

# Isolation of Biofilm-Forming Staphylococci from the Bulk-Tank Milk of Small Ruminant Farms in Greece

Daphne T. Lianou, Charalambia K. Michael, Nikolaos Solomakos, Natalia G. C. Vasileiou, Efthymia Petinaki, Vasia S. Mavrogianni, Athina Tzora, Chrysoula Voidarou, and George C. Fthenakis

**Table S1.** Details of multivariable models employed for the evaluation for potential associations of the recovery of biofilm-forming staphylococci from the bulk-tank of milk in 444 small ruminant farms in Greece.

Outcome	Variables ( <i>n</i> )	
	offered to the multi-variable models	required in the final models
Isolation of biofilm-forming staphylococci - only farms applying machine-milking	4 <sup>1</sup>	(a) Temperature of cleaning water of the milking parlour, (b) Frequency of milk collection from the tank
Isolation of biofilm-forming staphylococci - farms applying hand-milking	3 <sup>2</sup>	(a) Season of the year when sampling was carried out, (b) Frequency of milk collection from the tank, (c) Use of teat disinfection after milking

<sup>1</sup> Season of the year when sampling was performed, Temperature of cleaning water of the milking parlour, Frequency of milk collection from the tank, Use of teat disinfection after milking; <sup>2</sup> Season of the year when sampling was performed, Frequency of milk collection from the tank, Use of teat disinfection after milking.

**Table S2.** Cumulative information collected from 444 small ruminant farms in a countrywide investigation in Greece, classified according to animal species in the farms.

	Sheep flocks ( <i>n</i> = 325)	Goat herds ( <i>n</i> = 199)
Management system applied in the farm	Intensive: 44, semi-intensive: 140, semi-extensive: 116, extensive: 25	Intensive: 9, semi-intensive: 29, semi-extensive: 61, extensive: 20
No. of female animals in the farm	$\bar{X}$ : 325 ± 13 ewes	$\bar{X}$ : 237 ± 20 does
Type of milking	Machine-milking: 255, hand-milking no: 70	Machine-milking: 66, hand-milking: 53
Annual frequency of changing teatcups	Once: 130, less than once: 125	Once: 29, less than once: 37
Temperature of cleaning water of the milking parlour	$\bar{X}$ : 70 ± 0.5 °C	$\bar{X}$ : 70 ± 1.0 °C
Frequency of milk collection from the tank	Daily: 82, at least every two days: 243	Daily: 36, at least every two days: 83
Daily number of milking sessions	2 (1 – 3)	2 (1 – 3)
Use of teat disinfection after milking	Yes: 52, no: 273	Yes: 12, no: 107
Administration of ‘dry-ewe’ treatment at the end of the lactation period	Yes: 53, no: 272	Yes: 15, no: 104
Annual frequency of systemic disinfections in the farm	$\bar{X}$ : 3.6 ± 0.2 occasions	$\bar{X}$ : 4.3 ± 0.5 occasions
Month into the lactation period at sampling	0-1 <sup>st</sup> : 23, 2 <sup>nd</sup> -5 <sup>th</sup> : 138, 6 <sup>th</sup> -9 <sup>th</sup> : 147, over 9 <sup>th</sup> : 17	0-1 <sup>st</sup> : 8, 2 <sup>nd</sup> -5 <sup>th</sup> : 60, 6 <sup>th</sup> -9 <sup>th</sup> : 43, over 9 <sup>th</sup> : 8

39 **Table S3.** Results of univariable analysis for associations of recovery of biofilm-forming staphylococcal isolates from bulk-tank milk with the season of the year when sampling was performed  
40 in 444 small ruminant farms in Greece.

Recovery of biofilm-forming staphylococcal isolates ( <i>n</i> = 203)				No recovery of biofilm-forming staphylococcal isolates ( <i>n</i> = 241)				<i>p</i>
Season of the year when sampling was performed								
Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	
18	50	82	53	23	61	69	88	0.040

**Table S4.** Isolation of biofilm-forming staphylococci from small ruminant farms during a countrywide investigation in Greece, in accord with the temperature of the water used to clean the milking parlour.

Temperature ranges	Proportion of farms from which biofilm-forming staphylococci were isolated	<i>p</i> -value	Median (interquartile range) number of biofilm-forming staphylococci isolated per farm	<i>p</i> -value
< 49 °C	88.9%	0.0002	1 (0)	< 0.0001
50 °C – 69 °C	47.9%	0.62	0 (1)	0.70
70 °C – 89 °C	39.0%	0.005	0 (1)	0.005
≥ 90 °C	56.0%	0.29	1 (1)	0.34

**Table S5.** Results of univariable analysis for associations of recovery of biofilm-forming staphylococcal isolates from bulk-tank milk with management practices applied in 444 small ruminant farms in Greece.

Recovery of biofilm-forming staphylococcal isolates ( <i>n</i> = 203)				No recovery of biofilm-forming staphylococcal isolates ( <i>n</i> = 241)				<i>p</i>
Management system applied in the farm								
Intensive	Semi-intensive	Semi-extensive	Extensive	Intensive	Semi-intensive	Semi-extensive	Extensive	0.36
24	81	83	15	29	88	94	30	
No. of female animals in the farm								
≤ 165	166 - 330	331 – 500	> 500	≤ 165	166 - 330	331 – 500	> 500	0.41
66	78	30	29	79	79	49	35	
Type of milking								
Machine-milking		Hand-milking		Machine-milking		Hand-milking		0.96
147		56		174		67		
Annual frequency of changing teatcups								
Up to annually		Less often than annually		Up to annually		Less often than annually		0.53
70		77		89		85		
Temperature of cleaning water of the milking parlour (°C)								
< 49	50 – 69	70 – 89	≥ 90	< 49	50 – 69	70 – 89	≥ 90	0.0004
16	46	71	14	2	50	111	11	
Frequency of milk collection from the tank (days)								
Daily		At least every two days		Daily		At least every two days		0.05
45		158		73		168		
Daily number of milking sessions								
One	Two	Three		One	Two	Three		0.65
2	167	34		3	205	33		
Use of teat disinfection after milking								
Yes		No		Yes		No		0.17
34		167		30		213		
Administration of ‘dry-ewe’ treatment at the end of the lactation period								
Yes		No		Yes		No		0.30
35		168		33		208		
Annual frequency of systemic disinfections in the farm								
Median: 2 (interquartile range: 2)				Median: 2 (interquartile range: 2)				0.38

Month into the lactation period at sampling							
0-1 <sup>st</sup>	2 <sup>nd</sup> -5 <sup>th</sup>	6 <sup>th</sup> -9 <sup>th</sup>	Over 9 <sup>th</sup>	0-1 <sup>st</sup>	2 <sup>nd</sup> -5 <sup>th</sup>	6 <sup>th</sup> -9 <sup>th</sup>	Over 9 <sup>th</sup>
16	89	89	9	15	109	101	16
							0.68

**Table S6.** Parametres of the bulk-tank milk of 444 small ruminant farms in Greece in accord with the isolation of biofilm-forming staphylococci from the milk.

Parameters	Isolation of biofilm-forming staphylococci	Isolation of non biofilm-forming staphylococci	No isolation of staphylococci	ls means (sem)	<i>p</i> -value
Sheep flocks ( <i>n</i> = 325)					
Somatic cell counts <sup>1</sup>	0.575 × 10 <sup>6</sup> (0.518 × 10 <sup>6</sup> - 0.638 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.503 × 10 <sup>6</sup> (0.430 × 10 <sup>6</sup> - 0.587 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.385 × 10 <sup>6</sup> (0.333 × 10 <sup>6</sup> - 0.442 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.479 × 10 <sup>6</sup> (0.013 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	< 0.0001
Total bacterial counts <sup>1</sup>	499 × 10 <sup>3</sup> (372 × 10 <sup>3</sup> - 676 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	378 × 10 <sup>3</sup> (245 × 10 <sup>3</sup> - 575 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	308 × 10 <sup>3</sup> (240 × 10 <sup>3</sup> - 398 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	387 × 10 <sup>3</sup> (1 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	0.046
Fat content <sup>2</sup>	6.08% ± 0.07%	6.17% ± 0.11%	6.26% ± 0.08%	6.17% (0.03%)	0.22
Protein content <sup>2</sup>	4.42% ± 0.02%	4.46% ± 0.03%	4.42% ± 0.02%	4.43% (0.01%)	0.59
Lactose content <sup>2</sup>	4.19% ± 0.02%	4.24% ± 0.03%	4.21% ± 0.02%	4.22% (0.02%)	0.41
Goat herds ( <i>n</i> = 119)					
Somatic cell counts <sup>1</sup>	0.879 × 10 <sup>6</sup> (0.764 × 10 <sup>6</sup> - 1.009 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.871 × 10 <sup>6</sup> (0.689 × 10 <sup>6</sup> - 1.104 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.777 × 10 <sup>6</sup> (0.647 × 10 <sup>6</sup> - 0.935 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.842 × 10 <sup>6</sup> (0.013 × 10 <sup>6</sup> ) cells mL <sup>-1</sup>	0.53
Total bacterial counts <sup>1</sup>	633 × 10 <sup>3</sup> (417 × 10 <sup>3</sup> - 955 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	723 × 10 <sup>3</sup> (372 × 10 <sup>3</sup> - 1413 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	507 × 10 <sup>3</sup> (339 × 10 <sup>3</sup> - 759 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	608 × 10 <sup>3</sup> (1 × 10 <sup>3</sup> ) cfu mL <sup>-1</sup>	0.62
Fat content <sup>2</sup>	4.78% ± 0.15%	4.38% ± 0.26%	4.93% ± 0.20%	4.73% (0.09%)	0.25
Protein content <sup>2</sup>	3.20% ± 0.03%	3.22% ± 0.05%	3.27% ± 0.06%	3.25% (0.02%)	0.56
Lactose content <sup>2</sup>	4.77% ± 0.04%	4.82% ± 0.05%	4.67% ± 0.06%	4.74% (0.01%)	0.14

<sup>1</sup> mean (95% confidence intervals); <sup>2</sup> mean ± standard error of the mean.