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Detection of *Mycoplasma bovis* carrier animals using tonsil samples – preliminary results

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Background

Mycoplasma bovis causes a variety of diseases and has a major impact on animal welfare and on the cattle industry. However, diagnosing of clinically healthy carrier animals is challenging, generally employing mucosal smear (PCR/culture) or serum (antibodies) samples.

Pathogens are known to persist in the nasopharyngeal area. It has been reported that bovine tonsils can be reservoirs for *Pasteurella haemolytica* infection of the nasopharynx. (1). *Mycoplasma bovis* was found after 28 days from the nasopharyngeal samples from almost all of the calves in a feedlot study, from clinically ill and asymptomatic calves (2).

The aim of the study was to evaluate the utility of sampling pharyngeal and palatine tonsils for *M. bovis* diagnosis in diseased or healthy carrier animals.

Methods

We sampled pharyngeal and palatine tonsils of 30 clinically healthy and diseased bovines; in eight animals only palatine and in one animal only pharyngeal tonsils were sampled. Tonsil tissue was disrupted using ceramic beads in a MagNA Lyser instrument (Roche). DNA was isolated according to manufacturer's instructions using QIAamp DNA Mini Kit and tissue protocol (Qiagen). Real time PCR targeting the oppD gene was used to detect *M. bovis*. Samples were cultured in Friis broth and oppD real time PCR was used to detect *M. bovis* growth. Pairwise comparison was done with results from tonsils and results of lung (n=21) and other tissue samples (n=7) of the same animals.

Results

Ten of the sampled animals showed clinical signs related to *M. bovis*, mainly pneumonia Eight of these diseased animals were PCR-positive in tonsil samples and culture-positive in lung samples. Two animals had PCR-positive tonsils, but lung samples were culture-negative.

Amongst the 20 animals without clinical signs, both tonsils and lungs were culture-positive in three animals, and in one animal the tonsils were PCR -positive (no other tissues were analyzed).

Table 1. Comparing results of the *Mycoplasma bovis* analyzation of the tonsils, lung and other tissues of 30 animals from 18 farms. Samples either taken at autopsy or organs sent from the abattoir or the farm.

No.	Signs[1]	Lesions[2]	Age (mth)	Farm	Farm status[3]	Tonsil		Lung	Other
						Culture[4]	PCR[5]	Culture	Culture
1	0	0	>12	A	0	-[6]	neg	-	-
2	0	L	>12	B	0	neg	-	neg	-
3	0	0	>12	B	0	neg	-	neg	-
4	0	0	>12	B	0	neg	-	neg	neg
5	0	0	7-12	B	0	neg	-	neg	-
6	0	0	≤6	C	0	-	neg	-	-
7	0	0	≤6	D	0	-	neg	-	-
8	0	0	>12	E	0	-	neg	-	-
9	0	0	>12	F	0	-	neg	-	-
10	0	L	>12	G	1	neg	-	neg	-
11	0	U	>12	H	1	-	neg	neg	neg
12	0	0	>12	I	0	-	neg	-	-
13	0	0	>12	J	0	-	neg	-	-
14	0	0	>12	K	0	-	neg	-	-
15[7]	0	0	≤6	L	1	-	neg	neg	-
16	0	L	≤6	M	1	-	neg	neg	-
17	0	0	>12	N	0	-	pos	-	-
18	0	0	≤6	N	1	pos	pos	pos	pos
19	0	L	>12	O	1	pos	-	pos	-
20	0	L	>12	O	1	pos	-	pos	-
21	1	0	≤6	H	1	-	pos	pos	pos
22	1	L	≤6	H	1	-	pos	pos	-
23	1	L	≤6	H	1	-	pos	neg	-
24	1	L	≤6	H	1	-	pos	pos	-
25	1	L, J	≤6	H	1	-	pos	pos	pos
26	1	L	≤6	H	1	-	pos	pos	-
27	1	L, E	≤6	P	1	-	pos	pos	pos
28	1	L, E	≤6	P	1	-	pos	pos	pos
29	1	L	7-12	Q	0	-	pos	neg	-
30	1	L	≤6	R	0	-	pos	pos	-

[1] Signs; 1=The animal had signs related to *M. bovis* (pneumonia, mastitis, ear or joint infection); 0= the animal did not have signs related to *M. bovis.*

[2] Lesions; 0= Pathological lesions indicative of *M. bovis* were not found (lung, joints, udder or ear); lesions found in the lung (L), joint (J), udder (U), ear (E). Other lesions are not mentioned.

[3] Farm status; 1= Farm was known to be *M. bovis* –positive before the samples were taken.

[4] Culture from palatine tonsils

[5] PCR of either palatine or pharyngeal tonsil or both, positive if either tonsil is positive

[6] Not available

[7] Calf that died itself without any prior signs.

Conclusions

Tonsil samples can be used to detect *Mycoplasma bovis* in carrier animals. However, more research is needed to determine their sensitivity in comparison to other tissues in healthy carrier animals.

References

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