

Atypical *Brucella inopinata*-Like Species in 2 Marine Toads

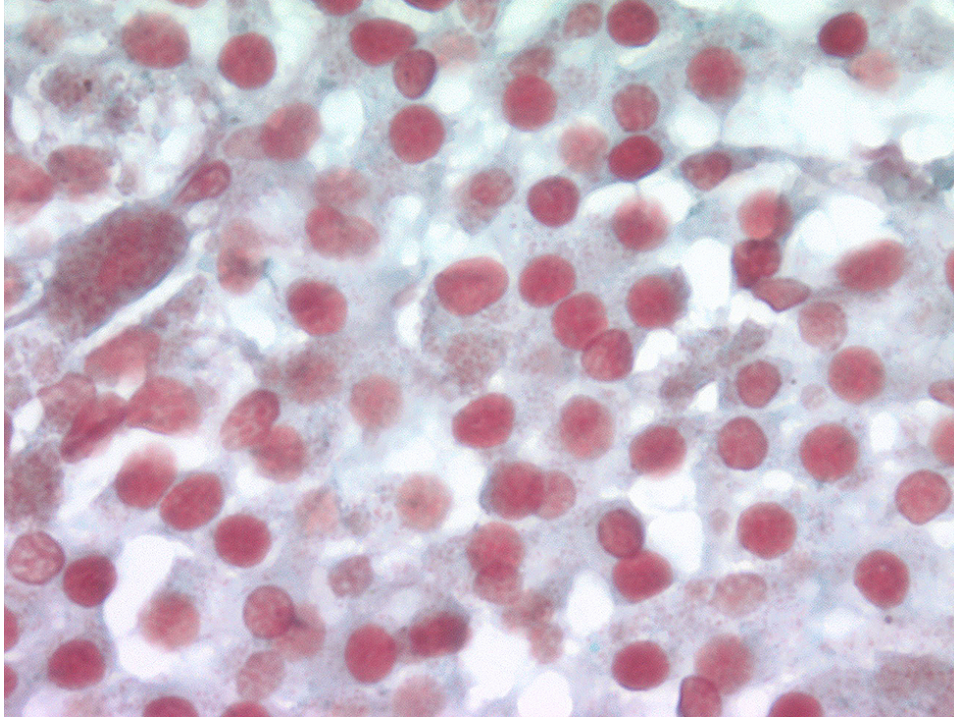
Appendix

Methods

We used a Qiagen DNeasy tissue kit (Qiagen, <https://www.qiagen.com>) to extract DNA from suspect *Brucella* sp. We used the extracted DNA for a Laboratory Response Network real-time assay and partial 16S rDNA and recA PCR using previously published primers (1,2). We treated PCR products with ExoSAP-IT Express (Thermo Fisher, <https://www.thermofisher.com>) and used both forward and reverse primers for sequencing at Eurofins Genomics (Eurofins Genomics, <https://www.eurofins.com>). We trimmed the resulting sequences and analyzed the consensus sequence with NCBI BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the nucleotide database. The biochemical characterization of the bacteria included lead acetate, oxidase, catalase (Remel, <http://www.remel.com>), and gel formation tests (gel formation of the bacterial isolate suspension in 1% phenolized saline) (3).

References

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2. Kiupel M, Desjardins DR, Lim A, Bolin C, Johnson-Delaney CA, Resau JH, et al. Mycoplasmosis in ferrets. *Emerg Infect Dis*. 2012;18:1763–70. [PubMed](#) <https://doi.org/10.3201/eid1811.120072>
3. Lawaczeck E, Toporek J, Cwikla J, Mathison BA. *Brucella canis* in a HIV-infected patient. *Zoonoses Public Health*. 2011;58:150–2. [PubMed](#) <https://doi.org/10.1111/j.1863-2378.2010.01334.x>



Appendix Figure. Case 1: Gram stain of numerous intracytoplasmic *Brucella* sp. coccobacilli within macrophages, which were also acid-fast negative (not shown).