**Background:** *Mycobacterium haemophilum* is a rare and emerging nontuberculous mycobacteria (NTM). It normally causes localized or disseminated systemic diseases, particularly skin infections and arthritis in severely immunocompromised patients. There have been 5 cases of *M. haemophilum* ocular infections reported in the literature. Only 1 case presented with scleritis with keratitis. Here, we reported 2 cases of *M. haemophilum* scleritis.

**Case presentation: Case 1:** A 52-year-old Thai female with rheumatoid arthritis presented with scleritis. Conjunctival scraping was carried out and the culture result was positive for *M. haemophilum*. Despite receiving systemic and topical antibiotics, her clinical symptoms and signs worsened. Surgical debridement was performed. After surgery, the lesion was significantly improved and finally turned to conjunctival scarring. **Case 2:** A 32-year-old healthy Thai male without underlying disease presented with nodular scleritis and keratouveitis with multiple radial keratoneuritis. Surgical debridement of the scleral nodule was performed. Initial microbiological investigations were negative. Herpes ocular infections was suspected. Topical antibiotics, oral acyclovir, low-dose topical steroids and systemic steroids were started. The scleral inflammation subsided but later the keratitis relapsed, requiring corneal biopsy. Histopathology of the specimen revealed acid-fast bacteria and *M. haemophilum* was identified by polymerase chain reaction (PCR) and sequencing. The diagnosis of Mycobacterial keratitis was made. Although using the combination of systemic and topical antibiotics, his clinical status progressively deteriorated. Multiple therapeutic penetrating keratoplasties were required to eradicate the infection. No recurrence was found during the 1-year follow-up in both cases.

**Conclusions:** *M. haemophilum* can cause scleritis and keratitis, even in immunocompetent host. Radial keratoneuritis is first described in *M. haemophilum* keratitis. NTM keratitis should be considered in the differential diagnosis of patients with radial keratoneuritis. Increased awareness and early diagnosis using appropriate culture conditions and molecular techniques are important for the proper treatment of this infection. Prompt surgical intervention appears to be vital for successful management of *M. haemophilum* scleritis and keratitis.

**Keywords:** Nontuberculous mycobacteria, *Mycobacterium haemophilum*, Scleritis, Keratitis, Radial keratoneuritis, Case report
Background

Nontuberculous mycobacteria (NTM), also known as atypical mycobacteria or environmental mycobacteria, are aerobic, non-motile, non-spore forming bacilli that are naturally found in water, soil, food, and air [1]. Although ophthalmic infections from these opportunistic pathogens are infrequent, its prevalence and variety have been increasing over the past few decades [2]. The highest number of recent clinical reported on NTM ophthalmic infections are of keratitis, followed by endophthalmitis, cutaneous periocular infection, scleritis, dacycystitis and canaliculitis, orbital infection, uveitis, and conjunctivitis respectively [2, 3]. Most are caused by rapidly growing mycobacteria (Runyon group IV), particularly *Mycobacterium chelonae*, *M. abscessus*, and *M. fortuitum* groups [2, 3]. However, slowly growing mycobacteria (Runyon groups I-III) such as *M. avium complex*, *M. szulc*, *M. avium*, *M. gordae*, *M. haemophilum*, *M. kansas*, *M. flavescens*, *M. marinum*, *M. nonchromogenicum*, *M. triviale*, *M. asiaticum* or NTM of unknown species have also been reported to infect human eyes [2, 3].

*M. haemophilum* is a slow grower and traditionally classified as Runyon group III. It mainly causes cutaneous and subcutaneous infections, septic arthritis, osteomyelitis, pneumonia, and disseminated infection in immunocompromised hosts. Cervicofacial lymphadenitis is another common manifestation in immunocompetent children [4]. This organism rarely affects the eye. There have only been 5 cases of *M. haemophilum* ophthalmic infections reported in the literature. Two cases presented with endophthalmitis [5, 6], 1 case presented with scleritis with keratitis [7], 1 case presented with filamentary keratopathy and conjunctival mass [8], and 1 case presented with dacycystitis [9]. Here, we reported 2 cases of *M. haemophilum* scleritis. One of them also had keratitis with radial keratoneuritis as a presenting sign. Written informed consent was obtained from both patients to report their clinical data.

Case presentation

Case 1

A 52-year-old Thai female who presented with redness and irritation in her right eye for 1 month was referred to our hospital after 1 week of administration of topical ofloxacin and a combination of chloramphenicol and dexamethasone eye drops without remission. She had underlying rheumatoid arthritis which was well controlled with tofacitinib, methotrexate, and naproxen. She did not have any history of previous ocular trauma or surgery. Her best corrected visual acuity (BCVA) was 20/20 in both eyes. Slit-lamp biomicroscopy revealed multiple conjunctival pustules and severe, sectorial scleral inflammation with overlying conjunctival epithelial defects, extending from the 8 to 10 o’clock position and minimal punctate epithelial erosions at the inferior cornea (Fig. 1a). There was no anterior chamber or vitreous reaction. Fun- dus examination was unremarkable. B-scan ultrasonography showed no evidence of posterior scleritis. Left eye was normal except for minimal punctate epithelial erosions similar to those observed in the right eye. A diagnosis of infectious scleritis was clinically made.

Conjunctival scraping was carried out. Pus collected from the lesion was stained with Gram stain, 10% potassium hydroxide (KOH), acid-fast and modified acid-fast stains. It was also cultured on blood agar, chocolate agar, Sabouraud’s dextrose agar, thioglycollate broth, Löwenstein-Jensen (LJ) medium and Mycobacteria Growth Indicator Tube (MGIT, Becton Dickinson, Heidelberg, Germany). The specimens were also submitted for polymerase chain reaction (PCR) testing. The smears demonstrated no organism. Empirical treatment was started with topical 0.5% moxifloxacin eye drops 2 hourly and oral moxifloxacin (400 mg) once daily.

The clinical picture did not improve over the following 2 weeks and the initial cultures were all negative. New and larger pustules appeared in the same area. Surgical debridement consisting of conjunctival dissection, de-roofing the pustular lesions and scraping the scleral base of the lesions was performed. Postoperatively, topical 5%
amikacin and 0.5% moxifloxacin eye drops were given every hour.

Three weeks later, mycobacterial isolate was detected in standard MGIT. The presence or absence of *M. tuberculosis* complex was evaluated using the *M. tuberculosis* complex specific antigen test (BD MGIT TBc Identification Test, Beckton Dickinson, Sparks, MD, USA) [10]. When negative for *M. tuberculosis* complex, the mycobacterial strain was identified using pan-mycobacterial 16S rRNA gene PCR [10]. At the same time, universal primers targeted to the 16S rRNA gene were used for the optimizing of real-time PCR assay [11]. After the results showed NRM PCR positive, amplified DNA fragments were commercially sequenced from Macrogen Inc., South Korea using ABI 3730XL sequencers. The sequence data obtained were analyzed using the software program BioEdit 7.0.9 [11]. The results were compared with the sequences from the GenBank database, confirming *M. haemophilum*. In vitro antimicrobial susceptibility testing demonstrated that the isolate was sensitive to multiple antimicrobial agents, such as amikacin, rifabutin, rifampicin, clarithromycin, ciprofloxacin, moxifloxacin, and linezolid; and had intermediate susceptibility to doxycycline. The patient was placed on systemic antibiotics including oral rifampicin, clarithromycin, ciprofloxacin, and amikacin; and topical antibiotics including 0.5% moxifloxacin, 5% amikacin and 1.5% azithromycin. Oral tofacitinib and methotrexate were replaced with hydroxychloroquine for her underlying rheumatoid arthritis to minimize the adverse effects of immunosuppression.

Two days after intensive anti-nontuberculous mycobacterial treatment, markedly diffuse conjunctival hyperemia and edema as well as central corneal epithelial defect without infiltration developed (Fig. 1b). Toxic medicamentosa was diagnosed. Topical amikacin and azithromycin eye drops were stopped and topical lubricants were given. After the continuation of systemic antibiotics and topical moxifloxacin eye drops for 6 weeks, the conjunctival pustules and scleral inflammation resolved. Only conjunctival and thinned corneal scar were seen (Fig. 1c). Her BCVA was unaffected. No recurrence within the first year of follow-up was detected.

**Case 2**

A 32-year old healthy Thai male presented with pain and redness in the left eye for 5 weeks. He denied any history of previous ocular trauma, surgery, or contact lens wear. His BCVA was 20/20 in both eyes. Intraocular pressure (IOP) was 15 mmHg in the right eye but increased to 41 mmHg in the left eye. Slit-lamp biomicroscopy of the left eye demonstrated conjunctival congestion with a 2 × 2 mm scleral nodule in the temporal quadrant adjacent to the limbus, with dilatation of the superficial and deep episcleral vessels (Fig. 2a). The nodule was firm, immobile, and tender to palpation. Few radial keratoneuritis in the upper temporal cornea with intact intervening stroma and overlying epithelium were observed (Fig. 2b-c). There was 3+ anterior chamber reaction [12] with some small to medium-sized, non-pigmented keratic precipitates (KPs) centrally (Fig. 2b-c). The rest of the ocular examination was unremarkable. Confocal microscopy showed enlarges corneal nerves, but no cysts or obvious abnormalities were seen. Clinical examination of the right eye was normal. Infectious nodular scleritis with keratouveitis was suspected. Surgical debridement of the scleral nodule was performed. The specimens were stained with Gram staining, KOH, acid-fast and modified acid-fast stains and plated on blood agar, chocolate agar, Sabouraud’s dextrose agar and thioglycollate broth. The smears revealed no organism. The patient was placed on a regimen of 0.5% moxifloxacin eye drops 2 hourly and topical IOP-lowering drugs.

Systemic examination and laboratory work-up for autoimmune diseases, including complete blood count, C-reactive protein, serum electrolytes, renal and hepatic functions, urine analysis, serum rheumatoid factor, cytoplasmic and perinuclear antineutrophilic cytoplasmic antibodies, antinuclear antibodies, angiotensin

![Fig. 2](image-url)
converting enzyme, syphilitic serology, serology for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus, X-ray of chest and sacroiliac joint showed normal findings.

One week after treatment, there was no significant clinical improvement except for a decrease in IOP. The initial scleral cultures were all negative. Herpes ocular infection was suspected. Topical steroids, oral steroids and oral acyclovir (400 mg) 5 times a day were added. Symptoms and signs gradually improved and all medications were slowly tapered. However, scleral inflammation relapsed after cessation of steroid therapy, once-daily dosing of topical steroids was then maintained.

Six months later, the patient developed central corneal edema overlying an area of medium-sized, non-pigmented KPs, with some degree of superficial, gray-white, dry stromal infiltrates (Fig. 3a). There was 1+ anterior chamber reaction [12]. An aqueous tap was obtained and samples were sent for PCR analysis for human herpes simplex virus type 1 and 2, varicella zoster virus, cytomegalovirus, and Mycobacterium tuberculosis. The PCR results were all negative. A presumptive clinical diagnosis of recurrent herpes simplex keratouveitis was made and topical steroids and oral acyclovir therapy were restarted.

One week after treatment, he developed multifocal paracentral stromal infiltrates at varying depths without epithelial defect, along with recurrent radial keratoneuritis and worsening of the anterior chamber inflammation (Fig. 3b). Conjunctival hyperemia was observed with no scleral nodule. Diagnostic corneal biopsy was performed after corneal scraping did not yield positive results and the lesions progressed. Histopathologic examination of the corneal tissue revealed the presence of acid-fast bacilli. Cultures failed to isolate any organisms, but PCR testing identified M. haemophilum. Anti-nontuberculous mycobacterial therapy including hourly topical 0.5% moxifloxacin, 5% amikacin and 1.5% azithromycin; and oral rifampin, clarithromycin, amikacin and moxifloxacin were begun.

Despite aggressive treatment, the corneal lesions became enlarged and rapidly progressed with dense stromal infiltrates and 2 mm hypopyon (Fig. 3c-d).
Therapeutic penetrating keratoplasty was performed. Regrafting was required twice due to the recurrence of infection in the corneal grafts. Topical and systemic antibiotics were continued for 1 year until the lesion completely resolved. He underwent the 4th penetrating keratoplasty 6 months after antibiotic discontinuation to improve his vision. No recurrence was found during the 1-year follow-up. He achieved a final BCVA of 20/70.

**Discussion and conclusions**

To date, 15 reports of NTM scleritis have been published in the literature. The infections were caused by *M. cheloneae* (17), *M. fortuitum* (8), *M. abscessus* (1), *M. marinum* (1), and *M. haemophilum* (1) [7, 13–26]. *M. haemophilum* ocular infection was first described in 2007 in a man with graft-versus-host disease who presented with filamentary keratopathy and conjunctival mass and another man with post-cardiac transplantation who presented with purulent endophthalmitis [5, 8]. Since then, it has been 3 more case reports of *M. haemophilum* ocular infection. Here, we present 2 cases of *M. haemophilum* scleritis and one of them also had keratitis with radial keratoneuritis as a presenting sign. The details of all 7 cases were summarized in Table 1.

Diagnosis of *M. haemophilum* ocular infection is challenging. The majority of patients with this infection had underlying immunocompromised conditions, including diabetes mellitus, use of immunosuppressive agents, and young age [5–9]. However, one of our 2 cases was an immunocompetent host. It is interesting that our cases and most cases of *M. haemophilum* scleritis did not have predisposing surgeries, such as scleral buckling, pterygium excision, cataract surgery, intravitreal injection, and pars plana vitrectomy. Conversely, almost all cases of other NTM scleritis were directly preceded by surgical interventions [2]. Therefore, a high-index of suspicion is needed to diagnose this infection even in immunocompetent patients without history of accidental or surgical ocular trauma.

The insidious onset of *M. haemophilum* scleritis (4 and 5 weeks in our cases) appeared to be similar to that of other NTM scleritis, which generally varied, ranging from 1.5 weeks to 172 months [2, 3]. There was also no notable difference in the clinical features of *M. haemophilum* scleritis compared to other infectious scleritis [2, 3, 27]. Additionally, the identification of *M. haemophilum* is often difficult and delayed because *M. haemophilum* is a slow-growing acid-fast bacilli and requires special culture techniques and media different from those of most pathogenic NTM species [4]. False-negative stains and cultures occasionally occurred such in our second case, possibly due to inadequate specimen or smear preparation, fastidious/nonviable organisms or inappropriate culture conditions [28]. Thus, *M.

**Table 1** Summary of the reported cases of *Mycobacterium haemophilum* ocular infection

| No. [ref] | Age/sex | Underlying disease | Diagnosis | Exposure to topical steroids | Onset (wk) | Medications Systemic | Topical | Duration of medications | Surgery | Outcome |
|-----------|---------|-------------------|-----------|-----------------------------|-----------|----------------------|--------|------------------------|---------|---------|
| 1 [8]     | 55/M    | GVHD*, DM         | Dry eye, FK, conjunctival mass | No          | 8         | Moxifloxacin, clarithromycin, valacyclovir, clindamycin | Preservative-free lubricants, N-acetylcysteine 4 times a day | Not reported | Conjunctival biopsy | Cured |
| 2 [5]     | 66/M    | Post cardiac transplantation* | Endophthalmitis | Yes | N/A | Azithromycin, gatifloxacin, doxycycline, rifabutin* | None | 10 months | Vitrectomy, Enucleation | Failed |
| 3 [9]     | 9/F     | Healthy           | Dacryocystitis | No | N/A | Clarithromycin, rifampin | None | Not reported | Extensive excision | Cured |
| 4 [6]     | 66/M    | DM, HT, DLP, post multiple glaucoma surgeries | Endophthalmitis | Yes | 16 | Azithromycin, doxycycline, rifampin | Levofloxacin | 12 months | Vitrectomy | Phthisis bulbi |
| 5 [7]     | 65/F    | DM                | Scleritis and keratitis | N/A | N/A | Imipenem, clarithromycin, levofloxacin, rifampin, linezolid* | None | 4 months | Enucleation | Failed |
| 6 (case 1)| 52/F    | Rheumatoid arthritis* | Scleritis | Yes | 4 | Rifampin, clarithromycin, ciprofloxacin, amikacin | Moxifloxacin, amikacin, azithromycin | 1.5 months | Debridement | Cured |
| 7 (case 2)| 32/M    | Healthy           | Scleritis and keratitis | Yes | 5 | Rifampin, clarithromycin, amikacin, moxifloxacin | Moxifloxacin, amikacin, azithromycin | 12 months | Debridement, multiple PKs | Cured |

* = on immunosuppressants, ** = the mode of application was not stated, DLP Dyslipidemia, DM Diabetes mellitus, FK Filamentary keratopathy, GVHD Graft-versus-host disease, HT Hypertension, N/A Data not available, PK Penetrating keratoplasty
haemophilum can be incorrectly identified as other organisms. Furthermore, some cases were misdiagnosed as viral infection or immune-mediated disease, leading to unnecessary use of steroids similarly to our second case [5, 6].

It is nevertheless intriguing that microbiological diagnosis was achieved by routine culture methods for Mycobacterium species in Case 1. Essentially, M. haemophilum is unique among Mycobacterium species owing to its specific growth requirements [29]. It prefers a lower growth temperature (30°-32°C) than other NTM (35°-37°C) and needs iron supplements such as hemin or ferric ammonium citrate in both liquid and solid media [4, 29]. Accordingly, the growth of M. haemophilum in conventional liquid MGIT medium at 35°C in Case 1 was likely supported by the sufficient iron provided by blood from the biopsied conjunctival tissues. Because most ocular NTM infections are calcitrant to treatment and recur after cessation of therapy, long-term treatment with combination therapy with ≥2 medications is suggested. Systemic antibiotics may also be used. Although there is no consensus on the antibiotics of choice for M. haemophilum, previous studies showed that slow-growing NTM were susceptible to tuberculosis drugs, whereas rapid-growing NTM were susceptible to macrolides, fluoroquinolones, and aminoglycosides [3]. Recently, a combination of new-generation fluoroquinolones, rifampin, and new macrolides is recommended for treating M. haemophilum [5-7]. Topical steroids are contraindicated [3, 30]. Multiple topical and systemic antibiotics; and surgical interventions for both diagnostic and therapeutic purposes were applied in our 2 cases. The prognosis of NTM scleritis and keratitis could be guarded even with proper treatment [2, 3, 30]. Fortunately, due to early surgical treatments, our patients had favorable visual outcomes.

To the best of our knowledge, little information has been published regarding diagnosis and treatment of M. haemophilum scleritis and keratitis. These 2 cases highlight the rare but emerging pathogen that can infect the eye in several different ways. This is also the first report of radial keratoneuritis in NTM keratitis. Real-time PCR assay is a valuable tool for the early detection of the organism.

In conclusion, M. haemophilum can cause scleritis and keratitis, even in immunocompetent host. Radial keratoneuritis could be observed in NTM keratitis. The diagnosis of M. haemophilum ocular infection may be underdiagnosed because of unawareness among clinicians and challenges in identification of the organism in routine laboratories. The clinician’s vigilance must be increased to early recognize and promptly manage this infection. Consultation with a microbiologist and infectious disease specialist is essential to ensure accurate and timely diagnosis and effective treatment. Early surgical intervention appears to be vital for successful management of M. haemophilum scleritis and keratitis.
Abbreviations
NTM: Nontuberculous mycobacteria; BCVA: Best corrected visual acuity; KOH: Potassium hydroxide; LJ: Löwenstein-jensen; MGIT: Mycobacteria growth indicator tube; IOP: Intraocular pressure; KPs: Keratic precipitates; PCR: Polymerase chain reaction

Acknowledgements
Not applicable.

Authors’ contributions
All authors were qualified for authorship, based on contribution to: design of the study (PP, TS, KL); conduct of the study (PP, KL); collection (PP, KL, PH); management (PP, TS, KL); analysis (PP, TS, KL, PH); interpretation of the data (PP, TS, KL, PH, PS); manuscript preparation (PP, KL); review and approval of the manuscript (PP, TS, KL, PH, PS). All authors read and approved the final manuscript.

Funding
None.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
The study was approved by the ethics committee of Mahidol University School of Medicine and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each individual prior to enrollment in this study.

Consent for publication
Written informed consent to publish this information was obtained from study participants.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Ophthalmology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Rama VI Rd., Rajathevi, Bangkok 10400, Thailand.
2Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Received: 2 July 2020 Accepted: 17 September 2020
Published online: 23 September 2020

References
1. van Ingen J, Boeree MJ, Dekhuijzen PN, van Sooijen D. Environmental sources of rapid growing nontuberculous mycobacteria causing disease in humans. Clin Microbiol Infect. 2009;15:888–93.
2. Khie W, Sheheiti H, Fatih MA, Hamam RN. Nontuberculous mycobacterial ocular infections: a systematic review of the literature. Biomed Res Int. 2015;2015:164989.
3. Moorthy RS, Valluri S, Rao NA. Nontuberculous mycobacterial ocular and adenal infections. Surv Ophthalmol. 2012;57:302–35.
4. Lindeboom JA, van Coppenraet LE, van Sooijen D, Prins JM, Kuijper EJ. Clinical manifestations, diagnosis, and treatment of Mycobacterium haemophilum infections. Clin Microbiol Rev. 2011;24:701–17.
5. Modi D, Pyatetsky D, Edward DP, Ulanowski LJ, Purcell KL, Tessler HH, et al. Mycobacterium haemophilum: a rare cause of endophthalmitis. Retina. 2007;27:1148–51.
6. Pintpuwadol W, Sarunket S, Boonsopon S, Tesavibul N, Choopong P. Late-onset postoperative Mycobacterium haemophilum endophthalmitis masquerading as inflammatory uveitis: a case report. BMC Infect Dis. 2018;18:70.
7. Nookeu P, Angkasekwina N, Foongladda S, Phoomponph P. Clinical characteristics and treatment outcomes for patients infected with Mycobacterium haemophilum. Emerg Infect Dis. 2019;25:1648–52.
8. Millar MJ, Buillard C, Balachandran C, Maloof AJ. Mycobacterium haemophilum infection presenting as filamentary keratopathy in an immunocompromised adult. Cornea. 2007;26:764–6.
9. Zuercher B, Waridel F, Monnier P, Cherpiol J. A case of dacryocystitis due to M. haemophilum. Int J Pediatr Otorhinolaryngol Extra. 2011;16:261–4.
10. Simon A, Onya O, Mazza-Stalder J, Nicod L, Gilbert G, Katia J. Added diagnostic value of 16S rRNA gene pan-mycobacterial PCR for nontuberculous mycobacterial infections: a 10-year retrospective study. Eur J Clin Microbiol Infect Dis. 2019;38:1873–81.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.