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A concentration technique for obtaining viable cysts of blastocystis hominis from faeces

V Zaman
Aga Khan University, viqar.zaman@aku.edu

K Z. Khan
Aga Khan University

A Concentration Technique for Obtaining Viable Cysts of Blastocystis hominis from Faeces

Pages with reference to book, From 220 To 221

V Zuman, K.Z. Khan (Department of Microbiology, Aga Khan University, Karachi.)

Introduction

Blastocystis hominisis a common parasite implicated in a variety of intestinal problems[1]. Oral-faecal route of transmission has been postulated but the cystic phase which is presumably the infective stage of the parasite, has only recently been described using electron microscope[2]. However, little information is available on the light microscopic morphology of the cyst. There are two possible reasons for this. The first is the relatively small size of the cyst, as compared to some other intestinal protozoa and the difficulty in separating it from the non-cystic stages of the parasite. The concentration technique described in this paper ovemomes this problem and separates the cysts from the non-cystic stages, there by enabling its easy recognition in faecal samples.

Materials and Method

The concentration technique used is as follows:

1. Approximately 5 gm of faeces was thoroughly mixed in 15-20 ml of distilled water and sieved through gauze to remove coarse particles.
2. Sediment was re-suspended in 15-20 ml of distilled water and centrifuged at 300g for 20 minutes. This was repeated 3 times.
3. The centrifuged deposit was mixed in 1 nil of distilled water and layered on 10 ml of Ficoll-Paque (Pharmacia) column. This was centrifuged at 2000g for 20 minutes.
4. Blastocystis cysts banded about 0.5 cm below the top along with other protozoan cysts such as Entamoeba, Jodamoeba and Giardia. Most, but not all, of the bacteria and other faecal debris got pelleted at the bottom of the tube.
5. The concentrated material in the band was carefully removed with a pasteur pipette and re-centrifuged in 20 ml of distilled water to remove Ficoll-Paque. Suspension of cysts was now available for establishing cultures and studying morphology in electron and light microscope. The essence of the technique is the use of distilled water which breaks up all the non-cystic stages of the parasite. These include the vacuolar, the granular and the ainoeboid forms.

Results

The main morphological features of the cyst in the light microscope are as following:

1.Shape - Using phase-contrast the cyst appeared as a sharply demarcated polymorphic, but mostly oval or circular dense body, surrounded by an outer membranous layer (Figure).

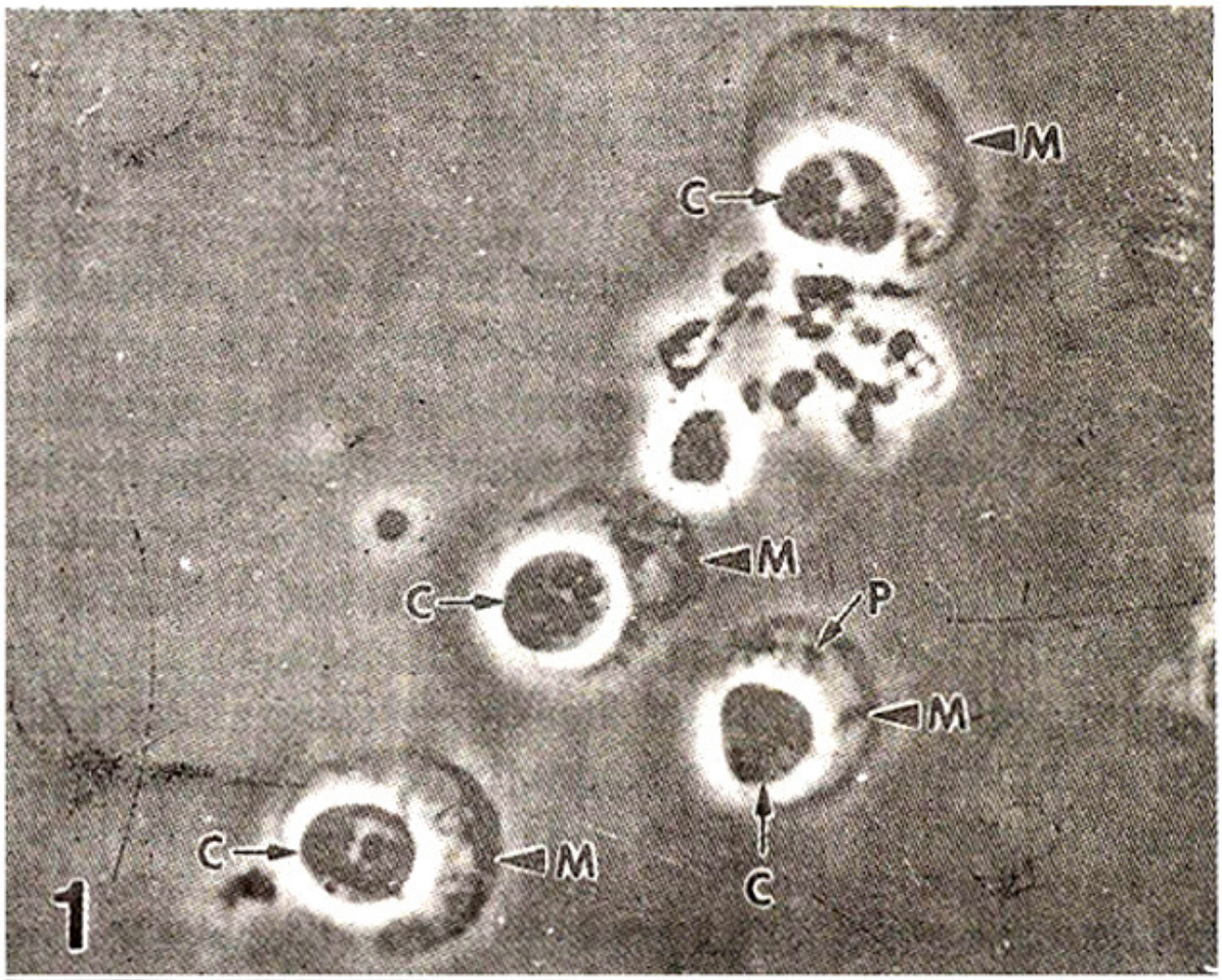

Figure. A concentrated suspension of cysts from faeces. Four cysts are visible in this field. Note the absence of all the other stages of the parasite and relatively bacteria free background. Phase contrast X1200. C=cyst, M=outer membranous layer, P=particulate matter.

Without phase- contrast the membranous layer was not easily visible. The space between the membranous layer and the cyst usually showed fine particulate matter.

2. Size -20 cysts were measured with and without the membranous layer. With the membranous layer the mean diameter was 12.65gm (SD=l .14). Without the membranous layer (cyst only) the mean diameter was 6.65um (SD=2.32).

3. Cyst contents - A number of large and small circular bodies were visible inside the cyst The electron-microscopy of the parasite reveals large mitochondria, so some of these were probably mitochondria and others nuclei. Their relative numbers could not be assessed accurately.

Discussion

One of the diagnostic features of the cyst under the electron microscope is the fibrillar layer which loosely surrounds the cyst. The term fibrillar layer was originally used by Boreham and Stenzel[1], as it appeared to be made up of fibre like material. The outer membranous layer seen in the light microscope is the same as the fibrillar layer and confirms the observation that we were dealing with the cyst and not any other stage of the parasite. The particulate matter seen between the cyst and the outer

membranous layer is also seen in the electron microscope. The size of the cyst in the light microscope is larger than that seen in the electron microscope (3-5um). This is probably due to the flattening of the specimen because of the pressure of the cover glass during light microscopy. The viability of the cysts is maintained in this technique which is very useful as the cysts can be cultured for various other studies.

References

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2. Stenzel, D.J. and Boreham, PF.L A cyst like stage of Blastocystis hominis. Int.J.Parasitol., 1991;21:613-15.