

Table. Serologic findings of an autochthonous case of dengue fever, Buenos Aires, February 2007

Date (days after onset)	MAC-ELISA*	Plaque reduction neutralization test (90%)						
		Saint Louis encephalitis virus	West Nile virus	Yellow fever virus	Dengue 1 virus	Dengue 2 virus	Dengue 3 virus	Dengue 4 virus
2007 Jul 7 (16)	+	<20	<20	<20	80	<20	80	<20
2007 Apr 13 (53)	ND	<20	<20	<20	40	<20	640	<20

*Immunoglobulin M antibody-capture enzyme immunoassay with suckling mouse dengue virus antigen mixture of dengue 1, dengue 2, dengue 3, and dengue 4 serotypes. ND, not determined.

Most of the patients whose cases were diagnosed in Buenos Aires, including 5 who required hospitalization, were referred to Muñiz Hospital. Built a century ago, Muñiz Hospital comprises a number of independent pavilions surrounded by a spacious garden, where mosquitoes thrive, especially in summer. Thus, vector-borne infection in this case might have occurred either in Muñiz Hospital, in the Federal District, or in the southern city suburb, where the patient lives and works.

Until recently, dengue had not been suspected in patients with a fever living in the Buenos Aires area in the absence of a recent history of travel to an endemioepidemic area. Confirmation of our case was evidence of local circulation of dengue virus. Thereafter, serum testing became recommended in Buenos Aires for acute febrile illness, among other dengue surveillance interventions in the area. More recently, epidemiologic surveillance of febrile illness has been strengthened country-wide upon the recent reporting of yellow fever cases in Argentina (8).

No circulation of dengue virus was reported in Buenos Aires during the first 10 epidemiologic weeks of 2008. However, vector control measures should be strengthened to minimize the risk of infective persons triggering an epidemic of dengue or other flavivirus disease.

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DOI: 10.3201/eid1409.080143

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Naegleria fowleri in Well Water

To the Editor: *Naegleria fowleri*, a protozoan found in hot springs and warm surface water, can cause primary amebic meningoencephalitis in humans. A survey of drinking water supply wells in Arizona determined that wells can be colonized and may be an unrecognized source of this organism that could present a human health risk.

N. fowleri is a free-living amebic flagellate found in warm bodies of water such as ponds, irrigation ditches, lakes, coastal waters, and hot springs and can cause primary amebic meningoencephalitis. Humans come into contact with *N. fowleri* by swimming or bathing, particularly in surface waters. The ameba enters the nasal passages, penetrates the nasopharyngeal mucosa, and migrates to the olfactory nerves, eventually invading the brain through the cribriform plate (1). From 1995 to 2004, *N. fowleri* killed 23 persons in the United States (2), includ-

ing 2 children in the Phoenix, Arizona, area in 2002, who had been exposed to well water but had not consumed it (3). There have been 6 documented deaths in 2007, all in warmer regions (Arizona, Texas, Florida) (4).

Although *N. fowleri*'s presence in surface waters is well documented (5,6), no previous studies on its occurrence in wells have been conducted. We studied high-volume drinking water wells operated by municipal utilities or private water companies in the greater Phoenix and Tucson, Arizona, areas. Previous data from 500 wells in the region showed temperatures ranging from 13°C to 46°C. Typical well discharges ranged from hundreds to >3,780 L per minute. Well depths varied from 100 m to >300 m.

Well water samples were collected by using 1-L sterile polyethylene bottles at or near the wellhead before disinfection by well owners or utilities (7). In phase 1, samples were collected after wells were flushed until the water was clear. During phase 2, samples were collected as water was turned on from spigots at or near wellheads (initial) and after a 3-borehole volume had flushed through the system (purged). Additional wells were sampled during this phase. Samples were tested for temperature, pH, turbidity, chlorine residual, conductance, coliforms, heterotrophic bacterial plate counts (HPC), and *Escherichia coli* following standard methods (7).

To test for viable amoebae, we spread aliquots on nonnutrient agar seeded with *E. coli* at 37°C (3,8). We placed scrapings from the advancing front of subsequent amoeba plaques in distilled water to identify enflagellation (5); however, precise species identification was not possible. Live amoebae

were therefore harvested for PCR analysis to specifically identify *N. fowleri*. We chose PCR over the mouse pathogenicity test because other *Naegleria* species that are nonpathogenic in humans are lethal in mice (8). The genotype of isolates was not determined because all of the described genotypes found in the United States have been shown to be pathogenic in humans (9).

To concentrate trophozoites/cysts, we gently agitated samples for 2 minutes and then centrifuged and filtered them through polyethylene filters (2- μ m pore; Millipore, Bedford, MA, USA). A 10- μ L volume of concentrate was used as a template for nested PCR (3,8) (triplicate tests were conducted immediately and after a 2-week 37°C incubation). Positive and negative PCR products were frozen at -80°C, coded to prevent bias, and shipped to Francine Marciano-Cabral at Virginia Commonwealth University for confirmation by cloning and sequencing (3).

The general microbial quality of the wells was as follows: 73 (51%) had >500 HPC/mL; 8 (5.5%) were positive for coliforms; none were positive for *E. coli*. Oils used to lubricate well motors may result in the growth of HPC in well water (10). *N. fowleri* feeds on heterotrophic bacteria in water and could multiply in the well casing. This may explain *N. fowleri*'s colonization of wells.

The recent association in Arizona between unchlorinated drinking water and the transmission of *N. fowleri* suggests that groundwater has been an unrecognized source of this organism. PCR detected *N. fowleri* DNA in 11 (7.7%) of 143 wells. Of 185 total samples, 30 (16.2%) tested positive for *N. fowleri* (Table). The organism was

most often detected after the wells had been purged (17.9% purged vs. 10.0% initial samples), suggesting that *N. fowleri* was present in the aquifer or was released from the well casing or column during pumping. The wells testing positive for *N. fowleri* ranged in temperature from 21.9°C to 37.4°C (average 29.0°C; median 29.5°C).

The live trophozoite form was confirmed in only 1 well, though 11 of 143 wells tested positive according to PCR. This discrepancy may be due to the low occurrence of trophozoites in water or to differences in assay volumes for detection of live trophozoites (0.75 mL) versus PCR (30 mL equivalent unconcentrated volume). PCR is also more sensitive, capable of detecting 100 organisms/L in an unconcentrated sample (8); however, PCR did not determine if the amoebae were infectious. Although PCR can determine the species by using primers for a specific gene sequence not found in other *Naegleria* species, it cannot determine the life stage (cyst/trophozoite). Trophozoites are believed to be the infectious form of the organism (1); nonetheless, cysts can be equally harmful because they may revert to trophozoites under optimal conditions (1). The surprisingly common occurrence of *N. fowleri* in drinking water wells suggests that groundwaters may be an unrecognized human health threat.

Acknowledgments

We thank the *Naegleria* advisory panel, composed of the Arizona Department of Environmental Quality and 2 Arizona drinking water utilities.

The University of Arizona's National Science Foundation Water Quality Center and the Arizona Technology and Research Initiative Fund provided financial support for this study.

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Table. *Naegleria fowleri* in well water samples, Arizona

Sample type*	No. (%) positive
Initial	4/40 (10)
Purged	26/145 (17.9)
All	30/185 (16.2)

*Samples were collected before and after purging 3 borehole volumes. PCR was used to test samples.

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DOI: 10.3201/eid1409.071076

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Popular and Scientific Attitudes Regarding Pandemic Influenza

To the Editor: Blendon et al. (1) described a survey of public attitudes regarding Americans' willingness and ability to follow the advice of public health officials during a severe influenza pandemic. The authors' results, however, can only be considered indicative if Americans' perceptions of pandemic influenza during the next pandemic are comparable to those associated with the hypothetical event they imagined while participating in the survey by Blendon et al.

By asking respondents to imagine a "severe outbreak" of "a new type of flu," the authors likely portrayed to survey participants an image of pandemic flu as an event starkly different from ordinary flu seasons. Although such a contrast reinforces popular perceptions of pandemic flu as a catastrophic event (2), it is not supported by historical studies which show that, in terms of deaths, recent pandemics have been comparable to (3) or less deadly than (4) ordinary influenza seasons.

A gap thus exists between the perceptions and reality of pandemic influenza. Although the authors described pandemic flu as an "unfamiliar crisis" that "many of the respondents may not have been familiar with," in actuality, 39% of survey respondents were ≥ 50 years of age and therefore had firsthand experience of 1 or more

past pandemics. (The last 2 pandemics occurred in 1957 and 1968; a pandemic was predicted in 1976, but never materialized.) Whether those respondents were aware that they had lived through past pandemics is a question with important implications for the survey results, but unfortunately, this understanding was not queried by the authors. For example, would all of the 94% of respondents who reported a willingness to isolate themselves at home for 7–10 days if that were recommended by health authorities—in effect, "voluntarily" placing themselves in quarantine—also be willing to do so during a pandemic no more severe than ordinary influenza?

If even those who have experienced pandemics do not recall them as particularly memorable events, it calls for a rethinking of public communication strategies with respect to influenza. Perhaps a first step is to acknowledge that as the past 2 pandemics have not been public health crises, the next pandemic may likewise also not be a crisis.

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DOI: 10.3201/eid1409.080647

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