INFECTION AND IMMUNITY, Jan. 1990, p. 252–253
0019-9567/90/010252-02$02.00/0

Vol. 58, No. 1

NOTES

Identification of Outer Oocyst Wall Proteins of Three *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) Species by ^{125}I Surface Labeling†

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Received 18 August 1989/Accepted 26 September 1989

Autoradiography of oocyst wall surface proteins of three *Cryptosporidium* spp. revealed common bands at 285 to 290, 145 to 148, 120, 57, and 32 kilodaltons (kDa). *Cryptosporidium baileyi* and *C. muris* share proteins at 180, 100, 80 to 81, 29, and 18 to 19 kDa; *C. baileyi* and *C. parvum* share one protein at 46 to 47 kDa; and *C. muris* and *C. parvum* share a protein at 67 to 69 kDa. Additional protein bands, each unique to one species, were also observed.

Cryptosporidium spp. (Apicomplexa: Cryptosporidiidae) are 4- to 9-μm protozoa that infect the gastrointestinal and respiratory tracts of a variety of species of animals, including humans. Because of the medical and economic significance of one species, *Cryptosporidium parvum*, individuals in several research laboratories are now examining the prevalence of the parasite in feces and surface waters by relying predominately on recently developed immunofluorescent techniques (6–9). Although it is generally not known whether these monoclonal antibodies can cross-react between various *Cryptosporidium* spp., at least one commercially available diagnostic kit utilizes a monoclonal antibody against *C. parvum* that is also capable of cross-reacting with *C. meleagridis* and *C. muris* but not *C. baileyi* (Hydrofluor Combo; Meridian Diagnostics Inc., Cincinnati, Ohio). Because it is important to be able to differentiate pathogenic from innocuous species in the environment, we designed a study to determine which polypeptides occur on the outer oocyst surface wall and which may be common among conspecifics.

Oocysts of *C. parvum* KSU-1 were obtained originally from the feces of a naturally infected 3-week-old calf in March 1987 (11). The isolate has been passaged 3 to 4 times per year in 1- to 2-week-old goats (*Capra hircus*) by oral inoculation with 2.0×10^6 oocysts. Goats were housed in stainless steel cages, and feces were gathered 6 to 9 days postinoculation from collecting pans beneath the animals. Fecally derived oocysts were strained through a series of brass sieves to a final exclusion of 53 μm, concentrated into a crude pellet by a single centrifugation for 30 min at $1,000 \times g$, and stored at 4°C in fresh 2.5% (wt/vol) aqueous $K_2Cr_2O_7$ solution. Oocysts of *C. muris* were obtained from the feces of a naturally infected steer near Caldwell, Idaho. Feces containing oocysts were strained and concentrated as described above for *C. parvum*. Prior to use, oocysts were washed twice in 0.5% (vol/vol) aqueous Nonidet P-40 and concentrated by flotation in a sucrose solution (specific gravity, 1.30). Oocysts of *C. baileyi* were cultivated and harvested from chicken embryos as described previously (4) and stored in phosphate-buffered saline at 4°C for ≤2 weeks until use.

Oocysts were purified from remaining fecal debris or embryonic cells by density gradient centrifugation with a discontinuous CsCl gradient (2) and were stored in phosphate-buffered saline at 4°C for ≤2 weeks. Following purification, oocyst surface proteins were labeled with ^{125}I and water-soluble Bolton-Hunter reagent (10). Oocysts were then suspended in phosphate-buffered saline to the desired concentration, and 10 μg of each of the protease inhibitors aprotinin, leupeptin, phenylmethylsulfonyl fluoride, *N*-*p*-tosyl-L-lysine chloromethyl ketone, and L-1-tosylamido-2-phenylethylchloromethyl ketone per ml was added. Parasites were frozen at −75°C for ≤60 days until use.

Parasite proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 5 to 15% gradient gel by using a discontinuous buffer system (3). Prior to use, oocysts were frozen at −75°C and thawed (three cycles) to rupture walls and were solubilized in reducing buffer containing sodium dodecyl sulfate and β-mercaptoethanol. The equivalent of 6×10^6 *C. parvum*, 4.5×10^6 *C. muris*, and 5×10^6 *C. baileyi* oocysts were added to individual lanes. Molecular weight standards were coelectrophoresed in separate wells for comparative purposes. Bands were visualized by the enhanced silver-staining method (1), followed by drying and autoradiography with Kodak XAR X-ray film.

The total protein profile of freeze-thawed sporulated oocysts of the three *Cryptosporidium* species is shown in Fig. 1. Although autoradiography revealed vague similarities in both total number and molecular size distribution of oocyst surface proteins among the three species, a number of differences were noted (Table 1). Oocysts of *C. parvum* appeared to possess the fewest detectable surface proteins (17 in all), whereas *C. muris* possessed 18 and *C. baileyi* possessed 18. At least five surface proteins with molecular sizes of 285 to 290, 145 to 148, 120, 57, and 32 kilodaltons

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† Kansas Agricultural Experiment Station contribution no. 90-57-J.

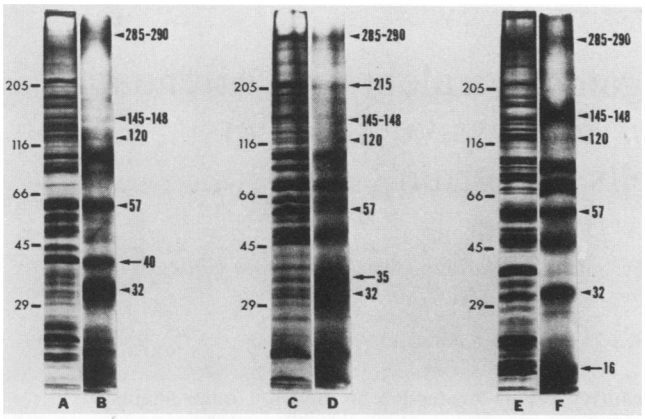

FIG. 1. Sodium dodecyl sulfate-polyacrylamide (5 to 15% acrylamide) gradient gel profiles of three *Cryptosporidium* spp. Lanes A, C, and E, Silver-stained profiles of *C. baileyi*, *C. muris*, and *C. parvum*, respectively; lanes B, D, and F, ^{125}I autoradiograms of oocyst wall surface proteins of the three parasite species. Symbols: ◀, surface proteins that all three parasites appear to have in common; ⬅, potentially unique proteins. Molecular size markers (left of lanes A, C, and E) and sizes of representative proteins are in kilodaltons.

(kDa) appeared to be common to all three species (Fig. 1, arrowheads; Table 1). Both *C. baileyi* and *C. muris* shared five distinctive proteins with molecular sizes of 180, 100, 80 to 81, 29, and 18 to 19 kDa. *C. baileyi* and *C. parvum* shared one distinctive protein at 46 to 47 kDa. *C. muris* and *C. parvum* shared a protein at 67 to 69 kDa not found on *C. baileyi* oocysts. Several labeled protein bands, each unique to its species, were also observed (Table 1), some of the more prominent of which are indicated in Fig. 1 (arrows). Several of the large-molecular-size bands seen in the silver-stained profile of *C. muris* (215, 247, and 272 to 274 kDa; Fig. 1, lane D) are lightly present on the autoradiogram negatives of the other two species but failed to reproduce on the prints.

Lumb et al. (5) recently reported ^{125}I labeling of the outer oocyst walls of five isolates of *C. parvum*. Bands at 15.5, 32, 42, 47.5, 79, and 96 kDa were consistently present for all isolates, whereas several proteins of other molecular sizes at 52, 62, 116, and 132 kDa were present in some but not all isolates. Our results found three surface proteins at 16, 32, and 46 to 49 kDa, which correspond to the 15.5-, 32-, and 47.5-kDa bands of Lumb et al. (5). Our 73-kDa band may correspond to the 79-kDa band of Lumb et al. (5), but we noted no 42- or 96-kDa bands. Although the 32-kDa band has been suggested as a possible candidate for environmental probes (5), our results suggest that the protein is probably common to all three species. On the basis of our study, surface polypeptides at 16, 25 to 27, 37, 72 to 73, 86, and 240 kDa are potentially unique to *C. parvum*, and monoclonal antibodies against these epitopes may offer the best chance of producing a unique detection assay for environmental samples.

This research was supported by a grant from Bioserve Space Technologies (NASA NAGW-1197) and by the Kansas Agricultural Experiment Station.

TABLE 1. Oocyst surface proteins of *Cryptosporidium* spp.

Molecular size (kDa)	Relative autoradiograph exposure intensity of protein from[a]: *C. baileyi*	*C. muris*	*C. parvum*
285–290	++	++	++
272–274	+	+	+
247	+	+	+
240	−	−	+
215	+	++	+
180	+	+	−
145–148	+	+	++++
120	+	+	+
100	+++	+++	−
86	−	−	+++
80–81	+	+	−
72–73	−	−	++
67–69	−	+++	+++
62	−	+++	+++
57	+++	++	+++
49	−	+++	−
46–47	+	−	++
40	+++	−	−
37	−	−	+
35	−	+++	−
32	++++	++	+++
29	+	+	−
25–27	−	−	+
21–22	+	−	−
20–21	+	−	−
18–19	++	+++	−
16	−	−	++++
15	+++	−	−
14	−	+++	−

[a] Symbols: −, band not present; +, low; ++, moderate; +++, high; ++++, very high.

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