

M. ulcerans is known to be associated with wetlands, and the Kouilou River environment is certainly suitable for its spread (10). Identification of this zone as a high-risk area for Buruli ulcer disease will help the Ministry of Health improve early detection, biological confirmation, and treatment programs. In the other regions, active and continuous surveillance is necessary to establish a detailed map of the villages and areas where Buruli ulcer disease is endemic; such information would enable the implementation of targeted control activities. However, active surveillance in Congo has substantially declined since 2011. Our findings support the reactivation of such surveillance campaigns to ensure the early identification and confirmation of Buruli ulcer cases and to improve patient management.

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Rapid Metagenomic Diagnostics for Suspected Outbreak of Severe Pneumonia

To the Editor: Recent outbreaks of severe pneumonia or acute respiratory distress syndrome (ARDS) have attracted much public interest. Given current awareness levels, clinical personnel and health officials must rapidly and adequately respond to suspected outbreaks to prevent public disturbances. We report a case that highlights the potential of next-generation sequencing (NGS) to complement conventional diagnostics in such scenarios.

On March 29, 2013, a police officer (patient 1) was admitted to the emergency department of the University Medical Centre Hamburg-Eppendorf in Hamburg, Germany, because of ARDS. The patient was given mechanical ventilation; all diagnostic test results for pathogens commonly known to cause pneumonia were negative (www.virus-genomics.org/supplementaries/EID1406.pdf). Although treatment with antimicrobial drugs was immediately initiated, the patient died 6 days later of multiple organ failure.

On April 5, a second police officer (patient 2) from the same county was admitted to the same emergency department because of ARDS. He was transferred to the intensive care unit and given mechanical ventilation. Similar to the situation for patient 1, diagnostic test results were negative, prompting the news media to suspect an outbreak of a novel or mutated virus (1,2). Especially because of simultaneous outbreaks of avian influenza and infections with Middle East respiratory syndrome coronavirus in other parts of the world, these reports caused serious concern among the public and health officials.

After the death of patient 1 and hospitalization of patient 2, we subjected

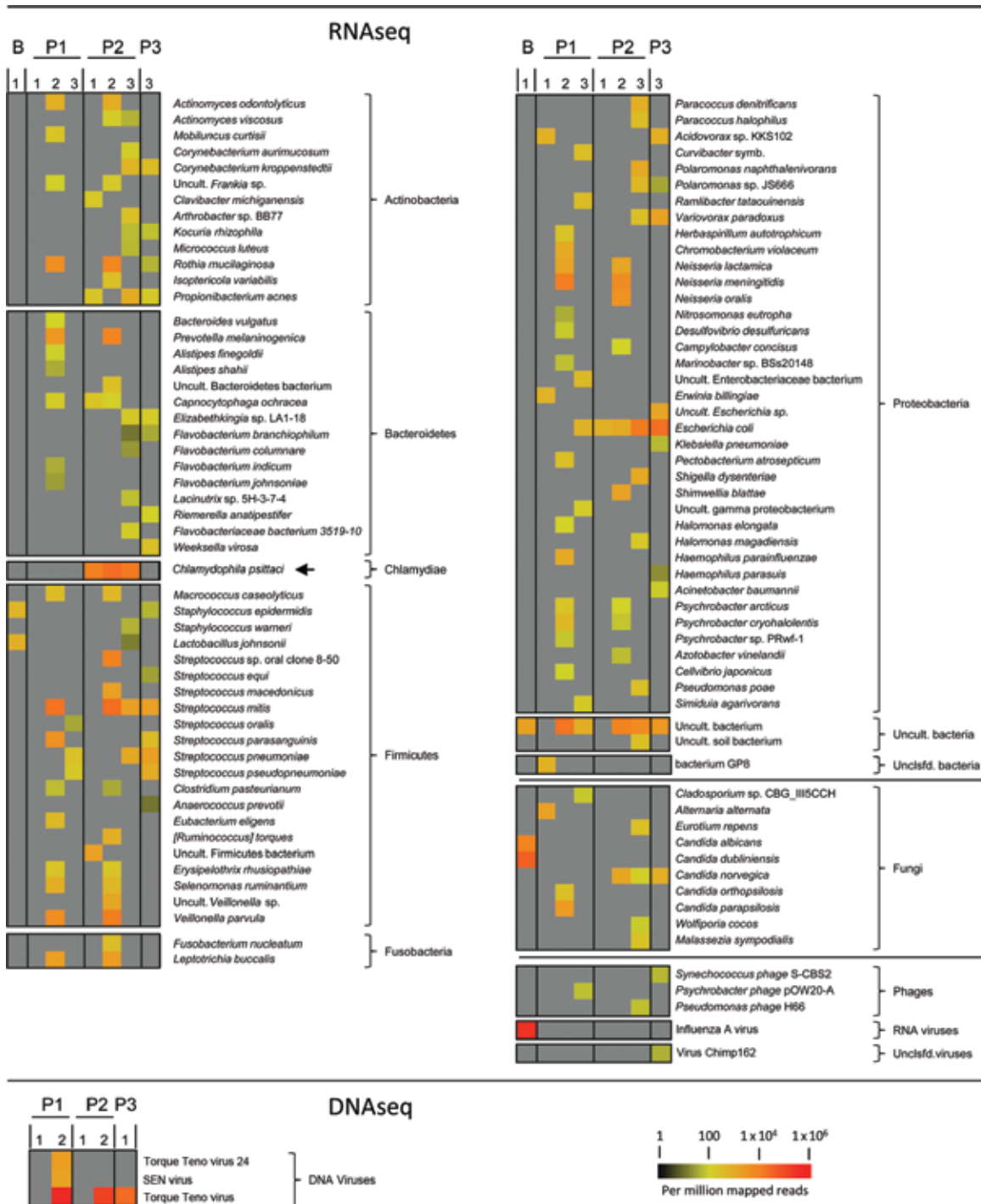


Figure. Next-generation sequencing of RNA (RNaseq) and DNA (DNaseq) isolated from bronchoalveolar lavage (BAL) samples from 3 patients with severe pneumonia, northern Germany. Shown are data from BLASTN (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis of de novo assembled sequence contigs (www.virus-genomics.org/supplementals/EID1406.pdf). Relative abundance of contig reads mapping bacterial, fungal, or viral species is indicated by a heat map (scale bar). White areas indicate that no reads were detected. Diagnostic samples were obtained from 3 patients (lanes P1, P2, and P3). Lane B, control BAL sample (analyzed by using RNaseq only) from an influenza patient; lane 1, MS: analysis on the Illumina MiSeq platform (www.illumina.com/systems.ilmn); lane 2, HS: analysis on the Illumina HiSeq platform (www.illumina.com/systems.ilmn); lane 3, HS dpl., RNA samples depleted of human rRNA before analysis on an HiSeq instrument. *Chlamydia psittaci*, which was unequivocally detected in all samples from patient 2 but not in samples from the other patients, is indicated by an arrow. Symb., symbiont; Uncult., uncultured; Unclsfd., unclassified; SEN virus, strain of Torque teno virus.

nucleic acids extracted from bronchoalveolar lavage (BAL) specimens from both patients to NGS by using a MiSeq sequencer (www.illumina.com/systems.ilmn). To enable rapid and unbiased detection of bacterial and viral agents, we did not enrich specific sequences. The entire workflow (www.virus-genomics.org/supplementaries/EID1406.pdf) was completed within 50 hours.

First-line analysis clearly identified *Chlamydophila psittaci* in the RNA sample from patient 2, but no sequences of obvious pathogenic origin were detected in samples from patient 1 (Figure). *C. psittaci*, an intracellular bacterium, can be transmitted by inhaling aerosolized secretions or feces from infected birds (3). Person-to-person transmission of this bacterium is rare (4,5). Ornithosis, a disease characterized by severe pneumonia and influenza-like symptoms, might develop in persons infected with this bacterium. Because of the rarity of the disease, standard diagnostic panels usually do not include *C. psittaci*. After 11 days of antimicrobial drug treatment, the condition of patient 2 improved, and the patient was transferred to a general hospital ward.

On April 29, a third police officer (patient 3) who had been sharing office space with patient 2, came to the hospital with symptoms of pneumonia. After antimicrobial drug treatment, his condition rapidly improved and the patient was discharged on day 7.

At admission of patient 3, samples from all 3 patients were analyzed at greater read depth by using a HiSeq system. RNA sequencing identified commensal bacteria in all samples, but *C. psittaci* was present only in samples from patient 2 (Figure). Likewise, subsequent high-throughput 16S rRNA sequencing (6), PCR, and serologic analysis unequivocally identified a *C. psittaci* infection in patient 2, but not in patients 1 or 3 (www.virus-genomics.org/supplementaries/EID1406.pdf).

We did not detect viral pathogens in any samples. At the DNA level, most nonhost reads originated from nonpathogenic single-stranded DNA anelloviruses (7). No RNA viruses were found, although influenza A(H3N2) virus was readily identified in a MiSeq analysis of a control BAL sample from a patient with a diagnosis of influenza A (PCR cycle threshold 32) (Figure). Furthermore, pairwise BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) did not reveal the presence of unknown sequence contigs that were shared among the patients, as would be expected in case of infection with a novel viral agent. Together with the confirmed *C. psittaci* infection in patient 2, the absence of a common pathogen signature strongly suggests that the cases were unrelated.

We used a comprehensive metagenomic approach to resolve cases suspected of representing an ongoing outbreak. The method used enabled diagnosis of a *C. psittaci* infection within a reasonable timeframe to allow for timely clinical intervention. These findings strongly suggest that NGS methods can complement conventional diagnostics (8–10) and also highlight their potential to aid clinical personnel and health agencies in making appropriate decisions during suspected outbreaks. Clearly, however, NGS-based methods will have to be further standardized and validated before their full potential in diagnostic settings can be realized.

The absence of pathogenic sequences in patients 1 and 3 might suggest that their clinical symptoms had noninfectious causes. However, although samples were collected during the acute phase of clinical symptoms, and despite our ability to detect an influenza A infection in controls, we cannot fully exclude the possibility that a potentially causative pathogen present at low levels might have evaded detection. Thus, systematic and correlative studies evaluating the sensitivity of NGS-based detection

methods in different diagnostic entities are urgently needed.

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Respiratory Infection with Enterovirus Genotype C117, China and Mongolia

To the Editor: Enteroviruses (EVs) are small, nonenveloped viruses of the family *Picornaviridae* (1). EVs are classified into 12 species according to the molecular and antigenic properties of their viral capsid protein (VP1). To date, 7 species are known

to infect humans, including EV-A to EV-D and rhinovirus A, B, and C (www.picornastudygroup.com/taxa/species/species.htm)

EV-C117 was a newly found EV-C genotype. It was identified in a nasopharyngeal sample from a hospitalized child, 3 years and 9 months of age, with community-acquired pneumonia in Lithuania in 2012 (2,3). However, aside from this case, little is known about the prevalence and clinical significance of EV-C117. Here, we report the detection of EV-C117 in children in China and Mongolia with respiratory tract infections (RTIs).

During March 2007–March 2013, we screened for EV-C117 in respiratory samples from patients with RTIs in China and Mongolia, including nasopharyngeal aspirates collected from 3,108 children in China who had lower respiratory tract infections when they were admitted to Beijing Children's Hospital (4) and swab samples from 2,516 patients in Mongolia with influenza-like illness (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/20/6/13-1596-Techapp1.pdf). Respiratory viruses in samples from China were screened by using multiplex PCR and single PCR assays as described (4). Samples from Mongolia were screened by using the FTD Respiratory Pathogens Multiplex Assay Kit (Fast-track Diagnostics, Luxembourg City, Luxembourg). EV-positive samples were further genotyped by using reverse transcription PCR (RT-PCR) and primers sequentially targeting the VP1 region (5), the 5'-untranslated region (5'-UTR)/VP4/VP2 region (6) and the 5'-UTR (7). A 394-nt amplicon corresponding to the 5'-UTR of EVs was obtained from 10 children in China; a 598-nt amplicon corresponding to the 5'-UTR/VP4/VP2 region was obtained by RT-PCR from 5 children in Mongolia. Blastn analysis (www.blast.ncbi.nlm.nih.gov/Blast.cgi) of PCR amplicons showed that only amplicons detected in 2 children from China

(patients BCH096A and BCH104A) and 2 children from Mongolia (patients MGL126 and MGL208) had the highest similarity (95%–98%) to the EV-C117 prototype strain LIT22.

To further confirm that these 4 strains belong to EV-C117, we attempted to amplify the full-length viral genome sequences. However, we only obtained full-length viral genome sequences from the 2 strains found in patients from China (GenBank accession nos. JX560527 [patient BCH096A], and JX560528 [patient BCH104A], respectively). For the remaining 2 strains from Mongolia, MGL126 (5'UTR/VP4/VP2: KF726102; VP1: KF726100) and MGL208 (5'UTR/VP4/VP2: KF726103; VP1: KF726101), we obtained the sequence of the 5'-UTR/VP4/VP2 region and VP1 gene. Phylogenetic analysis of these sequences showed that they all belonged to genotype EV-C117 (Figure, panels A and B).

Virus isolation for EV-C117 by using Vero and H1-HeLa cells was unsuccessful. Through blastn and phylogenetic analyses, we also found that the previously identified EV-C strain HC90835 (EU697831, from Nepal) (8), EV-C104 strain CL-C22 (EU840734, EU840744, and EU840749, from Switzerland) (9) and a rhinovirus strain SE-10-028 (JQ417886, from South Korea), also belong to EV-C117 (Figure, panel A), indicating that EV-C117 is widely distributed geographically. Because a large proportion of EV infections are subclinical or mild (1), the prevalence of EV-C117 should be further estimated by using serologic investigations in general populations.

The VP1 sequences of the EV-C117 strains isolated in China and Mongolia were 89.9%–95.6% (nt) and 95.2%–98.3% (aa) identical to the EV-C117 prototype strain LIT22 (patient JX262382). Alignment analysis of amino acid sequences showed differences between strains isolated in this study and LIT22, i.e., Ser¹⁵ (strains