CONTRIBUTION TO THE LABORATORY DIAGNOSIS OF HUMAN CRYPTOSPORIDIOSIS

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SUMMARY

Human cryptosporidiosis is an infection caused by Cryptosporidium spp, a coccidial protozoan of emergencial pathogenicity and responsible for severe and prostrating watery diarrhea, mainly in immunocompromised patients. Smears of stools submitted to concentration and staining by carbol fuchsin technique has been used in our laboratory as a diagnostic procedure for cryptosporidiosis. The long time observing the smears in the microscope due to the small size of the forms and the low contrast of the staining led us to introduce some modifications in the original protocol for the acid-fast staining. The smears were treated with the carbol fuchsin solution for 3 minutes as recommended by LENNETTE et al., 1985 and the solution of the ethyl alcohol 70%-chloride acid 0.5% was used instead of the ethyl alcohol-sulfuric acid 5% recommended by HENRIKSEN & POHLENZ, 1981. Smears were treated with the discoloration solution for 2 minutes. These modifications promoted a better washing out of the excess of carbol fuchsin therefore increasing the dye efficiency. In such conditions, the visualization of protozoan oocysts on the slides examined became easier. It’s worthwhile to emphasize that these modifications offer advantages when time and accuracy are concerned.

KEYWORDS: Cryptosporidium spp; AIDS; diagnosis

INTRODUCTION

Cryptosporidiosis is an infection caused by a protozoan parasite named Cryptosporidium spp, that colonizes epithelial cells of the gastrointestinal and respiratory tracts of vertebrates and is related, mainly, to enteric diseases in human beings.

The genus Cryptosporidium contains several species that were identified in a vast sort of hosts including mammals, birds, fishes, and reptiles. Although there are nineteen species described so far only Cryptosporidium muris and Cryptosporidium parvum are associated with mammals. Nevertheless, considering the difficulties in the specific identification through the use of immunologic tests and molecular biology and the positive confirmation, so far, of existing species, the term Cryptosporidium spp has the preference when regarded to those secluded from humans.

The first cases of human cryptosporidiosis were diagnosed by intestinal and rectum biopsies. Before 1978, the parasite used to be diagnosed histologically only in tissue sections and observed in the epithelial surface of the intestine by electron microscopy or by staining with hematoxylin and eosin, Giemsa, periodic acid-Schiff, or toluidine blue. POHLENZ et al., 1978 described the finding of Cryptosporidium oocysts in fecal material of young calves, since then the diagnosis of this protozoan has become essentially coprologic, although it shows some limitations.

Diagnostic methods for detection of oocysts in stool were already described by using agglutination techniques of latex particles covered with antibody anti-Cryptosporidium, direct immunofluorescence and enzyme-linked immunosorbent assay. Although there are several sensitive and specific immunoserologic tests they require technical skills and sophisticate equipments. Therefore, these methods are not used routinely in the majority of laboratories. Fecal smears, stained by carbol fuchsin (acid-fast staining) were proposed as an alternative method to detect Cryptosporidium spp oocysts in stools.

In this report, the carbol fuchsin method was modified to further improve its performance in the diagnosis of cryptosporidiosis.

MATERIAL AND METHODS

Fecal samples of HIV-positive patients, with and without diarrhea, were analyzed. Patients were followed up regularly by the Special Health Service DST/AIDS program in Araraquara, SP, Brazil.

Samples were preserved in phosphate-buffered saline containing 10% formaldehyde and concentrated by using the formol ether or the formalin ethyl acetate techniques. Smears were prepared on glass slides, dried and methanol fixed. Stools of young pigs containing oocysts of Cryptosporidium spp were used as positive control.
Samples were treated with carbol-fuchsin solution for 3 minutes. The discoloration procedure was realized with ethyl alcohol 70%-chloride acid 0.5% for 2 minutes. Smears were washed with running water and counterstained with solution of methylen blue at 1% for 1 minute. After the final wash with water, slides were dried at room temperature. Slides were analyzed by optical microscopy (Jenaval - Carl Zeiss) using 250x and 1000x magnification (immersion).

RESULTS

The acid-fast stain method modified by us in this report confirmed the presence of Cryptosporidium spp oocysts in fecal samples of HIV-positive patients. Oocysts can be seen as an intense pink-reddish body in contrast with the blue background (Fig. 1 and 2).

Results obtained in this study revealed an effective improvement in the oocysts detection when compared to the acid-fast staining method described previously (Fig. 3 and 4).

DISCUSSION

Cryptosporidiosis was initially diagnosed in intestinal biopsy. At present the diagnosis is basically coprologic, although not all routine techniques have been proved effective. The staining methods described

Fig. 1 and 2 - Cryptosporidium spp oocysts in fecal smear. Acid-fast staining using alcohol-chloride acid solution (250x and 1000x).

Fig. 3 and 4 - Cryptosporidium spp oocysts in fecal smear. Acid-fast staining using alcohol-sulfuric acid solution (250x and 1000x).
so far are based on the alcohol-acid fast property of coccidia oocysts. These methods when used in smears of concentrated stools have been proved to be very efficient in the detection of oocysts, increasing the diagnostic value of coprologic tests11.

In the literature, a large number of coprologic techniques are based on the direct examination of wet smears or smears treated with lugol7, phenic fucsin3, methanamine silver1, nigrosin18, auramine-rodamine2, acidrine orange4. Giemsa2, methylene blue/eosin4, safranin-methylene blue and modified periodic acid-Schiff11. These staining techniques are not specific to Cryptosporidium and therefore are not used routinely in the coprologic diagnosis of cryptosporidiosis.

The diagnosis of cryptosporidiosis was improved with the discovery of the acid-fast property of Cryptosporidium oocysts. There are several staining techniques based on the original Ziehl Neelsen staining10,11,14,15. Also, some modifications were proposed to eliminate the general staining techniques based on the original Ziehl Neelsen staining. The modified acid-fast staining, used in this investigation of Cryptosporidium spp when using a 250x magnification (Fig. 3) or in a 1000x magnification and greater visual accuracy to detect the oocysts of species other than Cryptosporidium spp and Cryptosporidiosis. These methods when used in smears of concentrated stools have been proved to be very efficient, reliable and feasible at any laboratory of clinical diagnosis (Fig. 4). The modified acid-fast staining, used in this investigation was confirmed using a magnification of 1000x (Fig. 2). The modifications described in this report decreased the microscopy utilization time and made the slide analysis quicker and less tiring.

To compare the results obtained with this modification with those generated by using the original technique, smears were also submitted to a discoloration with alcohol sulfuric acid solution. Using the original technique, smears were also submitted to a discoloration with alcohol sulfuric acid solution. The modified acid-fast staining, used in this investigation has shown to be effective, reliable and feasible at any laboratory of clinical analysis, due to the easy execution and low cost.

RESUMO

Contribuição ao diagnóstico laboratorial da criptosporidiose humana

A criptosporidiose humana é uma infecção causada pelo Cryptosporidium sp, um protozoário coccídio de patogenicidade emergente e responsável por severa e prostrante diarreia aquosa em humanos, principalmente em indivíduos imunodeprimidos. O diagnóstico, feito através da utilização de esfregaços de fezes submetidos a técnicas de concentração e coloração específica pela fucsina-carbólica tem oferecido bons resultados em nosso laboratório. Tendo em vista o longo tempo despendido para a observação dos esfregaços considerando-se a pequenez das formas e o contraste da coloração, realizamos modificações no procedimento técnico da coloração ácido-resistente que resultaram em sensível melhoria das preparações: a fucsina-carbólica passou a ser deixada sobre o esfregaço por período de 3 minutos (LENNETTE et al., 1985) e procedeu-se a substituição da solução de álcool-ácido sulfúrico a 5% (HENRIKSEN & POHLENZ, 1981) por solução de ácido clorídrico a 0,5% em álcool etílico 70%, por cerca de 2 minutos (contribuição original). Estas alterações promoveram melhor remoção do excesso de fucsina-carbólica, aumentando a eficiência da etapa de descoloração e consequentemente otimizando o contraste do processo de coloração. Nestas condições, as lâminas examinadas em microscópio ótico em aumentos de 250x e 1000x tiveram uma visualização dos oocistos do protozoário facilitada, sendo os mesmos observados em contraste delineado com pigmentação intensa de cor rosa-avermelhada contra coloração de fundo azulado. Vale destacar que estas modificações oferecem vantagens de rápido processamento do material e facilidade de visualização do protozoário, diminuindo o tempo de microscopia, tornando a análise das lâminas mais rápida e menos cansativa, agilizando o diagnóstico.

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REFERENCES


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