Short reports

Modified detergent Ziehl-Neelsen technique for the staining of *Cyclospora cayetanensis*

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Abstract

Cyclospora cayetanensis is a cause of prolonged diarrhoea, mainly in travellers. Laboratory diagnosis may be achieved by a number of methods such as the staining of faecal smears by the modified Ziehl-Neelsen (ZN) technique. Safer methods using this technique have been described for the staining of acid fast bacilli and cryptosporidia by replacing the phenol content of the carbol fuchsin stain with various concentrated detergents. In this report the technique was modified slightly using a non-concentrated detergent and applied to the staining of oocysts of *C. cayetanensis*. It was found that oocysts of *C. cayetanensis* do not stain using the modified detergent ZN method when compared with similar preparations containing oocysts of *Cryptosporidium* spp.

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Keywords: *Cyclospora cayetanensis*, Ziehl-Neelsen stain, detergent.

Cyclospora cayetanensis is an established cause of prolonged diarrhoea. Cases of infection have been reported worldwide but may be seen most frequently in individuals who have returned from Central and South America or South and South East Asia. Laboratory diagnosis may be achieved by examining wet mount preparations of faeces under direct light microscopy, by examining wet mount preparations for auto-fluorescence under ultraviolet epifluorescence¹ or by staining faecal smears by the modified Ziehl-Neelsen (ZN) technique.² The latter may be used in some laboratories for the diagnosis of cryptosporidiosis. The same technique may be easily applied to screening for *C. cayetanensis*³ even though screening may be easier by using ultraviolet epifluorescence.⁴

A safer modified ZN method for the staining of acid-alcohol fast bacilli has been described.⁵ The technique involves the substitution of phenol used in the carbol fuchsin solution with a concentrated liquid organic detergent. Phenol is both cytotoxic and carcinogenic. The substitution of phenol for detergent has also been described for use in the modified ZN method for the staining of cryptosporidia.⁶ The technique has not yet been described for the staining of oocysts of *C. cayetanensis*.

Methods

Two separate lots of carbol fuchsin solution were prepared, one containing phenol, the other containing detergent. The former was prepared according to the method of Casemore,² whilst the latter was prepared as follows: 0.2 g carbol fuchsin powder was added to 20 ml absolute alcohol and mixed well. After the former was completely dissolved, 80 ml distilled water was added and gently mixed, followed by 30 ml Amway LOC Regular detergent. Acid-alcohol and malachite green components of the staining technique were also prepared as previously described by Casemore.²

Faecal smears of a sample known to contain oocysts of *C. cayetanensis* and *Cryptosporidium* spp., respectively, were then stained following the method of Casemore,² but using the detergent carbol fuchsin solution as opposed to the phenol carbol fuchsin solution. Control staining was performed in parallel by using smears of the same samples and staining them following the method of Casemore² using the phenol carbol fuchsin solution. The staining methods were repeated at least 10 times to ensure that consistent results were obtained.

Results

Using the modified detergent ZN method, oocysts of *C. cayetanensis* completely resisted staining and appeared as non-refractile cysts against a blue-green background. They could often be seen due to the differentiation from the background. However, they were also often missed due to the lack of staining. Oocysts of *Cryptosporidium* spp. stained pink by this method.

Using the modified phenol ZN method, oocysts of *C. cayetanensis* stained variably. Some stained dark red with a mottled appearance, some stained pink, whilst others did not stain at all and appeared as non-refractile glassy spheres against the blue-green background. Oocysts of *Cryptosporidium* spp. stained dark pink and the staining was less variable.

Discussion

The modified detergent ZN stain did not perform at all well when compared with the results seen using the modified phenol ZN. This was because oocysts of *C. cayetanensis* did not hold the detergent carbol fuchsin stain. A number of detergents have been used for this technique

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including Amway LOC High Suds and BDH Neutracon. Unfortunately, the former is no longer available but LOC Regular, which is a less concentrated formula, was used at a higher concentration than has been previously described to overcome this difference. It is suggested that although the method may be cheaper and is safer than the modified phenol ZN due to the absence of phenol, it should not be used for the modified ZN staining of *C cayetanensis*. The technique should not therefore be applied to the staining of cryptosporidia because incidental findings of *C cayetanensis* may be missed. Although the presence of oocysts of *C cayetanensis* could be detected in the preparations, the technique could not be used for screening purposes in a diagnostic laboratory because the possibility of error would be too great. Oocysts of *Cryptosporidia* spp. have been shown to stain well by others⁶ and did so satisfactorily in this study. However,

the intensity of staining was not as good as seen in the modified phenol ZN. The auramine-phenol technique is probably more widely used for the staining of cryptosporidia. *C cayetanensis* does not stain well by this technique and therefore a separate procedure should be followed for the laboratory diagnosis of this organism.

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Gelatinous degeneration presenting as a preleukaemic syndrome

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Abstract

Gelatinous degeneration of marrow is a rare histological disorder associated with chronic debilitating diseases, such as anorexia nervosa, AIDS and postchemotherapy aplasia. Solid tumours have been associated with this condition but it has been reported in only two patients with leukaemia. In these cases leukaemia and gelatinous degeneration were diagnosed simultaneously. In the case reported here, a 48 year old man, gelatinous degeneration was the only histological finding observed more than two years before the diagnosis of acute myelogenous leukaemia with monosomy 7. The significance of hyaluronic acid deposition remains uncertain. Two hypotheses have been put forward: (1) that gelatinous degeneration occurs during tissue repair; and (2) that gelatinous degeneration inhibits haemopoiesis by altering the microenvironment of the bone marrow. In the case reported here, the presence of monosomy 7 suggests that myelodysplasia was the underlying disorder which finally evolved into acute leukaemia.

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Keywords: gelatinous degeneration, acute myelogenous leukaemia, monosomy 7.

Gelatinous degeneration is generally diagnosed in bone marrow biopsy specimens by the presence of a focal or generalised extracellular deposition of a gelatinous material, identified as hyaluronic acid, in association with fat atrophy and marrow hypoplasia. This disorder has been referred as serous atrophy, mucoid degeneration and starvation marrow.¹ Gelatinous degeneration has been classically observed in association with chronic debilitating disorders, such as anorexia nervosa, starvation, malignancy, chronic infections, systemic lupus erythematosus, and myxoedema.¹⁻⁵ Recently, it has been widely reported in patients with AIDS and a variant form of the classical degeneration has been reported after the administration of chemotherapy.⁶⁻⁸

In the case reported herein, gelatinous degeneration was diagnosed in a previously healthy man, with no evidence of an underlying disorder. To our knowledge, this is the first reported case in which gelatinous degeneration preceded the diagnosis of acute myelogenous leukaemia (AML).

Case report

A 48 year old healthy, well nourished, white man was admitted to hospital in August 1992 because of fever and pancytopenia. He worked as a driver for a chemical company, smoked

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