The use of Lactophenol Cotton Blue (LCB) stain for wet mount preparation of feces

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In this study, Lactophenol Cotton Blue (LCB) was used to demonstrate intestinal parasites by microscopic examination of feces and this stain was compared with Lugol's iodine and eosin stains. A sedimentation procedure was used for concentration of 502 fecal specimens. Three preparations of each specimen was stained with each of the stains and examined microscopically, initially by a low-power (10x) and then a high power (40x) objective of a light microscope. Of 502 specimens, 96 (19.1%) specimens yielded pathogenic and 73 (14.5%) specimens yielded nonpathogenic parasites. Of the 96 pathogenic parasites, 81 (84.4%) were Giardia lamblia, 7 (7.3%) were Hymenolepis nana, 4 (4.2%) were Taenia species, 3 (3.1%) were Enterobius vermicularis and 1(1%,) was Trichuris trichiura. The distribution of nonpathogenic parasites was as follows: 34 (46.6%) Entamoeba coli, 18 (24.7%) Endolimax nana, 12 (16.4%) Iodamoeba butschlli and 9 (12.3%) Chilomastix mesnili. The cysts or ova of all pathogenic and nonpathogenic parasites that were identified with lugol and eosin stains could be detected with LCB. Thus, LCB is considered to be an alternative stain that may be used for the routine parasitologic examination of feces. [Turk J Med Res 1997; 15(1): 26-28]

Key Words: Intestinal parasites, Diagnosis, Lactophenol Cotton Blue

Parasitic diseases have a worldwide distribution and continue to cause significant morbidity and mortality; particularly in developing countries. In Turkey parasite infections are still important. Since clinical features are not specific for the agent, the diagnosis of intestinal parasitic infections is dependent upon the macroscopic and microscopic examination of feces. For microscopic examination direct wet mount preparation, wet mount after concentration and permanent staining procedures can be performed on stool specimens. For the definitive diagnosis of parasites, several stain solutions have been used. These include Quensel stain, Lugol's, Dobell and O'Connors, D'Antoni's iodine solutions and Nair's buffered methylene blue (1). Besides these methods Parije et al (2) reported for the first time, the evaluation and use of LCB as a temporary staining agent in the wet mount preparation of stools for the demonstration of parasites.

In this study, we used LCB, eosin and Lugol's iodine for the microscopic examination of feces to demonstrate the parasites and compared the results. Also we determined the frequency of intestinal parasites in stool samples that were sent to our laboratory.

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MATERIALS AND METHODS

In this study, 502 fecal specimens were examined. The specimens were collected into wide mounted, plastic containers and promptly transported to the laboratory for examination. The sedimentation technique was used as a concentration procedure. The wet mounts were prepared by adding a drop of the sediment to a drop of lugol, eosin and LCB stains on a glass microscope slide and placing a coverslip on the mixture. The preparations were microscopically screened initially by a low-power (10x) and then by a high power (40x) objective of a light microscope. Pathogenic and nonpathogenic parasites and their staining features were recorded (1,3).

RESULTS

Direct microscopic examination of 502 fecal specimens stained with iodine, eosin and LCB showed cysts and ova of various pathogenic parasites in 96 (19.1%) and nonpathogenic parasites in 73 (14.5%) specimens.

The distribution of pathogenic parasites is given in Table 1 and nonpathogenic parasites in Table 2.

LCB, like iodine, stains internal structures of cysts and ova, thus faciliating the recognition and identification of them in stools. LCB stained Taenia eggs (Fig 1), E. vermicularis eggs (Fig 2) and G. lamblia cysts (Fig 3) and other parasites could easily be detected and identified.

When stained with eosin, protozoan cysts were light red and were faintly visible, whereas helmintic ova were clearly discernible.

Table 1. The distribution of pathogenic parasites

Parasite	n	%
G. lamblia	81	84.4
H.nana	7	7.3
Taenia spp.	4	4.2
E. vermicularis	3	3.1
T. trichiura	1	1
Total	96	100

Table 2. The distribution of nonpathogenic parasites

Parasite	n	%
Entamoeba coli	34	46.6
Endolimax nana	18	24.7
Iodamoeba biitschlii	12	16.4
Chilomastix mesnili	9	12.3
Total	73	100

With all the stains, the bile-stained helmintic ova stained brown, red or blue and lost their natural color.

The recognition of *I.butschlli* was facilitated with iodine, as this protozoan has a glycogen vacuole and stains brown with iodine.

In LCB wet mounts polymorphonuclear leukocytes (PMNL) were few or absent in stool preparations. As PMNL's lysed the accurate identification of-cysts or ova was obtained.

Also in the LCB wet mounts, vegetative cells, mucus, artifacts etc. were stained deep blue and could easily be detected (Fig 4).

DISCUSSION

As in other developing countries, in Turkey, parasitic infections are still frequent and cause significant public health problems. The diagnosis of most parasitic infections is dependent upon the laboratory. Direct microscopic examination is an important component of parasitologic examination of feces. The microscopic examination of fecal specimens is routinely performed in all medical centres and feces are screened for parasites by either experienced or sometimes by inexperienced laboratory staff. Incorrect laboratory diagnosis is shown to cause the misdiagnosis of parasitic infections. Since parasitic infections are not clinically specific and other diagnostic tests are not widely used, these infections become widespread and chronic, leading to such problems as retarded development of children (1,4).

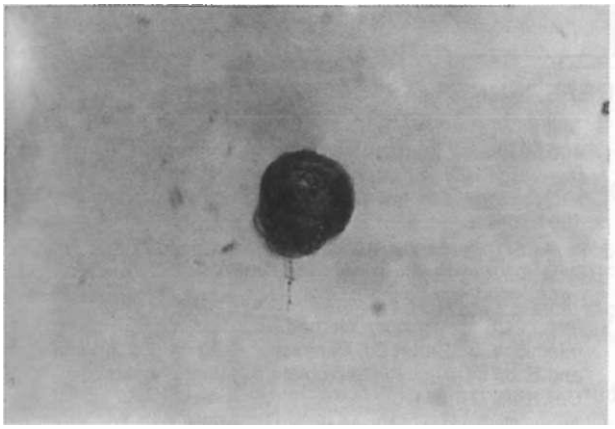

Figure 1. LCB-stained Taeniae egg (magnification, x400)

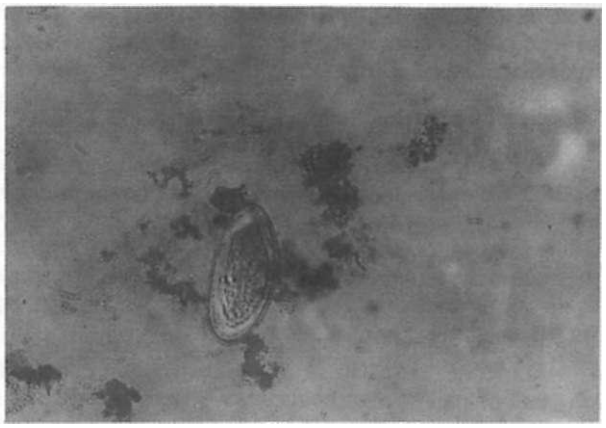

Figure 2. LCB-stained E.vermicularis egg (magnification, x400)

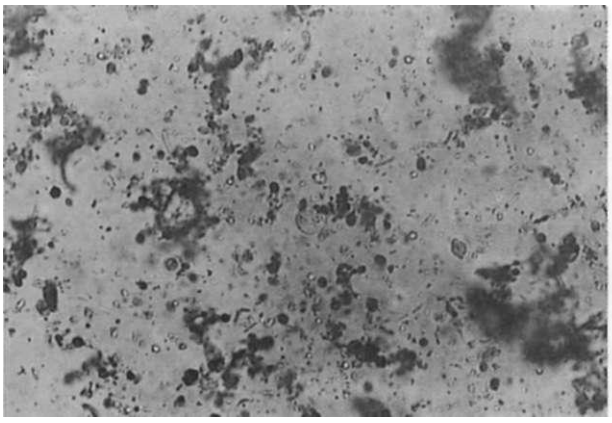

Figure 3. LCB-stained Giardia lamblia cyst (magnification, x400)

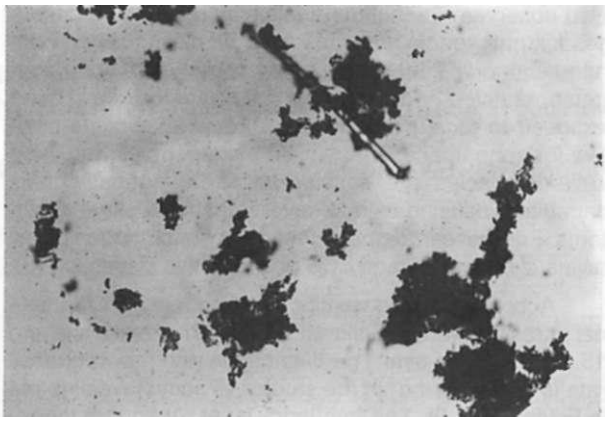

Figure 4. LCB-stained fecal material (magnfication, x100)

In the routine parasitology laboratories, the use of unstained preparations in physiological saline helps to demonstrate especially the trophozoites. However, definitive diagnosis of cysts and trophozoites is difficult, since internal structures are often poorly visible. To overcome this disadvantage several solutions have been used. These are Quensel stain, Lugol's, Dobell and O'Connors, D'Antoni's iodine solutions and Nair's buffered methylene blue. Lugol's iodine is the one that's most commonly used (1).

In our study, the iodine stained all helmintic ova, whereas protozoan cysts were poorly visible. Protozoan cysts possessing glycogen vacuoles could easily be defined by Lugol's iodine solution.

In the examination with eosin, protozoan cysts were not distinctive, but helmintic ova were clearly visible.

LCB is a stain routinely used in wet mounts of fungal cultures for microscopic examination and it was used for parasitologic examination for the first time by Parija et al. (2). In our study, the use of LCB made screening of stool specimens for parasites easier, because it is difficult to overlook parasites stained blue even with a low-power objective. This is particularly important because LCB allows screening of the fecal specimens rapidly without causing any eyestrain and leads to accurate identification of parasites. This will be an advantage especially for unexperienced laboratory staff. Also LCB contains phenol and lactic acid, that kill viable trophozoites, cysts and ova. This is also important since the examination of fecal specimens becomes more safe when the infectivitiy is reduced.

On the other hand, confusion of cysts or ova with PMNL's was prevented because they had been lysed by the stain. Also since the artifacts had been deeply stained more accurate identification could be made. Artifacts in the sediment may be confused with protozoan cysts or helmintic ova, especially in direct wet mounts or wet mounts after concentration (1)-

The only disadvantage of LCB, which we observed was that natural colored helmintic ova were stained blue. This problem had occurred in both the other stains, as well.

In this study, besides the staining properties of pathogenic parasites, those of nonpathogenic parasites were also observed. Even though this may seem to be unnecessary, pathogenic parasites must be distinguished from nonpathogenic species. Also, the presence of nonpathogenic species indicates that the person has been exposed to fecal contamination. Fecal specimens positive for nonpathogens may yield pathogens as well. Also several species are considered to be nonpathogenic capable of causing mild to severe gastrointestinal symptoms. For these reasons detection of nonpathogenic parasites is important, and LCB provided this distinction (5).

According to our results, the frequencies of intestinal parasites were as follows: 84.4% protozoan cysts and 15.6% helmintic ova. The distribution and the frequency rate is similar to that in the studies of some investigators in Eskişehir (6-9). The frequency is less than that reported by Doğan et al (10). However Doğan's study was performed in nursing homes and child care centers, where conditions increased parasitic infections.

We found the LCB wet mount to be simple and easily available. Its use aided in the detection and identification of protozoan cysts and helmintic ova in stools. Thus LCB may be used for routine microscopic examination of stools in parasitology laboratories as an alternative stain.

Dışkının parazitolojik incelemesinde laktofenol pamuk mavisinin kullanımı

Bu çalışmada, barsak parazitlerinin belirlenmesi amacıyla dışkı örneklerinin direkt mikroskopik incelemesinde laktofenol pamuk mavisi kullanılarak, bu boya lugol ve eozin boyalarıyla karşılaştırıldı. Bu amaçla 502 dışkı örneği çöktürme yöntemi ile yoğunlaştırılarak her üç boya ile x10 ve x40 büyütme ile incelendi. 502 örnekten 96 (%19.1)'sında patojen, 73 (%14.5)'ünde apatojen parazitler saptandı. 96patojen parazitten 81 (%84.4)'i Giardia lamblia, 7 (%7.3)'si Hymenolepis nana, 4 (%4.2)'ü Taenia spp., 3 (%3.1)'ü Enterobius vermicularis ve 1 (%1)'i Trichuris trichiura idi. Apatojen parazitlerin ise 34 (%46.6)'ü Entamoeba coli, 18 (%24.7)'i Endolimax nana, 12 (%16.4)'si Iodamoeba butschlli, 9 (%12.3)'u Chilomastix mesnili idi. Yapılan direkt mikroskopik incelemelerde laktofenol pamuk mavisi kullanılarak, lugol ve eozinle boyanan örneklerde görülen patojen ve apatojen tüm parazit kist ve yumurtaları kolaylıkla tanımlanabildi. Sonuç olarak; laktofenol pamuk mavisinin dışkının parazitolojik incelemesinde rutin boyalara alternatif olabilecek özellikte bir boya olduğu ve kullanılabileceği kanısına varıldı. [T Klin Araştırma 1997; 15(1):26-28]

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