Acanthamoeba spp. in Urine of Critically Ill Patients

To the Editor: Free-living amebae are ubiquitous protists able to endure extreme temperature and pH in diverse environments (*1*). In past decades, interest in them increased as causes of infections, such as keratitis (*2*) and granulomatous encephalitis (*3*). *Acanthamoeba* spp. can harbor pathogenic microorganisms as endosymbionts, such as bacteria (e.g., *Legionella* spp., *Pseudomonas aeruginosa*, and *Vibrio cholera*), fungi, and mycobacteria (*4*).

The occasional observation of amebae in urine specimens in a clinical microbiology laboratory (by L.C.S.) motivated our study. To estimate the prevalence of *Acanthamoeba* spp. in urine, on March 3 and 4, 2008, we collected urine samples from indwelling urinary catheters of all critically ill patients in the intensive care units of a tertiary care 2,000-bed university hospital (Hospital das Clínicas, University of São Paulo). Medical records were evaluated for patient age, sex, underlying diseases, length of hospital stay, use of central venous catheter, mechanical ventilation, antimicrobial drug use, and duration of urinary catheterization.

Chemical urinalysis was performed by using a urine dipstick (Uriquest; Labtest Diagnostica, Lagoa Santa, Minas Gerais, Brazil), and urine leukocyte and erythrocyte counts were performed. Samples were examined microscopically and cultured for amebae. Amebae were characterized on the basis of morphologic criteria (cyst morphology and trophozoite shape and motility) (*5*). For ameba culture, 10 mL of urine was centrifuged at 2,500 rpm for 5 min. The supernatant was discarded, and the sediment (1 mL) was added to 5 mL of brain heart infusion (Oxoid, Cambridge, UK). Cultures were incubated at 25°C for 48 hr and microscopically examined. For this study, finding trophozoites on direct examination or growing *Acanthamoeba* on culture were considered a positive result for the patient.

Pipet tips (10), vacuum containers (10), plastic 15-mL tubes (10), syringes (10), glass slides (20), and tubes containing medium (10) were submitted for direct examination and culture for ameba to ensure that they were not contaminated. Urine samples were cultured for bacteria and fungi. Urine samples were submitted for bacterial and fungal direct examinations, and cultures were performed (*6*). If found, organisms were identified by morphologic, biochemical, and Gram stain characteristics.

Data from patients with and without *Acanthamoeba* spp. were compared. For dichotomous variables, we used χ^2 to calculate odds ratios and 95% confidence intervals. For continuous variables, the Mann-Whitney test was used. Results were significant at $p \leq 0.05$.

A total of 63 urine samples were evaluated; 17 (26%) were positive for *Acanthamoeba* spp. (Table). All samples of the control materials and medium tested showed negative results.

The high prevalence of *Acanthamoeba* spp. in the urine of critically ill patients is difficult to explain. Although *Acanthamoeba* spp. can cause severe infections, amebae also carry pathogenic microorganisms (*4*). Bacteria may serve as food for amebae, but other interactions exist; for example, bacteria take advantage of the protection offered by amebae, especially in the cystic form (*4*). *P. aeruginosa*, *Escherichia coli*, and *Proteus mirabilis* can infect free-living amebae (*7*). The presence of *Acanthamoeba* spp. in critically ill patients may be advantageous to potentially pathogenic bacteria in the urine, protecting them against antimicrobial drugs, disinfectants, and host immunity. In this sense *Acanthamoeba* spp. may be a reservoir for pathogenic bacterial agents in severely ill patients or, as Khan described, a "Trojan horse for bacteria" (*1*). However, we found no association between the presence of *Acanthamoeba* spp. of bacteria and fungi in the urine.

Biofilms are attractive niches for *Acanthamoeba* spp. that may provide nutritional requirements and protection against disinfectants and antimicrobial drugs (*1*). After 7 days in a patient, most urinary catheters contain a biofilm (*8*). However, we found no association between duration of catheterization and presence of *Acanthamoeba* spp. Also, *P. aeruginosa* isolates from clinical infections have shown more virulence toward *Acanthamoeba* spp. than environmental samples (*9*).

Another possible explanation is the direct pathogenic activity of *Acanthamoeba* spp. In a study to determine whether ameba-associated microorganisms are a cause of nosocomial pneumonia, in 5 of 210 cases, *Acanthamoeba* sp. was considered the only cause of infection (*10*). In our study, patients positive for *Acanthamoeba* spp. had a higher mean and median of urine leukocytes and erythrocytes, suggesting aggression by the amebae. On the other hand, even these higher counts among positive patients are considered relatively low. Cardiovascular disease, cancer, and diabetes were associated with carriage of *Acanthamoeba* spp., which may occur in more severely ill patients. As a final possibility, *Acanthamoeba* spp. in the urine could have no role at all and may even reflect contamination during catheterization.

This study has limitations. It was a small, preliminary investigation designed only to evaluate the presence of *Acanthamoeba* spp. in urine. However, our findings should lead to further studies to increase knowledge about the role of free-living amebae in nosocomial infections.

Table. Univariate analysis of variables potentially associated with *Acanthamoeba* spp. in urine samples from critically ill patients, Hospital das Clínicas, University of São Paulo, Brazil, March 2008*

Variable	Sample positive for *Acanthamoeba* spp.†	Sample negative for *Acanthamoeba* spp.‡	OR	95% CI	p value
No. (%) patients					
Male sex	10 (59)	28 (61)	0.92	0.30–2.95	0.88
Antimicrobial drug use	13 (77)	35 (76)	1.02	0.28–3.78	0.97
Use of mechanical ventilation	10 (59)	23 (70)	1.43	0.46–4.40	0.53
Presence of central venous catheter	12 (75)§	33 (72)	1.18	0.32–4.34	0.80
Urine culture positive for bacteria/fungi					
Any count	9 (53)	21 (46)	1.34	0.44–4.09	0.61
$>10^5$ CFU/mL	8 (47)	13 (28)	2.26	0.72–7.12	0.16
Underlying diseases					
Cardiovascular	15 (88)	26 (57)	5.77	1.06–41.34	0.02
Infectious diseases	10 (59)	21 (46)	1.70	0.48–6.09	0.35
Cancer	9 (53)	9 (20)	4.63	1.20–18.40	0.01
Diabetes mellitus	5 (29)	3 (7)	5.97	1.02–38.03	0.02
Renal Insufficiency	2 (12)	6 (13)	0.89	0.11–5.81	0.89
Acute abdomen	2 (12)	4 (9)	1.40	0.16–10.44	0.71
Trauma	1 (6)	11 (24)	0.20	0.01–1.74	0.11
Respiratory	1 (6)	5 (11)	0.51	0.02–5.24	0.55
Neurologic	1 (6)	4 (9)	0.66	0.03–7.19	0.71
Others	8 (47)	19 (35)	1.26	0.36–4.45	0.68
Age, y					
Mean (SD)	62.4 (13.9)	54.7 (16.2)			0.05
Median (range)	64.6 (20.5–77.5)	53.4 (17.7–80.8)			
Length of hospital stay, d					
Mean (SD)	16.3 (12.8)	13.5 (11.3)			0.45
Median (range)	11 (2–43)	9 (1–48)			
Length of ICU stay, d					
Mean (SD)	6.9 (9.0)	9.4 (10.4)			0.18
Median (range)	3.0 (1–31)	5.5 (0–47)			
Duration of urinary catheterization, d					
Mean (SD)	8.3 (9.8)	10.4 (9.4)			0.17
Median (range)	4.0 (1–33)	8.5 (1–33)			
Leukocyte count in urine per high-power field					
Mean (SD)	10.9 (17.4)	3.59 (10.53)			0.009
Median (range)	3.5 (0–60)	1.0 (0–70)			
Erythrocyte count in urine per high-power field					
Mean (SD)	35.8 (42.6)	17.7 (27.9)			0.03
Median (range)	18.5 (0–150)	4.0 (0–120)			
Urine pH					
Mean (SD)	5.4 (0.8)	5.8 (1.1)			0.18
Median (range)	5 (5–8)	5 (5–8.5)			

*OR, odds ratio; CI, confidence interval.
†n = 17 except as indicated.
‡n = 46.
§n = 16.

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Leonilda C. Santos, Maura S. Oliveira, Renata D. Lobo, Hermes R. Higashino, Silvia F. Costa, Inneke M. van der Heijden, Mauro C. Giudice, Atalanta R. Silva, and Anna S. Levin

Author affiliations: Itaipu Binacional and Universidade do Oeste do Paraná, Paraná, Brazil (L.C. Santos); and University of São Paulo, São Paulo, Brazil (M.S. Oliveira, R.D. Lobo, H.R. Higashino, S.F. Costa, I.M. van der Heijden, M.C. Giudice, A.R. Silva, A.S. Levin)

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Address for correspondence: Anna S. Levin, Rua Banibas, 618, São Paulo-SP 05460-010, Brazil; email: gcih@hcnet.usp.br

Ranavirus Outbreak in North American Bullfrogs (*Rana catesbeiana*), Japan, 2008

To the Editor: Ranaviruses (family *Iridoviridae*) are emerging pathogens of farmed and wild amphibians and cause high mortality rates in these animals (*1*). These viruses are associated with massive population decreases of some species (*2*,*3*); outbreaks have been reported in the United States, Asia, Micronesia, and Europe. At the general meeting held by the International Epizootic Office in May 2008, iridoviruses of amphibians were added to the list of pathogens of wildlife that should be monitored (www.oie.int/aac/eng/Publicat/Cardsenglish/Ranavirus%20card_final.pdf, www.oie.int/eng/normes/fcode/en_chapitre_2.4.2.htm, and www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-viruses.htm). We report an outbreak of ranavirus disease in amphibians in Japan.

A mass die-off of wild North American bullfrog (*Rana catesbeiana*) larvae was discovered in a 1,000-m^2 pond in western Japan. The die-off lasted from September 10 through October 20, 2008, with an epidemic peak on September 20, during which several thousand carcasses were collected daily. No dead adults of *R. catesbeiana* or other amphibian species were found. Fish (families Cyprinidae and Gobiidae) in the pond were unaffected.

Clinical signs in frogs were depression; lethargy; palpebral hyperemia; abdominal edema, petechiae, and erythema on the ventral surface; skin ulcers; limb and tail necrosis; and emaciation. Pathologic changes were similar in all larvae. At necropsy, subcutaneous edema, body cavity effusions, and swollen and friable livers were observed. Histologic examination showed extensive glomerular necrosis with renal tubular hyaline droplet degeneration (online Appendix Figure, available from www.cdc.gov/EID/content/15/7/1146-appF.htm) and various degrees of hepatic cell degeneration and necrosis. Myxosporidia were not observed within any renal tubules. Electron microscopy showed cytoplasmic ranavirus-like particles within glomerular endothelial cells. These particles were icosahedral with a diameter of ≈120 nm. Bacterial colonies were observed on the skin and within multiple organs in some larvae examined. These colonies were interpreted to be opportunistic organisms and microbial cultures were not performed.

PCR with primers M153 and M154 (*4*) amplified a ranavirus-specific gene encoding major capsid protein (MCP) from 18 bullfrog specimens. DNA sequences (584 nt, which did not include primer-annealing regions) obtained from 5 PCR products randomly selected by direct-sequencing were identical. These sequences showed highest similarities with those of *R. catesbeiana* virus TW07–440 (GenBank accession no. FJ207464); only 1 nt difference was observed and this difference resulted in an amino acid substitution. Amplifications with several sets of primers (M68/M69, M70/M71, M72/M73, M84/M85, and M151/M152) (*4*) and sequencing were conducted.

We determined MCP DNA sequences of 1,472 nt that included the complete coding region (nt positions 17–1408, 1,392 nt) and proximal flanking regions. Sequences were deposited in the DNA Data Bank of Japan/GenBank/European Molecular Biology Laboratory DNA databases under the accession no. AB474588. Phylogenetic analysis showed that virus detected in this study, designated RCV-JP, showed greater similarity to TW07–440 virus than to other ranaviruses, including tadpole edema virus (*5*), frog virus 3 (*6*), and *R. catesbeiana* virus Z (*7*). Liver tissues of fish (*Gnathopogon*