A heterogeneous population of motile brucellae out of the frog pond

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From systemically diseased African bullfrogs (Pyxicephalus edulis), 21 small Gram-negative motile coccobacilli were isolated and primarily identified as Ochrobactrum anthropi by API 20NE and VITEK2. In contradiction to this tentative identification, isolates were positive for the Brucella specific genetic marker bcsp31 and the insertion sequence IS711 in PCR analysis. Subsequent 16S rRNA gene sequencing revealed two different genotypes. Type A was 100% identical to the sequence of Brucella inopinata. Type B was closely related to B. inopinata (99% similarity) but additionally carried an insertion of 44 nucleotides with the closest match (95% similarity) to Ochrobactrum spp. Phylogenetic reconstructions using eight markers in a Multi Locus Sequence Analysis (MLSA)- and 16 loci in a Multi Locus Variable Number Tandem Repeat Analysis (MLVA) both placed the isolates in a cluster related to B. inopinata and other non-classical and potentially novel Brucella species. Molecular analyses clearly illustrated five distinct lineages with three major clades and two singleton isolates. In-depth phenotypic characterization also showed that the amphibian strains are markedly distinct from hitherto known Brucella species. Comparative whole genome analysis of the most diverse strains from the previous analyses verified affiliation to the genus Brucella but also revealed significant genetic differences within the group. Four representative but molecularly and phenotypically diverse strains were used for in vitro and in vivo infection experiments. They all readily multiplied in macrophage-like murine J774-cells and their overall intramacrophagic growth rate was comparable to that of B. inopinata, and slightly higher than that of B. microti. In the Balb/c murine model of infection the amphibian strains replicated both in spleen and liver but were less efficient than B. suis 1330. However, they were able to survive in the mammalian host up to 12 weeks depending on the strain investigated.

The heterogeneity of the described novel strains might hamper a single species description but the features of the amphibian isolates suggest that these novel lineages represent missing evolutionary links between a soil associated ancestor and the modern host-adapted pathogenic Brucella species.