

Diagnosis of brucellosis using PCR

Wei Ling Yu
Klaus Nielsen

OIE Reference Laboratory for Brucellosis
ISO 17025 Accredited

Canadian Food Inspection Agency



1. Species:

Seven species are currently recognized within the genus *Brucella*: *B. melitensis*, *B. abortus*, *B. suis*, *B. neotoma*, *B. ovis*, *B. canis* and *B. microti* based on differences in pathogenicity and in host preferences.

Recently, two new species, *B. pinnipedialis* and *B. ceti* have been proposed according to the classical criteria of host preference and specific molecular markers.

“The gold standard” for Laboratory detection of *Brucella* and species identification is based largely on bacterial isolation and phenotypic characterization. This process is lengthy and labour-intensive and has been associated with a heightened risk of laboratory- acquired infection. To overcome these problems, nucleic acid amplification has been explored for rapid detection and confirmation of the presence of *Brucella* spp.

2. The roles of PCR-based diagnostics:

- For screening large populations of livestock to identify infected herds.
- For confirming the presence of infection in herds and identifying the species of *Brucella* involved.
- For identifying epizootic strains to help epidemiologists trace-back infections to their sources.

3. The PCRs for diagnosis brucellosis:

Conventional PCRs such as PCR-restriction fragment length polymorphism (RFLP) analysis, single PCR, multiplex PCR, Real-Time PCR and PCR-ELISA.

Molecular Typing methods: multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) and other methods.

4. primer design for identification of *Brucella*:

A number of nucleic acid sequences have been targeted for the development of *Brucella* genus-specific PCR assay, such as 16s RNA, the 16S-23S intergenic spacer region, omp2, and bcsp31 which is a frequently described PCR target for the diagnosis of human brucellosis. PCR identification of *Brucella* strains at the species or biovar level has been more challenging.

A source of DNA polymorphism is provided by the distribution of insertion sequences (ISs). An insertion sequence, named **IS711 or IS6501**, has been identified in the genus *Brucella*. Several PCR test which take advantage of IS711 distribution in the chromosome have been developed.

Cultured bacteria, tissues, blood samples, etc, **Brucellosis?**



A PCR to identify *Brucella* spp. uses B4, B5 primers and a PCR to identify terrestrial species or Marine species *Brucella* uses primers for bp26 gene and flanking sequence .



If positive results for terrestrial *Brucella*, then modified AMOS PCR is performed to identify which species:

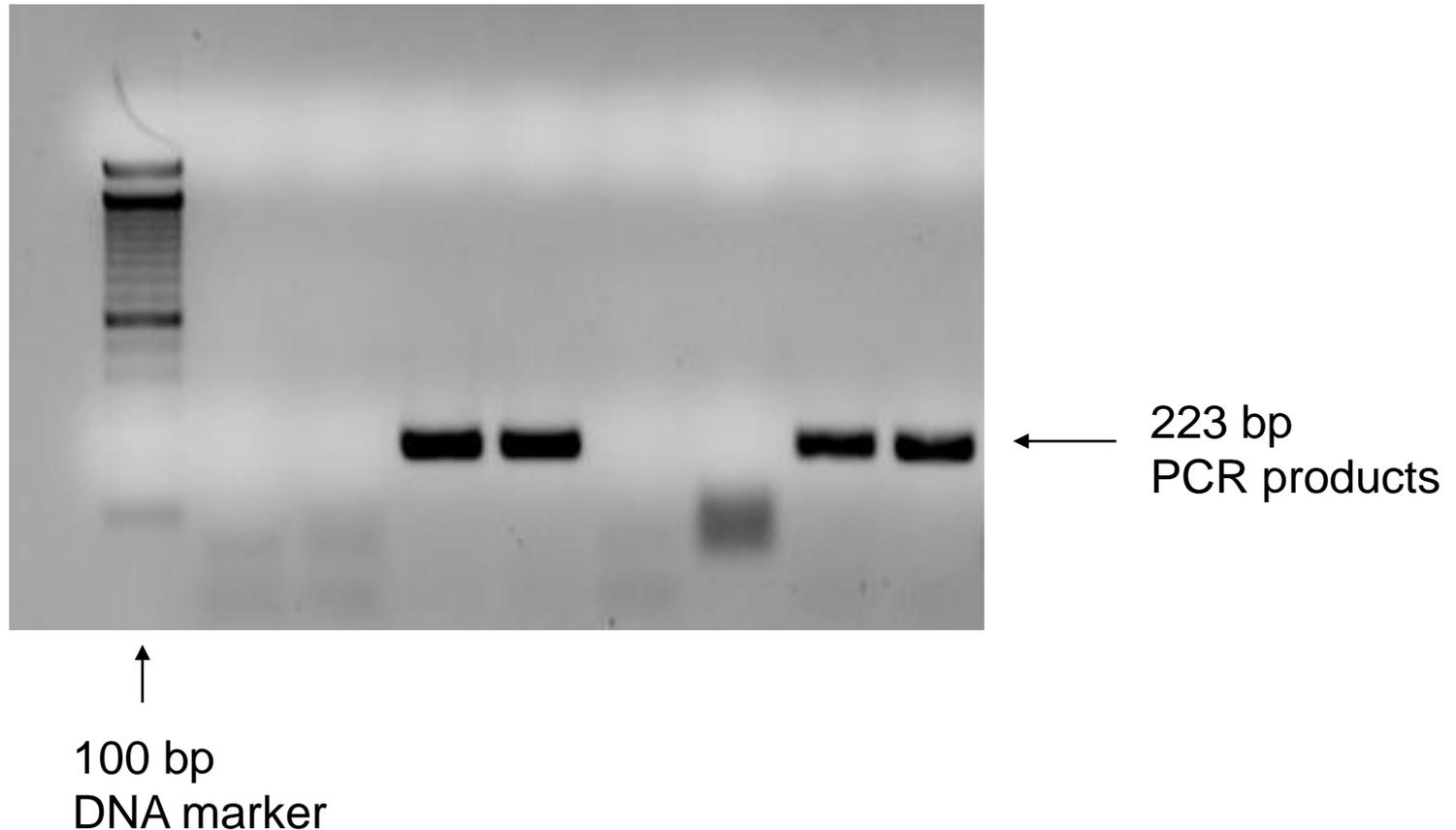
B. abortus, *B. melitensis*,

B. suis, *B. ovis*, *B. canis*, and

B. neotoma (some biovars can not be identified).

If positive results for marine species of *Brucella*, 4 specific PCRs are performed to identify with species: *B. pinnipedialis*, *B. ceti* .
(not clear how many species)

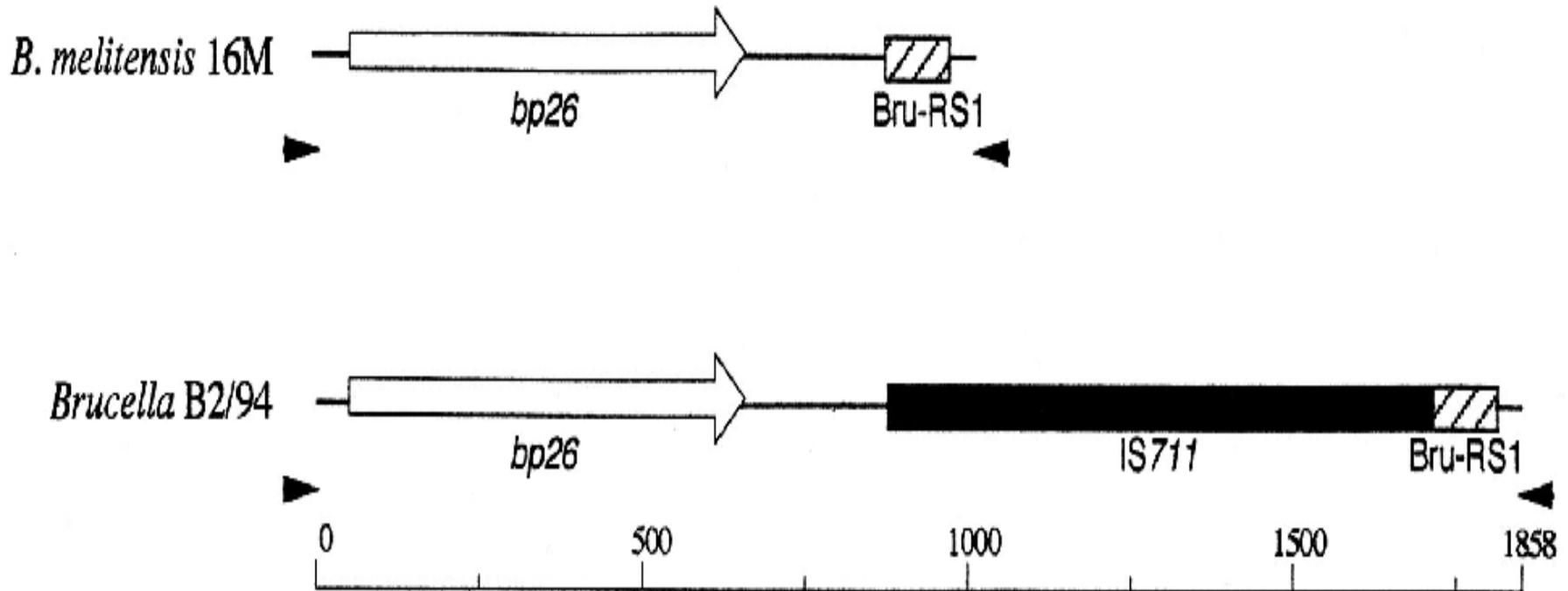
5. General *Brucella* PCR to identify *brucella spp.* using *B4, B5 primers from* tissue samples.



6. A *Brucella* PCR uses primers 26A and 26B for *pb26* gene and flanking regions to confirm and differentiate marine *Brucella* and terrestrial *Brucella*.

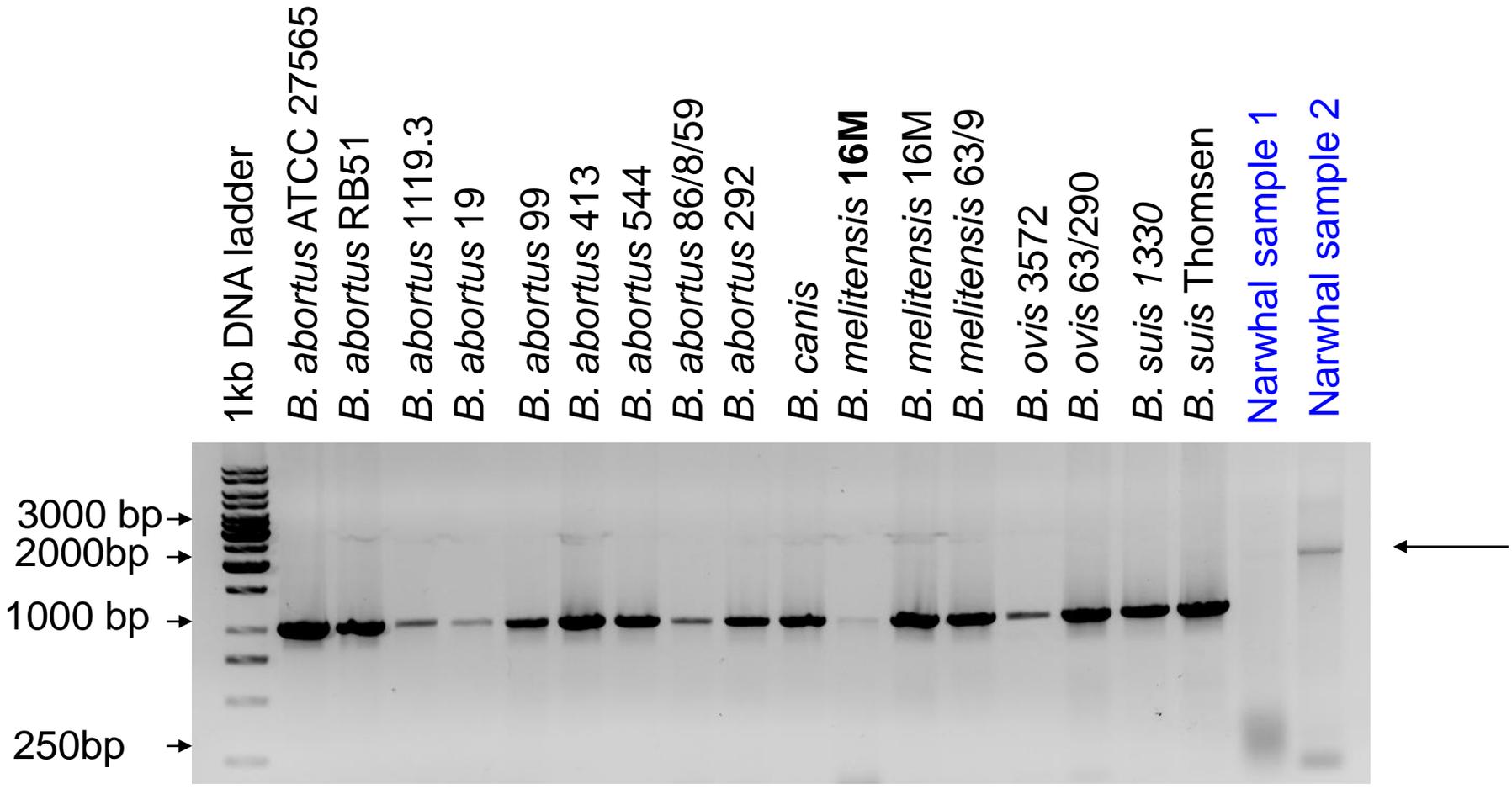
1900 bp PCR product is for marine *Brucella* and 1024 bp product is for terrestrial *Brucella*.

The IS711 copies are significantly higher in the marine mammal isolates than those in the seven classical terrestrial *Brucella* species, with the exception of *B. ovis*. A copy IS711 downstream of the *pb26* gene, adjacent to a Bru-RS1 element in all 34 marine mammal isolates was found but it was absent from the seven classical species and their biovars.



Schematic view deduced from nucleotide sequencing of the *bp26* gene and flanking regions of *B. melitensis* 16M and seal isolate B2/94. Arrowheads indicate the locations of the primers used for PCR. (Axel Cloeckert, 2000)





Marine Brucella PCR by using primers 26A and 26B.

PCR products derived from different *Brucella* strains and narwhal case 3024 by 1% agarose gel electrophoresis.

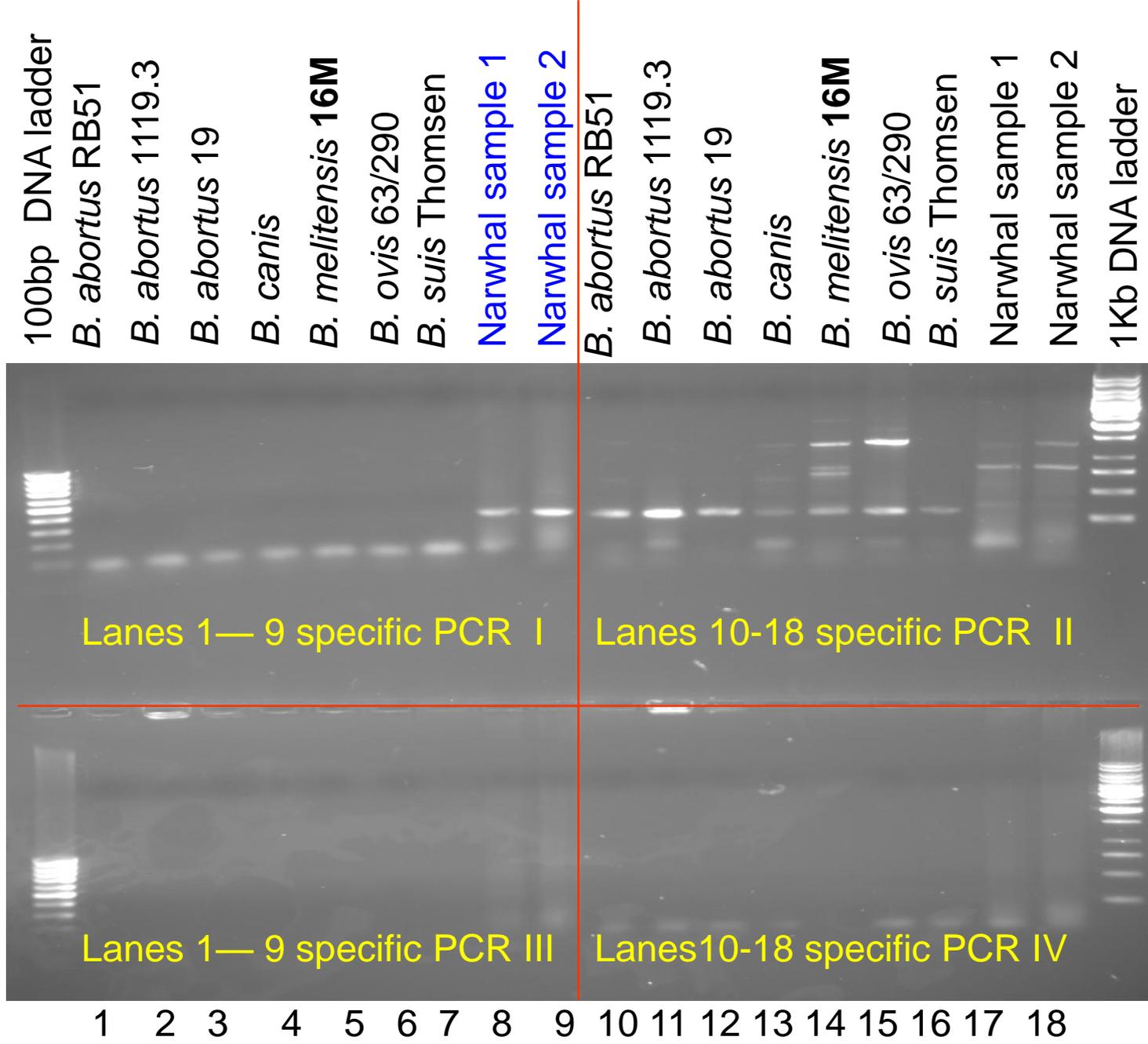
Four specific DNA fragment (I, II, III, and IV) generated by Infrequent restriction site-PCR(IRS-PCR) based on DNA polymorphism at the *omp2* locus and preferential hosts confirmed the marine *Brucella* classification.

- **Fragment I is specific for *B. pinnipedialis* for pinniped isolates based on marine *Brucella* positive.**
- **Fragments II, III and IV are for *B. ceti* for cetacean isolates based on marine *Brucella* positive.**

Fragment II consisted of the 5' end of the D- α -hydroxybutyrate dehydrogenase gene, annotated as BMEI0268 in the *B. melitensis* 16M genome. Fragment III consisted of the cytochrome B561 gene, annotated as BMEII 1073 in *B. melitensis* 16M genome. Fragment IV consisted of part of the penicillin-binding protein 1A gene, annotated as BMEI1351 in the *B. melitensis* 16M genome, followed by part of an IS711 element

Specific PCR I, II, III, and IV for Marine Brucella.

17ul of PCR products was loaded in 1% agarose.



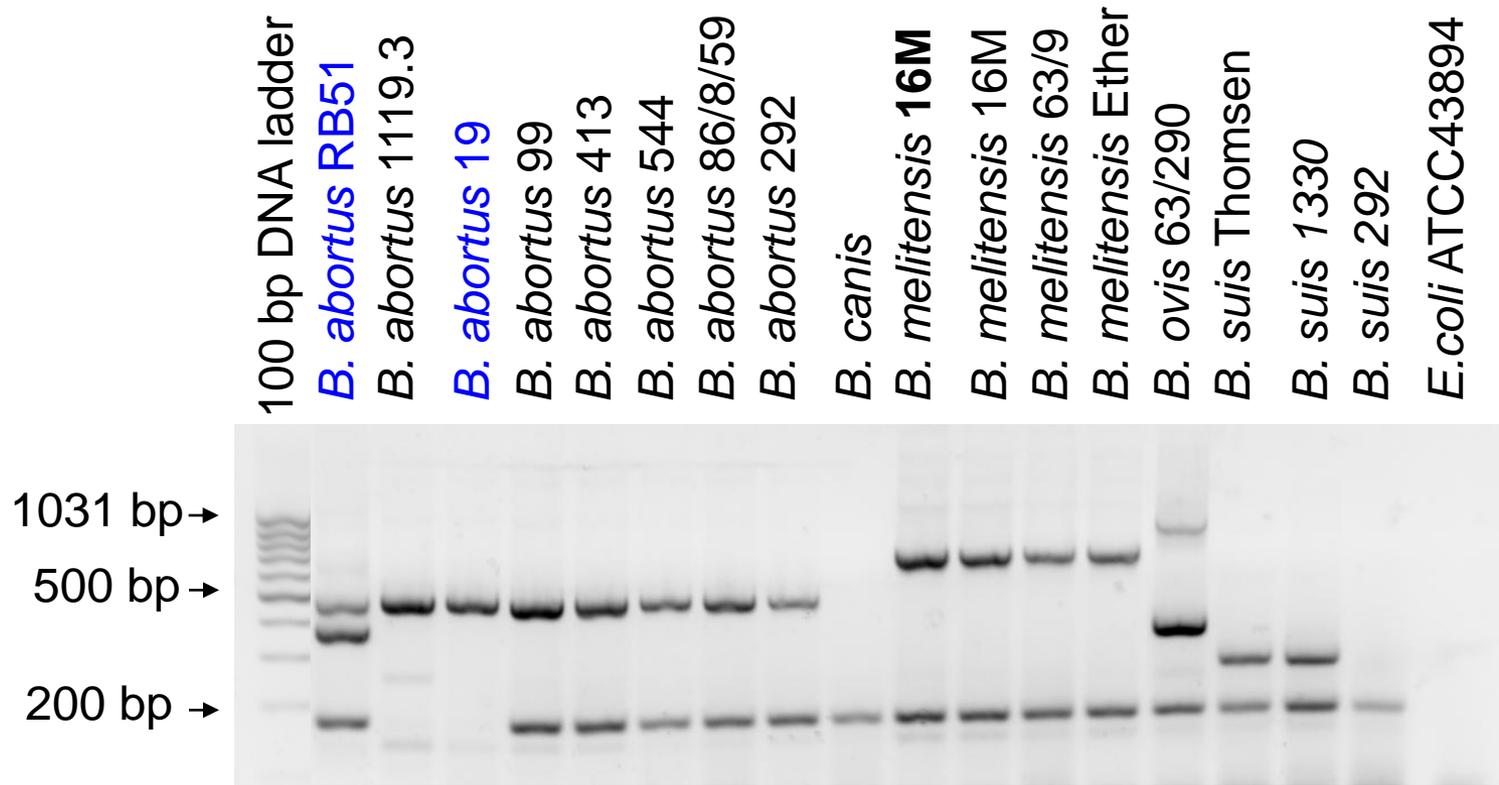
7. PCR by using IS711--- for terrestrial *Brucella spp.*

The IS711 copy number was determined to be 7 to 10 in *B. melitensis*, 6 to 8 in *B. abortus*, *B. suis* and *B. canis*, and more than 28 in *B. ovis*.

AMOS (AbortusMelitenisOvisSuis) PCR by using 8 primers

Differentiation was possible at the species level but some of the biovars could not be distinguished for terrestrial *Brucella*. It also can distinguish RB51 and strain19 vaccines.

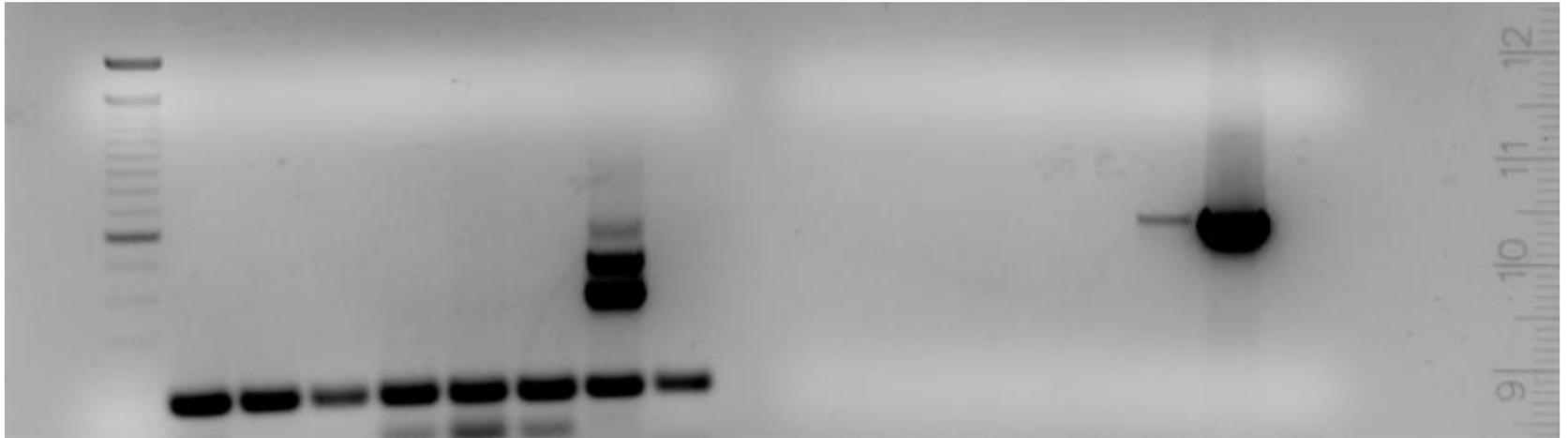
All *brucella spp.* have 178 bp PCR product **except vaccine S19**. *B.abortus* (biovars 1, 2, and 4) amplifies a 498 bp products; **vaccine RB51 has an extra 364 bp band**. *B.melitensis* (all biovars) amplifies a 731 bp product, *B.ovis* amplifies a 976 bp product, and *B.suis* (biovar 1) amplifies a 285 bp product.



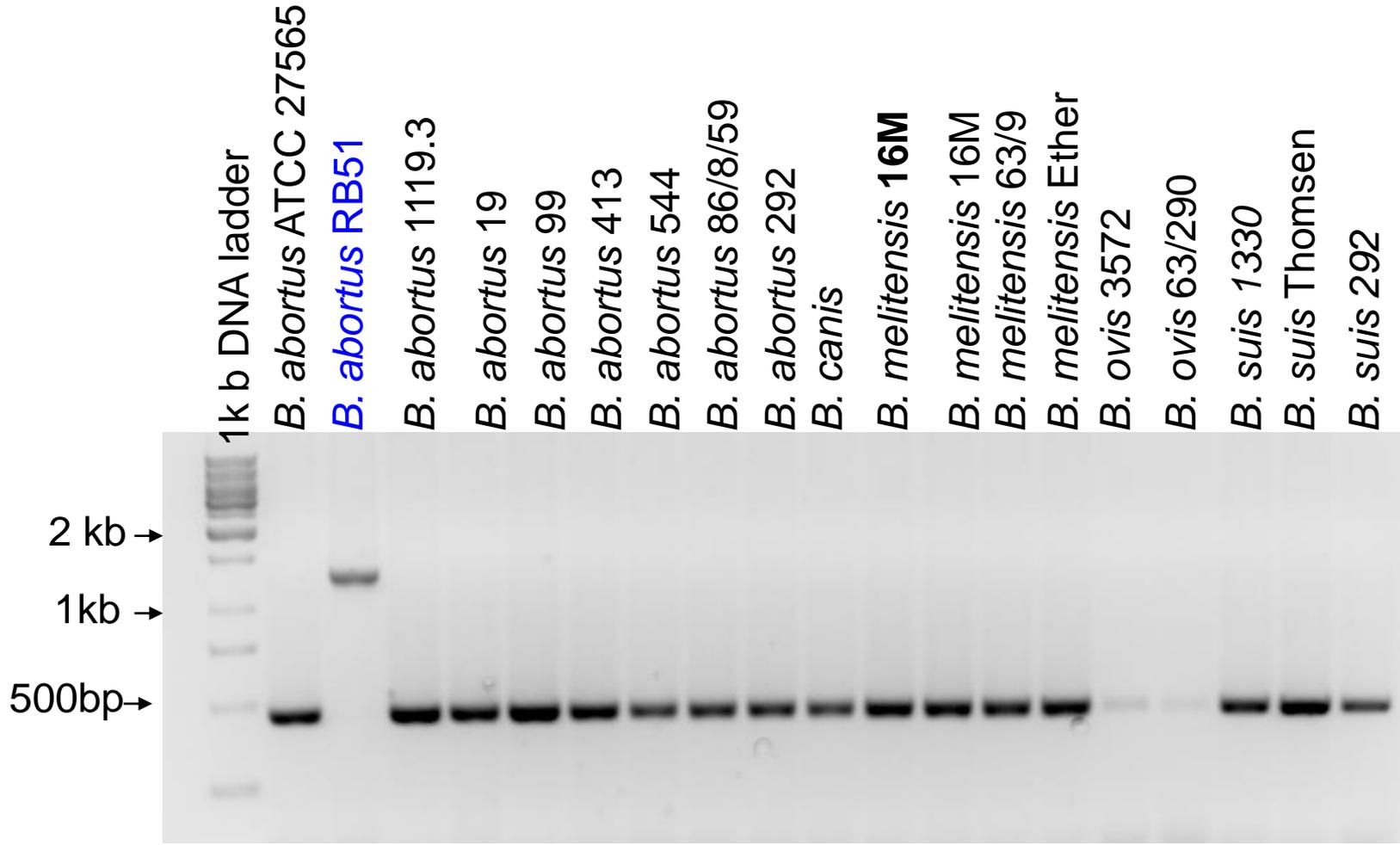
PCR products derived from different *Brucella* strains were analyzed by 2.5% agarose gel electrophoresis.

AMOS PCR results of caribou samples:

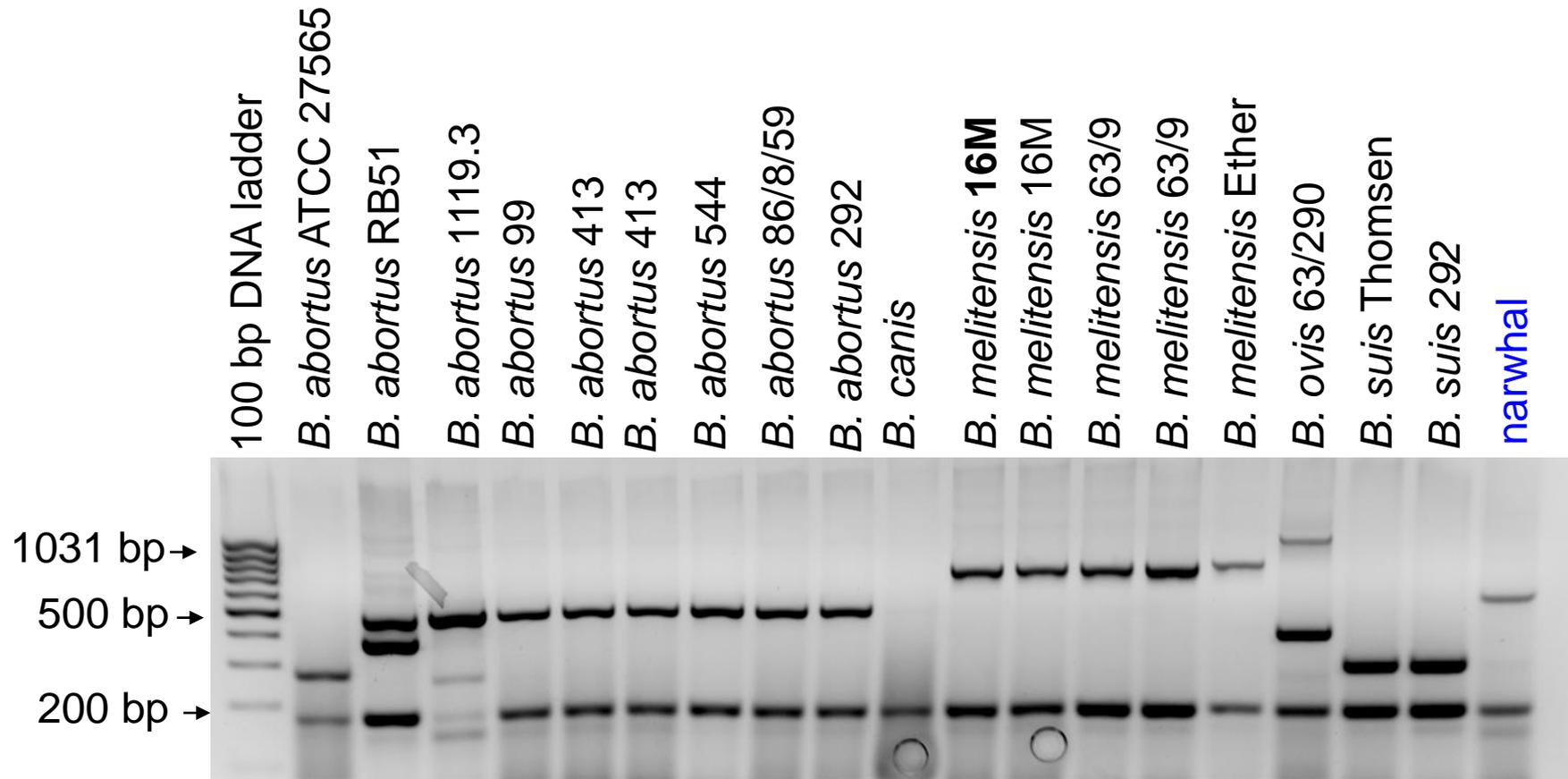
M 1 2 3



M:100bp DNA Marker; lanes 1, 2. 3: Caribou samples.



PCR products derived from different *Brucella* strains by using RB51 primers were analyzed by 1.5% agarose gel electrophoresis.

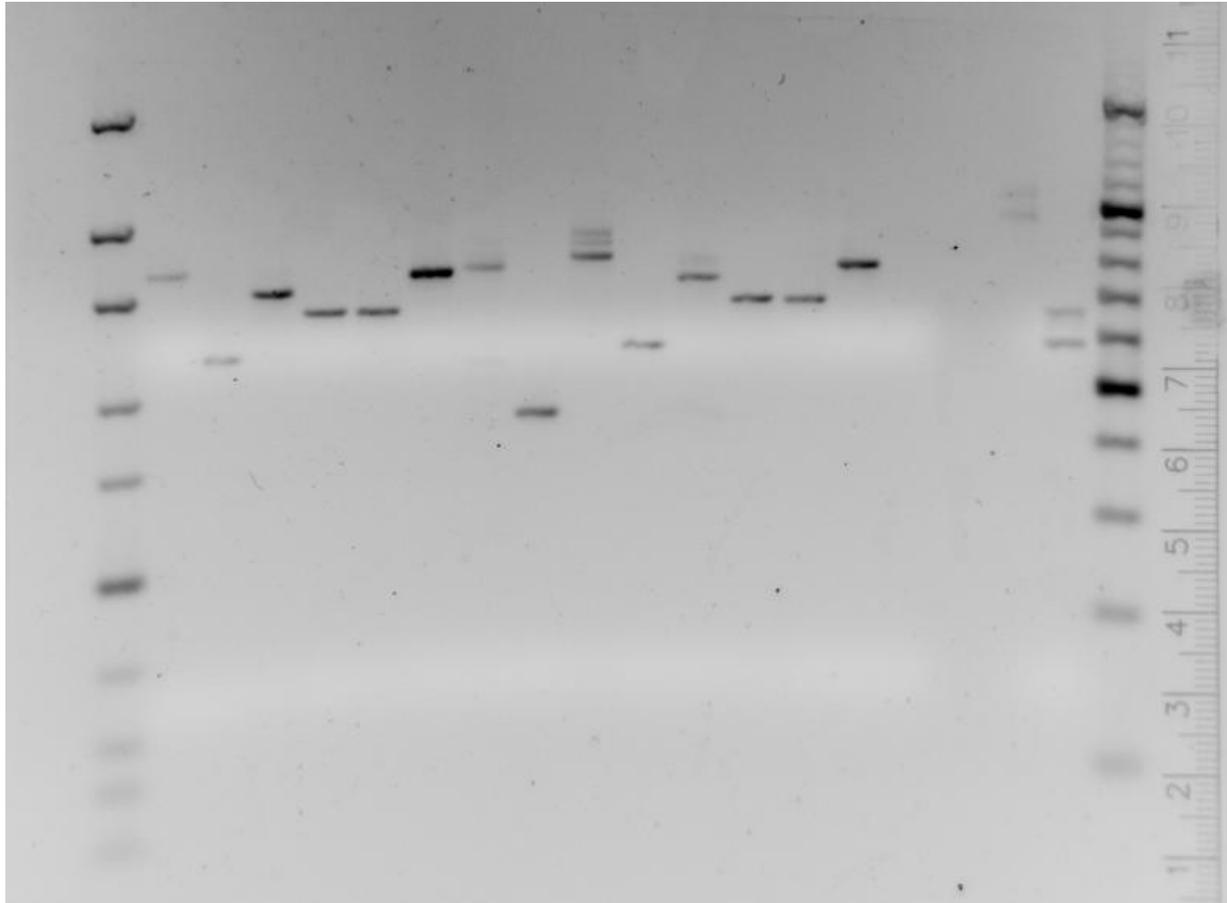


AMOS PCR products derived from different *Brucella* strains and narwhal case 3024 were analyzed by 2.5% agarose gel electrophoresis.

multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA), such as HOOFP-Prints-- a new method for fingerprinting *Brucella* isolates (B. Bricker, et al; 2003)

----we need some time to adapt them to diagnostic lab combined with other typing methods. Now we are doing HOOFP-Prints and another kind of VNTR method.

The results of HOOOF Prints by 3% MetaPhor Agarose



Summary

1. Diagnosis of brucellosis by PCR is relatively rapid, simple and accurate; It reduces danger to staff, no requirement for level 3 containment and the cost is reasonable.
2. PCRs combined can overcome each shortcoming of individual PCR and make them useful for various purposes.

