Epidemiology of bovine brucellosis using Probe-based Real-time PCR from whole blood samples in Colombia

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DNA Extraction Protocol: Whole blood collected in Ethylenediaminetetraacetic acid (EDTA) tubes was gently mixed, then 0.5 ml of whole blood were transferred to sterile 1.5 ml microtubes containing 1 ml of cell lysis solution (320 mM saccharose, five mM MgCl2, 1% Triton X-100, ten mM Tris-HCl [pH 7.5]). The microtubes were mixed by inverting three times and incubated for 10 min at room temperature. The microtube was inverted three more times during incubation and then centrifuged at 15,000x g for 2 min (5424 Eppendorf®, Enfield, Connecticut, USA). The supernatant was discarded without losing the cells pellet. The pellet was rewashed once with a cell lysis solution and twice with 1 mL of ultrapure Milli-Q® water. After the last supernatant was discarded, the microtube was vortexed to separate the pellet from the walls. Added 400 µl of nucleic lysis solution (60 mM NH4Cl, 1% sodium dodecyl sulfate and 24 mM Na2-EDTA [pH

8.0]), and slowly added 5 µl of proteinase K (1 mg/mL). Then was incubated for 30 min at 56°C and 500 RPM in Vortemp™ 56 incubator shaker (Labnet®, Edison, New Jersey, USA). After digestion, the samples were cooled at room temperature, then 100 µl of ammonium acetate (7.5 mM) was added, mixed, and followed by centrifugation at 15,000x g for 10 min. The supernatant containing DNA was carefully transferred to a microtube containing 1 mL of 100% ethanol at room temperature. The tube was gently mixed until DNA strands were observed. After that, the tube was centrifuged at 15,000x g for 10 min. The supernatant was discarded without losing the DNA pellet, and 1 ml of cold 70% ethanol was added to rinse the pellet. The microtube was inverted until the ethanol was completely dried. The DNA was resuspended in 60 µl of molecular biology grade DNase and RNase Free water (RPI®) and incubated at 65° C for 20 min. to improve hydration and to loosen DNA strands. The DNA was stored at -20 °C until the qPCR assay was performed.