INFECTIOUS DISEASES

Combining sampling techniques increases detection of carriers of *Mycoplasma bovis*

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INTRODUCTION

- *Mycoplasma bovis* is an economically important bacterium, that can cause important economic losses and impacts animal welfare of newly infected farms.
- Asymptomatic carriers are the main cause of introduction. Identification of carriers is difficult, also due to the intermittent shedding of the bacteria and the different carrying sites.

OBJECTIVE

- This study aimed to evaluate the relevance of combining different sampling sites to increase the possibility of detecting *M. bovis* carriers with RT-PCR.
- Furthermore, in line with previous research on SARS-COV-2, an evaluation was done if bilateral sampling of the nostrils could increase *M. bovis* detection.

MATERIALS AND METHODS

- Six herds with active *M. bovis* circulation within the two months before the study were selected: 5 dairy herds, and one semen collection center.
- In total, 55 cows and 15 bulls were conveniently selected.
- Samples of these animals were taken bimonthly for a year. A total of 359 nasal swabs (NS), 348 swabs of the genital area (GS) (258 vaginal swabs (cow) + 90 sheath swabs (bull)), 258 milk samples (MS) and 87 semen samples (SS) were taken.
- Most of the NS were taken in a single nasal opening. On 76 occasions, nasal swabs were obtained from the 2 nostrils independently.
- A PCR was performed on each sample individually using LSI VetMAX Mycoplasma bovis® (Thermo Fisher Scientific)

The detection of *M. bovis* carriers by PCR is increased if animals are tested in parallel in different sites, at least the nasal opening and the genital opening should be sampled. No semen tested positive for *M. bovis* in this study.

Furthermore, 8 out of 13 (62%) positive animals were only positive in a single nasal opening. As such, it is highly recommended that both nasal openings are swabbed when trying to identify *M. bovis* carriers.

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RESULTS

- Thirty-two animals out of seventy tested at least once positive during the year of follow-up.
- Of the 55 cows in the study, 36 never had a positive sample.
- Eighteen cows tested positive one time, on 1, 2 or all of their sample sites, while one tested positive at 2 different sampling times.
- The total sample results for the sampled cows can be found in **Fig 1.**

FIGURE 1. Positive predictive value calculation and positive test samples cows (n=20)

		Cow tested on				
		M	N	G	N+G	N+G+M
Cow positive on	Only milk	1	0	0	0	1
	Only nasal opening	0	8	0	8	8
	Only genital opening	0	0	6	6	6
	Milk & Genital opening	1	0	1	1	1
	Nasal & genital opening	0	2	2	2	2
	All 3 sample points	2	2	2	2	2
Total amount of animals testing positive when tested on specific matrix (n=20)		**4**	**12**	**11**	**19**	**20**
PPV		**20%**	**60%**	**55%**	**95%**	**100%**

PPV: positive predictive value

RESULTS

- Of the 15 bulls, 13 had *M. bovis* detected on at least 1 sample.
- Four animals were positive at 2 different sampling moments.
- In total, bulls tested positive for: 9*NS, 4*GS and 4*NS+GS. Interestingly, **none of the SS tested positive.**
- **An overview of the bull results can be found in Fig 2**.

FIGURE 2. Positive predictive value calculation and positive test samples bulls (n=17)

		Bull tested on				
		N	G	S	N+G	N+G+S
Bull positive on	Only nasal opening	9	0	0	9	9
	Only genital opening	0	4	0	4	4
	Only semen	0	0	0	0	0
	Nasal & genital opening	4	4	0	4	4
	All 3 sample points	0	0	0	0	0
Total amount of animals testing positive (n= 17)		**13**	**8**	**0**	**17**	**17**
PPV		**76%**	**47%**	**0%**	**100%**	**100%**

PPV: positive predictive value

RESULTS

From the samples taken separately from each nasal opening (n=76), 8 out of 13 animals testing positive for *M. bovis* only tested positive in a single nasal opening.

FIGURE 3. Samples taken from the left & right nasal opening

LEFT	RIGHT	N
Negative	Negative	63
Positive	Negative	3 (23,1%)
Negative	Positive	5 (38,5%)
Positive	Positive	5 (38,5%)
		13/76

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