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شبكة المعلومـــات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

﴿ قـسـم ﴾

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد اعدت دون آية تغيرات

يجب أن

تحفظ هذه الأفلام بعيداً عن الغبار

في درجة حرارة من 15 – 20 مئوية ورطوبة نسبية من 20-40 %

To be kept away from dust in dry cool place of
15 – 25c and relative humidity 20-40 %

بعض الوثائق الأصلية تالفة

بالرسالة صفحات

لم ترد بالأصـــل

Studies on Coccidian parasites (*Cryptosporidium*)

A Thesis

Submitted to The faculty Of Science, Cairo University

In Partial fulfillment of the requirement for the degree of Master of Science (M.Sc.) In Zoology
(Parasitology)

By
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Abstract

The separation of *Cryptosporidium parvum* with Cesium chloride collected the oocysts in one sharp zone *Cryptosporidium* antigens showed reaction using isoelectric focusing at PI 4.1 for English and Egyptian calves and two zones of reactions at PI 4.8 and 5 for Egyptian goats.

Using of SDS-PAGE showed 15 similar molecular weight bands at the range between 110 Kda and 15 Kda for *C. Parvum* (English and Egyptian calves. While *C.Parvum* from Egyptian goats showed only 5 bands at 110, 70, 54, 15 and 13 Kda. Western blotting was applied using homogenous 20% Gel *C.Parvum* (English calves strain) reacted with the posiutive sera in 5 bands (MWs, 120, 81, 54, 43 and 11 Kda). While the Egyptian calves reacted at 11 bands at MWs ranged from (160 and 11 Kda). *C. Parvum* (Egyptian goats strain) showed 6 clear bands at 94, 81, 70, 54, 14 and 11 Kda.

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I. Introduction

During the last decade, there has been increasing demands for consumption of meat, milk and milk products through out the whole world. Cattle, sheep and goats are one of the main source of meat, milk and milk products, wool and leather in Egypt. Calves and kids morbidity and mortality are serious problems in all countries where calves and goats are bred.

Parasites considered a very important pathogen agent for ruminants in Egypt causing great losses in the animal productivity or may led to their death. *Cryptosporidium* species is one of the major detectable enteric pathogen causing diarrhea and high morbidity in kids and calves (Nagy *et al.* 1983). Cryptosporidiosis is caused by the coccidian protozoa *Cryptosporidium*. It is the most world wide prevalent pathogen associated with diarrhea in young cattle and usually caused by *C. parvum* which is also responsible for infections in humans, specially in immunocompromised patients. *Cryptosporidium* is a protozoan parasite which completes its life cycle on the intestinal and respiratory surface epithelium of mammals, birds and reptiles (Tzipori, 1983). The intestinal species *Cryptosporidium parvum* is the one which most commonly cause ruminant cryptosporidiosis (Current, 1989). The first report of *Cryptsoporidium* specis infection in cattle appeared in 1971 (Pancieria *et al.*, 1971). However, the role of *Cryptosporium* species as primary enterpathogens was uncertain until 1980, when evidence from field studies showed that the parasite was capable of causing clinical diarrhea in calves in the absence of concurrent infection with the common viral or bacterial agents normally incriminated in outbreaks of neonatal calf diarrhea (Tzipori *et al.*, 1980). Caprine *Cryptosporidium* species infection was first reported in Tasmani, Australia in 1981 (Mason *et al.*, 1981) in a

2 week old kid. No bacterial or viral pathogen were recognised in gut content. It was firstly described in sheep by Barker and Cabonell (1974). *Cryptosporidium* has only received attention in the recent years as an entero-pathogen causing acute diarrhea in man and animals for the following reasons, firstly , the disease rapidly became known as a complication of host immuno-defficiencies , Secondly , it has apparent lake of host specificity among the mammalian species (Current, 1990) Thirdly , there is no known effective therapy for control of the cryprosporidial enteritis (Hill , 1990) .

Cryptosporidiosis in Egypt was investigated in man and animals by examination of direct faecal smears stained with modified Ziehl-Neelsen technique, Giemsa and safranin (Desoky *et al.*, 1989, Salem 1989, EL-Akabawy ; 1993 ; Wahba , 1994 , and Radwan , 1996).

Unfortunately, the antigenic structure of *Cryptosporidium parvum* isolated from Egyptian animals and its relation with antigenic structure of *Cryptosporidium* from other countries was not studied before,

This work is proposed to study:

1. The best way for separation and isolation of purified *Cryptosporidium* oocysts from fecal matter.
2. Characterization of *Cryptosporidium parvum* antigen using Isoelectricfocusing (IEF) and Sodium dodecyl Sulphate (SDS-PAGE).
3. Studying the antigenic difference between different isolates of *Cryptosporidium parvum* (Calf origin ; Egyptian and English strain; and goat origin; Egyptian strain;).
4. Identification of a diagnostic eptitope of *Cryptosporidium parvum* isolates using western blotting teechnique.

II. Review of literature

1. Isolation and Purification of *Cryptosporidium* oocysts

Heyman *et al.* (1986) developed a method to obtain purified *Cryptosporidium* oocysts from fecal samples. Oocysts were initially collected by centrifugation through a sucrose density gradient and further purified by passage through glass bead columns. The purified oocysts were antigenically active and sufficiently pure for immunological studies.

Waldman *et al.* (1986) found that *Cryptosporidium* oocysts were concentrated by an ether-phosphate-buffered saline sedimentation technique and then separated by density gradient centrifugation. This two-step method yielded highly concentrated oocysts largely free of bacteria and fecal matter.

Madore *et al.* (1987) eluted *Cyptosporidium* oocysts from the filter, and concentrated using centrifugation. The resultant pellet was then homogenized, sonicated, and placed on a sucrose gradient to separate oocysts from the sediment. The uppermost gradient layer was then examined by immunofluorescence using a labeled monoclonal antibody.

Kilani and Sekla (1987) described two new procedures for the purification of *Cryptosporidium*. The first, consisting of pretreatment of oocysts with sodium hypochlorite followed by concentration using a Percoll gradient. This technique was suitable for nucleic acid analyses. The second, a concentration of untreated oocysts using a Cesium chloride gradient. It was suitable for biochemical and immunological studies.

Lumb et al (1988) recovered *Cryptosporidium* oocysts by density gradient centrifugation from diarrheal feces of four human patients and one goat kid. Goat-derived oocysts were further treated with excystation medium and the excysted oocyst walls purified by isopycnic ultracentrifugation.

Arrowood and Sterling (1989) found that techniques for the large-scale isolation of *Cryptosporidium* oocysts and sporozoites, obtained from the feces of experimentally infected Holstein calves, were developed using discontinuous sucrose gradients and isopycnic Percoll gradients. The oocyst recovery method utilized 2 sequential discontinuous sucrose gradients followed by 1 Percoll gradient. Recovered oocysts were essentially free of debris and bacteria and represented 34% of the original oocyst suspension. Sporozoites were recovered from excystation mixtures on a single Percoll gradient. Sixty-three percent of the original sporozoites were recovered with 2.2% contamination by intact oocysts and virtually no oocyst walls

Upton *et al.* (1988) passed used Cesium chloride gradient-purified oocysts by passing them through 1.0-cm-diameter columns with lengths of 2.5, 5.0, and 10.0 cm at 23 C. Following column passage, oocyst viability was determined both in vitro by excystation and in vivo by the ability to establish infections in suckling mice. Oocysts were found to be retained by the pentaiodide resin in a linear fashion, probably by electrostatic interactions. Linear regression analysis revealed that 100% of the oocysts should be removed in such a manner using a column length of greater than or equal to 25.7 cm.

Regan *et al.* (1991) used different centrifugation speed to purify *Cryptosporidium* oocysts. Red blood cells were removed by differential

centrifugation (600 xg); merozoites remained in the supernatant. The merozoites were pelleted (2,100 xg) and washed in modified Hank's balanced salt solution deficient in Mg+2 and Ca+2. Percoll purification (density 1.070 g/ml and centrifugation speed of 22,000 xg for 30 min).

Rhee et al (1991) purified *Cryptosporidium* oocysts. Fecal samples from various animals using Sheather's flotation technique, Kinyoun's modified acid-fast staining, and osmic acid pre-fixed Giemsa staining.

Moodley *et al.* (1991) found that a comparative assessment of a modification of the Sheather's flotation technique and other commonly employed staining procedures proved the modified Sheather's technique to be most useful in identifying *Cryptosporidium* oocysts in diarrheal stools.

Suresh and Rehag (1996) evaluated three methods of isolating *Cryptosporidium parvum* oocysts from rat feces. Oocysts were initially isolated by sucrose density gradient centrifugation. They were further purified by passage through a glass bead column or a Percoll gradient or by dialysis. Although oocysts recovered from the glass bead column and by dialysis were relatively free of fecal debris, only oocysts recovered from the Percoll gradient were free of bacteria

Brush *et al.* (1998) found that the electrophoretic mobility of oocysts purified on a cold Percoll-sucrose gradient from feces was defatted with ethyl acetate (EAPS method), thus displaying the negative surface charge at neutral pH. The hydrophobicity of oocysts and two types of polystyrene beads was measured as a function of ionic strength by adhesion to polystyrene. Oocysts were purified by the DIS method.