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Ameba-associated Keratitis, France

To the Editor: Amebic keratitis is an aggressive infection usually associated with soft contact lenses, and its poor outcome can lead to a corneal graft (*1*). Ameba can host ameba-resistant bacteria (*2*) and serve as a source for numerous organisms to exchange DNA, adapt to changing environments, and become pathogenic to the host (*3*). However, mixed keratitis caused by amebae in association with ameba-resistant pathogens is rarely seen.

A 17-year-old woman who was myopic and had worn soft contact lenses for 3 years consulted our ophthalmology department for pain and redness of the left eye that had persisted for 2 weeks. No visual loss was reported, and a slit-lamp examination showed a millimetric epithelial defect associated with a round stromal infiltrate (Figure). No intraocular reaction was observed, and bacterial keratitis was diagnosed. Her condition improved after a 7-day topical fluoroquinolone treatment, and follow-up at 13 months showed only a slight superficial stromal scar. The patient reported that she had inappropriately worn monthly contact lenses for 3 months, cleaned and rinsed lenses with a commercial cleaning solution but diluted the solution with tap water, and washed her hands with tap water but did not dry them before handling the lenses.

Results of microbiologic analysis of a corneal scraping, which included molecular detection of ameba (18S rDNA), bacteria (16S rDNA), and herpesvirus (DNA polymerase), were negative. However, culture of contact lens storage case liquid in Page-modified Neff ameba saline enriched with heat-killed *Enterobacter aerogenes* (*2*) identified *Pseudomonas fluorescens* and *Stenotrophomonas maltophilia*, both of which were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (*4*); *Mycobacterium chelonae*, which was identified by *rpo*B gene sequencing; and *Acanthamoeba polyphaga*, which was identified by partial 18S rDNA sequencing.

Culturing this ameba in sterile peptone–yeast extract–glucose broth showed that it hosted 4 organisms. The first organism was a new intracellular Deltaproteobacterium provisionally named *Candidatus* Babela massiliensis (100% 16S rDNA sequence similarity with a reference strain [GenBank accession no. GQ495224], which was susceptible to 200 μg/mL rifampin and resistant to 20 μg/mL ciprofloxacin). The second organism was a new, unnamed, gram-negative, ciprofloxacin-susceptible (20 μg/mL) *Alphaproteobacterium* bacillus with 99% 16S rDNA sequence similarity (GenBank accession no. HM138368) with *Candidatus* Odyssella sp. and *Acanthamoeba* endosymbiont KA/E9 (*5*). The third organism was a new giant virus related to an *A. polyphaga* mimivirus (*6*) strain Lentille. The

fourth organism was a new virophage referred to as Sputnik 2 (*7*).

Acanthamoeba spp. are ubiquitous in tap water (*2,5*), including that used by contact lens wearers to wash hands before manipulating the lens (*8*). Tap water hosts many organisms, including bacteria (*2,5*); large DNA viruses (*9*); and the recently described Sputnik virophage, a virus that infects ameba-resistant large DNA viruses (*10*). We found 5 bacterial species, a giant virus, and a virophage in the contact lens storage case liquid for this patient.

Although *Acanthamoeba* spp. and *M. chelonae* are well-described agents of keratitis, other ameba-resistant organisms have not been associated with keratitis. However, 1 of the 2 new ameba-resistant bacteria isolated from the contact lens storage case liquid had a 16S rDNA sequence similar to that of the endosymbiont of an *Acanthamoeba* species isolated from the corneal sampling of a patient with keratitis in South Korea (*5*).

These data indicate the ubiquity of these emerging organisms and raise questions about roles of the ameba host and the symbiont as causative agents of keratitis. We therefore estimate that any of the organisms reported or any combination of these agents could have been involved in the keratitis that developed in the patient. Because the minute quantity of corneal scraping material prevented molecular detection, the relative contribution of each organism could not be specified.

This case illustrates that culturing ameba from ocular specimens and contact lens storage case liquid is mandatory for determining the diversity of pathogens potentially responsible for ameba-associated infections, such as keratitis, in patients who wear contact lenses. Ameba-resistant organisms have complex reciprocal interactions with the host, reminiscent of the mafia behavior.

The patient's practice of not drying her hands after cleansing them with tap water and diluting the contact lens cleansing liquid with tap water may have provided a route for contamination of the contact lens fluid with ameba-resistant, tap water–borne organisms. Patients should be informed of the risk for keratitis caused by water-borne, amebal-resistant pathogens. They should also be educated to avoid contact with tap water when manipulating contact lenses, to dry hands after washing them with water and soap, or to use antiseptics containing ≥70% alcohol for hand disinfection before contact lens manipulation.

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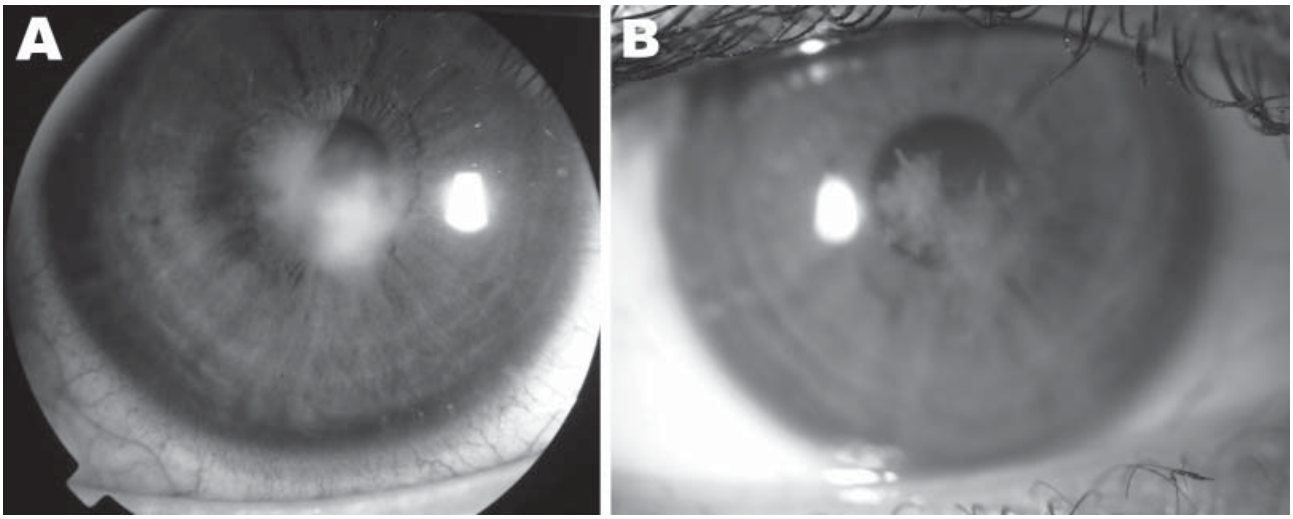

Figure. Ameba-associated keratitis in a 17-year-old woman (contact lens wearer), France, showing a paracentral corneal scar (A) and recovery at 13-month follow-up (B). Original magnification ×10. A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1306-F.htm).

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Human Herpesvirus 1 in Wild Marmosets, Brazil, 2008

To the Editor: Human herpesvirus 1 (HHV-1) infections in New World monkey species, especially in the Callithrichid family, have been described (*1–6*), but most reports have discussed experimental infections or isolated spontaneous infections in pet, zoo, or research animals. We report an outbreak of HHV-1 in wild marmosets (*Callithrix* spp.) in the city of Rio de Janeiro, Brazil.

In October 2008, the Empresa de Pesquisa Agropecuária received 5 marmosets (*Callithrix* spp.) from the Campo Grande district of Rio de Janeiro for necropsy. These animals were usually fed by residents of a condominium complex and were having neurologic signs and severe prostration, physiologic changes suggestive of herpesvirus infections. Euthanasia, followed by necropsy and histopathologic examinations to determine the cause of illness, were recommended.

The primary changes observed during necropsy were vesicular and necrotic plaques on tongues (online Appendix Figure, panel A, www.cdc.gov/EID/content/17/7/1308-appF.htm) and ulcerations in oral mucosa of all examined animals, as well as large lymph nodes of the cervical region, mainly retropharyngeal. Three animals showed marked brain congestion (online Appendix Figure, panel B). Other alterations were splenomegaly, lung congestion, and adrenomegaly.

Histopathogic examinations found superficial ulcerations of the tongue, variable in dimension, that showed fibrinopurulent exudates, mononuclear cell infiltrates on lamina propria, and balloon degeneration of epithelial cells. The brains had multifocal nonsuppurative meningoencephalitis with perivascular and vascular infiltrates of mononuclear cells and gliose foci (Figure, panels A, B). Adrenal glands had hyperemia, hemorrhage, perivascular infiltrates of mononuclear cells, and focal necrosis. Mild hyperemia and alveolar emphysema had occurred in lungs. The livers showed hyperemia and mild to moderate periportal infiltrates of mononuclear cells. Lymph nodes showed hemorrhages, lymphoid hyperplasia, and small foci of subcapsular necrosis. Hyperemia and decreased lymphoid cells population were present in the spleens. In addition, intranuclear inclusion bodies in cells of brains, peripherical nerves, tongues, and adrenal glands were observed. These changes were found in all animals. All changes were consistent with HHV-1 in nonhuman primates (*2–6,7,8*).

To confirm the diagnosis, immunohistochemical examination was done by using polyclonal antibody directed against HHV-1. We used the avidin–biotin–peroxidase complex method with Harris hematoxylin counterstain. Sections taken of the ulcerated oral lesions had intranuclear inclusion areas strongly marked by immunoperoxidase (Figure, panels C, D). HHV-1 infection was confirmed in the 5 marmosets.

Many reports have described human herpesvirus in New World monkeys. Most of the reports were of experimental or isolated spontaneous infections in pets (*1*,*2*), zoo (*3*), research (*4*,*5*) or wild animals (*6*). This is the second report of a naturally occurring infection in wild marmosets. Both infections occurred in the Grande Rio region, where *Callithrix* spp. imported from other Brazilian states were accidentally introduced. These species came to occupy a niche that once belonged to the golden lion tamarin (*Leontopitecus rosalia*) (*9*,*10*).

Humans are the reservoir and the natural host of human herpesvirus (*3–6*), which can be disseminated by direct contact, through sexual