VIRAL AND BACTERIAL PATHOGENS CAUSING BOVINE RESPIRATORY DISEASE IN ESTONIA

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OBJECTIVES

Though being a major health problem in cattle herds, the causal agents of bovine respiratory diseases are not profoundly studied in Estonia. Hence, the **aim of this study** was to examine the occurrence of bacterial, mycoplasmal and viral pathogens in the lower respiratory tract of calves in Estonia. All the microbes were analysed from tracheobronchial lavage (TBL) samples.

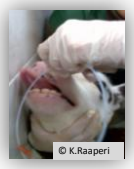
MATERIALS AND METHODS

Sample collection

This study was conducted in 2011-2012. From twenty cattle herds with acute BRD outbreak 4-5 diseased calves (age < 90 days) from each farm were chosen for closer examination (in total 100 animals). The clinical examination revealed that all of the calves had abnormal sounds on auscultation, and most had one or more of the following symptoms: fever, elevated respiratory rate, cough, nasal or ocular discharge or ear drop. Individual TBL samples were taken with a special instrument for collecting samples of the lower respiratory tract from calves (Bengtsson *et al.* 1998). 0,5 ml of the TBL was immediately transferred into mycoplasma D-medium. The rest of TBL sample were used for analysing bacteria and viruses.

Laboratory analyses

- For isolation and identification of bacteria in TBL samples conventional aerobic, microaerophilic and anaerobic bacterial cultivation and identification methods were used.
- Viral RNA/DNA was extracted from TBL samples by using the RTP DNA/RNA Virus Mini Kit (STRATEC Molecular, Berlin, Germany) according to the manufacturer's instructions.
- Extracted RNA/DNA was analysed by using different PCR protocols (Table 1).
- The mycoplasma D media was subsequently diluted in a 10-fold series up to 10^{-6} in D and F media and incubated at 37 °C for up to 14 days. DNA from isolated mycoplasmas was extracted with a 5% solution of Chelex (Sigma, USA). Identification of *M.bovis* and *M.bovirhinis* was done by species specific PCR (Table 1).



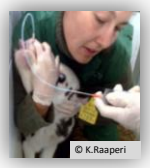


Table 1. Primers used for amplification and detection of viruses and mycoplasmas

Pathogen	Method	Primers	Sequence of primer	Size of amplicon, bp	Reference for primers
BCV	RT-PCR	BCV F BCV R	5'- GCC GAT CAG TCC GAC CAA TC -3' 5'- AGA ATG TCA GCC GGG GTA T -3'	407	Tsunemitsu et al 1999
BVDV	RT-PCR	Nr 324 Nr 326	5'- ATG CCC T/ATA GTA GGA CTA GCA -3' 5'- TCA ACT CCA TGT GCC ATG TAC -3'	288	Vilcek et al 1994
BoHV-1 and BoHV-5	PCR	PF PR	5'- CGG CCA CGA CGC TGA CGA -3' 5'- CGC CGC CGA GTA CTA CCC -3'	575/572	Esteves et al 2008
PIV-3	Real-time RT-PCR	piv3M1 piv3M2	5' - GCTCTGTTGAGGCAGCTCTGTTGAGGCAGGATTG - 3' 5' - ATTGATTGAGGAGCAAGTGCAACC - 3'	419	Autio et al 2007
BRSV	RT - PCR	BRSV F BRSV R	5'-AAC CGG CTT CCT TCA GTA GAG C -3' 5'- CAA TAC CAC CCA CGA TCT GTC C -3'	729	Larsen et al 1999
M.bovis	PCR	MboF2 MboR2	5'- GAA GAA AAA GTA GCA TAG GAA ATG AT -3' 5'- CGT CGT CCC CAC CTT CCT CCC G -3'	734	Johansson et al 1996
M.bovirhinis	PCR	Mbr-F Mbr-R	5'- GCT GAT AGA GAG GTC TAT CG-3' 5'- ATT ACT CGG GCA GTC TCC -3'	316	Kobayashi et al 1998

RESULTS

Table 2. Bacteria, viruses and mycoplasmas detected from 100 TBL samples from 20 herds

Sample	Pathogen	Positive samples (%)	Positive herds (%) n=20
TBL	*P.multocida*	33 (33%)	16 (80%)
	M.haemolytica	27 (27%)	11 (55%)
	A.pyogenes	3 (3%)	1 (5%)
	H.somni	4 (4%)	3 (15%)
	Pasteurella spp	13 (13 %)	7 (35%)
	BVDV	0 (0%)	0 (0%)
	BRSV	0 (0%)	0 (0%)
	BHV-1	0 (0%)	0 (0%)
	Corona virus	11 (11%)	7 (35%)
	PIV-3	66 (66 %)	20 (100 %)
	M.bovis	32 (32%)	17 (85%)
	M.bovirhinis	57 (57%)	19 (95%)

CONCLUSIONS

- First evidence on *M.bovis* in Estonia
- *P.multocida* and *M.haemolytica* were the most common bacterial pathogens. They were isolated from 33 % and 27% of TBL samples respectively
- PIV-3 was detected by PCR in 66 % of TBL samples
- BVDV, BRSV, BHV were not detected at all

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