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A New Modified Staining Technique for Demonstration of *Prototheca* Spp. Algae on Paraffin Sections First Record.

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THE ENVIRONMENTAL *Prototheca* algae under certain condition are responsible for protothecosis infection in animal and human. The detail pathology and pathogenesis of this type of infection was unclear. Light microscopic diagnosis of *Prototheca* spp. algae is difficult with the use of routine histologic H&E stains. The specific stains of fungusas periodic acid–Schiff (PAS) or Gomori Methenamine silver (GMS) were used and these stains are not enough for examination of algae. A new modified staining method was prepared. The main chemical constituents were Chromotrope 2R, Aniline blue and phosphotungestic acid. The new stain gives very sharp affinity for staining of intra and extra cellular of *Prototheca* spp.microalgae and sporangium in different stages of maturation. The new stain will help the pathologist to study the pathology and pathogenesis of this type of pathogenic algae and considered the first specific stain for algae in tissue section.

Keywords: Chromotrope 2R, Aniline-Blue, *Prototheca* algae, sporangium, sporangiospores.

Introduction

The chemical Chromotrope 2R has been used in the Gomori trichrome staining in tissues [1] and staining of eosinophil's [2], also it used for the staining of microsporidia in body fluids and stool samples [3]. Chromotrope-Aniline-Blue-staining were used to identify hyaline droplets in the rat kidney [4]. Hematoxylin and eosin (H&E) stainispoor for *Prototheca* spp. in tissue section [5]. In this note Imodify Chromotrope 2R –Aniline blue solution for staining of *Prototheca* spp. microalgae in paraffin sections for the first time.

Materials and Methods

Buffered formalin fixed tissue samples of kidney of natural infected animal with *Prototheca* spp. was confirmed the infection by using PCR techniques and ultra-structure examination. For histopathological examination staining of PAS and GMS were used for confirm the infection, but the results was not satisfied.

A new modified stain specific for *Prototheca* spp. algae was prepared; the chemical contents are Chromotrope 2R, Aniline Blue and phosphotungestic acid. The procedure of preparing the stock solution of the stain is simple, I mix well 1.0 g of Chromotrope 2R to 0.5 g of Aniline Blue and phosphotungestic acid and add 5 ml of glacial acetic acid mix and then add 100 ml distilled water. Working solution was Prepared by adding equal volume of stock solution to distilled water. Keep the stock solution in a dark bottle for 3 months at room temperature. The staining method of tissue sections are very simple, paraffin tissue sections 5-6 microns thick were attached to slides, dewaxed, and hydrated with distilled water. After hydration, sections are staining with routine H&E stains .Other section stained with freshly prepared working solution of the new modified stain for 20-90 minutes according to the type and thickness of the slide, then differential stain in acid alcohol, dehydrate with 95% ethanol wash in absolute alcohol for 5 minutes, and clear with xylene and mount with DPX. The section is ready for examination with research microscope.

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Result

The results indicated that H&E stain did not react with *Prototheca* algae in examined tissue and give bright color with *Prototheca* spp. (Fig.1). The new modified stain showed excellent affinity for *Prototheca* spp. microalgae in the degenerated contents of the epithelial cells of renal tubules, and taking red rouge color (Fig.2). The sporangium color is always from a blue or violet color to red, the small and growing sporangium taking faint to deep blue stain however, other mature one taking red color (Fig.3). These changes may be due to the duration time of staining, chemical contents and maturation of sporangium, and presence of sporangiospores. The disadvantages of the stain in our work that chromotrope 2R will stain red blood corpuscle with red color. The protocol of this stain is very simple and easy. The chemical ingredients of the dye is safe and non-expensive and will help the pathologist and mycologists to study the morphological characteristics of *Prototheca spp.* infection in tissues this stain will help us for understand some pathological and pathogenesis of this type of pathogen.

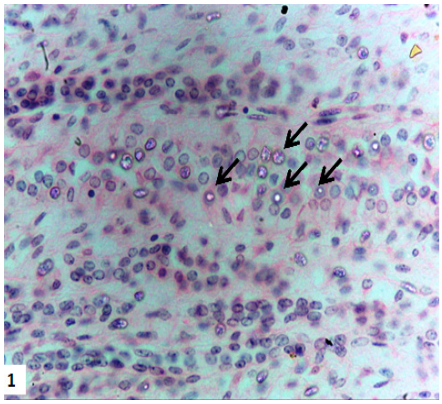

Fig. 1. Cross section of renal tubules showing poor staining of Prototheca algae (arrows). (H&E., X200)

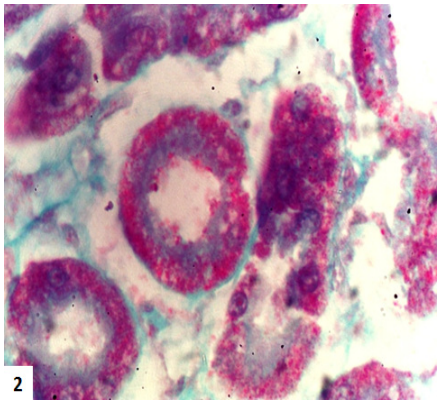

Fig. 2. Cross section of renal tubules showing heavy infection with intracellular micro algae. (Modified chromotrope2R-Aniline blue. X200).

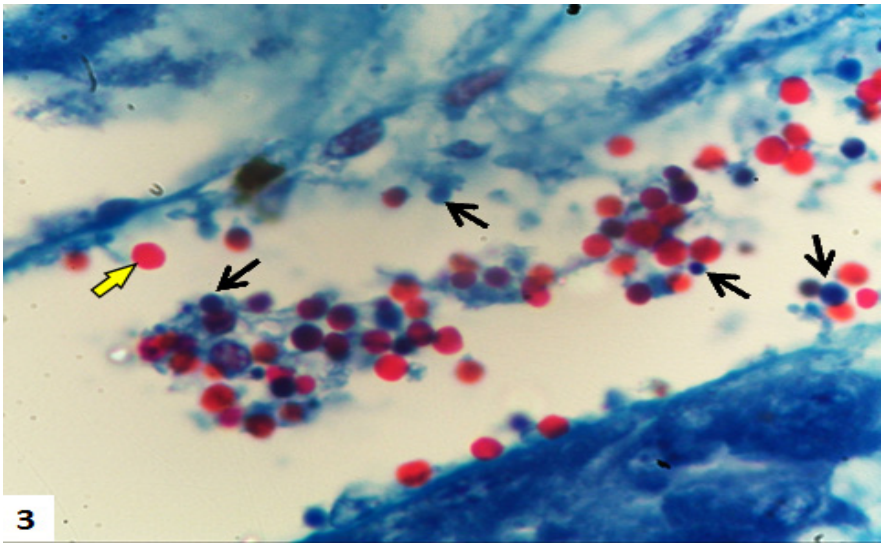

Fig. 3. Cross section of distal renal tubules showing heavy infection with different size and color of sporangium, the mature take red color(yellow arrow) and the small immature take blue in color (arrows). (Modified chromotrope2R-Aniline blue. X 400).

Conclusion

This new stain will help us to study protothecosis in animal and human in details with other specific attain as PAS and GMS and molecular identification.

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Conflicts of interest:

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the article.

This proposal has been submitted to the Academy of Scientific Research & Technology No. 2142 on December 2018 to obtain the patent and preserve the rights of the discoverer.

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طريقة معدله لصباغه الطحالب من نوع بروتوسيكا فى قطاعات انسجه البرافين -التسجيل الاول

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الطحالب وحيده الخليه من نوع بروتوسيكا تحت ظروف خاصة تحدث امراض لكل من الانسان والحيوان. لا توجد صبغه مخصصه لدراسه الطحالب فى الانسجه ولكن يعتمد على صبغات الفطريات وهى غير كافيه للفحص والدراسة تم تصميم صبغه متخصصه للطحالب وذلك لاول مرة . تم تقديم المقترح الى اكاديميه البحث العلمى بتاريخ ديسمبر ٢٠١٨ برقم ٢١٤٢ للحصول على براءة اختراع.