Microsatellite Analysis of the Human and Bovine Genotypes of *Cryptosporidium parvum*

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Cryptosporidiosis is a significant cause of foodborne and waterborne outbreaks of diarrheal diseases. Molecular typing tools have shown that two genotypes of *Cryptosporidium parvum* are responsible for these outbreaks [5, 9]. The human genotype parasites have so far been only found in humans, whereas the bovine genotype parasites have been found in farm animals and some humans [1, 2, 4-6, 9]. Higher resolution typing tools, however, are needed to identify outbreaks, and to track infection and contamination sources. In other eukaryotic systems, microsatellite (MS) sequences containing simple sequence repeats have been used in the development of high resolution genotyping tool [3, 7]. In this study, we characterized 9 microsatellite (MS) loci in the human and bovine genotypes of *C. parvum* and evaluated inter- and intra-genotype sequence diversities.

MATERIALS AND METHODS

Isolates of *C. parvum* bovine and human genotypes were used in this study. To compare the inter- and intra-genotype diversities, 8 human genotype and 7 bovine genotype isolates from different geographic areas were used in the initial analysis. *C. parvum* human genotype isolates used were HFL5 and HFL6 from a waterborne outbreak in Florida in 1995, HWA4 and HWA5 from a foodborne outbreak in Washington in 1997, HGA6 and HGA7 from a daycare outbreak in Atlanta, and HGM9 and HGM10 from pediatric cases in Guatemala City. *C. parvum* bovine genotype isolates used were BOH6 and BOH54 from naturally infected calves in Ohio, BID21 from a calf in Idaho, BWV65 from a calf in West Virginia, BIO1 and BIO2 from calves infected with two different lineages of the Iowa strain, and GCH1 from calves infected with an isolate from an AIDS patient. These isolates were chosen because of the presence of inter- and intragenotype diversities at other genetic loci. Additional 59 human genotype were used to confirm the intragenotype diversity at MS 1. Oocysts and DNA were isolated from the samples as previously described [8].

Nine MS loci were chosen for sequence characterization. These MS sequences were obtained by screening sequences from a genomic sequencing project of a *C. parvum* bovine isolate. Primers were designed from sequences flanking the MS repeats (Table 1). For all primer pairs, 35 cycles of PCR were performed. PCR products were sequenced from both directions as previously described [8, 9]. Each isolate was sequenced twice from two separate PCR assays to assure sequence accuracy. Sequences obtained from different isolates were aligned to evaluate diversities.

RESULTS AND DISCUSSION

All 9 MS primer sets amplified efficiently the bovine genotype isolates. Primers from MS loci 1-5 also amplified human genotype isolates consistently, whereas those from MS6-9 did not amplify the target MS region or failed to amplify all human genotype isolates. Differences in nucleotide sequences were observed between the bovine and human genotype isolates at MS 1-5 (Fig 1 A to E). These changes included both nucleotide switching and deletion, and occurred both in the simple sequence repeats and outside the repeat regions. In all cases, deletions involving MS repeats and non-repeats occurred in the human genotype isolates, resulting in shorter sequences and smaller PCR products for the human genotype parasites.

Table 1. MS primers used in the study.

Locus	Primers	Repeat
1	5'-GCCAATCGGGATGAAATCC-3' 5'-CACCAAATATTGAAGATAGAAG-3'	$(AT)_8(TA)_1$ $(AT)_{11}$
2	5'-GGCGGTAACATCTTTGCC-3' 5'-TCAAGACAAGACTAACAC-3'	$(AAT)_8$
3	5'-CCAATAGAAGTAGCTCTATCAAGGC-3' 5'-GCAATATTCAATGGTGTTGATTGG-3'	$(AAT)_7$
4	5'-GAAACTGTGATAAATATTCTGGT-3' 5'-GCGAATTTTCTTGATTTCGGTC-3'	$(A)_{14}$
5	5'-CCGGCTTGGGCGCCATTTC-3' 5'-GTAATAGTGACAATAACAG-3'	$(AAT)_9$
6	5'-AAAGTTAAGACAATTGAATGG-3' 5'-GTATCATATGATGAAAATAC-3'	$(T)_{13}$
7	5'-TTGGGAATGGAGTTAGTGAC-3' 5'-CCAATTGTTCTTGTCTTCC-3'	$(TAG)_{15}$
8	5'-GCTGAAGTGGAGTGTTTC-3' 5'-CTAACTGAATGATTATATGTAG-3'	$(AT)_{12}$
9	5'-CTAGATACACAGCCTAATCC-3' 5'-TTTGTCAATTTATGTGGACAC-3'	$(T)_{19}$

Intragenotype diversities were observed in MS 1. The human genotype isolates generated two types (A and B) of sequences. The A sequence had one less AT repeat than the B sequence (Fig 1A). Among the original 8 human genotype isolates used in the characterization, 4 isolates (HGA6, HGA7, HWA4, and HWA5) produced A sequence and 4 (HFL5, HFL6, HGM9,and HGM10) produced B sequence. Subsequent analysis with 59additional human genotype isolates showed that A type of sequence was the predominant (41/67). No other sequence variation was detected in the analysis with increased number of samples.

Intragenotype variation was also detected in the bovine genotype isolates. At the MS 6 locus, 6 of the 7 (except for BID21) original bovine isolates showed A sequence, which had 13 T in the MS repeat comparing to 12 T in the B sequence (Fig 1 F). Although no sequence diversity at MS 1 was observed within the bovine genotype when the original 7 bovine isolates were used in the analysis, subsequent analysis with 2 other isolates revealed that one isolate from Australia (H22) had a variant (B) sequence. The bovine B sequence had one more AT repeat than A sequence, and a deletion of T (as seen in the human genotype) in one the T repeat regions (Fig 1A).

The length polymorphism in MS between the human and bovine genotypes of *C. parvum* provides an opportunity to develop a simple PCR genotyping tool. This avoids the need of restriction digestion or sequence analysis of PCR products, which increases assay time and cost. We used the MS 1 primer set for such a purpose and found this technique satisfactory. Although the diversity in MS sequences may serve as a useful marker for the differentiation of the human and bovine genotypes of *C. parvum*, its current use in high resolution typing of *C. parvum* is limited. There is a need to characterize additional MS loci in order to obtain greater intragenotype variation.

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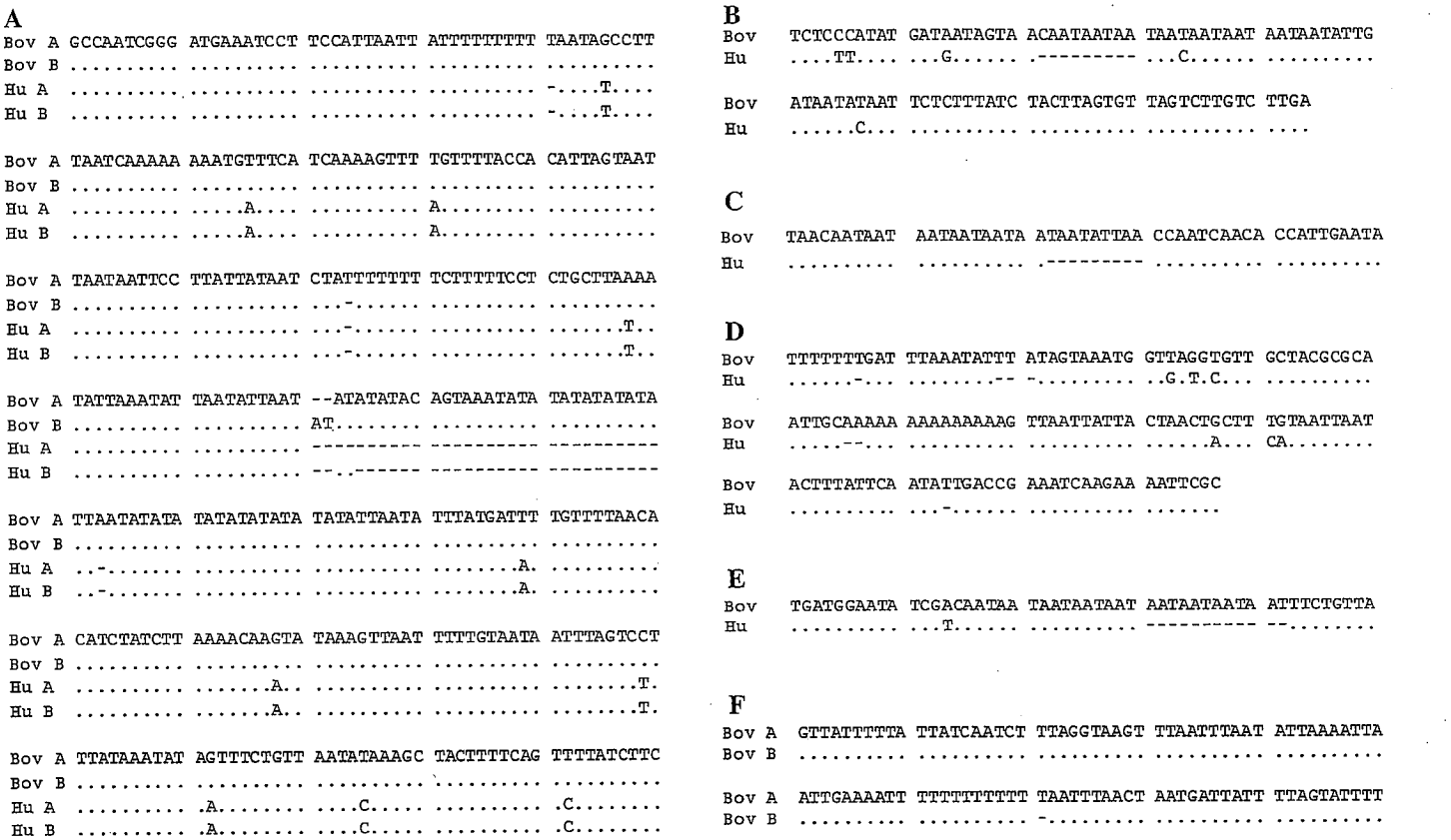

Fig **1.** Inter- and intragenotype variation in nucleotide sequences in 6 microsatellite loci of *C. parvum*. **A:** MS 1. **B**: MS 2. **C**: MS 3. **D**: MS 4. **E**: MS 5. **F**: MS 6. For each locus, only the polymorphic region is shown. Bov: *C. parvum* bovine genotype; Hu: *C. parvum* human genotype.

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