PREVALENCE OF CRYPTOSPORIDIUM IN RAWALPINDI/ISLAMABAD A COMPARISON OF SALINE, IODINE AND STAINED PREPARATIONS

Pages with reference to book, From 219 To 221

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ABSTRACT

Three hundred stool samples for routine ova and parasite examination were examined as saline and iodine wet preparations, the results were then compared with the Zeihl-Neelsen-modified Acid Fast staining (MAFS), as the oocysts are acid fast in character. Examination with saline preparation was unable to detect any oocyst, while iodine preparation yielded three oocyst and stained smears with MAFS revealed five positive stool samples (JPMA 40: 219, 1990).

INTRODUCTION

Cryptosporidium has only recently been recognised as a human pathogen[1,2]. The protozoa was recognised primarily as a cause of diarrhoea in domestic animals (goats, calves, lambs, chickens, cats and dogs) until the advent of AIDS[3]. Cryptosporidium gained attention as it causes profound, voluminous watery diarrhoea occasionally associated with cramps in AIDS patients[4]. It was occasionally seen in slaughter house workers or veterinarians who developed self-limiting diarrhoea that required no therapy1. However it may cause severe life threatening diarrhoea in elderly[5]. The prevalence of this protozoa has not been reported previously in Pakistan.

MATERIAL AND METHODS

Stool specimens submitted for routine ova and parasite examination were collected, and processed without concentrating the sample. Although the concentrating technique results in increase yield of the oocyst[6] but this method is not convenient in performing routine examination in most clinical laboratories[7,8].

Saline and iodine preparations

Smears were made directly by emulsifying a small portion of stool sample in a drop of normal saline and Lugol's iodine on separate glass slides. These wet preparations were covered by glass cover slip and examined under high power objective (x 40), with reduced light for a minimum of two minutes per preparation covering about 70-80 fields.

Stained preparations

Stool smears were made be emulsifying small portion of stool in a drop of normal saline and air dried. The smears were then fixed in formalin vapours for 20 minutes. Staining was done by cold carbol-fuchsin[9], retaining the stain on the smears for about 20 minutes without any heating. Decolourization was done with 10% sulphuric acid and counter-staining with malachite green. The smears after air drying were screened by using highpower objective (x 40) for atleast 3 minutes exploring about 70-80 fields (Table I).

TABLE I. Appearance of the oocyst and yeast after staining.

Procedure	Oocyst	Yeast
Saline	Refractifle-look	Non-refractile
Iodine	Colourless	Brown
MAFS	Pink	Green*

*Oocyst of cryptosporidium are acid-fast while yeast is not.

RESULTS

Of the total three hundred stool samples tested Modified Acid Fast Staining was able to pick five samples while by Iodine method we detected oocyst only in three stool samples. We failed to detect any oocyst of cryptosporidium by saline preparations. The overall prevalence rate of cryptosporidium as obtained by MAFS was 1.66% (Figures 1,2, Table II).

TABLE II. Percentage of positive samples by different methods.

Procedure	No. of Positive samples
Saline preparation	Nil
Iodine preparation	3 (1%)*
MAFS	5 (1.66%)

* Samples positive by Iodine method were among the five which were positive by MAFS.

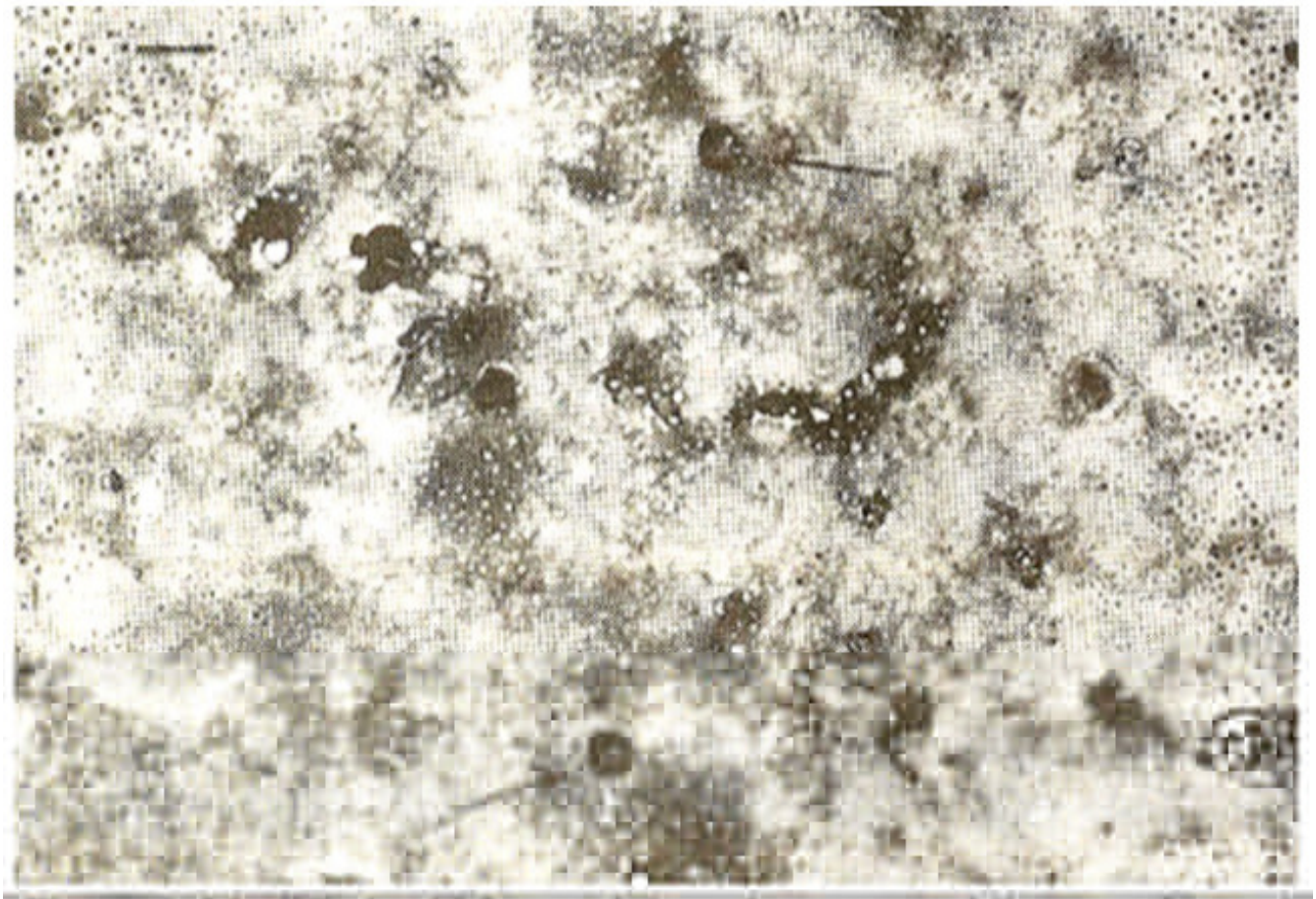

Figure 1. Modified acid fast staining of cryptosporidiumcyst (arrow)
Bar = 10 micron.

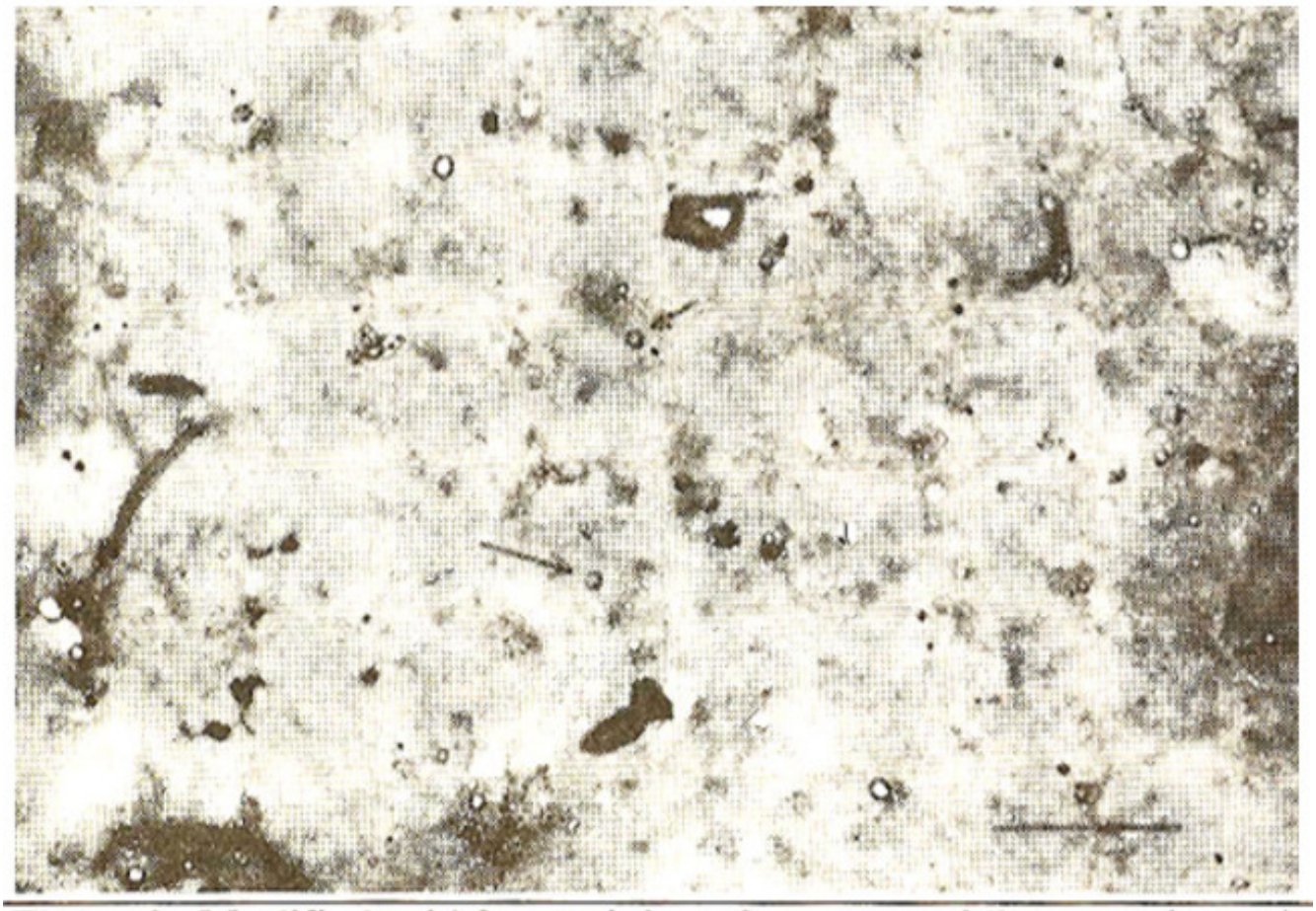

Figure 2. Modified acid fast staining of cryptosporidium cyst (arrow) Bar = 100 micron.

DISCUSSION

Human infection with cryptosporidium was first reported in 1976. In a 3 year old child previously in good health and with no evidence of altered immune status, in whom this organism produced a severe but self-limiting entero-colitis. In another case the infection affected a 39-years old man with bullous pemphigoid who in the course of treatment for the said disease with immunosuppressant (corticosteroids and cyclophosphomide) developed severe diarrhoea which cleared two weeks after discontinuation of the drugs[1]. Recent reports have, therefore, indicated that cryptosporidium species can cause diarrhoea not only in immuno suppressed or immunocompromised patients but also in immunocompetent persons especially in infants and children[1,10,11]. The size of our study was- small but its main scope was to bring in the awareness of the problem. Because the symptoms of cryptosporidiosis are not different from those of many other gastrointestinal infections[12] and because diarrheic stools in Pakistan are not routinely screened for cryptosporidium due to lack of awareness. The sudden awareness of this newly emerging pathogen has raised the question of whether cryptosporidium species is an unrecognised cause of diarrhoea in an otherwise healthy population; if the set up of our living favours this protozoa by having plenty of domestic animals, cattle colonies and poultry farms right in the cities, fmally in the month of Zilhij the animals of sacrifice live literally with us in our houses and compounds. Is the diarrhoea, which is always there immediately after Eid, due to overeating of meat or is it the cryptosporidiosis? It has now also been established that the protozoa can

also spread, via water supplies[13]. Considering the results, although the prevalence rate is not very alarming (1.6%) but it is also very important to mention that we have screened the general public, the samples did not represent the risk group (residents in or near cattle colonies slaughter house workers, people living near poultry farms etc.). Even then the figures are high as compared to the figures of developed western countries. According to published studies from developed countries including Australia, Canada, England and United States prevalence among people without any known risk factor has been extremely low, about 0.5%[14]. Considering our results we suggest MAFS as a method of choice (the best method is by Immunofluorescence technique). Although the procedure is a lengthy one but chances to miss a cyst in a saline or iodine preparation by a technician are more likely (may be due to recent exposure to this newly recognised parasite). We suggest screening of all the cases of infectious gastroenteritis in immunocompetent children by saline and iodine preparation and confirmation of suspected case by MAFS. Further work on the prevalence, incidence and seasonal variation of cryptosporidiosis in Pakistan is highly desired.

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