Mycoplasma bovis test performance and sample size to detect *M. bovis* positive farms

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Introduction

***Mycoplasma bovis* is a bacterium that infects bovines. It can cause problems like mastitis in dairy cows and otitis, arthritis and pneumonia mainly in younger animals. Problems, due to *M. bovis* increases in the developed world over the last decades. In New Zealand, large scale testing and measures are taken to eradicate *M. bovis*. However, also in other countries, handling *M. bovis* infections is a challenging job, since *M. bovis* can be present in the upper airways of bovines without giving clinical signs. Therefore, laboratory testing of animals is crucial to effectively fight *M. bovis* on farm level. However, different test methods can be used and animals of different age and in different numbers can be tested. This study describes the relative sensitivity of different test systems in non-clinical animals of *M. bovis* on farm level.**

Materials and Methods

The farms

Twenty Dutch dairy farms were included that had experienced an acute clinical *M. bovis* outbreak in dairy cows about eleven weeks before, which was characterized by arthritis or mastitis and was confirmed by laboratory testing. At time of testing, clinical signs of *M. bovis* infection were scarce to absent.

The animals

The performance of different laboratory test-systems was evaluated with samples of randomly selected animals of different age classes: calves (< 0.5 yr), young stock (0.5-2 yr) and dairy cows. Up to 12 calves, up to 14 young stock and up to 19 dairy cows were sampled.

The tests

PCR tests for *M. bovis* DNA were performed on conjunctival fluid swabs. Commercially available antibody ELISA tests (BioX) were performed on serum samples.
All farms had one or more test-positive animals in the large testing scheme.

The analysis

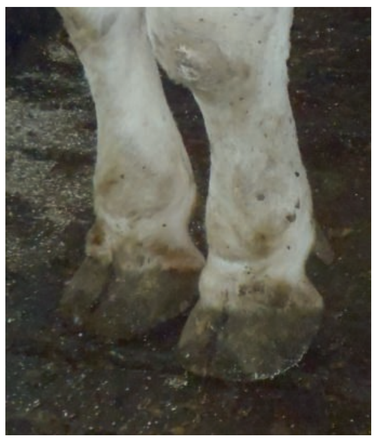
After receiving the results, probability calculations were done to determine the chance that the different farms would have tested positive if a random sample was taken with a size of 3, 5, 8 or 10 animals of that age group. Afterwards, the mean chance to test positive on herd level was calculated.

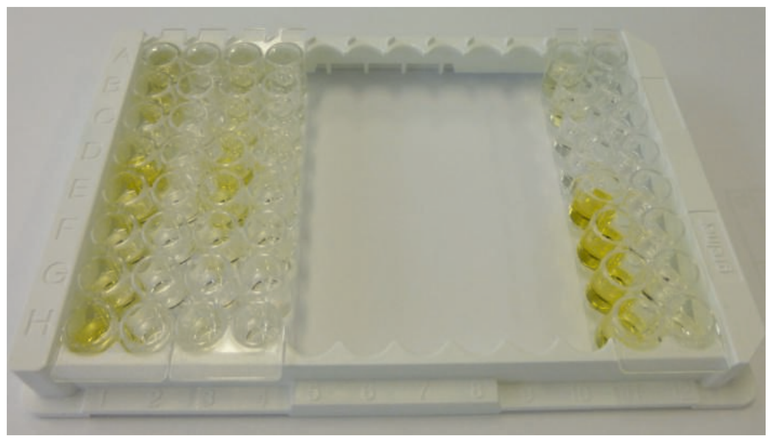
Results

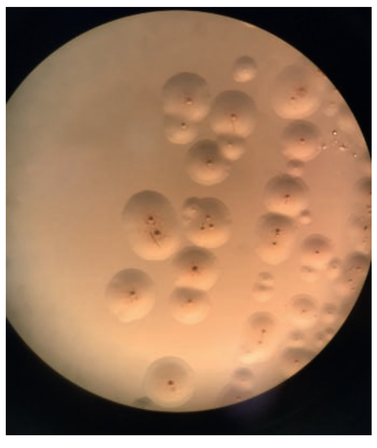
It appeared that prevalences of *M. bovis* DNA and *M. bovis* antibodies differed per farm and age group. Prevalence in ELISA was higher compared with PCR. Both PCR and ELISA prevalence were higher in calves compared to older age groups (significant for ELISA). Test sensitivity to detect *M. bovis* on farm level could be calculated if lower numbers of individual animals were tested, such as is suitable in routine settings. These results are given in the table. If sample sizes of 5 or lower are used, only ELISA testing of calves has sensitivity reaching 80% or more.

Table 1. Test sensitivity to detect an *M.bovis* infected farm depending on sample size (n), test method and animal group

		sample size (n)			
		3	5	8	10
ELISA	calf	0,72	0,83	0,91	0,94
	young stock	0,42	0,55	0,66	0,70
	cow	0,50	0,65	0,78	0,83
PCR	calf	0,46	0,55	0,61	0,63
	young stock	0,36	0,45	0,54	0,58
	cow	0,39	0,48	0,57	0,61







Conclusions

With regards to the testing of dairy farms for the presence of Mycoplasma bovis in non-clinical animals, it can be concluded that:

1. ELISA testing has a higher sensitivity compared to PCR testing
2. Calf testing has a higher sensitivity compared to testing of other age groups
3. A sample size of 5 animals is a minimum number to gain reliable results

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