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Comparison of the FeKal CON-Trate System with the Formalin-Ethyl Acetate Technique for Detection of Intestinal Parasites

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The ability of the FeKal CON-Trate system (Trend Scientific, Minneapolis, Minn.) to recover parasite ova, cysts, or larvae from 300 fecal specimens was compared with that of the Formalin-ethyl acetate sedimentation technique. One hundred stool samples had previously been determined to contain 129 parasite forms. The FeKal system detected 127 parasites (sensitivity, 98.4%), and the Formalin-ethyl acetate sedimentation technique detected 125 parasites (sensitivity, 96.9%). Higher counts of parasite forms were obtained for 11 of 14 species of parasites in specimens prepared by the FeKal system. Four specimens were negative by the Formalin-ethyl acetate method and two by the FeKal system. These false-negative observations occurred in specimens with low counts of parasites. There were no false-positive results.

The Formalin-ether sedimentation technique (2) for determining the concentration of intestinal parasites in stool specimens withstood modification for 30 years until the incendiary hazard posed by the use of diethyl ether was reduced by the substitution of ethyl acetate (1, 3, 4). Recently, Trend Scientific, Inc., Minneapolis, Minn., introduced a system that is based on the Formalin-ether sedimentation method but is essentially self-contained with centrifuge tubes, filters, solvent, and a mucolytic agent. We describe here a comparative study of the Trend FeKal CON-Trate system model no. FK40 stool concentration kit (FeKal) and our routinely used Formalin-ethyl acetate sedimentation (FEAS) technique.

We were unable to use randomly selected stool samples in this study since the proportion of samples found to be positive at this institution is about 11% per year. The cost of processing all specimens until we had a satisfactory number of positives was thus prohibitive.

MATERIALS AND METHODS

A total of 300 fecal specimens sent to the Clinical Parasitology Laboratory were used in this study. Of these specimens, 100 were known to be positive for parasite ova, cysts, or larvae by direct examination, FEAS, and a trichrome stain. The other 200 specimens had been determined to be negative by the same method. All samples (~3.0 g) were emulsified in 15.0 ml of 10% Formalin, and this was divided into equal volumes for examination by each method.

For FEAS (3), each stool emulsion was filtered through two layers of gauze into a 15.0-ml centrifuge tube and then centrifuged at 500 × *g* for 2 min. The supernatant was discarded, and the sediment was resuspended in 10.0 ml of water. Ethyl acetate (3 ml) was added to the suspension, and the mixture was shaken vigorously for 30 s. The specimen was then centrifuged as before. The plug of debris was loosened with an applicator stick and, together with the liquid, was carefully decanted. A cotton swab was used to wipe the inside of the tube to remove excess fluid. The remaining pellet, about 0.25 ml, was resuspended with a drop of Lugol's iodine solution, and a drop of material was applied to a slide, covered with a coverslip (22 by 22 mm), sealed with Vaspar, and examined in its entirety at ×100 and ×400 magnifications. All parasite forms were counted.

The instructions of the manufacturer on the package insert were closely followed for the FeKal system. Two drops of CON-Trate reagent A were added to the Formalin-fixed specimen, and the container was shaken vigorously for 1 min. A CON-Trate filter was inserted into the top of a 15-ml centrifuge tube provided with the kit. The fecal suspension was poured through the filter, the filter was removed and discarded, and saline was added to fill the tube. The specimen was centrifuged at 500 × *g* for 2 min. The supernatant was decanted, and the sediment was resuspended in 9.0 ml of 10% Formalin. Reagent B (3 ml) was added, and the tube was capped and shaken for 30 s with the tube inverted. The tube was then centrifuged at 500 × *g* for 2 min. The layer of reagent B, the plug of debris, and the aqueous layer were discarded, and the inside of the tube was wiped with a cotton swab. The remaining sediment was mixed with a drop of Lygol's iodine, and the specimens were examined as before.

RESULTS

One hundred specimens contained at least one parasite egg, cyst, or larva, and one specimen contained seven parasite species. The parasite most frequently seen was *Giardia lamblia* (40 specimens, 31%), and the least common parasites were *Clonorchis sinensis* (1 specimen, 0.8%), *Entamoeba hartmanni* (1 specimen, 0.8%), and *Hymenolepis nana* (1 specimen, 0.5%).

The total numbers of each parasite form seen for each species are given in Table 1. The results for helminth and protozoal forms are tabulated separately since the smaller protozoal cysts were more difficult to observe and identify without a trichrome-stained preparation. The FEAS technique gave a total count of 2,144 helminth eggs and larvae, and the FeKal method gave 2,807, an increase of 23.6%. For protozoal cysts the counts were 31,916 and 40,834 for the FEAS and FeKal systems, respectively, an increase of 22% for the FeKal system. There were a total of 129 observations since many of the 100 positive stool specimens were from patients with multiple parasitic infections. Fourteen species of parasites were detected by both methods. These were helminth ova or larvae and protozoal cysts. The FEAS technique revealed 34,060 parasites, and the FeKal system revealed 43,641, an increase of 22%. The FEAS technique

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TABLE 1. Recovery of parasite ova, cysts, and larvae by the FEAS and FeKal concentration methods

Parasite form	No. of known positive stool specimens	No. of parasites (no. of positive specimens)	
		FEAS	FeKal
Helminths			
Ascaris lumbricoides	11	959 (10)	1,643 (11)
Clonorchis sinensis	1	512 (1)	589 (1)
Hookworm	10	118 (8)	71 (9)
Hymenolepis nana	1	8 (1)	5 (1)
Strongyloides stercoralis	15	404 (14)	322 (15)
Trichuris trichiura	10	143 (10)	177 (10)
Protozoa			
Blastocystis hominis	2	232 (2)	980 (2)
Chilomastix mesnili	2	28 (2)	81 (2)
Endolimax nana	16	5,571 (16)	8,098 (16)
Entamoeba coli	13	1,618 (13)	2,351 (12)
Entamoeba hartmanni	1	2 (1)	4 (1)
Entamoeba histolytica	4	6,845 (4)	8,660 (4)
Giardia lamblia	40	17,033 (40)	19,813 (40)
Iodamoeba butschlii	3	587 (3)	847 (3)

missed four parasites detected by the FeKal method; these were one *Strongyloides* larva, one *Ascaris* egg, and two hookworm eggs. The FeKal system missed two parasites detected by the FEAS method; these were one *Entamoeba coli* cyst and one hookworm egg. The FEAS technique detected 125 of the 129 positive specimens (sensitivity, 96.9%). The FeKal system detected 127 positive specimens (sensitivity, 98.4%). No specimen was found to be negative by both techniques. There were no false-positive results among the 200 specimens previously determined to be negative.

Specimens which were positive by only one method were observed to have counts of a single organism (egg, cyst, or larva), indicating low levels of infection. It is more likely that these differences were due to chance.

We observed that the FeKal system provided a cleaner preparation with fewer particles of vegetable matter, and this may have permitted better visualization of parasite forms.

No parasite ova, cysts, or larvae were found in the 200 fecal specimens previously determined to be negative.

DISCUSSION

The greater overall sensitivity obtained in this study with the FeKal system appeared to be related to a more efficient filtration mechanism, which resulted in a cleaner preparation, and perhaps to the action of a mucolytic agent which may have freed parasite ova and cysts from clumps of mucus. The time taken to process specimens by the two methods differed greatly. Filtration of a specimen by the FEAS method was completed in 2 min, whereas the FeKal system required 30 min. The FeKal system is self-contained except for the Formalin and applicator sticks, and it is convenient because the tubes and filters are disposable. This advantage is offset by the cost of the kit, which is three times that of the materials used in the Formalin-ethyl acetate technique.

Although there were differences in the numbers of parasite forms revealed by the two methods, few organisms were missed by either of them and only when very low numbers of parasites were excreted. Neither test by itself was as sensitive as our standard methodology, which includes iodine- and trichrome-stained smears. This emphasizes the importance of the stained smear in examining stool samples for parasites. The trichrome-stained slide method is an additional test which provides more definitive identification and reveals forms, especially trophozoites, which are not amenable to detection by concentration procedures.

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