# CITA enrichment broth is suitable for the direct isolation of *Brucella* spp. from field contaminated samples and for direct PCR

Muñoz P.M. [1]*, De Miguel M.J.[1]*, Zúñiga-Ripa, A.[2] Moriyón I. [2], Blasco J.M.[1]

[1] Instituto Agroalimentario - Centro de Investigación y Tecnología Agroalimentaria de Aragón (IA2-CITA), Zaragoza, Spain; [2] Instituto de Salud Tropical. Departamento de Microbiología y Parasitología. Universidad de Navarra, Spain. * These authors contributed equally to this work.

The identification and typing of brucellae requires their bacteriological isolation. However, because these bacteria are easily overgrown by contaminants on isolation plates the usefulness of bacteriological culture is limited when samples are contaminated (a very common situation) or numbers of brucellae in tissues are low. To overcome these difficulties, the objective of this work was to develop an enrichment broth that would favor the growth of brucellae and could be combined with PCR for direct diagnosis and typing.

Based on existing knowledge, we assessed the effect of basal broth components in combination with detoxifying agents and antimicrobials to support the growth of the major *Brucella* species in the presence of bacterial and fungal contaminants common in field samples. In a first step, we determined the growth of representative strains of *B. melitensis, B. abortus, B. suis* and *B. ovis* and field contaminants (*Escherichia coli, Enterococcus faecalis, Streptococcus thoraltensis, Kocuria spp., Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*). For this, each basal broth was inoculated with $10^2$ CFU/mL of each bacterium and the colony forming units (CFU) and pH were determined after 4h, 24h, 48h, 72h and 6 days of incubation at 37ºC in a 10% $CO_2$ atmosphere. A selective broth containing Mueller-Hinton as basal component supplemented with yeast extract, newborn calf serum, CAPS [N-cyclohexyl-3-aminopropanesulfonic acid] and the antibiotics used in the selective CITA agar medium (amphotericin B, colistin, nitrofurantoin, nystatin and vancomycin) plus phosphomycin, inhibited all contaminants while allowing the growth of all *Brucella* strains up to $10^4$ to $10^8$ CFU/mL (depending on the strain and incubation time) after 48-72h.

Additional experiments on spiked samples (spleen and milk from sheep or pig) and tissues (lymph nodes and seminal vesicles) from naturally infected sheep or wild boar showed that CITA enrichment broth supported growth of *Brucella* spp. from less than 10 CFU/mL to more than $10^4$ CFU/ml after 48-72h of incubation, making possible their detection by direct PCR of the broth (Garcia-Yoldi et al. 2006).

This research was supported by INIA (project Bru-Epidia 291815-FP7/ERANET/ ANIHWA), the "Ministerio de Economía y Competitividad" of Spain (project AGL2014-58795-C4) and Consolidated Group of Aragón Government A14.

E-Mail of presenting author: pmmunnoz@cita-aragon.es