DISAGREEMENT BETWEEN MILK PATHOGENS PREVALENCE IN SEGREGATED MILKING COWS AND BULK TANK MILK IN AZOREAN PASTURES

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OBJECTIVES

The aim of the present study was to determine the apparent prevalence of a large wide of milk pathogens from BTM using a multiplex PCR method and compare it with the pooled samples from mastitic cows segregated at milking time from Azorean dairy herds.

METHOD AND MATERIAL

From all regions of San Miguel- Azores, 92 dairy herds considering a total of 5520 adult cows were used. The separation of mastitic cows, due to the presence of clinical mastitis (untreated or in treatment) or subclinical mastitis with high SCC detected by California Mastitis Test, at milking time was considered for herd inclusion on the present study.

A milk pooled sample from all quarters of these cows (mean = 3) were collected in each farm. In simultaneous, after milking all cows, a sample was collected from BMT. All final samples were collected using the Startcheck® sampling kit (HIPRA, Spain), according the manufacturer instructions, in order to test the presence of *Coagulase-negative staphylococci* (CNS), *Escherichia coli, S. aureus, Corynebacterium bovis, Enterococcus spp., Streptococcus dysgalactiae, Streptococcus uberis, Actinomyces pyogenes / Peptostreptococcus indolicus, Klebsiella spp., Streptococcus agalactiae and Serratia marcescens.* The extraction and amplification of the DNA were performed using a real time PCR (PathoProof TM Mastitis Complete-12 Kit; Thermo Fisher Scientific Inc., MA USA), with some modifications. A qPCR positive result was recorded when cycle threshold values were ≤ 37 and a sigmoidal amplification plot was obtained.

Figure 1. Dairy herds from São Miguel

RESULTS

In average, each farm presented 4.6 (CI95% from 4.3 to 4.9) and 5.6 (CI95% from 5.2 to 5.9) bacteria
species/group from SMC and BMT respectively. *Coagulase-negative staphylococci, Escherichia coli, Staphylococcus aureus, Enterococcus spp., Corynebacterium bovis and Streptococcus spp.* presented the higher apparent prevalence on both samples type (upper 95% IC > 50%). A high correlation of apparent prevalence from each pathogen between SMC and BMT samples were observed (Rho = 0.90; n = 11; P < 0.01) inter-herds. However, the agreements between these apparent prevalences, intra-herd, were always low (Kappa coefficient < 0.70).

Bacteria	Bulk tank milk	Milk pools from segregated cows	Kappa coefficient	McNemar-Bowker test
Coagulase-negative staphylococci	100.0%; n=92	100.0%; n=92	-	-
Escherichia coli	75.0% (65.3 to 82.7); n=69	73.9% (64.1 to 81.8); n=66	0.51 ± 0.10	P = 0.81
Staphylococcus aureus	62.0% (51.8 to 71.2); n=57	45.7% (35.9 to 55.8); n=42	0.51 ± 0.08	P = 0.002
Corynebacterium bovis	57.6% (47.4 to 67.2); n=53	50.0% (40.0 to 60.0); n=46	0.24 ± 0.10	P = 0.24
Enterococcus spp.	55.4% (45.3 to 65.2); n=51	42.4% (32.8 to 52.6); n=39	0.19 ± 0.10	P = 0.05
Streptococcus dysgalactiae	51.1% (41.0 to 61.1); n=47	27.2% (19.1 to 37.0); n=25	0.27 ± 0.09	P = 0.0002
Streptococcus uberis	41.3% (31.8 to 51.5); n=38	48.9% (39.0 to 59.0); n=45	0.30 ± 0.10	P = 0.22
Actinomyces pyogenes / *Peptostreptococcus* indolicus	41.3% (31.8 to 51.5); n=38	29.3% (21.0 to 39.3); n=27	0.27 ± 0.10	P = 0.05
Klebsiella spp.	37.0% (27.8 to 47.2); n=34	21.7% (14.5 to 31.2); n=20	0.29 ± 0.10	P = 0.008
Streptococcus agalactiae	32.6% (23.9 to 42.7); n=30	20.7% (13.6 to 30.0); n=19	0.26 ± 0.11	P = 0.03
Serratia marcescens	3.3% (1.1 to 9.6); n=3	n = 0	-	-

Table 1. Apparent prevalence (CI95%) and agreement (K coefficient) between bulk tank milk and pooled samples from mastitic cows.

CONCLUSIONS

In conclusion, high prevalences of bacteria species/groups were detected in BTM from Azorean pastures. Although the segregation of cows may be a potentially favorable practice, their number on each farm seem to be too small to be representative and consequently not an indicator of agents in BTM on the same herd. In consequence, a holistic approach on mastitis diagnosis, treatment and control should be considered in Azorean dairy farms.

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