of oocysts. The significance of these findings for human cryptosporidiosis has not been elucidated. Of primary interest is the question whether isolates of genotype H and C differ in virulence and susceptibility to drug treatment.

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