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First Incidence of the Ciliophoran Freshwater Fish Pathogen *Trichophrya piscium* Bütschli, 1899 as a Human Pathogen from Basrah, Iraq

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Abstract: The infective stage of the ciliophoran *Trichophrya piscium* Bütschli, 1899 attaches to the gill lamellae of fishes and transforms to a sessile adult stage (tentacle individual). Maxillary sinus aspirate was aspirated under general anesthesia from a 15-years old healthy looking female patient complained from recurrent left sided facial pain and nasal obstruction for about a year. A wet preparation of the sinus aspirate was examined microscopically, stained with methylene blue and photographs were taken before and after staining. A part of the specimen was also cultured. The wet preparation of the sinus aspirate revealed many motile larvae (buds) of the ciliophoran freshwater fish pathogen *T. piscium*, without the presence of any other pathogens, proved by both direct examination and culture. Before the patient respond partly to antibacterial medications until the recent diagnosis, the patient was kept on metronidazole course (500 mg three times daily) for 10 days which resulted in a complete resolution both clinically and radiologically. This study represents the first record of the ciliophoran *T. piscium* as a human pathogen from Basra, Iraq and consequently in freshwater fishes of Iraq with the goldfish *Carassius auratus* (Linnaeus, 1758) as the fish host. So far, two species of *Trichophrya* are known from fishes of Iraq.

Keywords: Sinusitis, *Trichophrya piscium*, Ciliophora, Basrah, Iraq.

Introduction

Acute or chronic rhinosinusitis is estimated to occur in 20% of human population at some time in their live (Howarth & Holmberg, 1995). Acute sinusitis is often associated with an upper respiratory tract viral infection, or less frequently, because of bacterial infection. It is usually either self-limited or well controlled with supportive treatment, and surgery is usually not needed. Although chronic sinusitis may be caused by many conditions, a major one is fungal infection and rarely by protozoan pathogens (Howarth & Holmberg, 1995; Perscott et al., 1996; Vennewald et al., 1999; Oud, 2009; Berenji et al., 2016).

The majorities of protozoan species are free living and can be found

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throughout the environment particularly freshwater or marine habitats. Many terrestrial protozoans can be found in decaying matter, soil and even on beach sand. Some are parasitic in plants or animals. Free living protozoans have little impact on human health. One example of free-living protozoans affecting human health is some free-living amebae which can cause disorders if introduced into the human host. Another means by which some free- living organisms can affect humans is indirectly through their effect on the environment by producing exotoxins, e.g. red tide or algal blooms (Deetz et al., 2003; Jung et al., 2004; Schuster & Visvesvara, 2004; Behets et al., 2007; Wiser, 2011). Species of the genus *Trichophrya* are widespread in nature of which *T. piscium* is known as freshwater fish pathogen and probably they are more common in ponds, bays and fish hatcheries (Calbertson & Hull, 1962; Hoffman, 1967, 1978; Corliss, 1979). The buds (larvae) of *T. piscium* are ciliated. They attach to the gill lamellae of fishes and transformed to sessile adult stages (Figure 1) which are known as tentacle individuals (Hoffman, 1978).

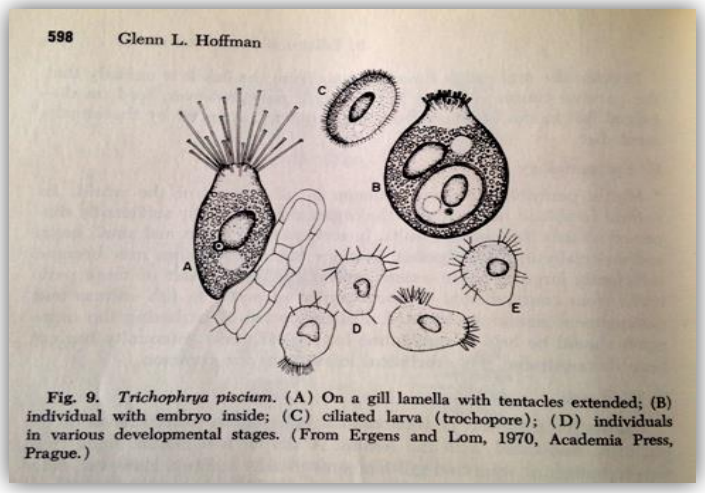

Figure 1: *Trichophrya piscium*, photograph from Hoffman (1978).

Up to date, this ciliophoran parasite has not been isolated from any fish species in Iraq (Mhaisen, 2018) and was not previously observed in humans. The present finding documents the first incidence of *T. piscium* attacking human body as an agent of human sinusitis.

Patient and Methods

A 15-years- old healthy female patient complained from recurrent left sided facial pain and nasal obstruction for about a year, at Al-Zubair district, west Basrah province. The patient was hospitalized (6th Sept., 2015) for general anesthesia, inferior meatal antrostomy (IMA) was performed and

aspiration from left maxillary sinus was done by which revealed the occurrence of an amber yellow fluid (about 10 ml) which was processed by for microscopy and culture.

A wet preparation of the maxillary sinus aspirate was examined microscopically. Numerous motile unicellular (transparent to translucent) ciliophoran parasites together with red blood corpuscles were observed and imaged. A drop of Loeffler's methylene blue stain was added beside the cover slip to stain the parasite and micrographs were taken again.

A drop of the sinus aspirate was inoculated on blood agar and on MacConkey's agar, streaked, incubated at 37 ºC (aerobically and under 5% CO_2) and then examined after 24 and 48 hours to observe any bacterial growth.

The ciliophoran parasite was identified following the criteria described by Calberton & Hull (1962), Hoffman (1967, 1978), Corrlis (1979) and Mogensen & Butler (1984).

Results

The result of direct Microscopy showed large number of ciliophoran larvae or buds (single, in pairs, chains or in clusters) with cilia showing vibrated movement together with moderate number of red blood corpuscles, but neither pus cells nor bacterial or fungal pathogens have been detected. The ciliophoran larvae were rounded with peripheral cilia, 15-20 µm in diameter (Figures 2B and 2C) or sub-globose to elongate, 20-35 µm long and 12-22 µm wide. The dark (melanin) granule in the present strain which is characteristic of ciliophorans was in low concentrations (Figure 2A). Some individuals were with prominent apical tentacles together with two fascicles of tentacles (auxiliary) as indicated in Figure 2A (arrow) or with three fascicles of tentacles and tufts of cilia at the opposite body site (Figure 3). Tentacles are up to 30 µm long but their number was difficult and resolve. Macronucleus is compact and globose, 8-14 µm in diameter (Figures 2A and 2C). The ciliated protozoan parasite was identified as *Trichophrya piscium*. Cultured sample did not show any bacterial pathogen.

Figure 2 A-C: Unstained wet preparation of sinus aspirate: a- Red blood corpuscle, b- Ciliated larvae of *Trichophyria piscium* at different stages.

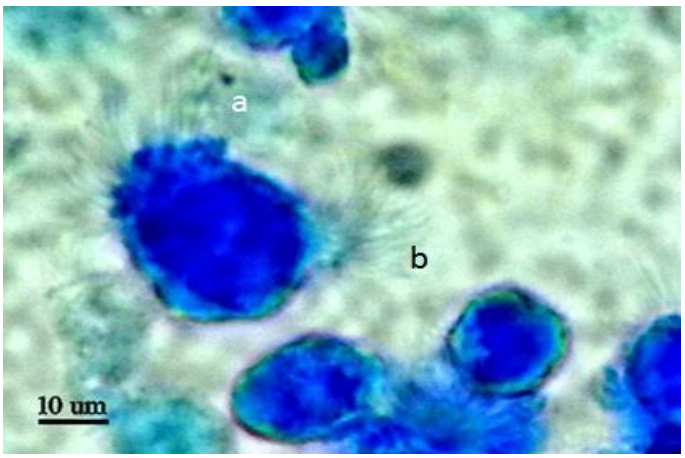

Figure 3: Methylene blue stained wet preparation of sinus aspirate showing the ciliated larvae of *Trichophyria piscium.*

Discussion

The nasopharynx serves as the reservoir for anaerobic bacteria as well as pathogenic bacteria that can cause respiratory infections including sinusitis. Many authors refer to bacteria, fungi, protozoans, molds and various viruses as agents of sinusitis (Cauwenberge & Ingels, 1996; Torres et al., 1996; Graville et al., 2004; Alho, 2005; Brook, 2005; Montone, 2007; Rupa & Thomas, 2013; Handra-Luca, 2015). Protozoan species as agent of sinusitis are rarely encountered, and if happened, they are usually found in immunocompromised patients (Dunand et al., 1997; Oud, 2009).

The freshwater fish ciliophoran pathogen *T. piscium* have not been previously reported as human pathogen and the present study is the first to describe a case of chronic sinusitis in a 15-years old healthy female patient complained from recurrent left sided facial pain and nasal obstruction for about a year. The patient suffering from a pain at the frontal and the maxillary parts which was mild to moderate. Clinical examination and nasendoscopy showed only congested left inferior and middle turbinate in comparison to the right side (Figure 4). There was neither facial swelling, nasal or postnasal discharge nor epistaxis. Plain sinus X-ray and CT scan showed only mucosal thickening of left maxillary sinus without air-fluid level or bony involvement (Figures 5A and 6). Before the patient responded partly to antibacterial medications until the recent diagnosis, the patient was kept on metronidazole course (500 mg three times daily) for 10 days resulted in complete resolution both clinically and radiologically (Figure 5B).

Figure 4: Congested nasal mucosa.

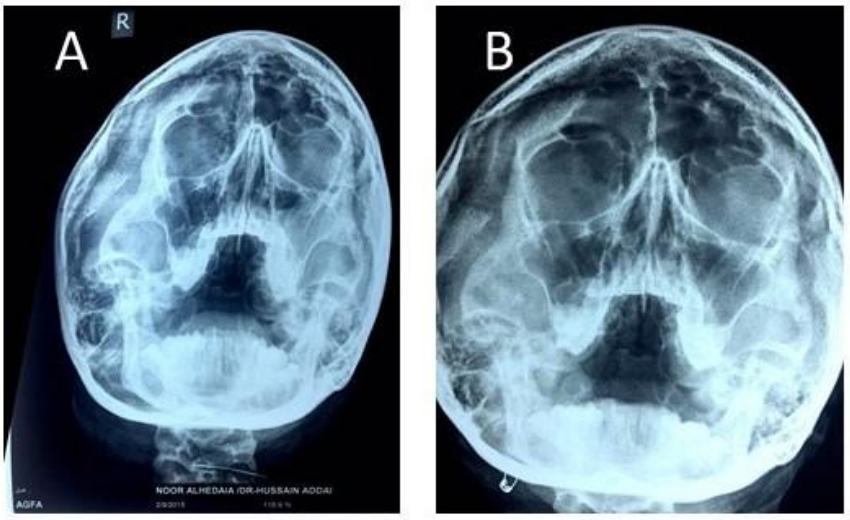

Figure 5: X-ray of maxillary sinuses. A- before treatment, B- after treatment.

Figure 6: CT scan of maxillary sinuses.

In conclusion, the present study reports the first case of *T. piscium* sinusitis in a female patient which is difficult to be explained, since she had no underlying disease. This patient had two gold fishes (*Carassius auratus*) in an aquarium from which is most likely the ciliophoran infective stage larvae (Figures 2 and 3) had found their way to her mouth (while changing the aquarium water by siphoning) and then infected her sinuses, or probably by eating undercooked infected fish which may also be a possible way of getting this parasite into her sinuses. The present finding provides an indirect

evidence on the first occurrence of *T. piscium* from fishes of Iraq based on information from the index-catalogue of parasites and disease agents of fishes of Iraq (Mhaisen, 2018). Previously, only one *Trichophrya* species was reported from fishes of Iraq which was *Trichophrya sinensis* Chen, 1955 from gills of the mugilid fish *Planiliza abu* from Babil drainage network, Babylon province (Al-Musawi & Al-Rubaie, 2017).

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