Characteristics of a Novel Type of Bovine Cryptosporidium andersoni

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We isolated oocysts that resemble Cryptosporidium andersoni from cattle grazing on a farm in Japan. The partial sequences of genes from the isolate were coincident with published sequences of genes of C. andersoni and whether all large-type oocysts from cattle are C. andersoni and whether subpopulations of C. andersoni exist (6, 16).

In this study, we characterized Cryptosporidium oocysts isolated from cattle by morphological, biological, and genetic analysis.

Fecal samples were collected from grazing cattle on a farm in Miyagi Prefecture in the northern part of the main island of Japan. As reference strains, we used C. muris RN66, which was originally isolated from a house rat (7) and Cryptosporidium parvum RN66 HNJ-1, which was isolated from a Japanese woman (12). These strains had been passaged in SCID mice in our laboratory. Oocysts used in this study were purified by the sugar centrifugal flotation method. DNA from each sample was extracted with MagExtractor-Genome (TOYOBO, Osaka, Japan) after five rounds of freezing and thawing of oocysts.

Three sets of primers were used to amplify fragments of genes, namely, 18S ribosomal DNA (rDNA) (5'- ACGCATCTCTGA-3' and 5'-CCAATGCAGATGCATCTC ATAA-3'), the gene for heat shock protein 70 (HSP70; 5'- ACAATGCGCCATTCAGGT-3' and 5'-GCTGGTGGA ATACCATCTAAA-3'), and the gene for Cryptosporidium oocyst wall protein (COWP; 5'-CTATGAAATCCTTGC CTTCAAGGT-3' and 5'-GTGGTTGGA ATACCATCTAAA-3'). We designed these sets of primers using OLGIO 5.0 (National BioScience Inc., Plymouth, Minn.), and they were based on sequences of a bovine strain of C. muris and a hyrax strain of C. muris (GenBank accession numbers: AF093496, AF221542, and AF161579).

Subcloned product amplification by PCR of 18S rDNA and of genes for HSP70 and COWP were sequenced on an automated sequencer (ABI 310; Applied Biosystems Japan Ltd., Tokyo, Japan). The sequence accuracy of data was confirmed by two-directional sequencing. By using our sequence data and data from Cryptosporidium species in GenBank, we performed distance-based analysis using Kimura's distance formula (9) and then we constructed a phylogenetic tree using MEGA (version 2.1) (10).

To assess the infectivity of the isolate, we inoculated 10⁶ purified oocysts orally into eight 4-week-old SCID mice. The feces of each mouse were collected, and the discharge of oocysts was monitored for 28 days. During the first survey of fecal samples from cattle after 23 days of grazing, we detected ovoid Cryptosporidium oocysts in feces from 6 of 113 cattle. Two of the six cattle died subsequently for unknown reasons. The four other cattle discharged oocysts for the entire grazing period (143 days). Long patent periods may be a characteristic of bovine Cryptosporidium, as reported previously (13, 14). We also detected oocysts in feces from 2 of 28 calves that were born during the grazing. The average dimensions of these oocysts ranged from 7.4 by 5.1 to 7.6 by 5.9 µm, whereas those of C. muris RN66 and C. parvum HNJ-1 are 8.1 by 5.1 µm and 4.8 by 4.2 µm, respectively. Postmortem examination of a 3-year-old cow revealed Cryptosporidium at various stages of development on the epithelial cells of the abomasum.

Amplification by PCR of 18S rDNA of C. muris RN66 and the isolate yielded products that were 1,253 and 1,255 bp long, respectively, and C. muris RN66 and the isolate yielded products of amplification of the genes for HSP70 (1,145 bp) and COWP (448 bp) of the same respective lengths. There were no differences in the respective nucleotide sequences of these three genes among isolates obtained from eight cattle. The nucleotide sequence of 18S rDNA from the isolate was identical to that from a bovine strain of C. muris (GenBank accession no. AF093496 [19]). Although only a sequence of 265 bp that was within the region we sequenced was registered for C. andersoni in GenBank (accession no. AJ275963 [16]), this sequence was homologous to that obtained from the new isolate. Partial sequences of genes for HSP70 and COWP from the isolate were coincident with published data for bovine isolates of C. muris (AF221542 [17]) and C. andersoni (AF266262 [20]).

For phylogenetic analysis, we constructed trees with the unweighted pair group method with arithmetic mean from aligned sequences of 18S rDNA and sequences of genes for HSP70 from various isolates of Cryptosporidium. In the case of 18S rDNA, two distinct clusters were formed in the genus Cryptosporidium: the first cluster consisted of two genotypes of

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C. parvum, and eight different genotypes of wrairi sporidium felis inoculated and discharged oocysts of each strain.

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REFERENCES


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<th>Mouse no.</th>
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a Values of sequence similarity were calculated by alignment of sequences of inoculated and discharged oocysts of each strain.