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SHORT COMMUNICATION

Brucella sp. sequence-type 27 associated with abortion in dwarf sperm whale *Kogia sima*

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Abstract

A dwarf sperm whale *Kogia sima* stranded alive along the Central Pacific Coast of Costa Rica. The whale, handled by tourists and local inhabitants, was weak, had buoyancy difficulties, and eventually aborted and died, showing severe necrotizing placentitis and other pathological signs. Both the mother and the fetus had antibodies against *Brucella* lipopolysaccharide. *Brucella* organisms were isolated from various tissues of both animals and were characterized. The bacterium genome corresponded to sequence-type 27 (ST27) and clustered together with other *Brucella* ST27 isolated in humans and cetaceans.

Keywords *Brucella ceti* · *Brucella* · Brucellosis · *Kogia sima* · Dwarf sperm whale · ST27 · Zoonosis

Cetacean brucellosis is an infectious disease caused by *Brucella* organisms (Guzman-Verri et al. 2012; Maquart et al. 2009). The number of cetaceans displaying brucellosis clinical signs remains unknown. However, many cetaceans have antibodies against *Brucella* organisms, suggesting that the infection is common (Guzmán-Verri et al. 2012; Isidoro-Ayza et al. 2014; Hernández-Mora et al. 2009). In Costa Rica, most of the dolphins stranding along the Pacific coast display neurobrucellosis and associated pathologies (Gonzalez-Barrientos et al. 2010). The most common agent causing cetacean brucellosis is *Brucella ceti* dolphin-type (Guzman-Verri et al. 2012; Hernández-Mora et al. 2009). Less common are *B. ceti* porpoise-type and *Brucella pinnipedialis* seal-type (Foster et al. 2007; Maquart et al. 2009). The fourth strain is the so-called "*B. ceti*" sequence-type (ST) 27, just reported in a few animals (Cvetnić et al. 2016; Duvnjak et al. 2017; Mackie et al. 2020; Ueno et al. 2020; Whatmore 2009; Whatmore et al. 2017). *Brucella* ST27 may have zoonotic relevance since strains of this ST have been found in humans with brucellosis (Sohn et al. 2003; McDonald et al. 2006); albeit, the sources of infections remain unknown.

The Kogiidae family comprises the dwarf sperm whale *Kogia sima* and the pygmy sperm whale *Kogia breviceps* extant species (Jefferson et al. 2015). The number of individuals is unknown because both species are rarely seen due to their undemonstrative shy behavior (Jefferson et al. 2015; IUCN 2020). Most of Kogia species' information has been obtained from strandings, often in cow-calf pairs (Jefferson et al. 2015; Manire et al. 2004). Some investigators have shown anti-*Brucella* antibodies in *K. sima* and *K. breviceps* (Ohishi et al. 2007; Hernandez-Mora et al. 2009) with no clinical signs of brucellosis.

A young adult female *K. sima* (204 cm and 80 kg) displaying weakness and buoyancy difficulties was stranded alive along the Central Pacific Coast of Costa Rica in March 2018. As with other stranded cetaceans in the country (González-Barrientos et al. 2010, Guzmán-Verri et al. 2012), the animal was intensely handled by local inhabitants and tourists

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(Fig. 1A). The whale was close to the last trimester of its first pregnancy. After 30 min of stranding, the animal aborted and died. The fetus, a female of 84.2 cm and 10 kg, never achieved breathing. The cow and the aborted calf were taken on ice to the National Service of Animal Health of Costa Rica for necropsy, histopathological, and immunohistochemical studies following previously described procedures (González-Barrientos et al. 2010).

Sampling is part of the National Brucellosis Control Program and Wildlife Program of the Costa Rican National Animal Health Service (CR-NAHS) and performed in agreement with the corresponding law "Ley de Bienestar de los Animales" (Ley N° 7451 1994) and to the International Convention for the Protection of Animals endorsed by Costa Rican Veterinary General Law on the CR-NAHS (Ley N°. 8495 2006). All procedures involving live *Brucella* followed the "Reglamento de Bioseguridad de la CCSS 39,975–0, 2012," after the "Decreto Ejecutivo #30,965-S," the year 2002 and research protocol 0045–17 approved by the National University, Costa Rica. According to the Biodiversity Law #7788 of Costa Rica and the Convention on Biological Diversity, the genetic resources were accessed under the terms of respect to an equal and fair distribution of benefits to those who provided resources under CONAGEBIO, Costa Rica, permit # R-CM-UNA-003–2019-OT-CONAGEBIO.

The whale showed moderate generalized lymphadenopathy and splenomegaly, with congested lungs containing multifocal parasitic granulomas. The uterus was distended with a light-yellow-creamy exudate, separating the chorioallantoic membrane from the endometrium. Over the chorioallantois were dozens of 1-mm diameter light-yellow necrotic foci (Fig. 1B). The uterus endometrial surface was multifocally dark red to tan (Fig. 1B inset). The late-term fetus presented moderate autolysis and had the lungs filled with aspirated keratin material, revealing in utero distress. The fetus's lungs, showing *Brucella* immunolabeling within macrophages, were diffusely atelectatic, with alveolar histiocytosis, moderate lymphoplasmacytic, and histiocytic interstitial pneumonia (Fig. 1C, D). The placenta was necrotic, presenting severe multifocal subacute histiocytic and neutrophilic infiltrate (Fig. 1E–G). The infected chorioallantois had moderate multifocal necrosis and ulceration of the trophoblasts, with multifocal to coalescing aggregates of macrophages and neutrophils deep in the stroma, close to the allantoic epithelium. There was intense *Brucella* immunolabeling in these inflammation areas

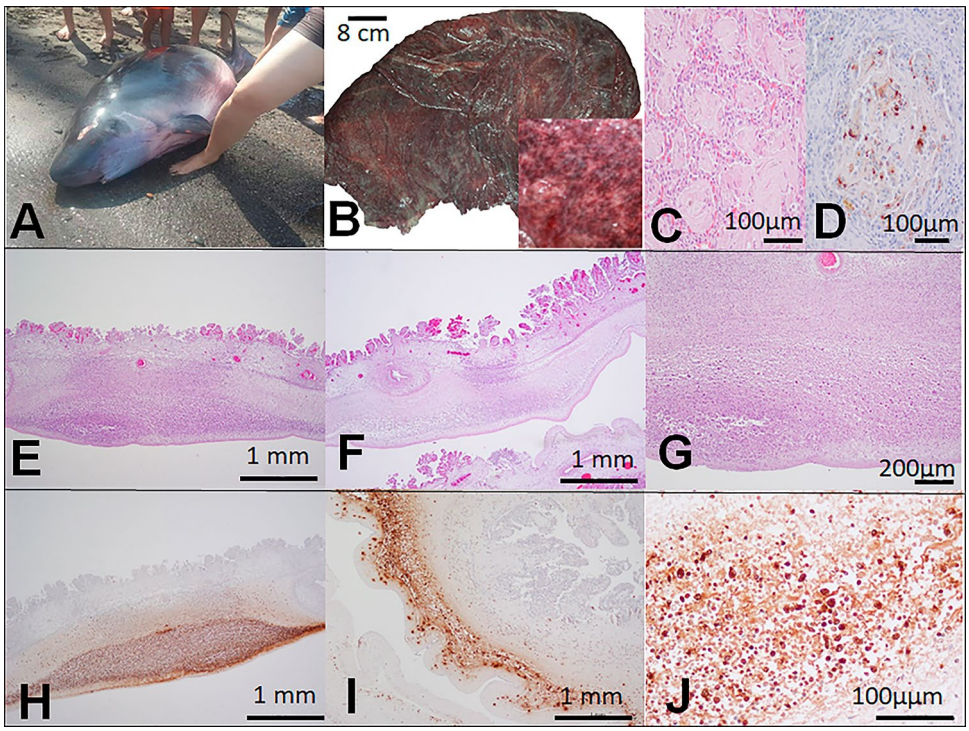

Fig. 1 Pathological findings in *K. sima* infected with *Brucella* sp. ST27. (**A**) Human contact with adult female dwarf sperm whale after abortion. (**B**) The gross aspect of the placenta displaying severe, subacute, and multifocal to coalescing necrosupurative placentitis. Insert shows severe multifocal hemorrhages in the endometrial surface of the uterus. (**C**) Haemotoxylin and eosin (HE) stain of fetal lung tissue shows distention of alveoli, large aggregates of keratin, and moderate alveolar histiocytosis. (**D**) Immunoperoxidase (IHC) against *Brucella* LPS shows intense positive immunolabeling of fetal alveolar histiocytes. (**E**, **F**) HE stain shows mild to moderate, multifocal ulceration of the trophoblast layer, and severe locally extensive infiltrates of macrophages and neutrophils in the chorion mesenchyme, extending close to the mesothelial lining. (**G**) HE stains show a close-up view of the inflammatory infiltrate. (**H–J**) IHC against *Brucella* LPS shows intense positive immunolabeling observed in macrophages and neutrophils infiltrating the placenta

and within the remaining trophoblasts (Fig. 1H–J). The cow showed enlarged spleen and lymph nodes with lymphofollicular hyperplasia and macrophages' infiltration in medullary sinuses and red pulp vascular spaces. The spleen had moderate extramedullary hematopoiesis. Central nervous system examination revealed mild lymphocytic, histiocytic, and plasmacytic perivascular infiltration, restricted to the thalamus, basal nuclei, and frontal cortex.

The Rose Bengal Test and competitive ELISA (Hernández-Mora et al. 2009) detected antibodies against *Brucella* smooth lipopolysaccharide (LPS) in sera and cerebrospinal fluid of both animals and the milk of the adult female. Following the detection of antibodies, we attempted to isolate *Brucella* organisms in all tissue samples using CITA and Farrell's medium, cultivated under a 10% CO_2 atmosphere at 37 °C, for 15 days (De Miguel et al. 2011). *Brucella* organisms were isolated from the cow's vaginal fluids, uterine fluids, chorioallantois, parietal cortex, and spleen. The fetal lungs, thymus, spleen, cerebellum, and gastric content also rendered *Brucella* organisms. We characterized the bacterial isolates following described procedures (Alton et al. 1988). The *Kogia* bacterial isolates displayed predominantly "A" LPS antigen and were differentiated from *B. ceti* dolphin ST26, *B. ceti* porpoise ST23, and *B. pinnipedialis* seal-type ST24/ST25 strains by their requirement of CO_2, and L-arabinose and D-xylose oxidation, but not D-galactose oxidation.

DNA extraction, Bruce-ladder multiplex PCR (Blm-PCR), high-resolution melting real-time PCR (HRM RT-PCR), RT-PCR, multiple loci-variable number of tandem repeats-16 loci (MLVA-16), and whole-genome sequencing analysis (WGSA) in Illumina platform were performed as described before (Suárez-Esquivel et al. 2017; Guzmán-Verri et al. 2019). MLVA-16 dendrogram was constructed using 593 MLVA bank database profiles (Grissa et al. 2008). Values obtained for each MLVA-16 marker are in Supplementary Data Sheet 1 and upload to microbes genotyping database (http://microbesgenotyping.i2bc.paris-saclay.fr/). WGSA data is accessible at the European Nucleotide Archive (ENA) (http://www.ebi.ac.uk/ena/), under the accession codes listed in the Supplementary Data Sheet 1. Other WGSA from various *Brucella* strains and *Ochrobactrum* spp., used for comparative purposes, were obtained from the NCBI Genome database. Multiple sequence alignment for phylogenetic reconstruction reads from two *Ochrobactrum* spp., and the 35 *Brucella* isolates from different hosts were aligned by bwa and mapped with SMALT v.0.5.8 against *B. abortus* 9–941, with an average coverage of 97.66%. Single nucleotide polymorphisms (SNPs) were called using samtools (Li et al. 2009), and 27,077 variable sites extracted using SNP sites (Page et al. 2016). We used the alignments for maximum likelihood phylogenetic reconstruction with RAxML v8 (Stamatakis 2014). The phylogenetic tree was rooted using *Ochrobactrum anthropi* ATCC49188 and *Ochrobactrum intermedium* LMG3301 and Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and microreact (Argimón et al. 2016) used for visualization of the phylogenetic tree.

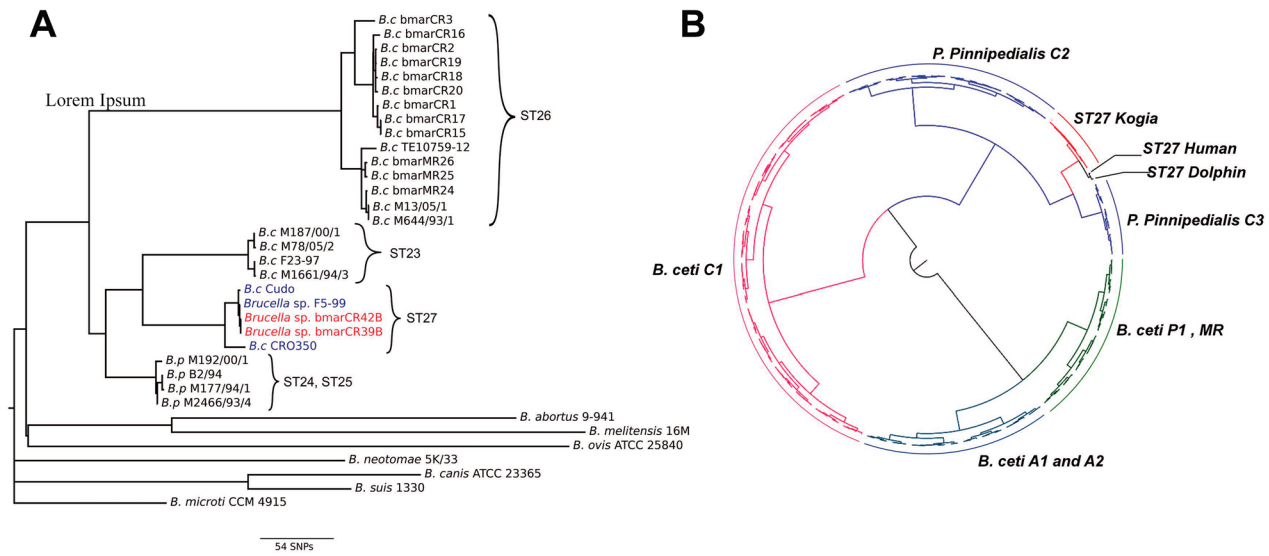

Fig. 2 WGS phylogenetic reconstruction and MLVA-16 dendrogram of *Brucella* isolates. (**A**) The tree was based on 27,077 SNPs of different *Brucella* WGS. Accordingly, the isolates related to marine mammals belong to seven ST categories (Whatmore et al. 2008). Highlighted in red are the *K. sima Brucella* ST27 bmarCR39B and bmarCR42B (accession numbers ERR3799635 and ERR3799636). *Ochrobactrum* sp., used as the tree's original root, was trimmed from the figure to increase tree resolution. Each cluster defining branch showed a 100 bootstrap value. (**B**) MLVA-16 dendrogram of different *Brucella* species and isolates. The *K. sima* isolate is in red close to the branch of the ST27 human isolate. We performed the analysis according to http://microbesgenotyping.i2bc.paris-saclay.fr/. For increased resolution, visit https://microreact.org/project/xaQYIdp96. *B. ceti* (*B.c*), *B. pinnipedialis* (*B.p*)

Although the Blm-PCR and RT-PCR confirmed that the isolate belonged to the *Brucella* genus, the HRM RT-PCR was inconclusive regarding species identification. MLVA-16 analysis clustered the bacterium close to other ST27 isolates (http://mlva.u-psud.fr/brucella/). Likewise, the WGS showed that *K. sima* isolates are close phylogenetic relatives to *Brucella* ST27 strains. Further, in silico multilocus sequence-type analysis confirmed the isolates as ST27 (Fig. 2).

We have previously reported *B. ceti* ST26 causing meningoencephalomyelitis, endocarditis, placentitis, and abortion in striped dolphins (*Stenella coeruleoalba*) stranded in the Pacific shores of Costa Rica (Gonzalez-Barrientos et al. 2010; Hernández-Mora et al. 2008; Suárez-Esquivel et al. 2017). Besides, we reported anti-*Brucella* antibodies in both *K. sima* and *K. breviceps* (Hernandez-Mora et al. 2008). However, this is the first report of *Brucella* ST27 recovered from a host of the Eastern Tropical Pacific in a dwarf sperm whale. Like ungulate brucellosis, the ST27 isolates caused lesions in the placenta, invaded the fetal organs, and induced abortion. However, in contrast to ungulates, *K. sima* presented severe pathological signs indicating that this bacterium is a resilient pathogen causing primary disease in this cetacean species. We have previously shown that tourists and local inhabitants commonly handled brucellosis infected cetaceans (Hernandez-Mora et al. 2008; Guzmán-Verri et al. 2012), and this was not the exception. Within this context, the description of *Brucella* sp. ST27 reservoirs is relevant since the sources of humans infected with ST27 strains remain unknown.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10344-021-01502-5.

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Declarations

Conflict of interest The authors declare no competing interests.

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