



## Application of Phase- Contrast Microscope for Detection of *Prototheca* spp. Microalgae in Tissue Section-First record (Note)

Youssef Fawzy Ahmed

Department of Animal Reproduction & A.I., Veterinary Research Division. National Research Centre. Dokki, Cairo, Egypt.



**P**HASE -contrast microscope was discovered in 1932 by the scientist Frits Zernike who won the Nobel Prize for Physics in 1953 for this scientific discovery. This technique is based on the fact that different parts of living cells have different densities and reflect the light with varying degrees and give different refractive parameters of the light. We used these phenomena to detect *Prototheca* spp. microalgae which are plant in origin and the wall contains sporopollenin which is biopolymers in nature and not found in animal tissue and this material is not in harmony with the contents of different animal cells. Therefore, it is possible to exploit this scientific phenomenon as a new method for the first time to identify *Prototheca* spp. microalgae in infected tissues. It has been possible to identify the infected tissue with *Prototheca* spp. microalgae with phase-contrast microscope as a simple rapid techniques, easy used for unstained slide. Also this technique may help the pathologist to study the life cycle and pathogenesis of this group of pathogen with the other specific standard methods for examination.

**Keywords:** Phase- contrast microscope, *Prototheca* algae, Microalgae.

### Introduction

Phase- contrast microscopy was used for examination of living activity of some protozoa as toxoplasma, ruminant microflora, and morphology of insects. Also evaluation of sperm [1] and ova of different animal species and examination of diatoms in animal tissue [2]. Now we use phase-contrast microscopy for detection of sporangiospores of *Prototheca* spp. in unstained tissue section.

### Method

Natural infected tissue of animal was confirmed infection with *Prototheca* spp. by using PCR techniques, also ultra-structure examination was confirmed the infection of *Prototheca* spp. algae in tissue. *Prototheca* spp. that detected in the infected kidney tissue showed bright color in the cytoplasm and nucleus and showed poor staining

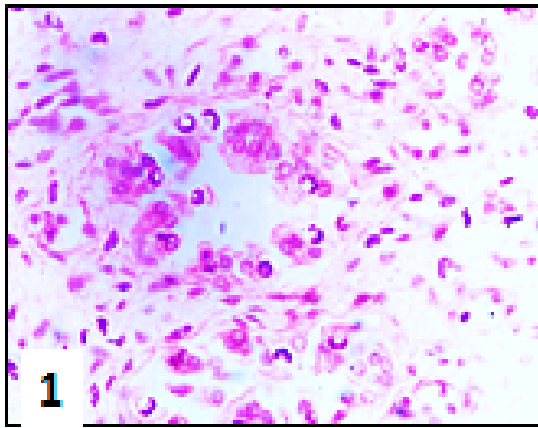
with Hematoxylin and Eosin (Fig.1). I used different stains as PAS and GMS for confirmation the infection. Paraffin tissue sections 5-6 microns thick were attached to slides, dewaxed, and the slide section was cleaned with xylene, the slide was covered with coverslip by using synthetic mounting medium as DPX, then the slide was examined under phase-contrast stain.

### Results

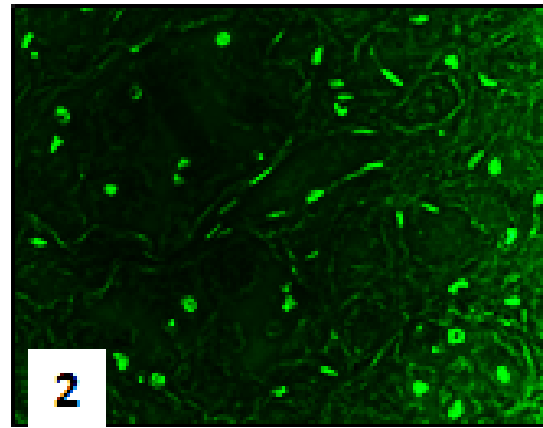
*Prototheca* spp. microalgae in both cytoplasm and nucleus of the renal tubules give positive reaction with phase- contrast microscope (Fig.2). However, the sporangium gives faint or negative results with phase contrast microscope.

### Conclusion

Phase –contrast microscope can used as a rapid simple tools for detection of *Prototheca* spp. microalgae in infected tissues. For my knowledge



**Fig. 1:** Cross section of renal tubules infected with *Prototheca* spp. showed poor staining. (H&E.x400).



**Fig. 2:** Cross section of renal tubules infected with *Prototheca* spp. showed positive with phase contrast microscope. (Unstained section, x400).

this method was considered the first to detect *Prototheca* spp. microalgae in tissue section by phase-contrast microscope. Other specific stains and ultrastructure and molecular examination were used for confirmation the infection.

#### **Acknowledgements**

The author is grateful to special unite (improvement of reproductive performance of farm animals), National Research Centre, Egypt, for the support of this work.

#### *Conflicts of interest*

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

#### **References**

1. Dina, El-Sayed, M. Rashad. DNA integrity and ultra-structures of epididymal spermatozoa in dromedary camels. M.V.Sc. Degree. Benha University. *Faculty of Veterinary Medicine* (2018).
2. Ahmed, Y. F. Unexpected presence of diatom in pathological specimens of fetal membrane of aborted cattle. Case Report. *Egypt. J. Vet. Sci.*, **47** (2), 111- 115 (2016).

## تطبيق مجهر الطور التباين للكشف عن فصيلة البروتوسيكيا *Prototheca spp* الطحالب الدقيقة في قطاعات الانسجة

يوسف فوزى احمد

أستاذ الباثولوجى وامراض الحيوان - قسم التكاثر الحيوانى- شعبه البحوث البيطريه- المركز القومى للبحوث- القاهرة - مصر

المجهر الضوئى ذو الأطوار المتباينة تم اكتشافه سنة ١٩٣٢ عن طريق العالم فريتس زيرنيكه (Frits Zernike) الذي حاز على جائزة نوبل للفيزياء عام ١٩٥٣ من أجل هذا الكشف العلمى . وتعتمد هذه التقنية أن الأجزاء المختلفة للخلايا الحية لها كثافات مختلفة. أى لها معاملات انكسار مختلفة للضوء وأجزاء الخلية لها معاملات انكسار أخرى مختلفة وعندما يسقط الضوء بزاوية معينة على الخلية فإن الأجزاء المختلفة للخلية تعكس وتكسر الضوء بدرجات متباينة وتعطي معاملات انكسار مختلفة للضوء وتظهر النتيجة أن بعض الأجزاء تبدو مضيئة تعكس الضوء بدرجة عالية وأخرى أقل إضاءة وبالتالي تتباين الإضاءة المنبعثة من الأجسام المختلفة وهذا يؤدي إلى إعطاء تباين - تغاير فى خلفيات العينة المراد فحصها، وبالرجوع الى طبيعته تكوين الطحالب وحيدة الخلية حيث أنها نباتية المنشأ أتضح أن جدارها يتكون من مادة الاسبوروبولينين وهى تتكون من مواد بيوبوليمر طبيعيه وهذه المادة لا تتجانس مع محتوى الخلية الحيوانيه وبالتالي امكن استغلال هذه الظاهرة العلميه كطريقه حديثه تستخدم لأول مرة للتعرف على الطحالب الميكروبيه . وامكن تحديد الانسجه المصابه بالطحالب بطريقه علميه سهله وبسيطه.