Isolation schema for *Brucella* spp.

Specimens
(Lymph nodes etc)

↓ Macerating samples

Preparation & Inoculation on selective medium (MBS)

↓ Cultivation at 37℃, 5% CO_2 for 3-5 days

Select suspect colonies
(Transparent, pinkish colonies)

↓ Cultivation at 37℃, 5% CO_2 for 3-5 days

Purified culture on blood agar
(Blood or brucella agar)

Catalase + / Oxidase + / Urea +

Biological methods
Classical biotyping method

- Agglutination with AMR sera
- Growth in dyes (thionin, basic fuchsin)
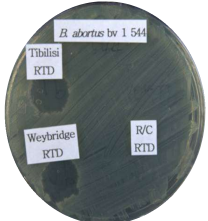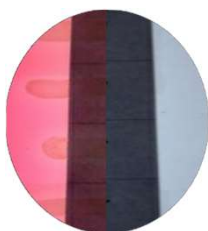- Lysis by phages (Tb, Wb, Iz, R/C)
- CO2 requirement

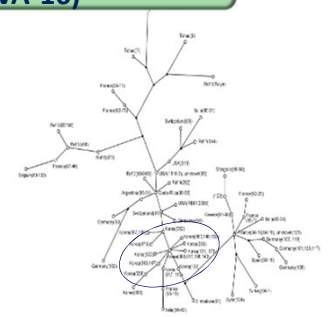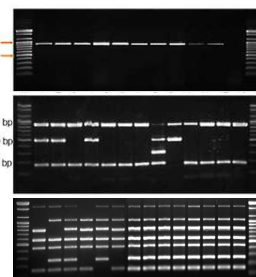## Molecular methods
Multiplex-PCR, MLVA-16

- 16S rRNA PCR (genus specific)
- B. abortus specific PCR (BaSS)
- Differential multiplex PCR
- Multi-locus VNTR analysis (MLVA-16)

Species	Colony morphology	Serum requirement	Lysis by phages Tb RTD	Tb 10⁴ RTD	Wb RTD	Iz RTD	R/C RTD	Oxidase	Urease activity	Preferred host
B. abortus	S	-	+	+	+	+	-	(+)	(+)	Cattle and other Bovidae
B. melitensis	S	-	-	(-)	+	-	-	+	+	Sheep and goats
B. suis	S	-	-	-	(+)	(+)	+	+	+	Bv.: swine / Bv. 2: swine, hare / Bv. 3: swine / Bv. 4: reindeer / Bv. 5: rodents
B. neotomae	S	-	-	+	+	+	-	-	+	Desert wood rat
B. ovis	R	+	-	-	-	-	+	-	Sheep
B. canis	R	-	-	-	-	+	-	+	Dogs
B. ceti	S	ND	(-)	(+)	(+)	-	(+)	+	Cetaceans
B. pinnipedialis	S	ND	(-)	(+)	(+)	-	(+)	+	Pinnipeds
B. microti	S	-	+	+	+	ND	+	Common voles
B. inopinata	S	ND	-	PL	ND	ND	ND	+	Unknown
B. papionis	S	PL	PL	-	ND	+	Unknown

Serological diagnosis for Brucellosis

Dairy cattle

Herd screen test
Milk-Ring test

(−)

(+)

Beef cattle

Individual test
Rose-Bengal test

(−)

(+)

Confirmative test
Tube agglutination test
ELISA

(−)

(+)

Positive herd
(Re-test after 30-60 days)

Negative herd
(Regular test; > 1 time/year)

(Individual)

Positive

Suspicious

Negative

Stamping out
(within 10 days)

Slaughter
(Recommend)

Additional test
(If necessary)

Fluorescence polarization assay (FPA)

Complement fixation test (CFT)

OIE