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PCR-based diagnosis and clinical insights into parasitic keratitis

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ABSTRACT

Purpose: This retrospective study aimed to investigate demographic characteristics, predisposing factors, and clinical outcomes in patients with parasitic keratitis.

Methods: Medical records of patients with molecularly confirmed *Acanthamoeba* or microsporidia, identified through corneal scraping specimens (collected between September 21, 2017, and June 27, 2023), were reviewed. Demographic data, clinical profiles, such as symptom duration before confirmed diagnosis, antiviral treatment pre-diagnosis, contact lens use, tap water and soil contamination, ocular trauma, and treatment regimens, were analyzed.

Results: Fifty PCR-confirmed cases included 35 *Acanthamoeba* keratitis (AK) and 15 microsporidia keratitis (MK). Of these, 23 males and 27 females, aged 8 to 81, showed a significant difference ($p = 0.02$) in the distribution of farmers between the AK and MK groups. Mean symptom durations pre-diagnosis were 27.6 days (range: 1–180) in AK and 11.47 days (range: 1–60) in MK. AK cases exhibited a higher prevalence of stromal involvement ($p < 0.05$) and contact lens use ($p < 0.001$), while more MK patients had a history of soil contamination ($p = 0.016$). Univariable analysis linked stromal keratitis, symptom duration, and pre-diagnosis antiviral treatment to prolonged time to stability. In the multivariable model, only symptom duration predicted extended time to stability, with an expected increase of 0.65 days for each additional pre-diagnosis day.

Conclusion: This study underscores the significance of parasitic keratitis in Southern Taiwan, emphasizing the necessity of incorporating PCR as an effective diagnostic tool to enhance the routine identification of these rare conditions, moving beyond reliance on standard conventional methods.

1. Introduction

Parasitic keratitis represents a rare and serious threat to eyesight, often resulting in unfavorable clinical outcomes. While the understanding of most parasitic pathogens is limited, *Acanthamoeba* and microsporidia have emerged as well-known protozoa over the past two decades.[1] Although traditionally classified as protozoans, microsporidia are now considered fungi.[2] Early stages of *Acanthamoeba* keratitis (AK) or microsporidial epithelial keratitis (MEK) typically exhibit coarse punctate epithelial keratitis, frequently misdiagnosed as viral keratitis. In contrast, advanced forms such as *Acanthamoeba* stromal keratitis (ASK) or microsporidial stromal keratitis (MSK) present as necrotizing stromal keratitis, resembling bacterial, fungal, or herpes keratitis.[3,4] Parasitic keratitis, encompassing AK and microsporidia keratitis (MK), demands a high level of clinical suspicion, and accurate diagnosis relies on specialized media and histological expertise. However, these conditions are prone to underdiagnosis, as routine microbiological examinations primarily focus on bacterial and fungal keratitis. Conventional culturing in special media is the standard method for diagnosing AK but is time-consuming. On the other hand, microsporidia, being obligate intracellular parasites, require specialized cell culture systems for

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List of abbreviations

AK	Acanthamoeba keratitis
MK	microsporidial keratitis
MEK	microsporidial epithelial keratitis
MSK	microsporidial stromal keratitis
NCKUH	National Cheng-Kung University Hospital
PCR	polymerase chain reaction
IRB	Institutional Review Board
IVCM	in vivo confocal microscopy

isolation. Due to the scarcity of cornea specimens and the need for expertise in ocular microbiology, direct microscopic examinations with various staining techniques are often not sensitive enough for detecting light parasitic infections. Since 2017, Huang et al. have developed DNA-based molecular techniques for diagnosing parasitic keratitis caused by *Acanthamoeba* and microsporidia at our institution.[5] At the National Cheng-Kung University Hospital (NCKUH), we offer a highly sensitive polymerase chain reaction (PCR) technique to support the clinical diagnosis of challenging parasitic corneal infections, aiming to expedite recovery.

Analysis on parasitic keratitis is crucial for both research purposes and clinical guidance. Additionally, the epidemiological patterns of microbial keratitis vary across countries, particularly between developed and developing nations. Therefore, local epidemiological research can provide valuable insights for clinical practice. In this study, we retrospectively analyze the demographic data, predisposing factors, and clinical outcomes of patients with parasitic keratitis who sought treatment at the NCKUH.

2. Method

This study adhered to the principles of the Declaration of Helsinki. The Institutional Review Board (IRB) and the Committee of Medical Ethics and Human Experiments at the NCKUH granted approval prior to the commencement of the retrospective review. The study spanned from September 21, 2017, to June 27, 2023, and involved the inclusion of medical records from patients with PCR-confirmed parasitic keratitis, specifically *Acanthamoeba* or microsporidia, based on specimens obtained from corneal scraping. The DNA-based molecular techniques employed at our institution for diagnosing *Acanthamoeba* and microsporidia infections were previously published.[5] Exclusion criteria for this study comprised patients with clinically suspected AK lacking PCR confirmation, those with irregular follow-up, essential missing data, or those showing clinical improvement without anti-amoebic therapy.

Corneal debridement scraping procedures, conducted using a sterilized #15 knife under biomicroscopy, were performed on cases with clinically suspected parasitic keratitis, as indicated in the medical charts. One portion of the scrape was subjected to conventional microbiological analyses in the laboratory, including direct microscopy (Gram stain, fungal stain, and acid-fast stain), and culture in *Escherichia coli* enriched non-nutritional agar for amebae cultivation. Simultaneously, another portion of the specimen was stored at −20 °C in a 1.5-ml sterile Eppendorf tube containing 1 ml saline for DNA extraction and subsequent PCR to detect parasitic pathogens. Demographic data, including age, sex, and occupation, were recorded. Clinical characteristics at initial presentation were categorized based on epithelial involvement, stromal involvement, presence/absence of hypopyon, and corneal perforation. Additionally, risk factors such as the duration from initial symptoms to diagnosis in our hospital, contact lens usage, tap water or soil contamination, ocular trauma before the ocular event, and antiviral treatment before parasitic keratitis diagnosis were documented. The clinical outcome was defined as the time it took for the disease condition to stabilize.

2.1. Statistical analysis

Chi-square test was employed to compare the demographics, clinical features, and risk factors of patients with AK and MK, as detailed in Tables 1–3. In Table 4, both simple and multiple linear regression analyses were utilized to assess the factors associated with the final time to stability for patients with AK or MK. All statistical analyses were conducted using commercially available software (SPSS ver. 19.0; SPSS Inc, Chicago, IL), and p-values of ≤ 0.05 were considered statistically significant.

3. Results

Fig. 1 displays the patient selection flowchart. A total of 54 PCR-proven specimens were identified at NCKUH between September 21, 2017, and June 27, 2023. For comparison, AK and MK cases were separated, with 38 and 16 cases, respectively. Cases with loss of follow-up and missing data were excluded in each group. One immunocompromised patient was excluded due to a treatment regimen not involving polyhexamethylene biguanide (PHMB) or chlorhexidine 0.02 %. Consequently, the final analyzed cases in the two parasitic keratitis groups were AK (n = 35) and MK (n = 15). The demographics data of the patients were summarized in Table 1. Of these 50 cases, 23 were male, and 27 were female. The age range for parasitic keratitis cases was 8–81 years old. There was a significant difference in sex distribution between the AK and MK groups. The predominant occupation in the AK group was student, while farmer was the most common occupation in the MK group. Additionally, there was a significant difference ($p = 0.02$) in the distribution of farmers between the AK and MK groups. Clinical features at the initial presentation, as shown in Table 2, revealed no significant differences between AK and MK cases, except for a higher incidence of stromal involvement in AK patients ($p < 0.05$). Only one AK case presented with a perforated cornea, leading to evisceration two days after diagnosis, while another AK case with corneal melting and hypopyon underwent therapeutic penetrating keratoplasty (TPK) six days after diagnosis. A single case of MSK eventually required TPK after two months of unsuccessful medical treatment. Risk factors, such as the duration of symptoms before confirmed diagnosis, antiviral treatment before diagnosis, contact lens wear, tap water contamination, soil contamination, and ocular trauma, were included in Table 3. The mean duration of symptoms before diagnosis was 27.6 days (range: 1–180 days) in the AK group and 11.47 days (range: 1–60 days) in the MK group. Notably, more AK patients had a history of contact lens wear (n = 24, 68.57 %) compared to MK patients (n = 1, 6.67 %; $p < 0.001$), while more MK patients had a history of soil contamination ($p = 0.016$). The distribution of time to stable condition among AK and MK patients is shown in Fig. 2.

Table 1
Demographic data on PK patients from September 2017 to June 2023.

Characteristics	Acanthamoeba Keratitis (n = 35)	Microsporidial Keratitis (n = 15)	P-value
Age (years)			
<18	11 (31.43)	1 (6.67)	0.079
≥18	24 (68.57)	14 (93.33)	
Sex			
Male	20 (57.14)	3 (20.00)	0.016
Female	15 (42.86)	12 (80.00)	
Occupation			
Farmer	5 (14.29)	9 (60.00)	0.002
Student	14 (40.00)	2 (13.33)	0.099
Others	16 (45.71)	4 (26.67)	0.345

Note: Data are number (%) unless otherwise indicated.
Abbreviations: PK, Parasitic keratitis.

Table 2
Clinical features of PK at initial presentation.

Characteristics	AK (n = 35)	MK (n = 15)	P-value
Infection depth			0.021
Epithelial	11 (31.43)	10 (66.67)	
Stromal	24 (68.57)	5 (33.33)	
Hypopyon	8 (22.86)	2 (13.33)	0.702
Perforation	1 (2.86)	0 (0.00)	1.000
Mixed infections	5 (14.29)	4 (26.67)	0.423
Affected Eyes			
Unilateral	32 (91.43)	13 (86.67)	0.629
Both	3 (8.57)	2 (13.33)	

Note: Data are number (%) unless otherwise indicated.
Abbreviations: AK, *Acanthamoeba* keratitis; MK, Microsporidia keratitis; PK, Parasitic keratitis.

Table 3
The risk factors associated with PK.

Characteristics	AK (n = 35)	MK (n = 15)	P-value
Presenting time (mean, range in days)	27.60 (1–180)	11.47 (1–60)	0.058
Antiviral treatment before diagnosis of parasitic keratitis	7 (20.00)	3 (20.00)	1.000
Contact lens wearing	24 (68.57)	1 (6.67)	<0.0001
Orthokeratology lens wearing	11 (31.43)	0 (0.00)	0.021
Poor contact lens practice	11 (31.43)	1 (6.67)	0.079
Tap water contamination	8 (22.86)	3 (20.00)	1.000
Soil contamination	3 (8.57)	6 (40.00)	0.016
Ocular trauma	4 (11.43)	1 (6.67)	1.000

Note: Data are number (%) unless otherwise indicated.
Abbreviations: AK, *Acanthamoeba* keratitis; MK, Microsporidia keratitis; PK, Parasitic keratitis.

Table 4
Linear regression analysis of variables influencing the time to reach a stable condition.

Variables	Univariable	Multivariable	
	Model	Model 1	Model 2
	Coef. (std)	Coef. (std)	Coef. (std)
Clinical features at initial presentation			
Stromal keratitis	51.13 (18.80)*	35.25 (18.15)	27.14 (19.56)
Hypopyon	37.95 (24.21)		3.30 (23.21)
Risk factors			
Duration of initial symptoms to diagnosis	0.86 (0.23)*	0.65 (0.24)*	0.54 (0.25)*
Antiviral treatment before diagnosis of parasitic keratitis	50.64 (23.71)*	19.98 (22.22)	21.14 (22.36)
Contact lens wearing	−11.49 (19.95)		−31.72 (24.04)
Tap water contamination	−20.47 (23.80)		
Soil contamination	−58.15 (24.42)*	−39.97 (24.00)	−53.04 (25.87)*
Ocular trauma	−3.89 (33.06)		

Note: Multivariable model, model 1 was adjusted those variables with the p-value less than 0.05 under the univariable analysis and model 2 was adjusted those variables based on the clinical and statistical significance. *p < 0.05.

In univariable linear regression, patients with stromal keratitis at the initial presentation, longer durations of symptoms before confirmed diagnosis, and antiviral treatment before the diagnosis of parasitic keratitis were associated with a longer duration to stable condition (Table 4). Patients with soil contamination were associated with a shorter duration to stable condition compared to those without soil contamination. However, other potential risk factors did not associate with a longer treatment duration. Under the multivariable linear model (Table 4), only the duration of symptoms before diagnosis was associated with a longer duration to stable condition. For every additional day of duration before diagnosis, the time to stable condition is expected to increase by 0.65 days. Even after adjusting for clinical and statistical differences, the duration of symptoms before confirmed diagnosis showed an association with a longer time to stable condition. Additionally, the soil contamination status still showed an association with a shorter duration to stable condition.

4. Discussion

Parasitic keratitis caused by *Acanthamoeba* and microsporidia has been prominently reported over the past two decades, although it remains a rare disease globally.[6] A recent study by Zhang et al. estimated the global incidence of AK to be 2.9 cases per million people, with a rising trend in some regions.[7] In developed countries, poor hygiene practices during contact lens use, ineffective contact lens disinfecting solutions against cysts, and exposure to water while wearing contact lenses are major contributors to AK. Conversely, in developing countries, exposure to contaminated water and ocular trauma are the primary predisposing factors in non-contact lens wearers.[8] MK is less understood, with reported studies primarily consisting of case series due to variations in its epidemiology.[9–13] MK typically presents as disseminated, whitish, coarse punctate epithelial lesions. Although rare, MSK can resemble herpes simplex virus stromal keratitis or fungal keratitis, characterized by slowly progressive stromal infiltrates, stromal edema, and keratic precipitates. Cases of MK lacking typical clinical manifestations pose challenges with delayed clinical visits, high misdiagnosis rates, and poor visual outcomes.[13] Therefore, while we observed a significant difference in sex distribution between AK and MK groups (Table 1), such findings may be attributed to underdiagnosed cases and small sample sizes. Further studies are needed to determine whether there are sex differences between AK and MK. In our demographic analysis, we also found that patients with a history of soil contamination exhibited a shorter duration to reach a stable condition. This suggests that a thorough and comprehensive medical history may unveil parasitic infections resulting from environmental exposure, potentially related to engaging in agricultural activities. This association was notable in MK patients, where a higher percentage were farmers in our study. Tailoring treatment based on such detailed histories may expedite clinical recovery. Given the rarity of these parasitic keratitis cases, specific culture methods for confirming the diagnosis are typically pursued only when there is a high level of clinical suspicion. Among our 50 patients with parasitic keratitis, one-fifth of the cases received antiviral treatment before a confirmed diagnosis. This observation underscores the challenge of distinguishing parasitic keratitis from herpetic keratitis under slit-lamp biomicroscopy, posing a perplexing issue for differential diagnosis in clinical settings.

While *Acanthamoeba* culture remains the prevailing gold standard for diagnosing AK in clinical practice, the culture-positive rate for *Acanthamoeba* has been reported to be approximately 67 %.[14] Microsporidia, on the other hand, are obligate intracellular, spore-forming parasitic fungi that are challenging to isolate through microbiological cultures and tend to exhibit resistance to antimicrobial therapy. The emergence of PCR has offered a valuable improvement for the timely diagnosis of intractable infectious keratitis.[15] However, it is crucial not to make arbitrary diagnoses solely based on PCR results, as there is still a potential for false positives attributed to contamination.[16] The diagnosis of parasitic keratitis should always be correlated with clinical manifestations and the clinical course. Instead of relying solely on timely culture results or the pathology of invasive corneal biopsy, combined laboratory testing, such as PCR or the use of in vivo confocal microscopy (IVCM), can aid ophthalmologists in making optimal clinical decisions and ensuring accurate diagnosis.[13,14,17,18] This approach facilitates treatment strategies that are more specific to parasitic keratitis, thereby expediting recovery.

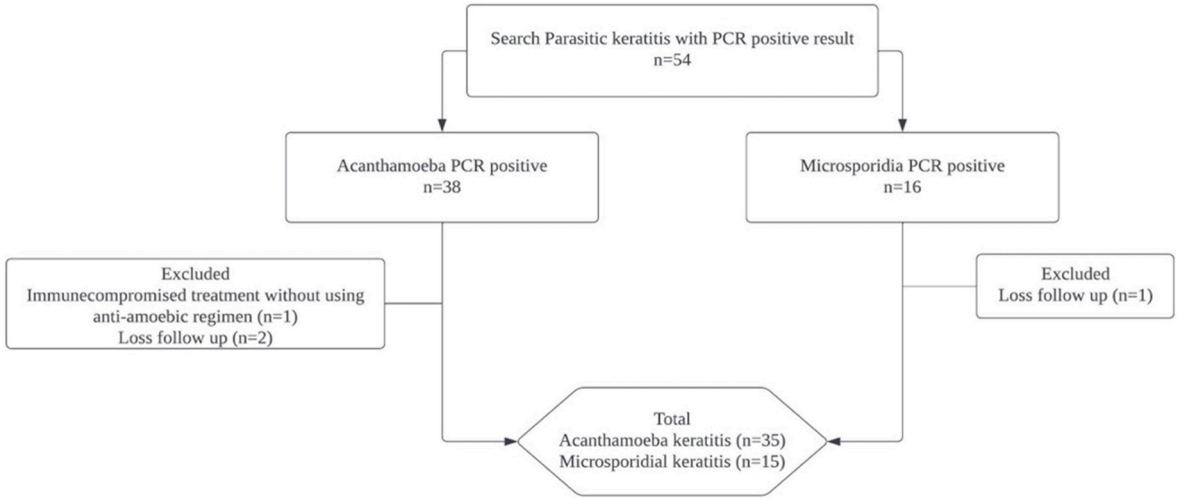

Fig. 1. Flow diagram depicting patient selection process for the study.

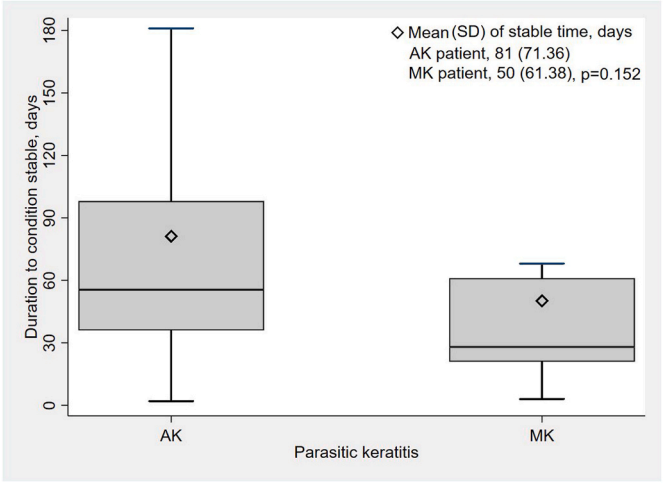

Fig. 2. The distribution of time to stable condition among AK and MK patients.

To our knowledge, both AK and MK share a common treatment goal, which is to eliminate the pathogen, whether it exists in the form of trophozoites, cysts, or spores. Currently, there is no standardized treatment protocol for MSK, and various drugs, such as fluoroquinolones, albendazole, itraconazole, metronidazole, propamidine isethionate, benzimidazole, fumagillin, voriconazole, PHMB, and chlorhexidine, either alone or in combination, have been reported.[9,10,13,19–21] In our study, all MK patients were treated with fluoroquinolones, yet one MSK patient eventually required TPK. This suggests that further investigation is needed to develop effective treatments for severe microsporidial infections in the future. Additionally, in our study, the duration of time to achieve a stable condition did not show a significant difference between the AK and MK groups. However, the mean duration of time to a stable condition in the AK group was 81 days, whereas in the MK group, it was 50 days. This trend could be explained by reviewing the clinical characteristics of AK and MK patients. A significant proportion of AK patients (68.57 %) presented with stromal involvement, while only 33.33 % of MK patients exhibited stromal involvement. Thus, the duration of time to achieve a stable condition could possibly be associated with the severity of the disease status at presentation.

In Taiwan, there is a high prevalence and incidence of myopia in children.[22] Overnight orthokeratology is increasingly employed as a method to control myopia, and the potential association between orthokeratology and AK should not be overlooked.[23] In our AK cases, a notable percentage (31.43 %) involved individuals using overnight orthokeratology, and they were diagnosed relatively early. These symptomatic younger patients were not referred for further evaluation until conventional treatments had proven ineffective. In comparison to soft contact lens users with poor hygiene practices, individuals using overnight orthokeratology in Taiwan typically undergo regular follow-up care. They are also diligently educated about the symptoms and signs of infectious keratitis by medical practitioners. We believe that these factors may contribute to the shorter duration of symptoms before referral for a confirmed diagnosis, consequently leading to a shorter recovery duration. However, it's noteworthy that there is no significant association between contact lens wearing and the time it takes for the condition to stabilize.

Our study has several limitations. Firstly, we focused exclusively on patients admitted to the Department of Ophthalmology at a single medical center, resulting in a limited number of cases. This limitation is attributable to the rarity of the disease, and as such, the findings may not be universally applicable. As AK is a relatively uncommon infectious disease, we could only identify 35 cases for inclusion in this study. Nevertheless, this number is non-inferior to the 64 cases treated at a tertiary hospital in China from 2000 to 2017, averaging only 4 cases per year.[24] The study may underestimate MEK cases, as most cases may resolve with empirical treatment of topical fluoroquinolone and may not necessitate referral.[4] The second limitation pertains to the study design. Our retrospective data collection relied solely on clinical records, external eye photographs, and laboratory data, introducing potential inaccuracies and documentation bias. Additionally, for cases without a culture result confirming the diagnosis of both AK and MK, the absence of in IVCM further limits our ability to aid in the reconfirmation of the diagnosis of parasitic keratitis. Thirdly, it is challenging to distinguish a genuine time trend from inherent variability with only two three-year time spans of data. Future studies should encompass at least three-time spans of data to establish a more robust trend line for analysis.

In conclusion, the findings from this retrospective study underscore the significance of parasitic keratitis in public health in Southern Taiwan. The results emphasize the importance of adopting PCR as an effective diagnostic tool to assist in the routine identification of these rare parasitic keratitis cases, surpassing reliance on standard conventional methods alone. Early diagnosis, as indicated by our results, correlates with a reduced treatment burden.

CRediT authorship contribution statement

Suan Hwang: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **I-Huang Lin:** Data curation, Formal analysis, Resources. **Chun-Chieh Lai:** Data curation, Formal analysis, Resources. **Fu-Chin Huang:** Data curation, Formal analysis, Resources, Supervision. **Sung-Huei Tseng:** Data curation, Formal analysis, Supervision, Validation. **Yi-Chen Chen:** Formal analysis, Software, Validation, Visualization. **Chung-Han Ho:** Data curation, Formal analysis, Software, Validation. **Wei-Chen Lin:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **Yi-Hsun Huang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Payment as a consultant/reviewer/evaluator

No.

Prior or repetitive publications

None.

Ethics approval

The Institutional Review Board (IRB)/the Committee of Medical Ethics and Human Experiments of National Cheng Kung University Hospital has approved before the initiation of the retrospective review (Approved no. B-ER-112-282).

Financial or commercial or proprietary interest

No.

Declaration of competing interest

No conflicting relationship exists for any author.

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