health concern in industrialized and resource-poor settings. Few reports are available from Africa, although hospital-associated ESBL producers have been described in Cameroon and the Central African Republic (*6*,*7*). ESBL-producing bacteria have been recovered from different sources in the community, including food and companion animals (*8*,*9*), and 1 recent study from India reported that a substantial number of tap water samples were contaminated with carbapenemase $bla_{\text{NDM-1}}$ producing organisms (*10*).

Kinshasa is the second-largest city in sub-Saharan Africa. In 2008, of its estimated 8.7 million inhabitants, only 46%had access to safe drinking water, and 23% had access to improved sanitation facilities according to the World Bank. Opportunistic pathogens in drinking water and poor sanitary conditions may increase the risk of developing infectious enterocolitis for consumers, especially for those who are immunocompromised. It can eventually lead to chronic intestinal carriage of multidrug-resistant organisms. The presence of ESBL producers in the intestinal flora could also lead to horizontal transfer of drug resistance genes from commensal flora to enteric pathogens. This emergence of ESBL-producing bacteria and further community-associated infections poses a public threat, especially in low-resource countries where surveillance is suboptimal and empiric treatment of invasive infections often includes third-generation cephalosporins.

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# Novel *Chlamydiaceae* Disease in Captive Salamanders

**To the Editor:** Although 2 major diseases of amphibians, chytridiomycosis and ranavirosis, have been relatively well studied, enigmatic amphibian disease and death not attributable to any of the known amphibian diseases frequently occur (*1*). We describe an apparently new disease in salamanders that is associated with a novel genus within the family *Chlamydiaceae*.

The salamanders seen in our clinic belonged to 1 of the following species: *Salamandra corsica*, the Corsican fire salamander (5 animals from 1 collection); *Neurergus crocatus*, the yellow spotted newt (11 animals from 3 collections); or *N. strauchii*, Strauch's

spotted newt (6 animals from 2 collections). All salamanders were captive bred; housed in breeding colonies in private collections in Elsloo and Eindhoven, the Netherlands, Munich, Germany, and Brugge, Belgium; and 1–3 years of age.

Disease was characterized by anorexia, lethargy, edema, and markedly abnormal gait. Mortality rate was 100%. Animals in these collections had no histories of disease. All animals were in good nutritional condition. Necropsy did not yield any macroscopic lesions. All animals had mild intestinal nematode or protozoan infections. Results of real-time PCRs for iridoviruses in liver and skin (*2*) or *Batrachochytrium dendrobatidis* fungus of skin (*3*) were negative for all animals.

We placed liver suspensions from the dead salamanders on Columbia agar with 5% sheep blood and tryptic soy agar and then incubated the samples up to 14 days at 20°C. No consistent bacterial growth was observed. Histologic examination of 2 Corsican fire salamanders and 1 yellow spotted newt revealed hepatitis in 1 of the Corsican fire salamanders and the yellow spotted newt. Hepatitis was characterized by high numbers of melanomacrophages and a marked infiltration of granulocytic leukocytes. Immunohistochemical staining for chlamydia (IMAGEN Chlamydia; Oxoid, Basingstone, UK) showed cell-associated fluorescently stained aggregates in liver tissue, suggestive of Chlamydiales bacteria. Transmission electron microscopic examination of the liver of a yellow spotted newt revealed intracellular inclusions containing particles matching the morphology of reticulate or elementary bodies of *Chlamydiaceae* (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1137-Techapp.pdf).

A PCR (*4*) to detect the 16S rRNA of all Chlamydiales bacteria, performed on liver tissue samples from all animals, yielded positive results in all 5 Corsican fire salamanders; in 4/7, 1/3, and 1/1 yellow spotted newts; and in 4/5 and 1/1 Strauch's spotted newts. For taxon identification, the 16S rRNA gene of the Chlamydiales bacteria was amplified and sequenced from the livers from 2 yellow spotted newts (1 from the collection in Elsloo, the Netherlands and 1 from the collection in Munich, Germany), 1 Strauch's spotted newt, and 5 Corsican fire salamanders.

The sequences shared >90% nt identity with the 16S rRNA gene of *C. abortus* B577 (GenBank accession no. D85709) and therefore can be identified as a member of the family *Chlamydiaceae* (*5*). The closest 16S rRNA similarity (92%) was observed with *C. psittaci* strain CPX0308 (AB285329). The sequence obtained from all spotted newt species specimens was identical (GenBank accession no. JN392920) but differed slightly (1%) from that obtained from the fire salamander species specimens (GenBank accession no. JN392919). These sequence differences point to the existence of multiple strains with possible host adaptation.

We determined the phylogenetic position of the novel taxon, named *Candidatus* Amphibiichlamydia salamandrae (online Technical Appendix), identified by using neighbor-joining analysis with Kodon software (Applied Maths, Sint-Martens-Latem, Belgium). The novel Chlamydiales forms a distinct branch in the well-supported monophyletic clade with the genera *Chlamydia* and *Candidatus* Clavochlamydia salmonicola (family *Chlamydiaceae*) (Figure). Maximum parsimony and unweighted pair group with arithmetic mean analyses yielded cladograms with the same topology (results not shown). Previous reports of members of the family *Chlamydiaceae* in amphibians concerned species occurring in other vertebrate taxa as well:

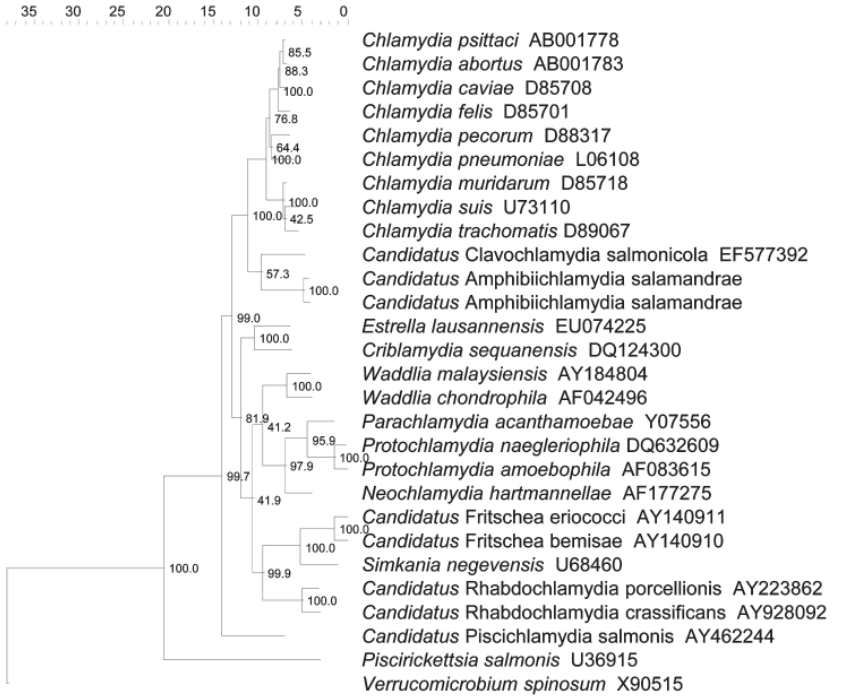


Figure. Topology of the novel amphibian *Chlamydiaceae* (*Candidatus* Amphibiichlamydia salamandrae) within the phylogenetic tree obtained by neighbor-joining and based on 16S rRNA gene data from representative species. Numbers show the percentage of times each branch was found in 1,000 bootstrap replicates. The tree has been rooted with *Verrucomicrobium spinosum* as outgroup. Scale bar indicates nucleotide substitutions per site.

*C. psittaci*, *C. pneumoniae*, *C. abortus*, and *C. suis* (*6*–*10*). To our knowledge, this member of the family *Chlamydiaceae* has been seen in amphibians, but not in other vertebrate hosts. The 16S rRNA analysis showed this taxon to belong to a clade with *Candidatus* Clavochlamydia salmonicola, a taxon found in fish. The phylogenetic position of the novel taxon in the family *Chlamydiaceae* thus roughly reflects the phylogenetic relation between the host species, providing evidence for host–bacterium co-evolution in the family *Chlamydiaceae*.

Although the results obtained are not conclusive with regard to the pathogenic potential of this novel genus and species of Chlamydiales, we were not able to attribute the clinical signs to any known disease. We therefore suggest that we discovered a novel bacterial taxon with possible considerable impact on amphibian health.

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# Novel Variant of Beilong Paramyxovirus in Rats, China

**To the Editor:** In 2003, two cDNA strands were identified in a human mesangial cell line during experimental screening for genes upregulated by angiotensin II (*1*). Sequence analysis showed that the strands were homologous to the matrix, fusion, and phosphoprotein genes of paramyxoviruses, suggesting the possibility of a novel paramyxovirus (*2*,*3*). Subsequent research found that these sequences, believed to originate from human kidney mesangial cell lines, were not amplifiable from such cell lines or human kidney samples but were amplifiable from a rat kidney mesangial cell line (*4*). Isolation and complete genome sequencing of the virus confirmed that it was a novel paramyxovirus of the subfamily *Paramyxovirinae*, named Beilong virus (BeV).

BeV is most closely related to J virus, discovered in autoculture of kidney tissue from a moribund house mouse, and Tailam virus from Sikkim rats (*5*,*6*). Because J virus and Tailam virus were found to originate in rodents and BeV was amplifiable from a rat kidney mesangial cell line, we hypothesized that BeV was a novel paramyxovirus originating in rats. To test this hypothesis, we conducted a territorywide molecular epidemiologic study of rats and other mammals to evaluate this novel paramyxovirus.

We tested 4,130 samples from 1,398 animals collected from various locations in Hong Kong, People's Republic of China, during September 2008–August 2009 (Table). These included 480 kidney, spleen, respiratory swab, and anal swab samples from 120 asymptomatic rats (105 brown rats [*Rattus norvegicus*] and 15 black rats [*R. rattus*]). To

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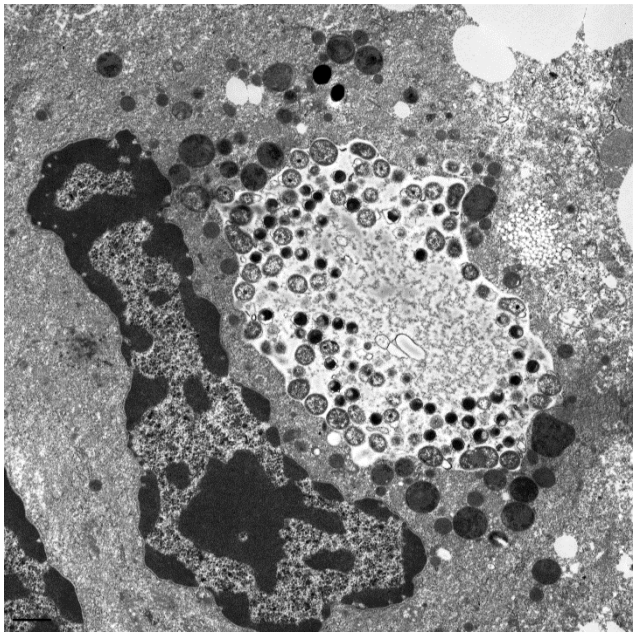
## Technical Appendix

### Description of *Candidatus* Amphibiichlamydia salamandrae

*Candidatus* Amphibiichlamydia salamandrae [Amphibii chlamydia N.L. n. Amphibia name of host class; L. fem. n. *Chlamydia* name of bacterial taxon; N.L. fem. n. *Amphibiichlamydia Chlamydia* from an amphibian; salamandra'e. L. gen. n. salamandrae of a salamander]

The provisional taxon "*Candidatus* Amphibiichlamydia salamandrae" contains intracellular bacteria that infect salamanders of the genera *Neurergus* and *Salamandra* in freshwater or terrestrial environments. The 16S rRNA gene of *Candidatus* Amphibiichlamydia salamandrae has been deposited in the GenBank under accession nos. JN392920 and JN392919. The 16S rRNA gene shows phylogenetic affinity toward the family *Chlamydiaceae*.



Technical Appendix Figure. Transmission electron micrograph of a liver section of a yellow spotted newt (*Neurergus crocatus*) showing an intracellular vacuole containing elementary bodies and reticulate bodies of *Chlamydia*-like organisms. Original magnification ×4,000.