

# CoVetLab: working together to strengthen European collaboration on *Mycoplasma bovis* and compare available diagnostic tools

## BACKGROUND

Different clinical presentations of *Mycoplasma bovis* disease predominate in European countries with significant economic and welfare impacts. *M. bovis* disease control relies on good husbandry and an early and reliable diagnosis. However, a lack of standardisation of approaches and diagnostic methods applied makes comparison of disease prevalence between countries difficult.

## AIMS

- A consortium of six European national veterinary institutes was established to share tools and expertise on *Mycoplasma bovis*.
- Objectives included hosting workshops and developing ring trials, including collating panels of DNA and serum samples, to evaluate available serological and PCR-based diagnostic tests.

## WORKSHOPS



A



B

- A. At Ruokavirasto in Kuopio to develop PCR and ELISA ring trials.
- B. Joint CoVetLab - Nordic Workshop on *M. bovis* in March 2018 at DTU, Lyngby was attended by 45 participants from the veterinary and scientific community from 10 countries.

## *M. bovis* PCR RING TRIAL

- Analytical specificity, sensitivity and comparability of seven different PCR methods used to detect *M. bovis* were assessed.
- All methods were in use by at least one of the participants.
- Five different DNA extraction methods, seven PCRs targeting four different genes and six different real-time PCR platforms.

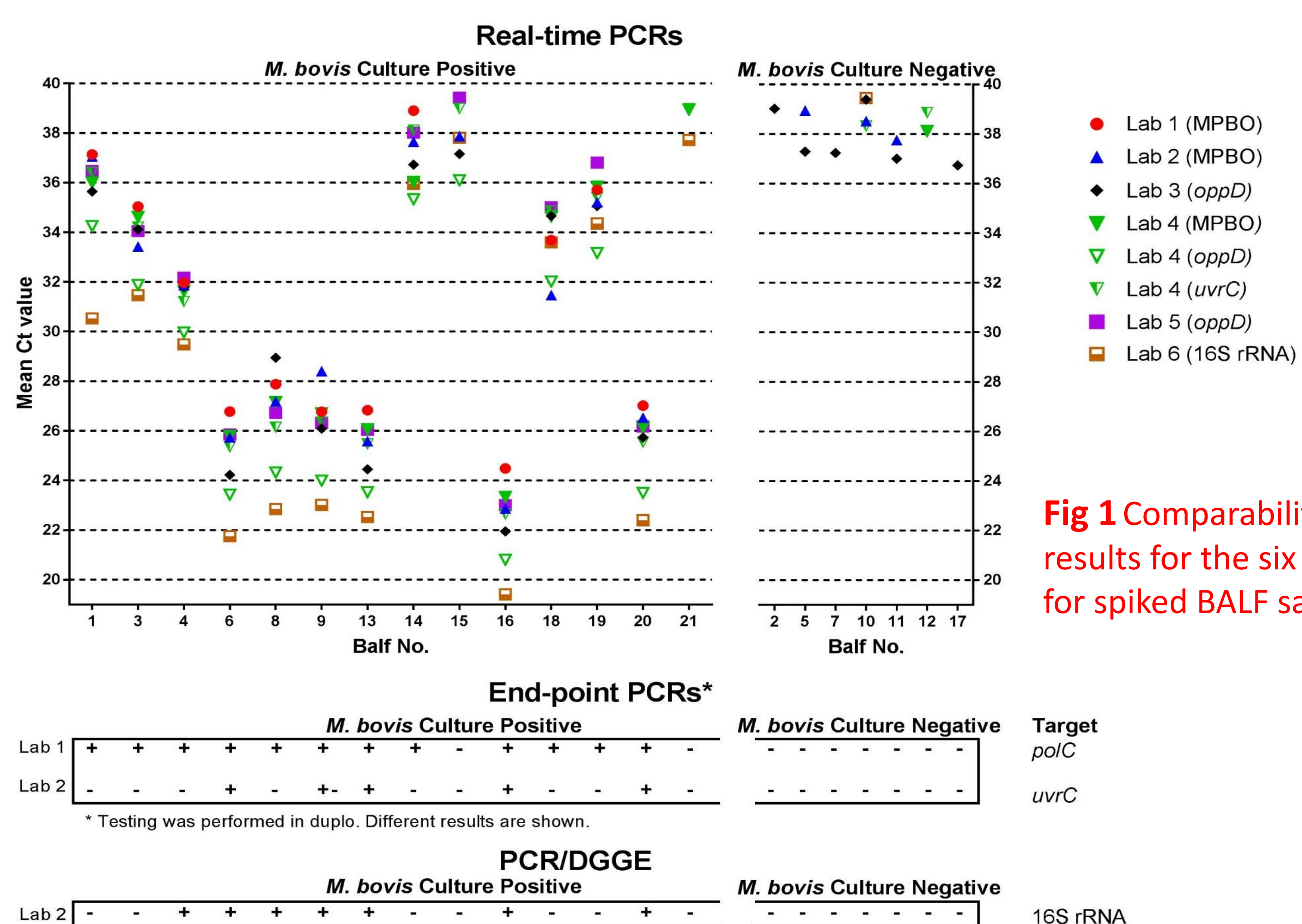


Fig 1 Comparability: PCR results for the six laboratories for spiked BALF samples

- Analytical specificity of the PCR methods was comparable, although only PCR-DGGE identified other bovine mycoplasmas.
- Limits of detection varied from 10 to 10<sup>3</sup> CFU/ml to 10<sup>3</sup> and 10<sup>6</sup> CFU/ml for real-time and end-point assays, respectively.
- Ct values for spiked bronchoalveolar fluid samples varied between laboratories and tests, without affecting result (Fig 1).

## *M. bovis* ELISA RING TRIAL

- Two commercial ELISA systems (ID screen® ELISA (Idvet, Grabels, France) and BIO K302 ELISA (Bio-X Diagnostics, Rochefort, Belgium)) were assessed by an inter-laboratory comparison.
- The sample panel (n=180) comprised sera from cattle from countries with high and low *M. bovis* prevalence.
- Sera were distributed to the six laboratories and tested according to a pre-defined plan.
- In-house assays were not included due to difficulties in minimising inter-laboratory variation.
- Immunoblot enabled statistical evaluation by latent class analysis.

	Informative priors		Uniform priors	
	Median	95% PCI	Median	95% PCI
<b>Sensitivity &amp; specificity</b>				
Sensitivity WB	0.918	[0.879; 0.950]	0.935	[0.892; 0.973]
Specificity WB	0.996	[0.987; 1.00]	0.999	[0.993; 1.00]
Sensitivity ID Screen®	0.935	[0.898; 0.965]	0.952	[0.910; 0.990]
Specificity ID Screen®	0.986	[0.976; 0.994]	0.994	[0.985; 0.999]
Sensitivity BIO K302	0.491	[0.447; 0.535]	0.493	[0.448; 0.538]
Specificity BIO K302	0.896	[0.872; 0.918]	0.879	[0.849; 0.905]
<b>Covariances</b>				
COV <sub>Se</sub> (WB*IDScreen®)	0.054	[0.024; 0.072]	0.038	[0.005; 0.074]
COV <sub>Sp</sub> (WB*IDScreen®)	0.008	[0.000; 0.018]	0	[0.000; 0.004]

Fig 2. Assessing sensitivity and specificity of the ELISA and immunoblot tests

- The ID Screen ELISA showed highest agreement with Western blot with higher precision and accuracy than the Bio K302 ELISA.
- Superior diagnostic sensitivity and specificity values were also achieved by the ID Screen® *Mycoplasma bovis* (Fig. 2).

## CONCLUSIONS

- Scientists from veterinary institutes in Europe collaborated on mutually agreed priorities concerning *M. bovis* diagnostics.
- A joint CoVetLab -Nordic Workshop extended opportunities to widen our network of scientists and present preliminary data.
- The PCR ring trial provided reassurance regarding the quality of diagnosis used in our laboratories.
- Although only commercial ELISA kits were included, differences in the sensitivity and specificity were obtained.
- Inter-laboratory studies are important for the robust assessment of performance of current and newly available tests.

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Anna-Maria Andersson<sup>6</sup> performed statistical analysis of the data

Look out for:

Wisselink *et al.* A European interlaboratory trial to evaluate the performance of different PCR methods for *Mycoplasma bovis* diagnosis. BMC Veterinary Research 2019;15:86. <https://doi.org/10.1186/s12917-019-1819-7>

Andersson *et al.* (in prep.). A European interlaboratory trial to evaluate the performance of three serological methods for diagnosis of *Mycoplasma bovis* infection in cattle, using latent class analysis.