

Novel Data About Unusual Location of *Toxoplasma Gondii* Within Active Microbial Community in the Anterior Chamber of the Eye of Egyptian Rural Children

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Abstract

In Egypt, many cases of granulomatous anterior uveitis consisting of single or multiple gelatinous nodules were detected in children living in rural areas. These lesions are believed to be waterborne and were previously attributed to flatworms' stage, showing some improvement after antiparasitic treatment. In a trial to explore the nature of these ocular lesions among rural Egyptian children, twenty surgically excised ocular lesions were subjected to transmission electron microscopy (TEM) examination. TEM results were combined with previous results of the metagenomic analysis performed for four cases out of the twenty samples, revealing the presence of *Toxoplasma gondii* (*T. gondii*), besides, a wide range of microbial communities, including variable species of fungi, bacteria, and archaea. The excised lesions ranged from 1 to 5 mm in size and demonstrated an extensive inflammatory cellular infiltrate. Using TEM, five out of twenty samples revealed active eukaryotic organisms with intact energetic cellular organelles, besides, numerous nuclei encircled within a syncytial layer and enclosed by a hyaline layer rich in mitochondria. Six samples showed inactivity in the cellular and the covering portions, while just inflammatory reaction was seen in the remaining nine samples. *Toxoplasma gondii* was found free within the distal part of the syncytium while, the proximal part showed the active synthesis of possibly extra polymeric substance, perhaps secreted by the microbial community. In a conclusion, *Toxoplasma gondii* has been detected among a microbial community in an atypical lesion in the eye. Further studies need to be sustained on genotype characterization, proteomic analysis, besides, the aquatic transmission of these mixed microbial species to the ocular tissues to clarify the reason behind such ocular illness.

Introduction:

In rural areas in Egypt, the Nile River and the irrigation canals are used for agriculture, drinking water, and for children's recreational activities. Thousands of Egyptian children are used to swimming and playing in the canals of the Nile River, exposing them to waterborne microbes [1, 2]. Among those children, many were reported to present with granulomatous anterior uveitis consisting of single or multiple pearl-like creamy white gelatinous nodules, which were almost seen in the inferior quadrant of the anterior chamber. These lesions were previously believed to be related to flatworms' life cycle stage, and as documented before, showed some improvement after antiparasitic treatment [3]. Analogous ocular lesions were reported earlier in children from India, and later from Southern Texas [4–6]. In these studies, members of the class Trematoda were accused of causing the lesions, applying histopathological and/or traditional molecular analysis using universal nonspecific primers.

In a trial to investigate the nature of these ocular lesions, metagenomic analysis was performed for a limited number of cases (under publication) to reveal surprisingly, by all measures the presence of *Toxoplasma gondii* (*T. gondii*) parasite among a community involving a wide range of microbial organisms, including fungal, bacterial and archaea within all the investigated samples. These initial results stimulated the curiosity of our team to explore what is going on inside these ocular lesions.

The present study aimed to visualize the organization and the ultrastructure of the microbial community within these ocular lesions utilizing transmission electron microscopy (TEM), including those initially investigated genetically.

Results:

Twenty children with ocular lesions (Figure 1) in the anterior chamber were involved. All patients had a history of swimming in the River Nile, where most of them presented in early autumn (September and October) with a history of swimming in river canals along with the summertime. It was noticed that the inflammation around the lesions was transiently improved after receiving anti-inflammatory. The size of the removed lesions ranged from 1 to 5 mm. High power microscopic examination for semi-thin sections stained by toluidine blue revealed dense inflammatory infiltration. Nine of these twenty samples showed just inflammatory reaction rich in eosinophils (Figure 2). While in the other eleven samples, inflammatory cellular infiltrates rich in eosinophils were seen enclosing a mass, which was similar to what is known as (Splendore–Hoepli phenomenon). Further, sectioning of these eleven samples revealed detailed structures, which were wedged between the extensive inflammatory reaction. Five of these eleven samples were reported to be clinically active, moving from one place to another inside the eye, especially after treatment, and appeared in semi-thin sections as a body with heterogeneous cellular components, enclosing a homogenous almost central portion. This body was enfolded by a clear homogenous thinner layer, that appears as a coat covering the whole structure (Figure 3A). In the other six samples, internal structures were seen, still with an obvious outer covering layer, but the body lost most of its cellular structure that was replaced by a hyaline homogenous structure, inactive lesions with no reported motility (Figure 3B).

The full details of bioinformatics results are under publication, but here we explore some scientific photographs not included in our study under publication. BioSankey diagram for one sample as an example showed a wide range of eukaryotic and prokaryotic organisms within the investigated mass (Figure 4). As shown in Figure 5, the Krona graphical representation of metagenomic analysis of two samples featured the classification and abundance of the whole microbial community including *T. gondii*, while the Krona graphical representation for species distribution within the Bacteria and Ascomycota communities are shown in Figures 6 and 7, respectively.

Electron micrograph for the ultra-thin sections of the five samples revealed active structure with clear intact cellular organelles. The cellular organelles were in the form of numerous mitochondria of variable size, which were found more in number, near and within the outer layer (Figure 8A), in addition to Golgi apparatus, rough endoplasmic reticulum, and free ribosomes (Figure 8B). All of these organelles and numerous nuclei were observed without any cellular boundaries but enclosed within a syncytial layer. This syncytium appeared full of active organelles and covered with a homogenous hyaline layer that seemed active as if it was in a dynamic cellular process.

Toxoplasma tachyzoites were seen only at the peripheral part of the lesion near the hyaline layer surrounding the lesion. Numerous mitochondria were seen near the parasitic stages, as well (Figure 9). The hypersecretory activity was also observed as numerous secretory vesicles, while abundant secretory granules were noticed within the distal cytoplasm near the outer hyaline layer. The graphical abstract was designed in a trial to elucidate the dynamic cellular process seen during the examination of these active structures.

While the other six lesions revealed signs of inactivity and necrosis of both the cellular and the covering portions. Within these inactive lesions, no clear cellular elements were observed, instead, areas of degenerated tissues were seen. In addition, the cellular activity of the outer layer, which was obvious in the active stages, was not seen. On the contrary, uneven boundaries with lost cellular activity were noticed (Figure 10).

Discussion:

The twenty children with ocular lesions enrolled in this study are just a sample of patients with similar lesions who frequently attended the ophthalmology clinics. Anti-inflammatory drugs were recommended as the first line of treatment, and the lesions were surgically removed for those not responding to medical therapy or those with recurrent lesions after treatment. The belief that prevailed in the earlier periods and the related research studies indicated a stage of one of the flatworms as the cause of these ocular infections [3–6]. Many Egyptian specialists in collaboration with their scientific teams accepted such assumption and recommended antiparasitic drugs alone, in combination with surgical aspiration, or other interventions in the management of such presumed trematode-induced anterior uveitis in children in different governorates Amin et al. [3] in Alexandria, El Nokrashy et al. [7] in Mansoura, Sadek et al. [8] in Al-Fayoum. The same concept was followed in India [4, 6]. Recently, in the Egyptian governorate, Sohag, Alsmman et al. [9] applied argon laser to the anterior chamber nodules with or without medical treatment to treat similar ocular lesions.

These preliminary identifications may have come from the fact that these studies relied on traditional methods of examination as histopathology or conventional PCR using universal nonspecific primers. In addition, findings as Splendore–Hoeppli phenomenon, microscopically observed within sections from the removed ocular lesions, may have been used to explain the investigators' views. Similarly, this phenomenon was also reported in all ocular lesions involved in this study. The reaction is characterized by the presence of dense eosinophilic amorphous material surrounding the causative microorganisms, which may be of fungal, bacterial, or parasitic nature [10]. Moreover, syncytial tissues, which are full of nuclei and cellular organelles, were visualized. This may have increased the belief that it might be the tegument of a parasitic stage related to class Trematoda.

The presence of cellular organelles is a vital feature of eukaryotes. In the present study, the ultrastructure of the ocular specimens revealed the presence of a functioning eukaryotic structure in 5 samples, evident by the characteristic organelles and an active synthesizing process within these organelles. These

eukaryotic structures were multinucleated with no distinct cell boundaries, forming a syncytium which was noticed distributed into distal and proximal parts in the active ocular lesions. The syncytium is a tissue characterized by cytoplasmic continuity with a large number of nuclei. Many organisms are distinguished by the presence of this unique structure as protists, plants, fungi, tegument of platyhelminths, and in some organs in animal species including humans [11]. In addition, cytoplasmic inclusions, which are generally diverse intracellular non-living substances, were also seen. These inclusions are possibly stored nutrients, secretory products, or pigment granules [12].

Ascomycota phylum of the kingdom Fungi was detected within the ocular lesions, with a wide range of bacterial species, applying metagenomic analysis. Fungi are known to have interconnecting syncytium, which is beneficial for colony development, transport of nutrients, and exchange of genetic substances, resulting in enlargement of colony size [13–16]. What is surprising in the results of our metagenomic analysis is the detection of *the T. gondii* parasite among such communities in this unusual ocular location for this parasite. *Toxoplasma* infections commonly affect the retina, resulting in retinochoroiditis and may involve the optic nerve, causing blindness [17, 18]. This can occur due to either congenital infection or acquired from the oral route that allows the parasitic stages to reach the ocular tissue via blood [19].

In this study, TEM examination revealed *Toxoplasma* tachyzoites among a microbial community enclosed by a hyaline material, possibly secreted by the microorganisms dwelling within the anterior chamber lesion. As demonstrated by the active secretory activity seen, this study suggests that this material is possibly extracellular polymeric substances (EPS) secreted by this microbial community. These EPS have been described as polymers synthesized biologically by numerous types of microorganisms, resulting in the formation of biofilms comprised mainly of polysaccharides, proteins, and DNA [20]. Protection is one of the vital functions of these EPS, which ensure a barrier between microbes and the external environment. This is guarding the community against drought and other factors. These polymeric substances facilitate the setup of nutrients (main carbon), energy source, adhesion, aggregation, communication with variable microorganisms [21, 22]. In the presence of bacteria, as evident in this study, bacterial polymers can act as virulence factors for pathogenicity [23]. Besides, it can protect the microbes against the host inflammatory reaction, as well as reactive oxygen species (ROS) [24, 25]. This may explain the survival of microbial colonies in an active productive status. In this work, the outer zones of the community “distal cytoplasm of the syncytium” showed a relatively large number of variable size mitochondria, which are necessary for metabolic activity and energy needed [26]. Thus, possibly denotes the activity and the absorptive capacity of the distal cytoplasm during the formation of the hyaline layer, by depositing the secretory EPS to this layer, perhaps as a protective covering layer. That was marked by the secretory vesicles and granules within the distal cytoplasm.

The mitochondrial activity, within the outer layer, may help the microbial community to acquire some nutrients from the host and/or to guard against the host's immune response. On the other hand, signs of cellular abnormalities (e.g., variable size or abnormal mitochondria) are seen in some areas, reflecting the overstress condition that faced these communities [27].

An aquatic transmission of the microbial community to the anterior chamber of the eye of Egyptian children was postulated in the present study. Water is very easily contaminated by cats' excreta full of the massive number of highly resisting *Toxoplasma* oocysts [28], resulting in outbreaks as reported in many developing countries [29]. Environmental changes, absence of host specificity, animal and bird movement, as well as children habitual behavior, may contribute to the aquatic transmission of such divergent microbial communities [30]. Once in the ocular tissues, microorganisms develop several strategies to survive including the production of EPS, yet the underlying procedures and the controlling pathways are still not well known. Many microorganisms produce EPS, and because of the great diversity we are still far from complete understanding, thus several opportunities remain for further explorations and discoveries [25].

In conclusion, our findings revealed the presence of *T. gondii* among a microbial community forming a syncytium-like structure, in an atypical lesion in the anterior chamber of the eye. Using TEM, active cellular activity was observed in active lesions. There is an urgent need for an ultimate understanding of the mechanisms behind such ocular illness. Further larger studies are required to obtain solid data, regarding genotype characterization, proteomic analysis, as well as aquatic transmission of these mixed microbial species to the ocular tissues.

Materials And Methods:

Ethical consideration

The study was approved by the Faculty of Medicine, Cairo University Institutional Review Board (IRB)/Ethics Committee and adhered to the guidelines of the Helsinki Declaration. Informed consent was obtained from the responsible guardians of the affected children.

Study population

The present study included 20 Egyptian children with pathological pearl-like anterior chamber lesions and active uveitis (Figure 1). They were attending Ophthalmology Department, Cairo University in the period from September 2019 to November 2020.

Clinical evaluation included full history taking, general medical examination, best-corrected Snellen visual acuity, slit-lamp inspection with a rating of the intraocular inflammation (SUN working group scale), as well as applanation tonometry, gonioscopy, and fundus biomicroscopy. Ultrasonographic examination was done according to clinical need.

Bioinformatics analysis

As a first step to explore the nature of the extracted lesions, an initial investigation was performed in the form of metagenomic analysis (under publication). According to Andrews [31], the quality of raw genomic reads was checked using FastQC, followed by Trimmomatic for filtration of reads [32]. Another quality check was done using again FastQC. Then according to Ewels et al. [33], MultiQC was performed to

aggregate FastQC results and sum up the quality matrices. To remove host reads, filtered reads were mapped against human GRCh38 assembly using BWA [34]. Reads related to different creatures were taxonomically allocated applying Kraken2 [35] against custom database retrieved from nr/nt NCBI [36] and then Krona and MultiQC tools were used to visualize the result [37].

Transmission Electron microscopy (TEM)

The extracted lesions were immersed immediately in a preservative solution; 5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 at -4°C. Samples were then washed three times with 0.1 M sodium cacodylate buffer then fixed in 1% osmium tetroxide within cacodylate buffer for two hours at -4°C. Dehydration was done, using propylene oxide over 90 minutes which was repeated twice. Tissue impregnation was done with equal parts of epoxy resin, epon 812 mixtures, and propylene oxide for 8 hours, then a mixture of 3/1 epoxy resin and propylene oxide for 8 hours, then in 2 changes of pure resin 8 hours in each change. Ultimately the samples were embedded in pure resin and were left for a full day (24 hours) in an oven at 40°C to solidify then for another 24 hours at 60°C for resin polymerization. For semi-thin sections' examination, an LKB ultra-microtome was used to cut the samples (0.5 µm) and stained them with toluidine blue, a preparatory step before the electron micrographic procedure. Then ultrathin sections were cut (80 nm) and stained with uranyl acetate for 30 minutes [38] to be followed by lead citrate for 15 minutes [39–40]. Ultra-thin sections were studied and photographed, using a JEOL 100S transmission electron microscope (Jeol; Tokyo, Japan) at 60 kV accelerating voltage.

Abbreviations:

TEM: Transmission electron microscopy; *T. gondii*: *Toxoplasma gondii*

Declarations:

Acknowledgments

Not applicable.

Funding

This study is self-funded.

Availability of data and materials

The data set used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

The whole team, with different specialties and the students from AFCM, has been working for nearly four years in continuous attempts to identify the type and nature of these ocular lesions in different ways. The

entire team participated in writing the initial research proposal, each regarding the specialty and role, reviewed the research, and agreed on its scientific content. Attempts are still ongoing due to the importance of research in terms of health, environment, and specialties as well. The following are just specific contributions per specialty. Parasitologists team [Amany A.A., A.I.E., Y.N.A., I.R.A., A.B.Y., M.A.E.] were responsible for sample collection, transferal, and preservation, examination, and analysis of TEM photos compiling the references writing the initial proposal and the manuscript. The 57357 Hospital team [A.S., A.A.D., O.S.] was responsible for all the work related to metagenomics and bioinformatics, including the compilation of related references, and writing of methods and results. Ophthalmologists team [K.G.A., A.H.N., AT.S.S., A.A.K.] were responsible for case detection and data collection, case management including treatment and surgical procedures, and follow-up. AFCM students [Adham A.A., M.A.S., A.M.N.] compiled the scientific material and collected the information about parasites that infect the eyes, and participated in organizing the collected data. M.A.E. acts as a guarantor and corresponding author.

Ethics approval and consent to participate

The study was approved by the Faculty of Medicine, Cairo University Institutional Review Board (IRB)/Ethics Committee and adhered to the guidelines of the Helsinki Declaration. An informed consent was obtained from the responsible guardians of the affected children.

Consent for publication

An informed consent was obtained for publication of patient's data including the eye lesions photographs.

Competing interests

All authors declare that they have no competing interests financial or otherwise.

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Figures

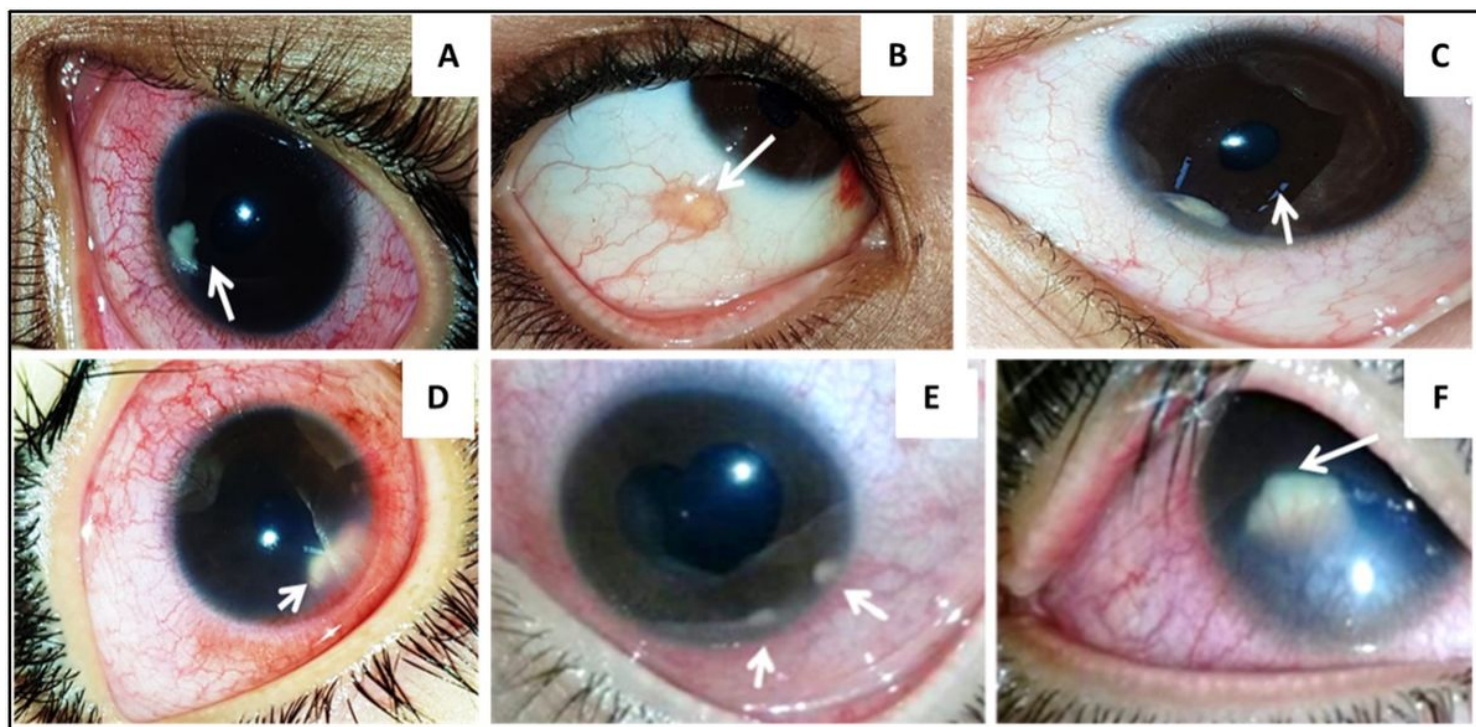


Figure 1

Ocular lesions in Egyptian children. Lesions were noticed slightly relocating, leaving an appendage-like structure (A, B, C, D & F), multiple fixed anterior chamber nodules in (E).

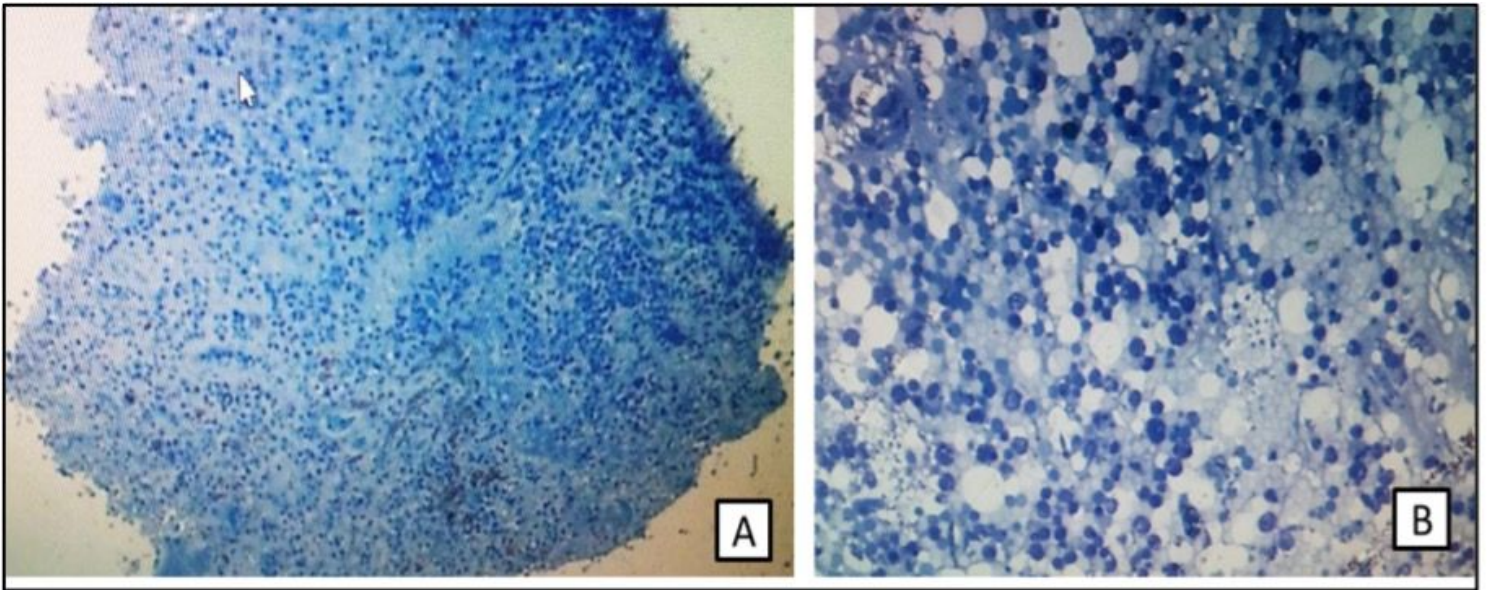


Figure 2

Inflammatory cellular infiltrate (X200 in A and x400 in B, toluidine blue-stained specimens).

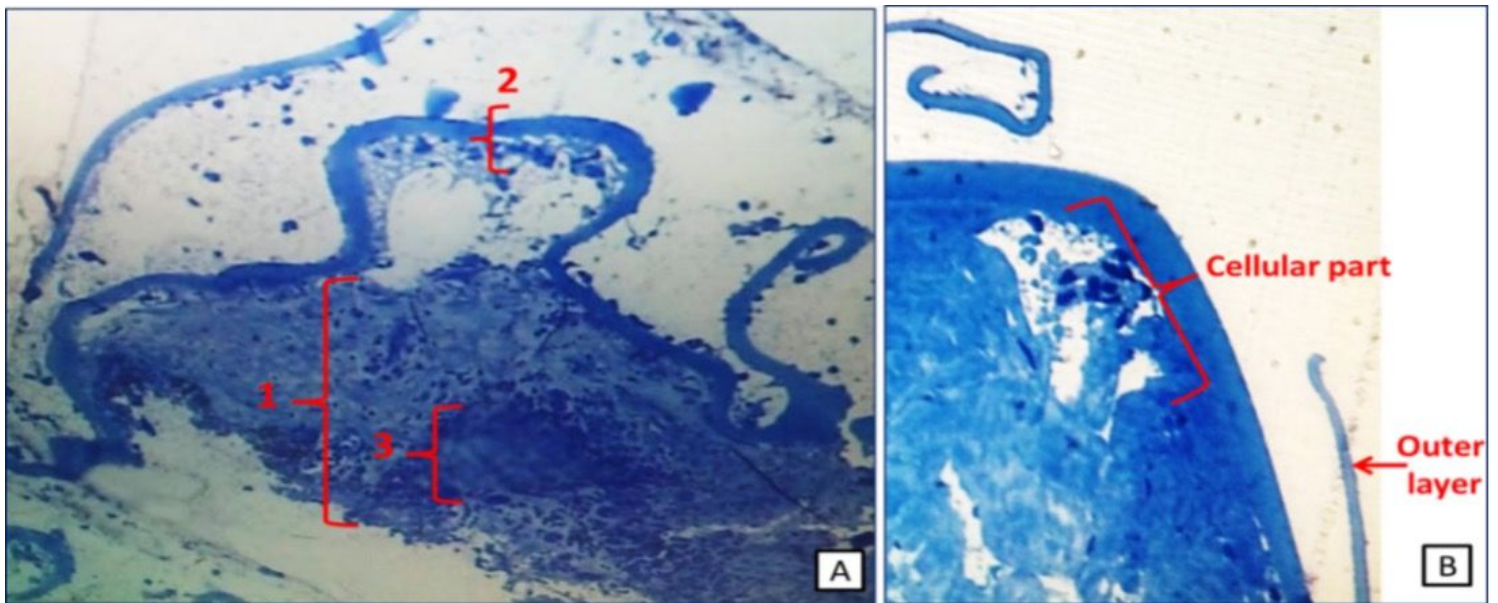


Figure 3

Semi-thin sections stained with toluidine blue. Active ocular lesion (A) appears as a body (1) enclosed by a hyaline homogenous cover (2), with a central, compact structure (3). Inactive ocular lesion (B) shows cellular degeneration with homogenous material instead of cellular structure, still covered by the outer thinner layer (X200).

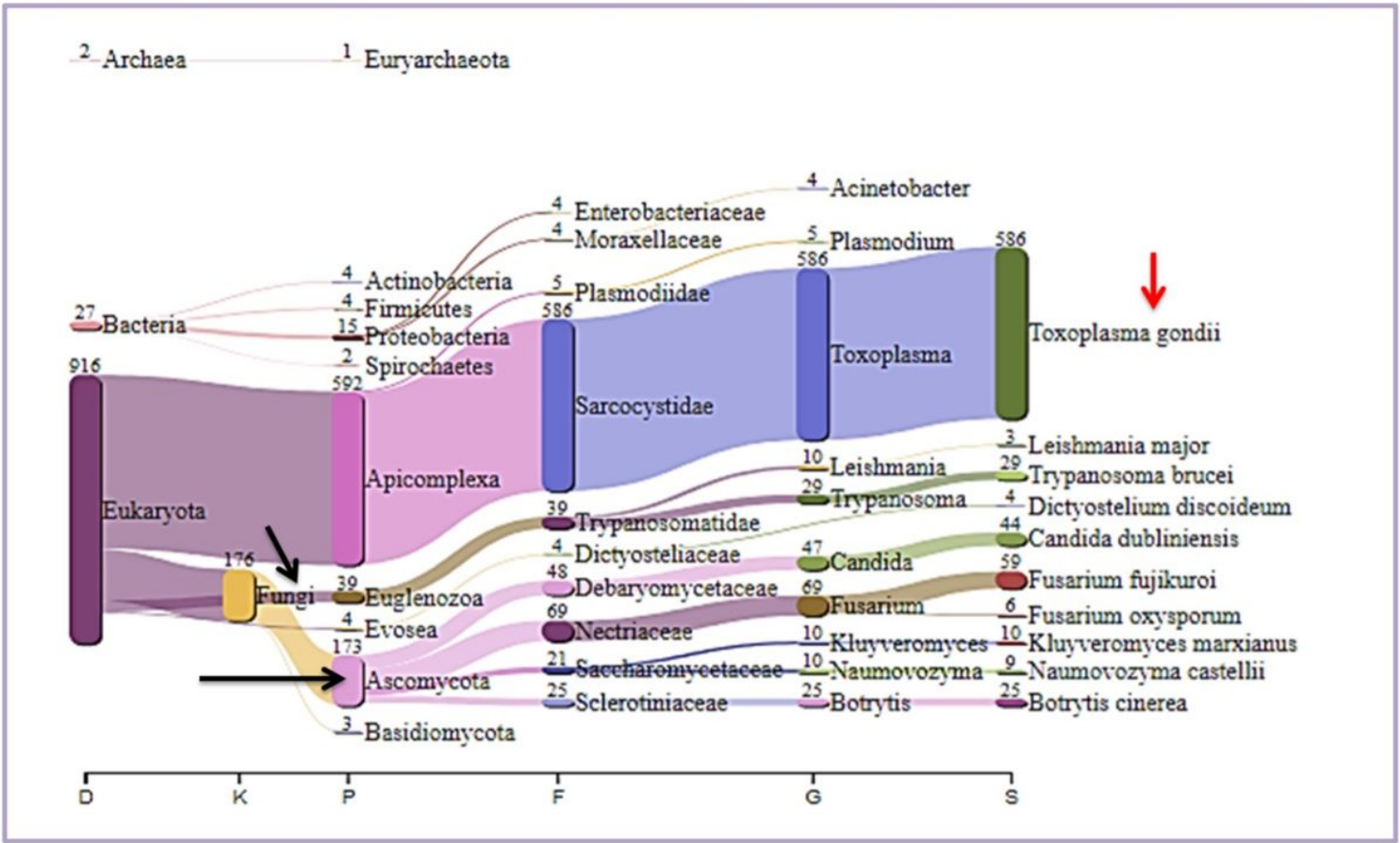


Figure 4

Biosankey diagram displaying Ascomycota (black arrows) and *T. gondii* (red arrow).

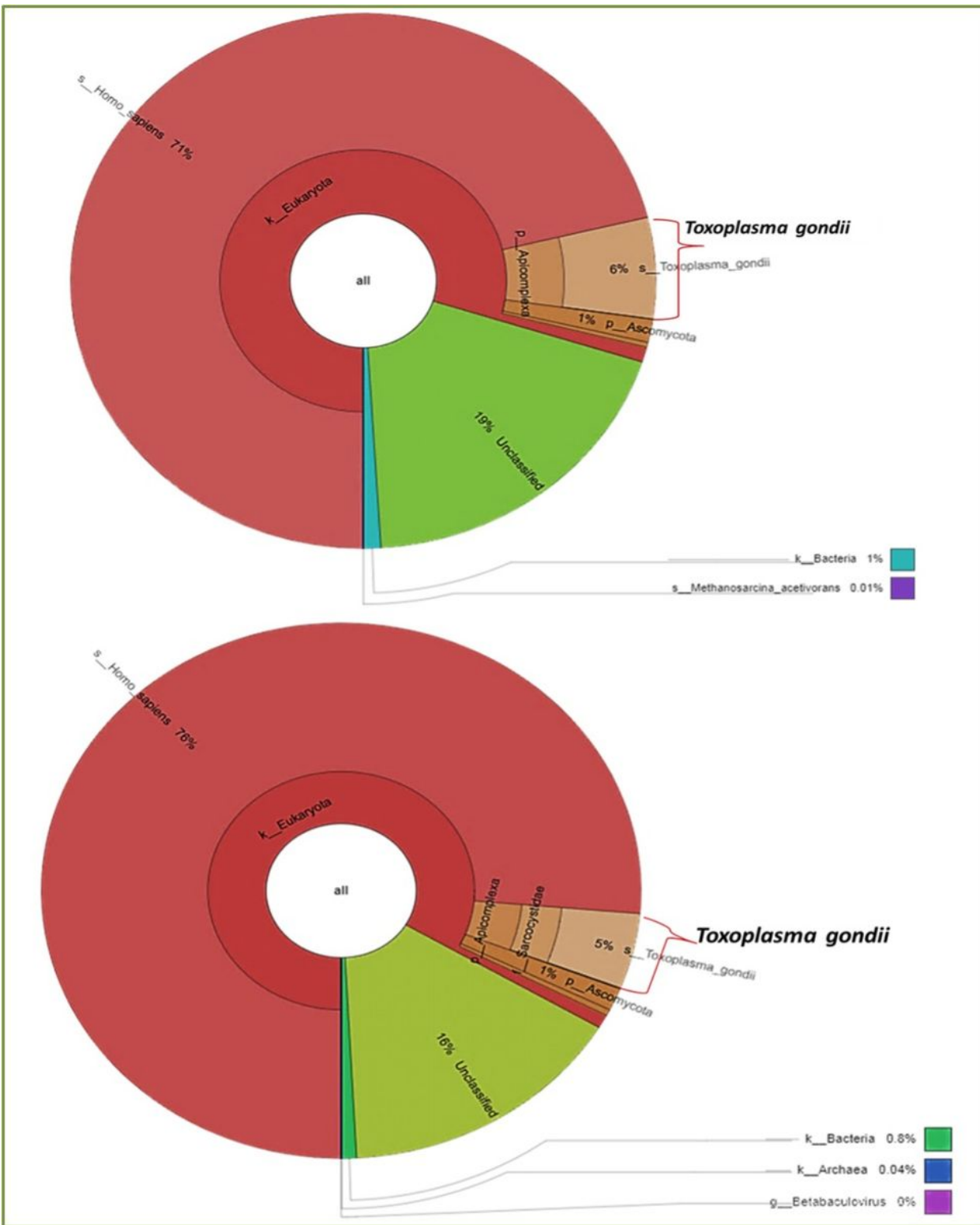


Figure 5

Krona graph of the microbial community including *T. gondii* in 2 samples of the ocular lesions.

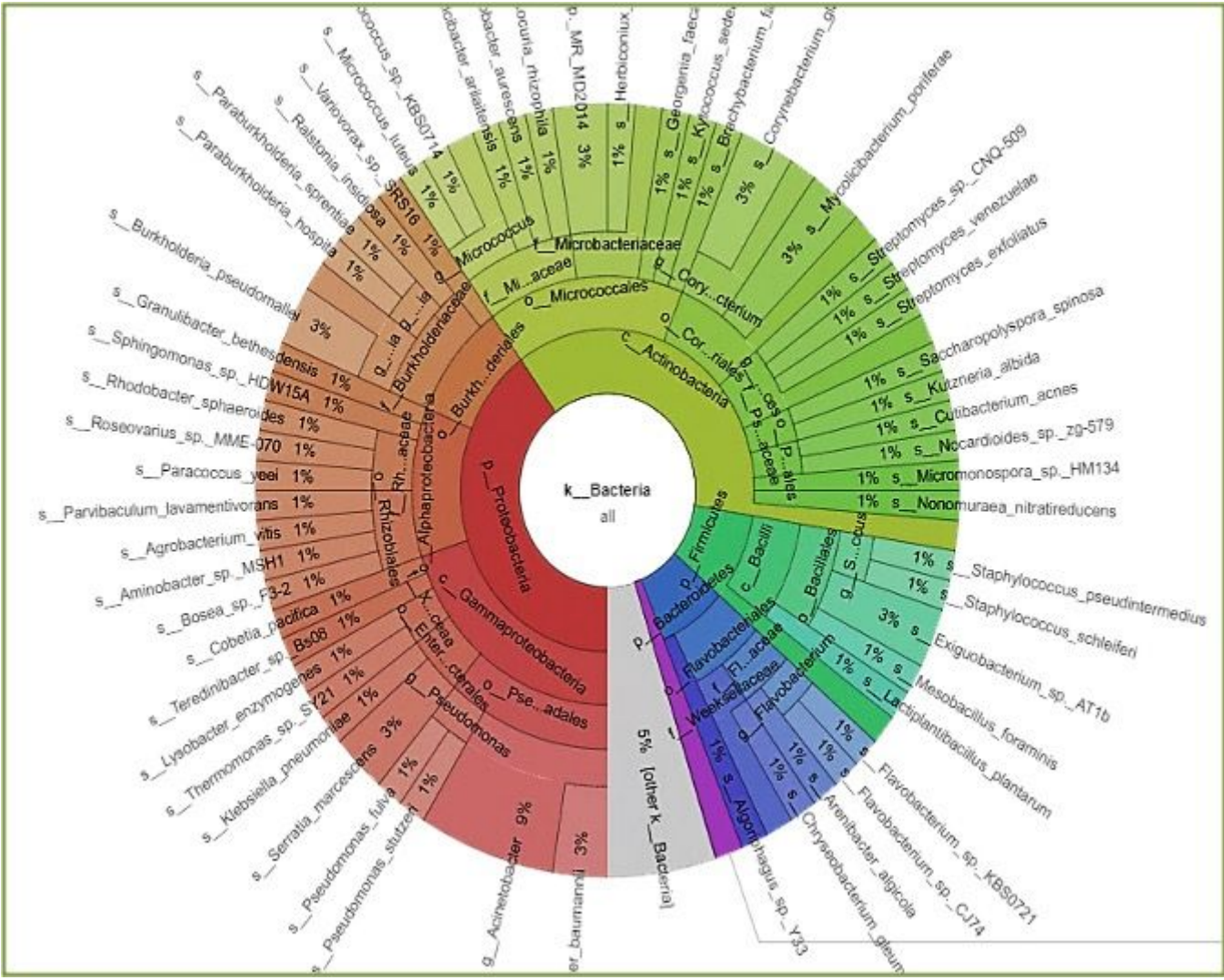


Figure 7

Krona graph of Bacteria species in a sample of the ocular lesions.

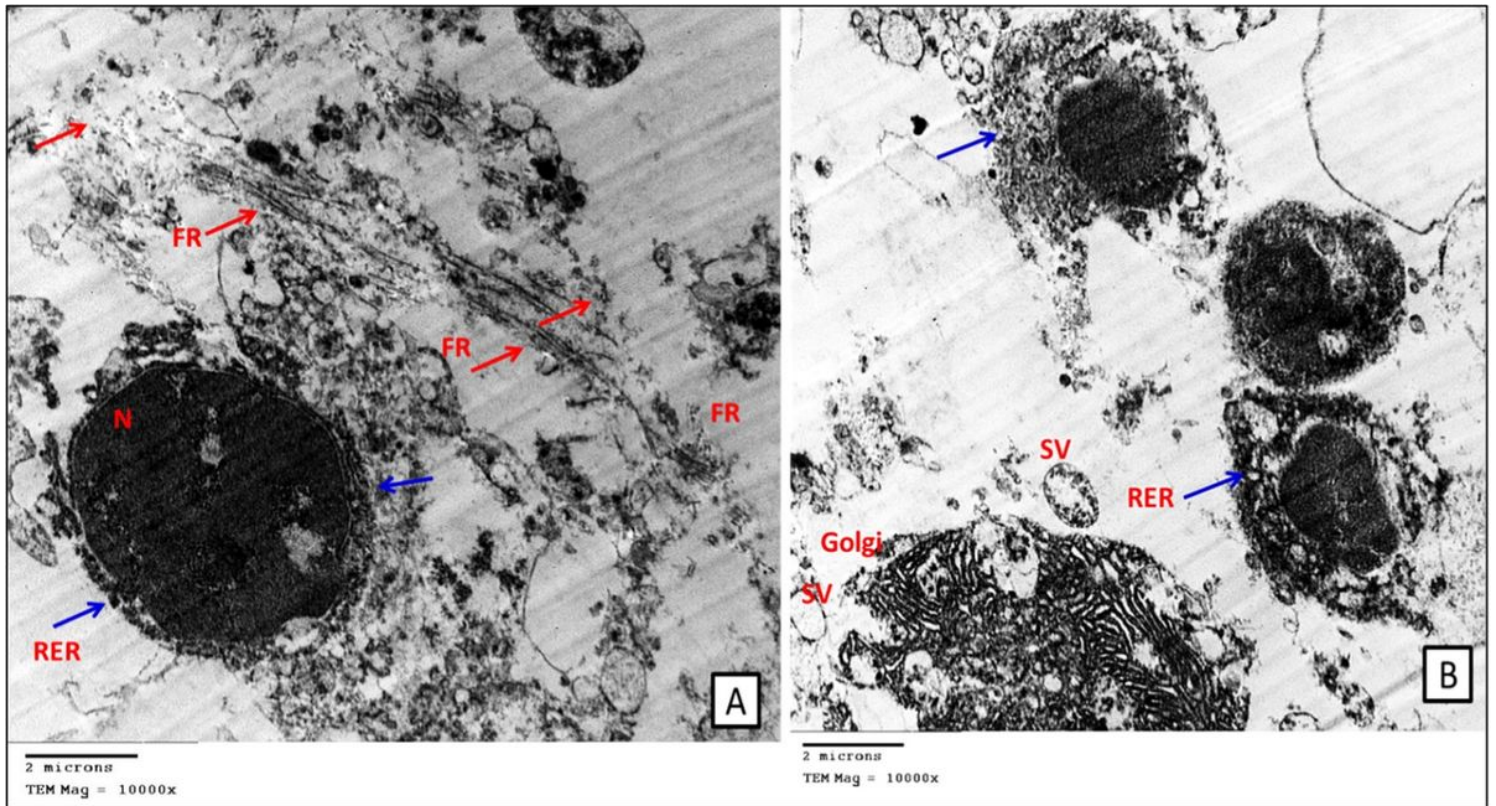


Figure 8

Active lesion by TEM indicating active protein synthesis. Rough endoplasmic reticulum close to one of the syncytial nuclei with dense heterochromatin (A). Golgi appears in an active state, releasing the modified secretory vesicles (B). Abbreviations: FR, free ribosomes; RER, rough endoplasmic reticulum; SV, secretory vesicles

Figure 9

Outer zone inactive lesion by TEM. In the distal cytoplasm of the syncytium, mitochondria seem to encircle a hyaline homogenous layer (A). *T. gondii* tachyzoites are seen with a clear nucleus, rhoptries, and dense granules (B). The outer layer (C&D) shows granular material with noticed mitochondrial activity. Abbreviations: DG, dense granules; HL, hyaline homogenous layer; M, mitochondria; N, nucleus; R, rhoptries; T, tachyzoites; SG, secretory granules; SV, secretory vesicles

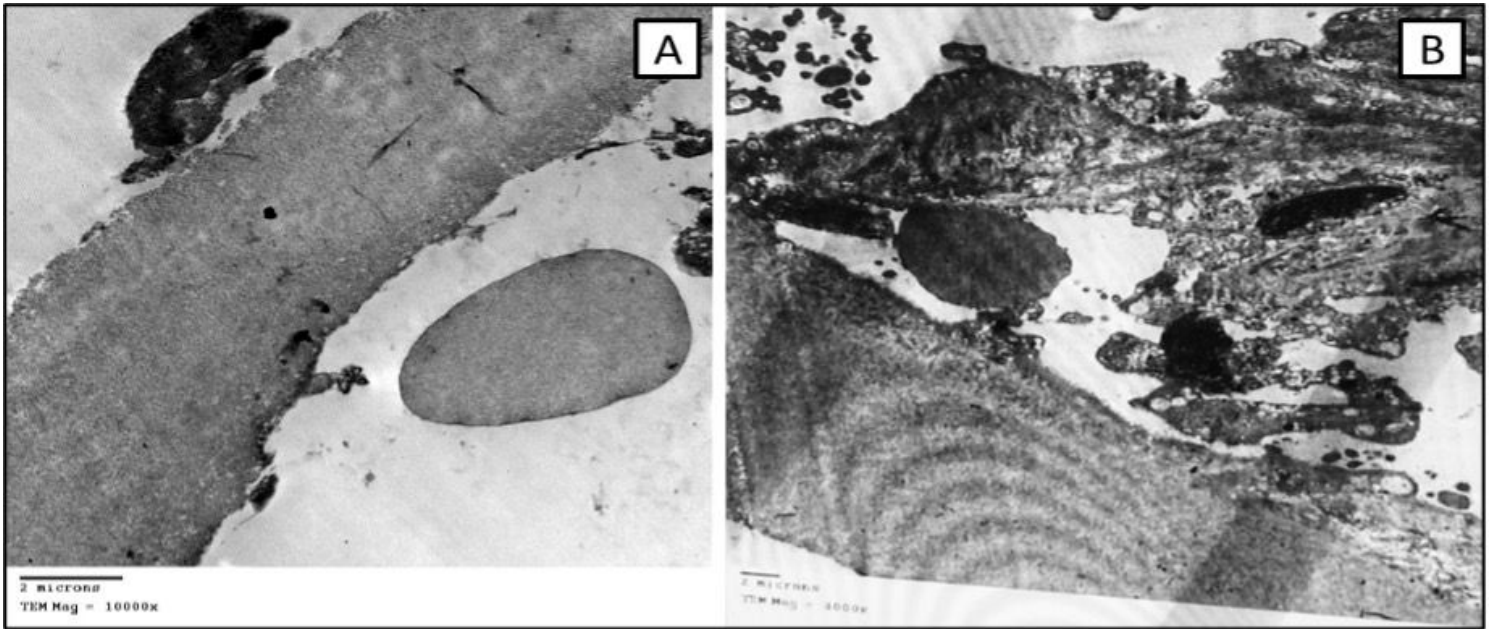


Figure 10

Outer zone in the inactive ocular lesions by TEM. The boundaries appear uneven without cellular activity (A), degenerative changes depicted in part of the lesion (B).

Supplementary Files

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- [GraphicalAbstract.jpg](#)