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

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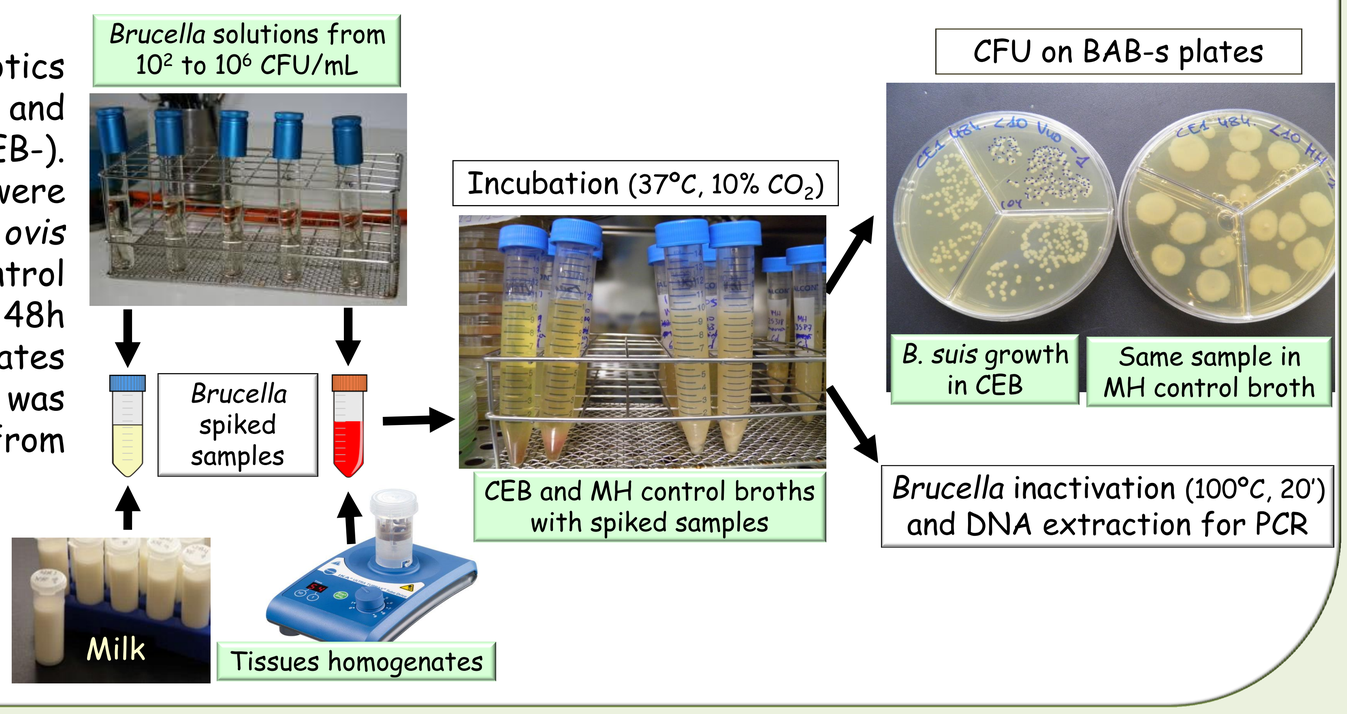
Identification and typing of brucellae require bacteriological isolation. However, these bacteria are easily overgrown by contaminants on culture plates, which limits the sensitivity of bacteriological diagnosis when field samples are contaminated (a common situation) or contain low *Brucella* numbers. The objective of this work was to develop an enrichment broth suitable for the growth of the main *Brucella* species that could be combined with a PCR for direct diagnosis and typing

Material and methods:

In a first step, we determined the optimal enrichment broth components for the growth of representative strains of *Brucella* and field contaminants (most often isolated from field samples on selective solid culture media -Figure 1-) by testing several commercial broths (Trypticase Soy, Mueller Hinton, Brain-Heart Infusion, Nutrient and Terrific Broth), supplemented with yeast extract, and containing different detoxifying and neutralizing agents (serum, active carbon, CAPS) and antibiotics. Each broth combination was inoculated with 10^2 CFU/mL of each strain (see below) and the colony forming units (CFU) and pH (critical factor for *Brucella* growth) determined after 4h, 24h, 48h, 72h and 6 days of incubation at 37°C in a 10% CO_2 atmosphere.

Mueller-Hinton with yeast extract, calf serum, CAPS and antibiotics (amphotericin B, colistin, nitrofurantoin, nystatin, vancomycin and phosphomycin) yielded optimal results (CITA enrichment broth–CEB-). Spleen and milk samples taken from healthy sheep and pigs were spiked with known CFU numbers of *B. suis, B. melitensis or B. ovis* strains and inoculated into both CEB and Mueller Hinton (control broth without antibiotics or CAPS). Samples were incubated for 48h to 6 days and CFU numbers determined on Blood Agar Base plates containing 5% sterile calf serum. Broth culture performance was further assessed with lymph node and seminal vesicle samples from naturally infected sheep and wildboars.

Bruceladder multiplex PCR was applied directly to broth samples after DNA extraction with Qiagen ® Qiamp DNA minikit ®.



Results:

CITA enrichment broth -CEB- inhibited all contaminants tested while allowing the growth of all *Brucella* strains from 10^2 up to 10^4-10^8 CFU/mL, depending on the strain and incubation time (Figure 1).

Table 1. *Brucella* growth in CEB inoculated with spiked spleen or milk or in tissue samples from naturally infected animals. Results in Mueller Hinton broth (without antibiotics or CAPS) are also shown as a control.

	Mean *Brucella* CFU/ mL in broth before and after incubation					
	CEB			MH		
Incubation time	Before	48h	6 days	Before	48h	6 days
Sheep spleen spiked with decreasing amounts of *B. melitensis* Rev 1	4×10^3	4×10^7	8×10^7	3×10^3	5×10^3+ Cont	Cont
	84	1×10^6	7×10^7	72	Cont	Cont
	7	1×10^5	2×10^7	6	Cont	Cont
Pig spleen spiked with decreasing amounts of *B. suis* bv2 field strain	8×10^3	4×10^7	7×10^7	6×10^3	9×10^3+ Cont	Cont
	1×10^2	9×10^5	6×10^7	97	Cont	Cont
	8	2×10^4	4×10^7	8	Cont	Cont
Sheep milk spiked with *B.ovis*	38	4×10^5	1×10^8	46	97 + Cont	Cont
Ram seminal vesicle naturally infected with *B. ovis*	23	4×10^3+ Cont	1×10^8+ Cont	26	Cont	Cont
			72h			72h
Wildboar lymphnode naturally infected with *B. suis* bv2	7×10^2	8×10^3+ Cont	5×10^5+ Cont	92	Cont	Cont

Cont: contaminant growth observed. Notice that CEB allowed *Brucella* multiplication even in presence of contaminants (this never occurred in MH control broth).

Figure 1. Growth curves of *Brucella* strains inoculated into CEB in presence of contaminants and incubated for 4h, 24h, 48h, 72h and 6 days (left panel). Growth curves for each contaminant in Mueller Hinton (MH) broth (without antibiotics or CAPS) are shown as control (right panel).

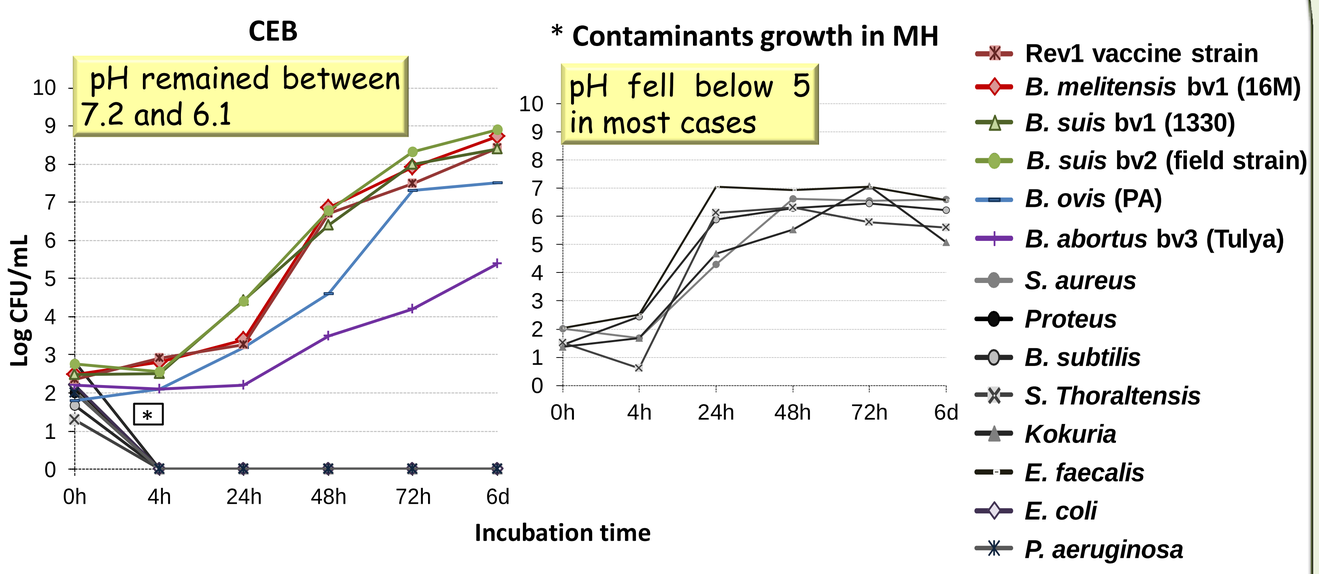
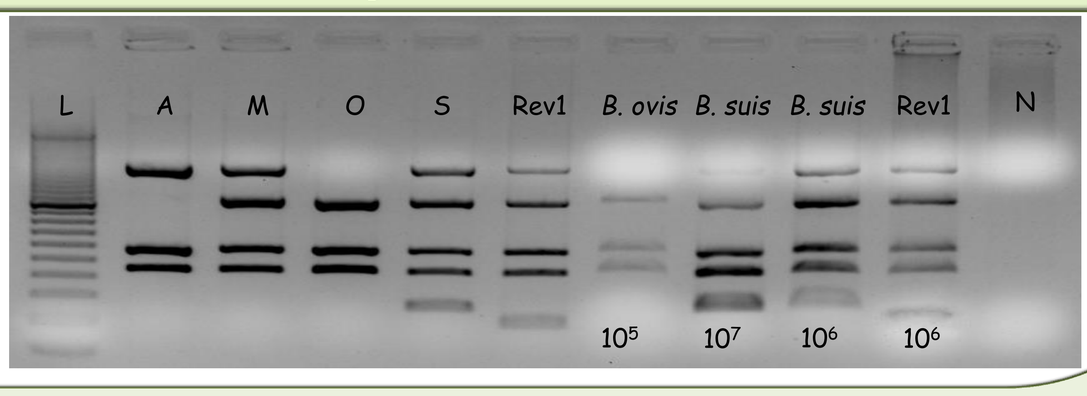


Figure 2. Bruceladder multiplex PCR. L, 100pb DNA ladder; DNA from pure cultures of *B. abortus* Tulya (A), *B. melitensis* 16M (M), *B. ovis* PA (O), *B. suis* 1330 (S) and Rev1; DNA from 48h-72h CEB broths inoculated with spiked samples (CFU/mL are shown below each lane). N: negative control (sterile CEB broth).



CONCLUSION: CITA selective enrichment broth (CEB) inhibited most contaminants while allowing *Brucella* spp. growth at high levels (over 10^4 CFU/ml) after 48-72h of incubation, making thus possible detection by PCR directly on the broth. Further experiments with field samples from both *Brucella*-free and naturally infected animals are in progress to assess the diagnostic sensitivity and specificity of CEB and its associated PCR

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