Microsporidia and Acanthamoeba: the role of emerging corneal pathogens

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Abstract

Parasitic organisms are increasingly recognized as human corneal pathogens. A notable increase in both well-defined *Acanthamoeba* keratitis and a more dramatic increase in reported cases of *Microsporidia* keratitis have suggested significant outbreaks of parasitic keratitis around the world. Historical and contemporary baselines as well as a familiar associated clinical presentation reinforce the significant outbreak of *Acanthamoeba* keratitis in the United States. The remarkable rise in cases of *Microsporidia* keratitis, however, lacks these established baselines and, further, describes a disease that is inconsistent with previous definitions of disease. While a well-defined, abrupt increase strongly suggests temporally related risk factors, most likely environmental, involved in the *Acanthamoeba* outbreak, the rise in *Microsporidia* keratitis suggests that increased awareness and improved diagnostic acumen are a significant factor in case ascertainment. Regardless, recent evidence indicates that both parasitic diseases are likely underreported in various forms of infectious keratitis, which may have unrecognized but significant implications in the pathogenesis of both primary protozoal and polymicrobial keratitis. Further understanding of the incidence and interaction of these organisms with current therapeutic regimens and more commonly recognized pathogens should significantly improve diagnosis and alter clinical outcomes.
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Introduction

Over the last decade, parasitic organisms have been increasingly associated with various forms of external ocular infections worldwide. The term parasite, strictly defined as an organism deriving benefit from a host while providing no benefit in return, could be applied to any corneal pathogen, but is usually reserved for protozoa or other more complex organisms. Several parasitic corneal pathogens have been well described, including *Onchocerca*, *Leishmania*, and *Trypanosoma*,[1,2] with recent attention focused on notable numbers of *Microsporidia* keratitis in South Asia and a more modest increase of *Acanthamoeba* keratitis in the United States as well as other developed countries.[3–10]

Clinically, parasitic infections are usually characterized as chronic and intractable presenting with non-specific findings masquerading as other infectious and non-infectious disease. Despite the recent outbreaks, these infections remain comparatively rare, requiring a substantial clinical suspicion to warrant the specific media and/or special histological expertise for diagnosis. Routine microbiological tests are insensitive. Finally, each of these infections traditionally requires uncommonly available drugs specifically effective against the individual pathogen for resolution.[11]

Altogether, these factors contribute to a delay in the diagnosis that may result in a clinical characterization of the 'classic' presentations used to define cases of parasite-related disease that is biased toward 'later' and poorly treatable disease at the expense of 'earlier' forms of the disease.[12] An examination of the recent increase in reports of protozoal disease offers an insight into a burden of disease previously unrecognized, but of potentially significant ocular and public health importance both locally and globally.

Recent increases in *Microsporidia* keratitis

Microsporidia describes a group of obligate intracellular organisms spanning several

genera, which reproduce within the host cell by forming spores, which are then released to infect neighboring cells. First described in a Sri Lankan boy in 1973, few further reports appeared in the literature until 1990 when it was first associated with HIV/AIDS infection.[13,14] Cases were defined as either a vision-limiting, diffuse epitheliitis (Figure 1a) found more commonly in immunocompromised hosts and associated with the genus *Encephalitozoon*, or a previously described stromal keratitis associated with the genera *Vittaforma* or *Trachipleistophora* in immunocompetent hosts. Both were chronic, intractable infections that would occasionally resolve with restoration of immune function and respond variably to topical fumagillin or systemic albendazole.[15–17] Isolation required incubation with host cells, not easily performed in most laboratories, or direct observation in microbiological smears. With a few exceptions,[18] *Microsporidia* keratitis remained primarily a disease of immunocompromised individuals until the first multiple case series of *Microsporidia* keratitis in immunocompetent individuals were reported from Singapore in 2003 and India in 2005.[10,19]

Subsequently in this geographic region, the number of reported cases increased dramatically with 134 cases reported in 4 years from 2 hospitals in Singapore and even greater numbers reported from various centers in India, which, at face value, suggested an outbreak of the disease.[8,9] The CDC outlines, however, 10 steps that are
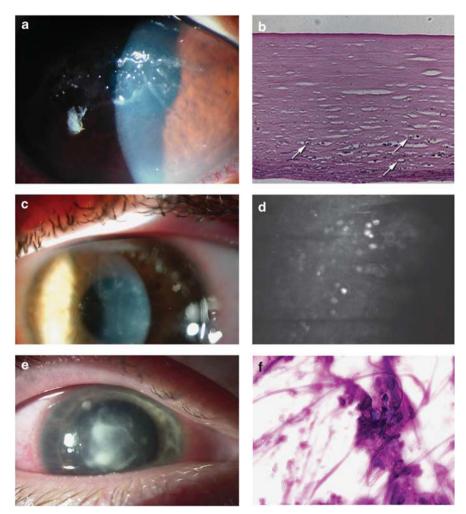

Figure 1 (a) A slit lamp photo of superficial microsporidial keratitis in an AIDS patient. (b) Photo of a histopathological specimen from a therapeutic penetrating keratoplasty performed for *Acanthamoeba* keratitis showing both full and empty cysts (arrows) in the posterior third of the stroma. (c) Slit lamp photo of epithelial *Acanthamoeba* keratitis with radial keratoneuritis. (d) Confocal microscopy of the same patient (c) demonstrating round double-walled cysts with a bright center. (e) A case of chronic stromal keratitis associated with *Acanthamoeba*. (f) Diff-Quick smear of a corneal scraping of the same patient (e) with a single characteristic double-walled cyst centrally with multiple pores.

integral to an outbreak investigation.[20] The initial steps involve confirming the existence of an outbreak, specifically, creating a case definition, confirming that the cases are 'real' and, thereby, establishing a background rate of disease. Although the vast majority of these recent cases reported are supported by histological confirmation, several features are inconsistent with our prior understanding of the presentation and course of *Microsporidia* keratitis. Specifically, these cases are almost exclusively found in immunocompetent individuals and, although previous cases were chronic, difficult to manage, and responded variably to only specific drugs, recent cases have resolved with a wide array of commonly available topical ophthalmic antibiotics[8] or with no therapy in a period of a few weeks.[21]

While the number of cases of *Microsporidia* reported has been remarkable, this new case definition suggests that a significant contribution of recognition bias may potentially exist. For example, its non-specific presentation and self-limited nature was routinely mistaken for seasonal outbreaks of presumed viral epidemic keratoconjunctivitis in India.[22] The shorter course, the self-limited nature of many, if not most, of these infections, and their response to non-specific empiric therapy would all be expected to further mask the number of true number of cases detected. This not only suggests that the more recent disease attributed to *Microsporidia* is currently underreported, but also likely to be previously more severely underreported. The strikingly different case definition makes it difficult to establish the existence or scope of a *Microsporidia* keratitis outbreak because of the lack of congruous, contemporary or historical controls utilizing the same case definition.

Clearly, the involvement of *Microsporidia* in corneal disease is more common now than previously understood, but to what degree this represents an outbreak or increased diagnostic acumen remains unclear. An accurate characterization of the disease burden and morbidity of *Microsporidia* keratitis and an understanding of its environmental source are important to quantify its unrecognized role in ocular disease and its future prevention. Efforts to control the disease may be most dependent on its perceived morbidity if the underlying source is constant and not easily modifiable.

Recent increases in *Acanthamoeba* keratitis

Similar to *Microsporidia* keratitis, *Acanthamoeba* keratitis was first described in 1973 with few additional cases reported until a strong association with soft contact-lens wear was recognized in the early 1980s.[23–25] Its subsequent history has, however, been characterized by a series of outbreaks in developed countries where contact-lens wear, still its most strongly associated risk factor, is common.[26–29] In the United States, analysis of an *Acanthamoeba* keratitis outbreak in the late 1980s calculated an acute incidence of approximately two cases per million contact lens wearers per year.[30] Similarly, comprehensive studies in the UK point to a much higher incidence of 17.53 per million rigid lens wearers and 21.14 per million soft lens wearers per year.[31] While no consistent background surveillance has been performed, a high level of detection at tertiary care facilities where the diagnosis could be made, appropriate treatment initiated and cases aggregated has been assumed because of its chronic nature and almost universal resistance to commonly available ophthalmic anti-infectives.

An increase in the number of cases of *Acanthamoeba* starting in 2003 in Chicago and 2004 elsewhere in the US has been noted in several recent studies.[5,32] Similar questions have been raised as to whether this increase constitutes an outbreak or a previously unrecognized background rate. Applying the same CDC criteria, the clinical aspect of the case definition for *Acanthamoeba* keratitis, although varied, has remained relatively consistent among past and present studies ranging from predominantly epithelial disease to deep stromal keratitis (Figures 1b–f) sometimes associated with extraocular manifestations and occurring predominantly in contact lens wearers. Gross calculations of incidence in the Chicago metropolitan area approach 20 cases per million contact lens users per year, an order of magnitude increase over the historical outbreak incidence of the late 1980s, but very similar to that seen in the UK.

The question as to whether these cases are 'real' is more controversial as the method of diagnosis in most prior US studies has relied heavily on confocal imaging (Figure 1d) with low rates of microbiological support (Figure 1f).[28,33] Our own experience with confocal microscopy has been very supportive of its use, however, it should be noted that it was not used as a the sole criteria for diagnosis and that culture rates were at least equal to if not exceeding those published in other international studies.[34] Confocal microscopy remains a useful tool for rapid diagnosis, but correlation varies significantly and should be individually validated.[35] Applying the stricter criteria of microbiological evidence alone, the increase in cases in Chicago would still represent a significant increase over previously understood rates in the United States. Further, the initial CDC surveys captured only culture-positive cases over the last decade indicating that the rise in these centers was real, applying this narrow diagnostic criterion within this time frame.[3,36] Taken together, a consistent case definition, confirmation of real cases, and various,

but consistent contemporary and historical background rates, it appears that the recent rise in cases of *Acanthamoeba* keratitis in the United States meets the criteria of a true outbreak.

Once established as an outbreak, two case–control investigations identified a significantly elevated risk from the use of AMO Complete MoisturePlus compared with other disinfection systems as well as some hygiene-related factors, including solution reuse (topping off), showering in lenses, and so on.[3,37] The abrupt rise in cases, however, makes it unlikely that a radical change in hygiene habits was solely responsible and, further, the large number of cases not using AMO Complete MoisturePlus still constituted a sizable increase over the known background rate of infection making it also unlikely the sole cause.[37] Focus on other contemporaneously introduced risk factors including the labeling change for the use of multipurpose solutions allowing the omission of lens rubbing as well as the introduction and subsequent market dominance of silicone hydrogel lenses in the contact lens market have similarly not yielded an explanation.[3,11] The lack of a specific lens-related risk factor and the significant increase across all solution systems and lens types suggests an increase in exposure to *Acanthamoebae* characteristic of water contamination seen with almost all previous outbreaks. Our ongoing studies in Chicago continue to demonstrate a geographic-based pattern of disease consistent with the domestic water distribution system as well as a higher rate of *Acanthamoeba* isolation from domestic taps than previously described.[38] As expected, although surveillance subsequent to the recall of AMO Complete MoisturePlus in 2007 demonstrated a modest decrease in the number of cases initially, the lack of reduction of *Acanthamoeba* keratitis cases to baseline levels has prompted another CDC case–control study that was initiated and completed earlier in 2011.

The potential role of unrecognized protozoal keratitis

As noted previously, the assumption has been, in the case of *Acanthamoeba* keratitis, that case ascertainment is high or nearly complete because all cases would eventually reach a site where case aggregation could occur.[11] Recent evidence suggests, however, that *Acanthamoeba* isolated in the presence of a bacterial keratitis may not require specific therapy for resolution. We found this to be the case in two of our own patients with pseudomonas keratitis, which resolved with topical antibacterial therapy alone before *Acanthamoeba* cultures were reported positive. This phenomenon has also been reported elsewhere on a larger scale.[39] Similar to the

altered case definition of recent cases of *Microsporidia* keratitis, these cases of *Acanthamoeba* keratitis are likely to be undetected as they did not require specific therapy and had none of the clinical characteristics 'classically' associated with *Acanthamoeba* keratitis.

For *Acanthamoeba* and *Microsporidia*, it would seem that the clinical significance of a self-limited or easily treatable infestation would be questionable. This may, however, be important for several reasons. If these organisms are non-pathogenic, then microbiological and especially indirect methods of diagnosing *Acanthamoeba* or *Microsporidia* keratitis, including molecular methods, would be difficult to validate. It is known that Acanthamoebal cysts survive poorly in axenic environments.[40] If antibacterial treatment can eliminate certain strains of *Acanthamoebae* either through elimination of its bacterial food source or, alternatively, through direct anti-*Acanthamoebal* activity, it would be important to test the anti-*Acanthamoebal* activity of ophthalmic antibiotics to better tailor empiric therapy of presumed infectious keratitis.

More importantly, perhaps, recent molecular studies have demonstrated that normal mucosal surfaces, including the eye, have a diverse biome undetectable through traditional microbiological methods.[41,42] The more common presence of undetected organisms, like *Acanthamoebae*, in routine infectious keratitis could significantly impact our understanding of its pathogenesis. Polymicrobial keratitis, in general, and in combination with *Acanthamoebae* specifically is known to pursue a more aggressive clinical course.[43] *Acanthamoebae* are well documented to be a co-pathogen with viral, bacterial, fungal, and other protozoal organisms as well as a host for a number of pathogenic bacterial endosymbionts, including pseudomonas and legionella.[44] The impact of these interactions on the often variable clinical course of *Acanthamoeba* keratitis and even routine bacterial keratitis may produce new therapeutic strategies to improve outcomes of infectious keratitis.

In summary, both *Microsporidia* and *Acanthamoeba* keratitis have a larger and increasingly recognized role in infections of the cornea. Recent evidence suggests that their true incidence may be significantly underreported, which not only underestimates their public health burden but also masks the scope of their associated morbidity. A better understanding of the modifiable risk factors involved in the individual outbreaks and of their role in corneal disease is needed.

Conflict of interest

The authors declare no conflict of interest.

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