Analysis of the complete genome revealed 92% similarity between the 2 isolates, and some genes confirm a remarkable variability (online Technical Appendix Figure, panel C). We constructed a phylogenetic tree (online Technical Appendix Figure, panel D) using the A56R gene sequence by the maximum-likelihood method and 1,000 bootstrap replicates in MEGA 6.02 (http://www.megasoftware.net). The analysis demonstrated a co-infection with viruses from both VACV-BR groups, such that the large-plaque clone clustered with group 2 VACV-BR isolates and the small-plaque clone clustered with group 1 VACV-BR isolates. We named these isolates Carangola eye virus 1 (small) and Carangola eye virus 2 (large).

Our study demonstrated the genetic and phenotypic variability between 2 viruses isolated from the same sample in a natural human co-infection with VACV. The viruses belong to 2 distinct VACV-BR groups, reinforcing and expanding previous work with other hosts (6–8). These results raise new questions about how co-infections with these viruses might change the aspects of an infection and its signs and symptoms, such as development of ocular vaccinia. Although cases of ocular vaccinia have been reported after vaccination and accidental laboratory infection (9,10), we proved the association and isolate VACV samples from a natural ocular vaccinia infection. The effort to understand singular aspects of VACV-BR co-infections should be increased, and further molecular and biologic characterizations of these samples should be conducted to identify and better understand the natural dynamics and signs and symptoms caused by VACV-BR.

About the Author
Mr. Lima is a PhD candidate at the Laboratório de Virus, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. His primary research interest is the poxviruses.

References

Address for correspondence: Maurício Teixeira Lima and Erna Geessien Kroon, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Dept Microbiologia, Lab de Virus, bloco F4, sala 258, Av. Antonio Carlos 6627, 31270-901 Belo Horizonte, Minas Gerais, Brazil; emails: maurili15@hotmail.com and ernagkroon@gmail.com

Estimation of Undiagnosed Naegleria fowleri Primary Amebic Meningoencephalitis, United States¹

Almea Matanock, Jason M. Mehal, Lindy Liu, Diana M. Blau, Jennifer R. Cope

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Primary amebic meningoencephalitis is an acute, rare, typically fatal disease. We used epidemiologic risk factors and multiple cause-of-death mortality data to estimate the number of deaths that fit the typical pattern for primary amebic meningoencephalitis; we estimated an annual average of 16 deaths (8 male, 8 female) in the United States.

¹Preliminary results of this study were presented at the Infectious Diseases Society of America Conference; October 8–12, 2014, Philadelphia, Pennsylvania, USA.


*N. fowleri* causes primary amebic meningoencephalitis (PAM); 0–8 laboratory-confirmed cases per year are documented in the United States (1). PAM causes <0.5% of diagnosed encephalitis deaths in the United States (2). Laboratory-confirmed PAM case-patients in the United States are a median age of 12 years and are identified primarily in southern states during July–September, and 79% are male (1,3). Many case-patients are identified postmortem; 4 known survivors have been reported in the United States (1,4). The signs and symptoms of PAM can be mistaken for other more common neuroinfections, such as bacterial meningitis and viral encephalitis (1,4). Because more than half of neuroinfectious deaths are unspecified (2), clinical expertise and diagnostic testing availability are limited, and true PAM incidence is unknown, concern is reasonable that PAM cases might not be diagnosed. In this study, we estimate the magnitude of potentially undiagnosed cases of PAM by applying previously identified epidemiologic risk factors to unspecified neuroinfectious deaths.

We created a list of codes from the International Classification of Disease, 10th revision (ICD-10), for unspecified possible neuroinfectious deaths by using previously published data (2), ICD-10 codes from death certificates of known PAM case-patients, and expert opinion. We selected codes from any location on the death record, not strictly the primary or immediate cause of death (http://www.cdc.gov/nchs/deaths.htm). We chose to start in 1999 when death certificate data were first coded by ICD-10 codes by epidemiologic risk factor for primary amebic meningoencephalitis among persons 2–22 years of age, United States, 1999–2010†

<table>
<thead>
<tr>
<th>ICD-10 code, disease</th>
<th>In high-incidence states‡ (%)</th>
<th>In July–Sept (%)</th>
<th>Male patients (%)</th>
<th>Female patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G03.9, Meningitis unspecified</td>
<td>Total 505 257 (51)</td>
<td>96 (19)</td>
<td>28 (6)</td>
<td>28 (6)</td>
</tr>
<tr>
<td>G04.9, Encephalitis, myelitis and encephalomyelitis, unspecified</td>
<td>479 112 (24)</td>
<td>135 (28)</td>
<td>33 (7)</td>
<td>26 (5)</td>
</tr>
<tr>
<td>R29.8, Other and unspecified symptoms and signs involving the nervous and musculoskeletal systems</td>
<td>264 112 (42)</td>
<td>63 (24)</td>
<td>8 (3)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>G00.9, Bacterial meningitis, unspecified</td>
<td>222 105 (47)</td>
<td>41 (18)</td>
<td>11 (5)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>A88, Unspecified viral encephalitis</td>
<td>154 92 (60)</td>
<td>40 (26)</td>
<td>15 (10)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>G06.2, Extradural and subdural abscess, unspecified</td>
<td>59 31 (53)</td>
<td>12 (20)</td>
<td>4 (7)</td>
<td>0</td>
</tr>
<tr>
<td>A87.9, Viral meningitis, unspecified</td>
<td>38 25 (68)</td>
<td>13 (34)</td>
<td>4 (11)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>A89, Unspecified viral infection of the central nervous system</td>
<td>6 2 (33)</td>
<td>3 (50)</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>A83.9, Mosquito-borne viral encephalitis, unspecified</td>
<td>1 1 (100)</td>
<td>1 (100)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

*Table. Unspecified neuroinfectious death ICD-10 codes by epidemiologic risk factor for primary amebic meningoencephalitis among persons 2–22 years of age, United States, 1999–2010†*

*Codes without cases (R83.5, A92.9, A85.2, A84.9, A81.9, A94, A06.6) not listed. ICD-10, International Classification of Disease, 10th revision. †The total provided is greater than the total number of cases because each case may have >1 ICD-10 code.‡Arizona, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, Missouri, Nevada, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, and Virginia.
(5). Viral causes (i.e., La Crosse and West Nile viruses) have a similar pattern, occurring during July–September (>80% of cases) and more commonly in males (3:2 male:female ratio) (6). An ICD-10 code for West Nile virus, A92.3, was added in 2005. There was only 1 case in our estimate that had the code Mosquito-borne viral encephalitis, unspecified (A83.9). Similar to PAM, cases of arbovirus disease could be included in even less-specific meningitis and encephalitis codes, illustrating that unspecified neuroinfectious deaths are likely caused by several pathogens.

Medical chart review and autopsies, not available for this study, would provide further information about the cause of death. Although this estimate likely captures more than just PAM cases for the reasons we have outlined, it might not capture all potential PAM cases. Reasons for an underestimate include inaccurate ICD-10 coding (7) and PAM cases that are outside the typical epidemiologic pattern (e.g., 2 cases in Minnesota [(8)] and out of season, such as adult cases linked to ritual nasal rinsing and sinus irrigation [(9,10)].

Although all available evidence points to PAM being a low-incidence disease in the United States, PAM remains a devastating and nearly universally fatal infection that erodes public confidence in the safety of everyday activities (swimming, using public drinking water) and increased stress on local public health departments that are already overextended. The reports of recent survivors indicate that timely diagnosis and early initiation of anti-amebic therapy may be instrumental in combating this deadly infection (4). Therefore awareness, evaluation of risk factors, testing, and early anti-amebic therapy provide the best opportunity for survival (1).

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About the Author
Dr. Matanock was an Epidemic Intelligence Service Officer in the Waterborne Disease Prevention Branch, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, at the time of this project and is now an epidemiologist in the Respiratory Diseases Branch, National Center for Immunization and Respiratory Diseases, CDC. Her research interests include disease detection and surveillance.

References
10. Centers for Disease Control and Prevention (CDC). Her research interests include disease detection and surveillance.

Address for correspondence: Jennifer R. Cope, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop C09, Atlanta, GA 30329, USA; email: bjt9@cdc.gov