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Mimivirus-like Particles in Acanthamoebae from Sewage Sludge

To the Editor: Mimivirus is a giant, double-stranded DNA virus. Its 650-nm diameter and 1.2-Mb genome make it the largest known virus (*1*). In 2003, mimivirus was isolated from a water cooling tower in Bradford, UK, after a pneumonia outbreak and was reported to infect *Acanthamoeba polyphaga* amebae (*2*). Subsequently, a small number of additional isolates have been reported (*3*).

Mimivirus has been associated with pneumonia, and this association was strengthened after antibodies to mimivirus were found in serum samples from patients with community- and hospital-acquired pneumonia and after mimivirus DNA was found in bronchoalveolar lavage specimens (*4*). More direct evidence of pathogenicity was illustrated when a pneumonia-like disease developed in a laboratory technician who worked with mimivirus and showed seroconversion to 23 mimivirus-specific proteins (*5*).

We report finding mimivirus-like particles during our molecular study of *Acanthamoeba* spp. abundance and diversity in final-stage conventionally treated sewage sludge from a wastewater treatment plant in the West Midlands, UK. Using metagenomic DNA extracted from the sludge (*6*), we estimated the abundance of *Acanthamoeba* spp. by using real-time PCR (*7*) and found it to be $\approx 1 \times 10^2$/g sludge. To assess species diversity, we amplified an *Acanthamoeba* spp.–specific 18S rRNA target, which resulted in products of ≈450 bp (*8*). PCR products were cloned and sequenced, revealing low *Acanthamoeba* spp. diversity with a predominance of clones most similar to *A. palestinensis* (22/25 clones), which fall within the T6 clade according to the classification of Stothard et al. (*9*). A small number (3/25) of clones showed closest similarity to acanthamoebae belonging to the T4 clade, which includes strains considered to be human pathogens, including some *A. polyphaga* strains.

Acanthamoebae were isolated from fully digested sewage sludge by inoculating diluted sludge onto cerophyl-Prescott infusion agar and subculturing onto nonnutrient agar plates streaked with heat-killed *Escherichia coli*. Cultures were incubated at 20°C and 30°C and examined under an Axioskop 2 microscope (Zeiss, Oberkochen, Germany) at 100× magnification; cells of interest were examined at 1,000× magnification. One clonal population of an *Acanthamoeba* sp. isolated at 20°C, which demonstrated typical trophozoite and cyst morphology, contained large numbers of particles either within vacuoles or within the cytoplasm (Figure). Vacuoles were densely packed with particles that appeared to be constantly moving; vacuole size varied from that typical of food vacuoles to large vacuoles that occupied most of the cell volume (expanded online Figure, panels B, D, and G, www.cdc.gov/EID/content/17/6/1127-F.htm). Because the particles were assumed to be bacterial pathogens, efforts were made to produce an axenic culture of the ameba isolate, and 16S rRNA PCR was performed to identify any intracellular bacteria. DNA was extracted by using a phenol chloroform method according to Griffiths et al. (*6*). However, no 16S rRNA PCR products were amplified.

Months later, an image review led to recognition of unusual arrangements of intracellular particles in a lattice-like structure in which each particle was surrounded by 6 others. Measurement of rows of particles, assuming tight packing, gave an average particle size of 620 nm. At this point, we realized that the particles were virus-like

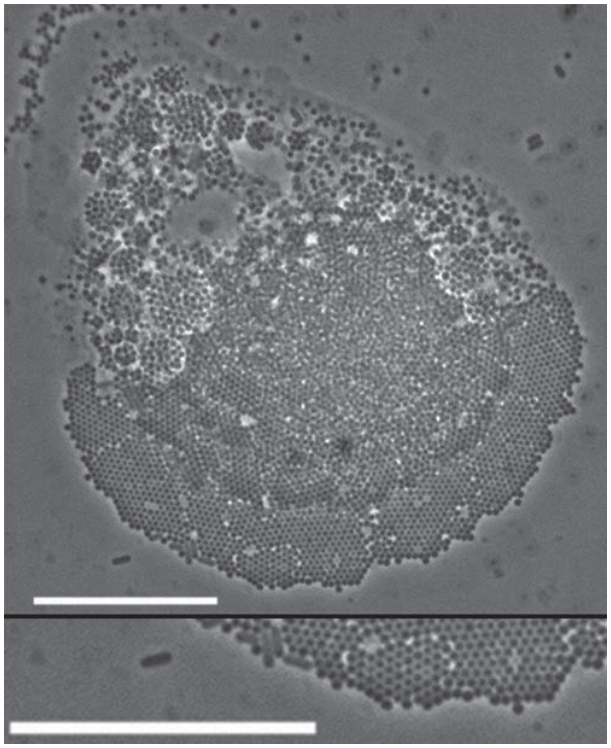

Figure. Light micrograph of acanthamoebae infected with mimivirus-like particles, showing cell packed with mimivirus-like particles. Enlargement of region of image shown at bottom of original image. Scale bar = 10 μm. An expanded version of this figure with additional images is available online (www.cdc.gov/EID/content/17/6/1127-F.htm).

and closely resembled mimivirus. Subsequent efforts to resuscitate the infected *Acanthamoeba* spp. culture were unsuccessful, and performing specific PCR for mimivirus sequences was not possible. Sewage sludge samples collected later were tested for mimivirus by using PCR (*4*); however, no amplification was observed, indicating either that mimivirus was present only transitorily, that mimivirus was below detection limits, or that the target primer sites were not conserved.

The density of virus-like particles within acanthamoebae cells was extremely high (Figure). The advantage of in situ observation of amebae on the surface on which they were cultivated is that the cell is not disturbed. The virus-like particles are arranged in tightly packed, flat sheets, indicative of an icosahedral structure. Toward the bottom of the Figure A, a single sheet of particles can be seen, corresponding to the hyaline zone at the anterior of the cell, and in the center of the cell are multiple layers of tightly packed virus-like particles. Toward the anterior of the cell, vacuoles containing particles were apparently being egested at the uroid. Dense 3-dimensional aggregations of particles (expanded online Figure, panel E) resembled previously described virus factories (*10*). Free mimivirus-like particles (expanded online Figure, panels B, D) indicate egestion by amebae or the result of amebal lysis, a phenomenon observed in cocultures. Prevalence of infection was high and infection was immediately obvious, even when cultures were observed at low magnification (100×).

Although we did not confirm the identity of the mimivirus-like particles by molecular methods or electron microscopy, the nature of the light micrographs enabled close examination of the particles. These particles demonstrated close similarity to mimivirus in size and shape as indicated by the lattice arrangement in which 1 particle was surrounded by 6 others, as seen previously (*10*). Our study illustrates that acanthamoebae that survive sewage treatment can harbor mimivirus-like particles, which could be disseminated to agricultural land and surface waters.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Rabies Immunization Status of Dogs, Beijing, China

To the Editor: In the People's Republic of China, >3,000 persons die of rabies each year; most were infected by dog bites (*1*). Since 2000, the dog population in Beijing has increased dramatically, and the exact vaccination coverage and immunization status of dogs are not known.

During 2006–2009, to assist with governmental rabies control, Fengtai District was selected as a geographically representative area in Beijing in which to conduct a survey of rabies antibody titers in domestic dogs. Blood samples were randomly collected from 4,775 dogs in Fengtai District, which account for 3% of all registered dogs in the district. Rabies virus neutralization antibody (VNA) titers were detected by fluorescent antibody virus neutralization (*2*). In brief, VNA titers ≥0.5 IU indicated positive immunization, implying that the dog had an adequate level of antibody, and VNA <0.5 IU indicated negative immunization (*3*). The data were analyzed by 2-tailed χ^2 test; $p<0.05$ was considered significant. Vaccination coverage and antibody levels were categorized either by dog's function (guard or pet) or residence (urban or suburban) (Figure).

Most dogs with a history of vaccination were positively immunized (68.1%) (Figure, bar A), compared with 16.4% in the unvaccinated group (Figure, bar B), demonstrating that compulsory immunization is crucial to rabies control (*4*). Of 944 dogs with unclear vaccination history, 221 (23.4%) (Figure, bar C) had adequate antibody levels, possibly from undocumented vaccination or contact with rabies hosts. However, for 2006, 2007, 2008, and 2009, immunization coverage in the district was 55.0%, 53.8%, 67.4%, and 54.4%, respectively, all below the >70% criterion recommended by the World Health Organization (*5*). The results imply that much work still needs to be done by the Beijing government, not only to meet the World Health Organization immunization baseline but also to keep risk for a rabies epidemic in Beijing low.

Immunization coverage ratios differed significantly ($p<0.05$) between guard (39.3%) and pet dogs (69.5%) (Figure, bars D, E) and between urban (81.7%) and suburban areas (27.6%) (Figure, bars F, G). Consequently, the number of negatively immunized guard dogs was 1.68× lower than that for pet dogs (Figure, bars D, E) ($p<0.05$), and the number of positively immunized dogs in urban areas was 2.5× higher than that in suburban areas (Figure, bars F, G) ($p<0.05$).

In Beijing, guard dogs are usually raised by villagers to protect the house, whereas pet dogs are usually raised by city dwellers who treat dogs as friends. As a result, in urban areas dogs are registered and vaccinated in a timely manner by authorized pet hospitals (*6*). In suburban areas, however, dog management is deficient. For example, guard dogs in suburban areas are sometimes not vaccinated because the owner or veterinarian cannot safely restrain the dog for vaccination.

According to our study, >10% of unregistered dogs with no clear history of vaccination are not vaccinated during yearly vaccination programs. In Beijing during 2007–2009, of 9 cases of rabies in humans, 6 were associated with stray dogs (*7*), and most stray dogs were found in suburban areas. Hence, strategies to either reduce stray dogs in the city or to get such dogs under official management (e.g., include stray dogs in compulsory annual vaccination programs) are urgently needed.

In our opinion, policies related to dog registration, vaccination recording, and vaccination strategies