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

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Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

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CoVetLab.org
Veterinary Public Health Institutes supporting each other

BACKGROUND

Different clinical presentations of disease caused by *Mycoplasma bovis* 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

- With assistance from CoVetLab.org a consortium of six European national veterinary institutes was established to develop a network of scientists and 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.
- One commercial kit, all other PCR assays were in-house tests.

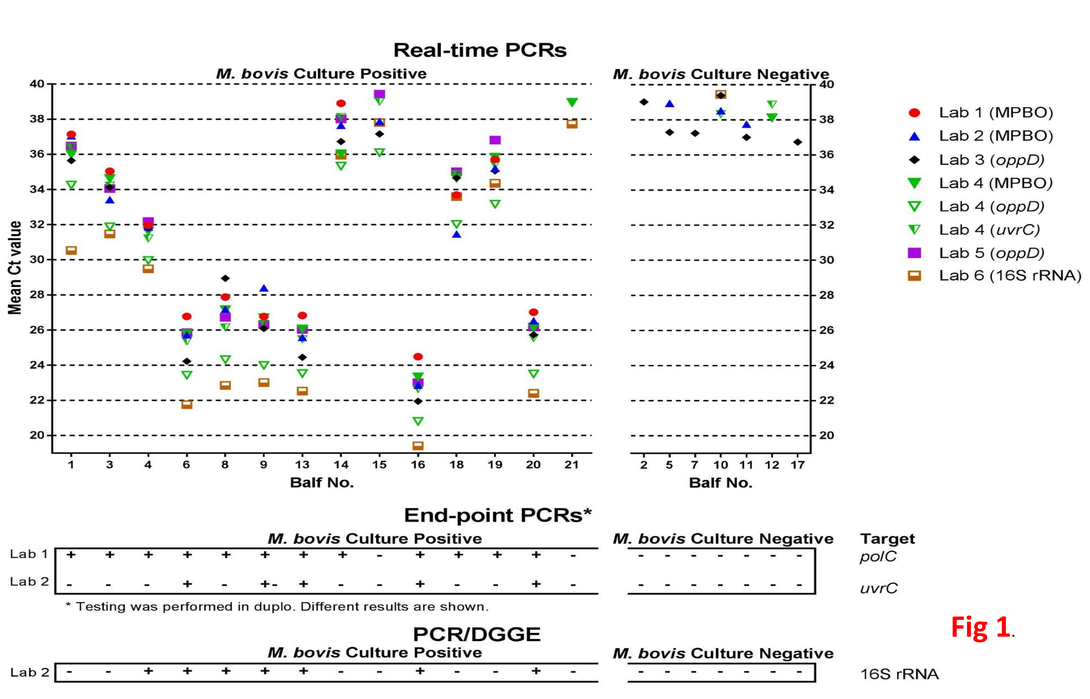

Fig 1.

- Analytical specificity of the PCR methods was comparable, although only PCR-DGGE identified other bovine mycoplasmas.
- Limits of detection varied from 10 to 10^3 CFU/ml to 10^3 and 10^6 CFU/ml for real-time and end-point assays, respectively.
- Ct values varied with naturally infected samples, both between laboratories and tests, without affecting interpretation (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 inter-laboratory comparison.
- The sample panel (n=180) comprised sera from cattle from five countries with high and low *M. bovis* prevalence.
- Standardised set of samples designed to reflect the range of samples encountered in all partner countries
- Sera were distributed to the six laboratories and tested as recommended by the suppliers of the test kits.
- Inter-laboratory variation associated with transferability of in-house assays precludes meaningful comparisons and so were not included in the analyses.
- Immunoblot enabled statistical evaluation by latent class analysis.

	Informative priors		Uniform priors	
	Median	95% PCI	Median	95% PCI
Sensitivity & specificity				
Sensitivity WB	0.930	[0.889; 0.966]	0.933	[0.887; 0.969]
Specificity WB	0.998	[0.990; 1.00]	0.999	[0.993; 1.00]
Sensitivity ID Screen	0.956	[0.916; 0.990]	0.958	[0.914; 0.994]
Specificity ID Screen	0.993	[0.983; 0.998]	0.994	[0.985; 0.999]
Sensitivity BIO K302	0.488	[0.444; 0.532]	0.488	[0.443; 0.533]
Specificity BIO K302	0.883	[0.854; 0.908]	0.880	[0.851; 0.906]
Covariances				
$Cov_{Se(WB*IDScreen)}$	0.037	[0.001; 0.071]	0.035	[0.003; 0.072]
$Cov_{Sp(WB*IDScreen)}$	0.001	[0.000; 0.006]	0.001	[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 analysis and performed with higher precision and accuracy than the Bio K302 ELISA (Fig 2).
- The diagnostic sensitivities of the ID Screen® *Mycoplasma bovis* and the Bio K302 ELISA were 95.6 % and 48.8 % respectively, with specificities of 99.3 % and 88.3 %, respectively.

CONCLUSIONS

- This CoVetLab project has enabled scientists from veterinary institutes in Europe undertaking *M. bovis* diagnostics to collaborate on mutually agreed priorities.
- A joint CoVetLab -Nordic Workshop extended opportunities to widen our network of scientists and present preliminary data.
- The comparison of PCR tests has provided reassurance regarding the quality of diagnosis, despite the different target genes and assays used in our laboratories.
- Although only commercial ELISA kits were included, differences in the sensitivity and specificity were obtained.
- Highlights the importance of inter-laboratory studies to assess performance of current and newly available tests.

- **References: Wisselink et al.** "A European interlaboratory trial to evaluate the performance of different PCR methods for *Mycoplasma bovis* diagnosis" accepted **BMC Veterinary Research.**
  Andersson et al. (in preparation).

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Anna-Maria Andersson[6] is acknowledged for her epidemiological analysis of the data