

**Table S1.** Details of 12 flocks included in a longitudinal study of subclinical mastitis.

Farm	Location	Management system	No. of ewes	Breed of ewes	Start of lambing period	Annual milk production per ewe	Machine - milking	Milking sessions daily	Sampling period
1	Achaea, Western Greece	Semi-extensive	130	Friesian	September	154 L	Yes	Two	4 visits: November 2019 to June 2020
2	Achaea, Western Greece	Semi-extensive	170	Friesian	October	235 L	Yes	Two	4 visits: December 2019 to June 2020
3	Achaea, Western Greece	Semi-extensive	180	Local	September	144 L	Yes	Two	4 visits: November 2019 to June 2020
4	Achaea, Western Greece	Intensive	235	Local	October	111 L	Yes	Two	4 visits: December 2019 to June 2020
5	Achaea, Western Greece	Semi-intensive	150	Lacaune	September	167 L	Yes	Two	4 visits: November 2019 to June 2020
6	Achaea, Western Greece	Semi-intensive	230	Chios	September	296 L	Yes	Two	4 visits: November 2019 to June 2020
7	Corinthia, Peloponnese	Semi-intensive	300	Local	September	293 L	Yes	Three	4 visits: November 2019 to June 2020
8	Corinthia, Peloponnese	Semi-intensive	85	Assaf	October	400 L	Yes	Two	4 visits: December 2019 to June 2020
9	Corinthia, Peloponnese	Intensive	600	Chios	October	183 L	Yes	Three	4 visits: December 2019 to June 2020
10	Corinthia, Peloponnese	Semi-intensive	230	Assaf	September	357 L	Yes	Three	4 visits: November 2019 to June 2020
11	Corinthia, Peloponnese	Semi-intensive	280	Lacaune	September	232 L	Yes	Two	4 visits: November 2019 to June 2020
12	Corinthia, Peloponnese	Semi-intensive	145	Local	October	152 L	Yes	Two	4 visits: November 2019 to June 2020

**Table S2.** Variables ( $n = 67$ ) evaluated for potential association with incidence risk of subclinical mastitis in a longitudinal study throughout the milking period in 12 sheep flocks in Greece.

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Management system applied in the flocks (description according to EFSA classification)
Availability of a waiting area before the milking parlour (yes / no)
Material of the floor of the barn (cement / soil / slatted wood / slatted metal / other)
Availability of straw bedding (yes / no)
Annual frequency of removal / clean-up of the straw bedding (no. of occasions)
Grazing land available to animals (acres per animal)
No. of ewes in the flock (no.)
Breed of ewes (description)
Average age of culling females (years)
Month of the start of the lambing season (description)
Total milk quantity per ewe obtained during the preceding milking period (litres)
Average number of lambs born per ewe (no.)
Total visits made annually by veterinarians to the flock during the preceding season (no.)
Use of laboratory diagnostic examinations in samples of milk preventively (yes / no)
Age of lamb removal from their dams (days)
Daily number of milking sessions (no.)
Duration of the dry-period (months)
Administration of selenium to pregnant ewes (yes / no)
Administration of 'dry-ewe' treatment at the end of the lactation period (yes / no)
Use of teat disinfection after milking (yes / no)
Total number of antibiotics used for treatment of clinical mastitis (description, no.)
Route for administration of antimicrobials (systematically / intramammary)
Vaccination against mastitis (yes / no)
Annual frequency of systemic disinfections in the farm (no. of occasions)
Years since initial establishment or most recent renovation of the milking parlour (no.)
Volume of the parlour (m <sup>3</sup> )
Material of the floor of the milking parlour (cement / tile / soil / other)
Type of milking parlour (fishbone / circular / linear parallel / linear one-sided / other)
Type of milking system (mobile / non-mobile)
Number of animal positions in the parlour (no.)
Number of available milking units per animal position (no.)
Provision of feed in the parlour (yes / no)
Availability of automated milk quantity measurement (yes / no)
Availability of milk quality indicators (yes / no)
Availability of milk flow indicators (yes / no)
System pulsation rate (p. min <sup>-1</sup> )
System pressure (kPa.)
System pulsation rate to pressure ratio
Type of flow line (low / high / other)
Frequency of check-ups of the system by farmer (description)
Annual frequency of check-ups of the system by technicians (no. of occasions)
Water cleaning of parlour after the milking sessions (yes / no)
Temperature of cleaning water (°C)
Use of detergent for parlour cleaning after the milking sessions (yes / no)
Frequency of changing teatcups (description)
Total number of wildlife species near the farm (description, no.)
Incidence of pregnancy toxemia during the preceding year

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Incidence of lameness during the preceding lactation period  
 Incidence of obstetrical problems during the preceding lactation period  
 Number of occasions of administration of anthelmintic drugs annually (no.)  
 Duration of grazing during the year (no. of months)  
 Provision of hay as fodder to animals (yes / no)  
 Average quantity of hay provided daily to animals during the preceding season (kg)  
 Provision of straw to animals (yes / no)  
 Provision of silage to adult animals (yes / no)  
 Provision of finished feed (concentrate) to adult animals (yes / no)  
 Provision of finished feed (concentrate) to adult animals throughout the year (yes / no)  
 Type of finished feed (concentrate) provided to adult animals (description)  
 Average quantity of finished feed (concentrate) provided daily to animals during the preceding season (kg)  
 Age of the farmer (years)  
 Length of animal farming experience of the farmer (years)  
 Level of education of the farmer (description)  
 Farmer by profession (yes / no)  
 Daily period of presence in the farm (hours)  
 Family tradition in farming (yes / no)  
 Presence of working staff in the flock (yes / no)

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**Table S3.** Details of multivariable models employed for the evaluation of predictors in a longitudinal study of subclinical mastitis among 12 sheep flocks in Greece monitored throughout a milking period.

Outcome	Variables Offered to The Multivariable Models ( <i>n</i> )	Variables Required in the Final Models
Incidence risk of subclinical mastitis	13	(a) Age of lamb removal from their dams, (b) Administration of selenium to pregnant ewes, (c) Vaccination against mastitis, (d) Years since initial establishment or most recent renovation of the milking parlour, (e) Type of flow line, (f) Frequency of changing teatcups, (g) Level of education of the farmer, (h) Presence of working staff in the flock
Incidence risk of staphylococcal subclinical mastitis	10	(a) Age of lamb removal from their dams, (b) Administration of selenium to pregnant ewes, (c) Vaccination against mastitis, (d) Years since initial establishment or most recent renovation of the milking parlour, (e) Frequency of changing teatcups, (f) Provision of finished feed (concentrate) to adult animals throughout the year, (g) Average quantity of finished feed (concentrate) provided daily to animals during the preceding season, (h) Level of education of the farmer, (i) Presence of working staff in the flock

**Table S4.** Incidence risk of subclinical mastitis, incidence risk of staphylococcal subclinical mastitis and recurrence risk of recurrence of subclinical mastitis among 12 sheep flocks in Greece monitored throughout a milking period.

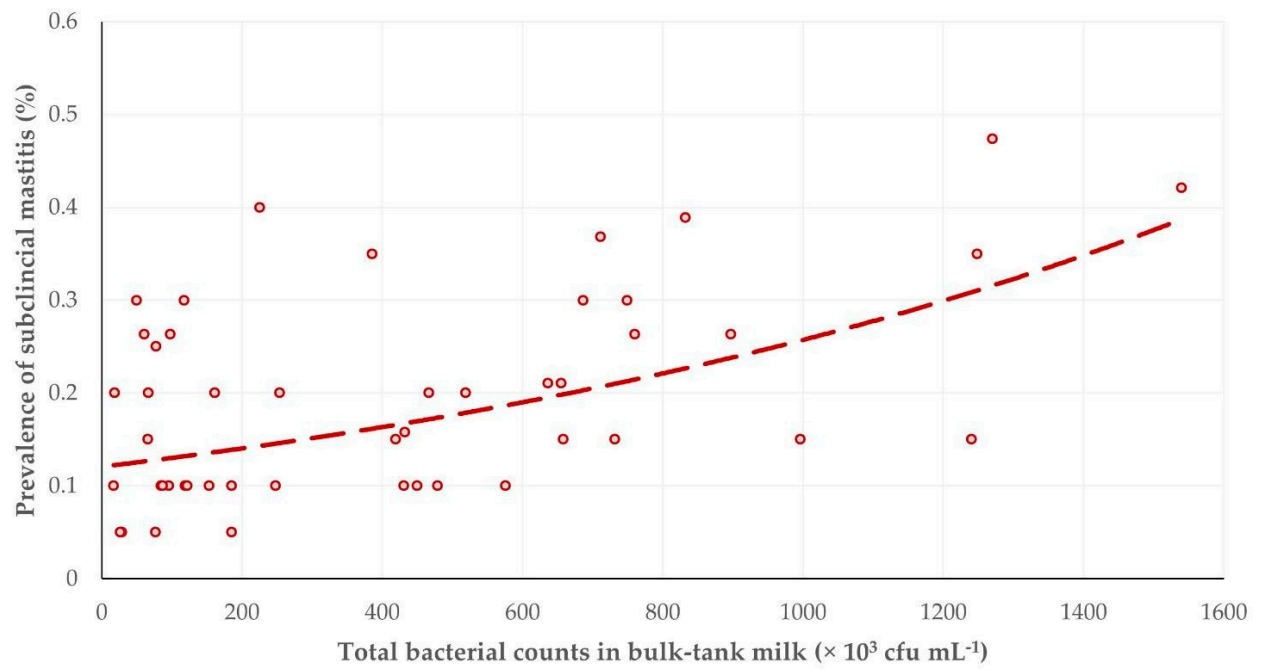
<b>Farm</b>	<b>Incidence Risk of Subclinical Mastitis</b>	<b>Incidence Risk of Staphylo-coccal Subclinical Mastitis</b>	<b>Incidence Risk of Recurrence of Subclinical Mastitis</b>
1	50.0%	50.0%	23.1%
2	65.0%	65.0%	42.9%
3	35.0%	35.0%	28.6%
4	25.0%	25.0%	33.3%
5	25.0%	25.0%	20.0%
6	75.0%	60.0%	25.0%
7	55.0%	50.0%	45.5%
8	25.0%	25.0%	44.4%
9	60.0%	55.0%	42.9%
10	65.0%	55.0%	75.0%
11	75.0%	75.0%	22.2%
12	65.0%	65.0%	44.4%

**Table S5.** Frequency of isolation of bacteria from cases of bacterial carriage among 12 sheep flocks in Greece monitored throughout a milking period.

<b>Bacterial Identity</b>	<b>No. (proportion) of Bacterial Isolates</b>
<i>Bacillus</i> spp.	4 (2.3%)
<i>Corynebacterium</i> spp.	2 (1.1%)
<i>Escherichia coli</i>	2 (1.1%)
<i>Micrococuss</i> sp.	1 (0.6%)
non-aureus staphylococci	148 (84.1%)
<i>Streptococcus</i> spp.	18 (10.2%)
<i>Trueperella</i> sp.	1 (0.6%)
Total	176

**Table S6.** Results of biofilm-formation by staphylococcal isolates from cases of subclinical mastitis or bacterial carriage among 12 sheep flocks in Greece monitored throughout a milking period.

Bacterial Identity	No. of Bacterial Isolates	No. (Proportion) of Biofilm-forming Isolates
Isolates from cases of subclinical mastitis		
<i>S. aureus</i>	38	32 (84.2%)
<i>S. capitis</i>	2	1 (50.0%)
<i>S. caprae</i>	6	3 (50.0%)
<i>S. chromogenes</i>	24	18 (75.0%)
<i>S. epidermidis</i>	28	11 (39.3%)
<i>S. equorum</i>	4	2 (50.0%)
<i>S. haemolyticus</i>	4	2 (50.0%)
<i>S. hominis</i>	5	4 (80.0%)
<i>S. lentus</i>	9	3 (33.3%)
<i>S. saprophyticus</i>	1	1 (100.0%)
<i>S. schleiferi</i>	1	0 (0.0%)
<i>S. sciuri</i>	3	1 (33.3%)
<i>S. simulans</i>	34	26 (76.5%)
<i>S. warneri</i>	2	1 (50.0%)
<i>S. xylosus</i>	18	12 (66.7%)
Total	179	117 (65.4%)
Isolates from cases of bacterial carriage		
non-aureus staphylococci	148	52
Total	148	52 (35.1%)
Grand total	327	169 (51.7%)



**Figure S1.** Scatter-plot of prevalence of subclinical mastitis and total bacterial counts in bulk-tank milk in 12 sheep flocks in Greece monitored throughout a milking period (dashed line is trendline).

**Table S7.** Results of biofilm-formation by staphylococcal isolates from bulk-tank milk among 12 sheep flocks in Greece monitored throughout a milking period.

Bacterial Identity	No. of Bacterial Isolates	No. (Proportion) of Biofilm-forming Isolates
Isolates from cases of subclinical mastitis		
<i>S. aureus</i>	9	7 (77.8%)
<i>S. carnosus</i>	1	0 (0.0%)
<i>S. chromogenes</i>	1	1 (100.0%)
<i>S. equorum</i>	2	0 (0.0%)
<i>S. haemolyticus</i>	2	1 (100.0%)
<i>S. lugdunensis</i>	2	0 (0.0%)
<i>S. pasteurii</i>	1	1 (100.0%)
<i>S. simulans</i>	5	4 (80.0%)
<i>S. xylosus</i>	3	1 (33.3%)
Total	26	15 (57.7%)



**Table S8.** Results (Spearman's rank correlation coefficients) of univariable analysis of variables ( $n = 67$ ) evaluated for association with the outcomes 'incidence risk of subclinical mastitis' and 'incidence risk of staphylococcal subclinical mastitis' among 12 sheep flocks in Greece monitored throughout a milking period.

Subclinical Mastitis			Staphylococcal Subclinical Mastitis	
Variables	Correlation Coefficient ( $r_{sp}$ )	$p$ -Value	Correlation Coefficient ( $r_{sp}$ )	$p$ -Value
Management system applied in the flocks	-0.078	0.81	-0.016	0.96
Availability of a waiting area before the milking parlour	n/r	n/r	n/r	n/r
Material of the floor of the barn	n/r	n/r	n/r	n/r
Availability of straw bedding	-0.399	<0.20	-0.398	>0.20
Annual frequency of removal / clean-up of the straw bedding	0.313	0.32	0.182	0.57
Grazing land available to animals	-0.336	0.29	-0.305	0.34
No. of ewes in the flock	0.248	0.44	0.195	0.54
Breed of ewes	0.015	0.96	-0.131	0.69
Average age of culling females	-0.340	0.28	-0.333	0.29
Month of the start of the lambing season	0.165	0.61	0.242	0.45
Total milk quantity per ewe obtained during the preceding milking period	0.284	0.37	0.145	0.65
Average number of lambs born per ewe	0.005	0.99	0.170	0.60
Total visits made annually by veterinarians to the flock during the preceding season	0.061	0.85	0.045	0.89
Use of laboratory diagnostic examinations in samples of milk preventively	0.149	0.64	-0.050	0.88
Age of lamb removal from their dams	-0.577	0.049	-0.656	0.021
Daily number of milking sessions	0.170	0.6	0.113	0.73
Duration of the dry-period	-0.035	0.91	0.012	0.97
Administration of selenium to pregnant ewe	0.494	0.10	0.426	0.17
Administration of 'dry-ewe' treatment at the end of the lactation period	0.049	0.88	0.147	0.65
Use of teat disinfection after milking	-0.296	0.35	-0.295	0.35
Antibiotics used for treatment of clinical mastitis	-0.110	0.73	-0.115	0.72
Route for administration of antimicrobials	0.115	0.72	0.044	0.89
Vaccination against mastitis	0.567	0.05	0.593	0.042
Annual frequency of systemic disinfections in the farm	-0.135	0.68	-0.177	0.58
Years since initial establishment or most recent renovation of the milking parlour	-0.539	0.07	-0.436	0.16
Volume of the parlour	0.299	0.35	0.204	0.53
Material of the floor of the milking parlour	0.177	0.58	0.135	0.68
Type of milking parlour	n/r	n/r	n/r	n/r
Type of milking system	-0.399	< 0.20	-0.398	> 0.20
Number of animal positions in the parlour	0.261	0.41	0.214	0.50
Number of available milking units per animal position	0.319	0.27	0.305	0.33
Provision of feed in the parlour	0.057	0.86	-0.056	0.86
Availability of automated milk quantity measurement	n/r	n/r	n/r	n/r
Availability of milk quality indicators	n/r	n/r	n/r	n/r
Availability of milk flow indicators	-0.222	0.49	-0.088	0.78
System pulsation rate	-0.468	0.12	-0.389	0.21
System pressure	-0.268	0.40	-0.237	0.46
System pulsation rate to pressure ratio	-0.073	0.82	0.010	0.98
Type of flow line	0.708	0.010	0.735	0.006

Frequency of check-ups of the system by farmer	0.112	0.73	0.261	0.41
Annual frequency of check-ups of the system by technicians	-0.163	0.61	-0.143	0.66
Water cleaning of parlour after the milking sessions	n/r	n/r	n/r	n/r
Temperature of cleaning water	-0.011	0.97	-0.091	0.79
Use of detergent for parlour cleaning after the milking sessions	n/r	n/r	n/r	n/r
Frequency of changing teatcups	0.470	0.12	0.579	0.049
Total number of wildlife species near the farm	-0.340	0.28	-0.266	0.40
Annual incidence of pregnancy toxemia during the preceding lactation period	-0.269	0.40	-0.170	0.60
Annual incidence of lameness during the preceding lactation period	-0.197	0.54	-0.284	0.37
Annual incidence of obstetrical problems during the preceding lactation period	-0.452	0.14	-0.363	0.25
Number of occasions of administration of anthelmintic drugs annually	-0.107	0.74	-0.046	0.89
Duration of grazing during the year	-0.195	0.54	-0.195	0.54
Provision of hay as fodder to animals	n/r	n/r	n/r	n/r
Average quantity of hay provided daily to animals during the preceding season	0.156	0.63	0.029	0.93
Provision of straw to animals	-0.395	> 0.20	-0.361	0.25
Provision of silage to adult animals	-0.028	0.93	-0.113	0.73
Provision of finished feed (concentrate) to adult animals	n/r	n/r	n/r	n/r
Provision of finished feed (concentrate) to adult animals throughout the year	0.567	0.05	0.762	0.004
Type of finished feed (concentrate) provided to adult animals	-0.186	0.56	-0.343	0.28
Average quantity of finished feed (concentrate) provided daily to animals during the preceding season	0.392	0.21	0.416	0.18
Age of the farmer	-0.173	0.59	-0.179	0.58
Length of animal farming experience of the farmer	-0.305	0.34	-0.185	0.57
Level of education of the farmer	-0.766	0.004	-0.718	0.008
Farmer by profession	0.222	0.49	0.354	0.26
Daily period of presence in the farm	-0.032	0.92	-0.029	0.93
Family tradition in farming	0.296	0.35	0.295	0.35
Presence of working staff in the flock	0.676	0.016	0.648	0.023

**Table S9.** Published papers derived from PhD theses cited in the reference list of the main text, to which readers may refer for specific information regarding the citations in the text.

PhD Theses Cited in the Reference List of the Main Text	Published Papers Derived from These Theses
	-D.T. Lianou et al. (2020). A detailed questionnaire for the evaluation of health management in dairy sheep and goats. <i>Animals</i> , 10:1489.
Lianou, D.T. <i>Mapping the Small Ruminant Industry in Greece: Health Management and Diseases of Animals, Preventive Veterinary Medicine and Therapeutics, Reproductive Performance, Production Outcomes, Veterinary Public Health, Socio-demographic Characteristics of the Farmers.</i> PhD thesis, University of Thessaly, Volos, Greece, 2023.	-D.T. Lianou, G.C. Fthenakis (2021). Dairy sheep and goat farmers: socio- demographic characteristics and their associations with health management and performance on farms. <i>Land</i> , 12:1358. -D.T. Lianou et al. (2021). Extensive countrywide field investigation of somatic cell counts and total bacterial counts in bulk-tank raw milk in sheep flocks in Greece. <i>Foods</i> , 10:268.
	-D.T. Lianou, G.C. Fthenakis (2022). Use of antibiotics against bacterial infections on dairy sheep and goat farms: patterns of usage and associations with health management and human resources. <i>Antibiotics</i> , 11:753.
	-D.T. Lianou et al. (2023). Data on mapping 444 dairy small ruminant farms during a countrywide investigation performed in Greece. <i>Animals</i> , 13:2044
Vasileiou, N.G.C. <i>Mastitis in Ewes Associated with Staphylococcus spp.: New Clinical, Epidemiological, Management, Microbiological, and Zoonotic findings and Evaluation of a Novel Vaccine Against the Disease.</i> PhD thesis, University of Thessaly, Volos, Greece, 2019	-N.G.C. Vasileiou et al. (2018) Extensive countrywide field investigation of subclinical mastitis in sheep in Greece. <i>Journal of Dairy Science</i> , 101:7297–7310. -N.G.C. Vasileiou et al. (2018) Slime-producing staphylococci as causal agents of subclinical mastitis in sheep. <i>Veterinary Microbiology</i> , 224:93–99. -N.G.C. Vasileiou et al. (2019) Evaluation of efficacy of a biofilm-embedded bacteria-based vaccine against staphylococcal mastitis in sheep - a randomized, placebo-controlled field study. <i>Journal of Dairy Science</i> , 102:9328-9344. -N.G.C. Vasileiou et al. (2019) Experimental study for evaluation of the efficacy of a biofilm-embedded bacteria-based vaccine against <i>Staphylococcus chromogenes</i> -associated mastitis in sheep. <i>Veterinary Microbiology</i> , 239:108480.