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Sampling techniques for the detection of *M. bovis* carriers

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CONTEXT AND OBJECTIVES

Mycoplasma bovis is a worldwide bacteria capable of causing severe disease such as pneumonia, arthritis, otitis and mastitis in cattle, resulting inevitably in important economic losses and reduced animal welfare in affected herds.

Purchase of asymptomatically infected cattle is considered one of the major pathways of introduction of *M. bovis* in a herd. It is therefore recommended to test animals before the introduction. However, detection of a carrier is problematic: no ideal testing strategy exists, due to *M. bovis* tendency to shed intermittently in different sites. Furthermore, testing methods are not ideal. Antibody ELISA testing allows the identification of animals previously exposed to the bacteria, but this technique is not very sensitive and does not allow the detection of carriers. PCR is very sensitive, but demands presence of the bacterial DNA on the samples taken which is difficult due to the intermittent shedding of *M. bovis*.

Given the difficulty of identifying carrier animals with current sampling techniques, this field study aimed to assess the relevance of different sampling sites, performed by practitioners.

MATERIAL AND METHODS

From 5 different Belgian herds (H1-5) having *M. bovis* isolated maximum 2 months before the start of the study, 55 dairy cows (11/herd) were selected. In parallel, 15 bulls from an artificial insemination centre (H6) were enrolled.

Samplings were schedulded every 2 months during one year (Periods M0/M2/M4/M6/M8/M10/M12). A total of 359 nasal swabs (N) and 348 genital swabs (G) (258 vaginal + 90 sheath) were performed using a Amies Agar Gel with Charcoal Transport Swabs while 258 milk samples (M) and 87 semen samples (S) were taken using sterile containers.

Most of the N were sampled from a single nasal opening per animal. Nevertheless, for 76 of them, the 2 nostrils were swabbed independently.

All samples were stored in the fridge and sent at the laboratory to be processed with a maximum delay of 2 days.

A PCR was performed on each sample individually using LSI VetMAX *Mycoplasma bovis*® (Thermo Fisher Scientific).

Herd	Animal +	Period	Sample(s) +
H1	1	M0	G
	2	M0	N
	3	M0	N
	4	M4	G
	5	M0	N
H2	6	M8	M
	7	M8	N
	8	M8	NG
	9	M8	NG
	10	M8	GM
	11	M8	N
	12	M8	NGM
	13	M8	N
	14	M8	NGM
H3	15	M0	G
	16	M0	N
H4	17	M4	G
	18	M4	G
H5	19	M6	N
		M8	G
H6	20	M3	NG
	21	M3	G
	22	M3	NG
	23	M3	N
	24	M3	G
	25	M1	N
		M3	NG
	26	M3	NG
	27	M3	N
	28	M3	N
	29	M3	N
		M5	NG
	30	M1	N
		M3	G
	31	M3	N
	32	M1	N
		M3	N

Table 1 : Time of sampling and Sample site positive on M. bovis PCR for each positive animal (N = NASAL ; G = GENITAL; M = MILK ; S = SEMEN)

RESULTS

- As shown in Table 1, 32 out of 70 animals (45,71%) tested had at least one positive sample on PCR during the study, distributed as follows : 5/11 in H1, 9/11 in H2, 2/11 in H3, 2/11 in H4, 1/11 in H5 and 13/15 in H6.
- Of the 55 cows, 18 were positive only once on 1, 2 or all of their samples while one tested positive at 2 different periods. In total animals tested positive on 8*N, 6*G, 1*M, 2*NG, 1*GM and 2*NGM.

  As summarized in Table 2.a, N, G or M sampling *on M. bovis* "carrier" cows allowed to identify respectively 12,24% (12/98), 11,11% (11/99) and only 4,04% (4/99) of them. While pooling N and G, PCR can detect 19,19 % (19/99) of these animals which is almost as sensitive as when the 3 types of sample N, G and M were tested 20,20% (20/99).

	Nasal (N)	Genital (G)	Milk (M)	NG (N and/or G)	NGM (N and/or G and/or M)
Samples on carriers	99	98	99	99	99
Positive samples	12	11	4	19	20
	12,12%	**11,22%**	**4,04%**	**19,19%**	**20,20%**

Table 2.a : Repartition of positive agreement of samples and pools of samples for the 19 carrier cows.

- Amongst the 15 bulls, 13 had *M. bovis* detected on at least 1 positive sample, of which 4 of them were positive at 2 different periods. In total, animals tested positive on : 9*N, 4*G and 4*NG. **Interestingly, no S samples tested positive.**

  As summarized in Table 2.b, sampling N, G or S on carrier bulls allowed to find back respectively 14,94% (13/87), 10,53% (8/76) and 0,00% (0/87) of carriers. While pooling N and G, PCR can detect 22,37% (17/76) of these animals.

	Nasal (N)	Genital (G)	Semen (S)	NG (N and/or G)	NGS (N and/or G and/or S)
Samples on carriers	87	76	87	76	76
Positive samples	13	8	0	17	17
	14,94%	**10,53%**	**0,00%**	**22,37%**	**22,37%**

Table 2.b : Repartition of positive agreement of samples and pools of samples for the 13 carrier bulls.

- As shown in Table 3, on the samples taken separately for each nasal opening (n=76), **8 out of 13 positive animals were only positive in a single nasal opening.**

  On the 26 nostrils (13 right + 13 left) from the 13 positive animals, 8 of them were negatives.

Nostril		#
Left	Right	
Neg	Neg	63
Pos	Neg	3
Neg	Pos	5
Pos	Pos	5

Table 3 : Repartition of results of animals swabbed on each nostril individually.

DISCUSSION AND CONCLUSION

- Detection of asymptomatic *M. bovis* carriers is essential to avoid the introduction of this pathogen. Nevertheless, except the serology ELISA test which is not very sensitive, there is no concrete recommendations in the field to sample asymptomatic animals due to the intermittent shedding from carriers on different sites.
- This study also confirmed the lack of sensitivity of the sampling to detect a *M. bovis* carrier. Ideally, a strict quarantine period during which **serial series of swabs** can be performed should be advised.
- Positive animals of the same herd were frequently carriers during the same period.
- Importantly, this study highlights the benefit of **combining at least a genital (vaginal or sheath) and bilateral nostril swabs**, which are relatively easy sites to test. Moreover, these samples are easy to pool leading to a reduced cost of testing.
- In case of historic mastitis known in the seller farm, **milk sample can be also realized**. By contrast, there is **no relevance to test semens**. These data are confirmed by all the other PCR on semen realized in our lab (0+/270 semen tested).

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