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Session 65.2



Milking order of healthy and subclinically mammary infected dairy ewes in mid lactation

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Milking order: Feasible Early Warning System (EWS) for mastitis detection in dairy ewes?.

Hypothesis:

- **Healthy and high welfare ranked ewes come earlier** to milking parlor (rewarded by oxytocin and concentrate).
- **Sick (mastitic) animals will come on delay** (malaise).

But, mastitis in small ruminants has a...

- Low prevalence for **clinical** (<5% on whole lactation).
- High prevalence for **subclinical** (>30%, mainly CNS).
- **Controversial link with SCC (>500·10³ cells/mL?).**
- Udder morphology and imaging (IR) show **past mastitis**.
- **IMI** (intramammary infection) needs to be assessed by **bacterial culture** (>3-5 CFU?).



Materials & Methods:

- UAB experimental farm: Lambing Sept 2022.
- **112 dairy ewes** (34 Manchega and 78 Lacaune) grazing and supplemented indoors with TMR.
- Milked twice daily (0700 and 1730 h) from parturition.
- Experimental period: **52 d in mid lactation (88 to 140 DIM)**.
- 2 x 12 milking parlor (DeLaval) with 12 electronic milk meters (MM25SG).
- Ewe e-ID with ceramic boluses (22 g, Datamars, ES) and 2 reading tunnels with ISO readers (DeLaval, SE).
- **Milking order and yield at each milking** (Alpro, DeLaval).
- **Milk composition and SCC** (ALLIC laboratory, ES) **by month**.
- **Bacterial culture on next week when $SCC > 500 \cdot 10^3$ cells**



Materials & Methods: Flock and milk recording

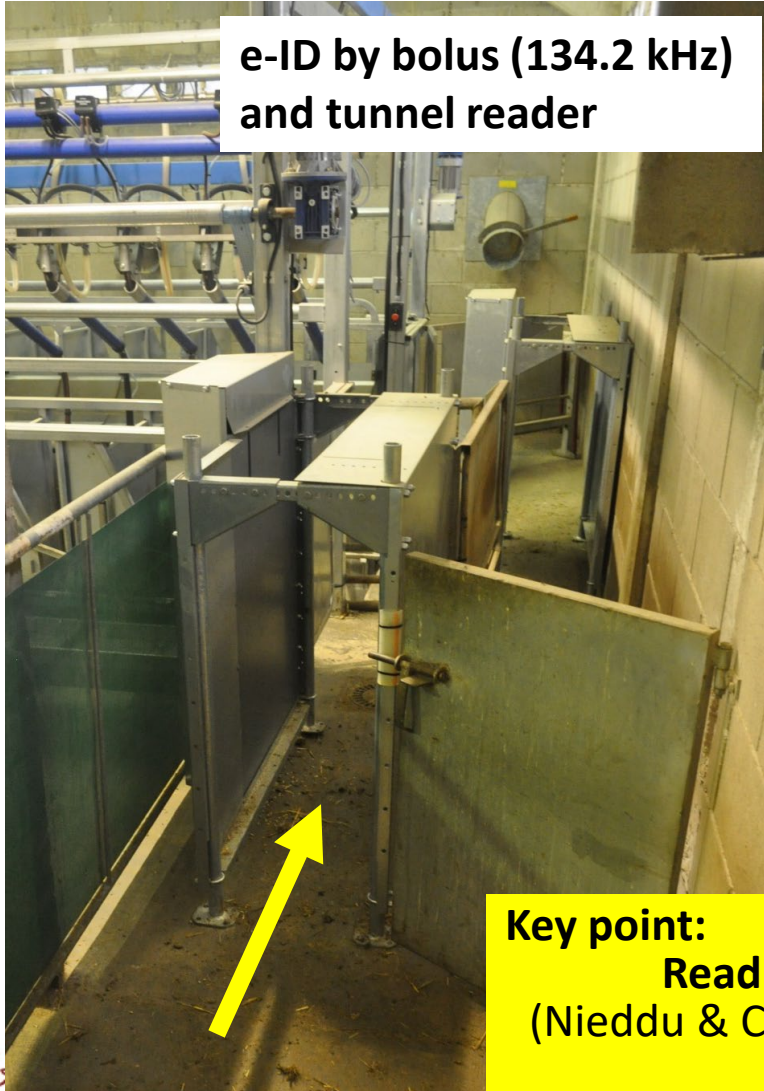


Milk sampling for
**composition and
SCC analyses**

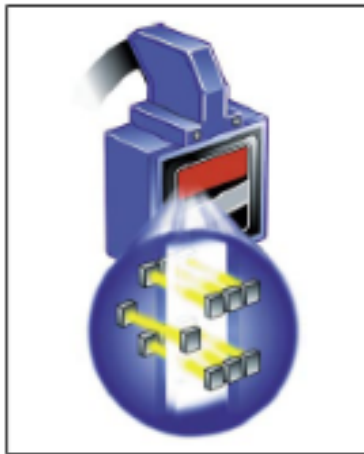


Materials & Methods:

Low frequency e-ID reading system and milk meters



Key point:
Reading efficiency 96-99%
(Nieddu & Caja, 2017) line errors.
Manual correction?



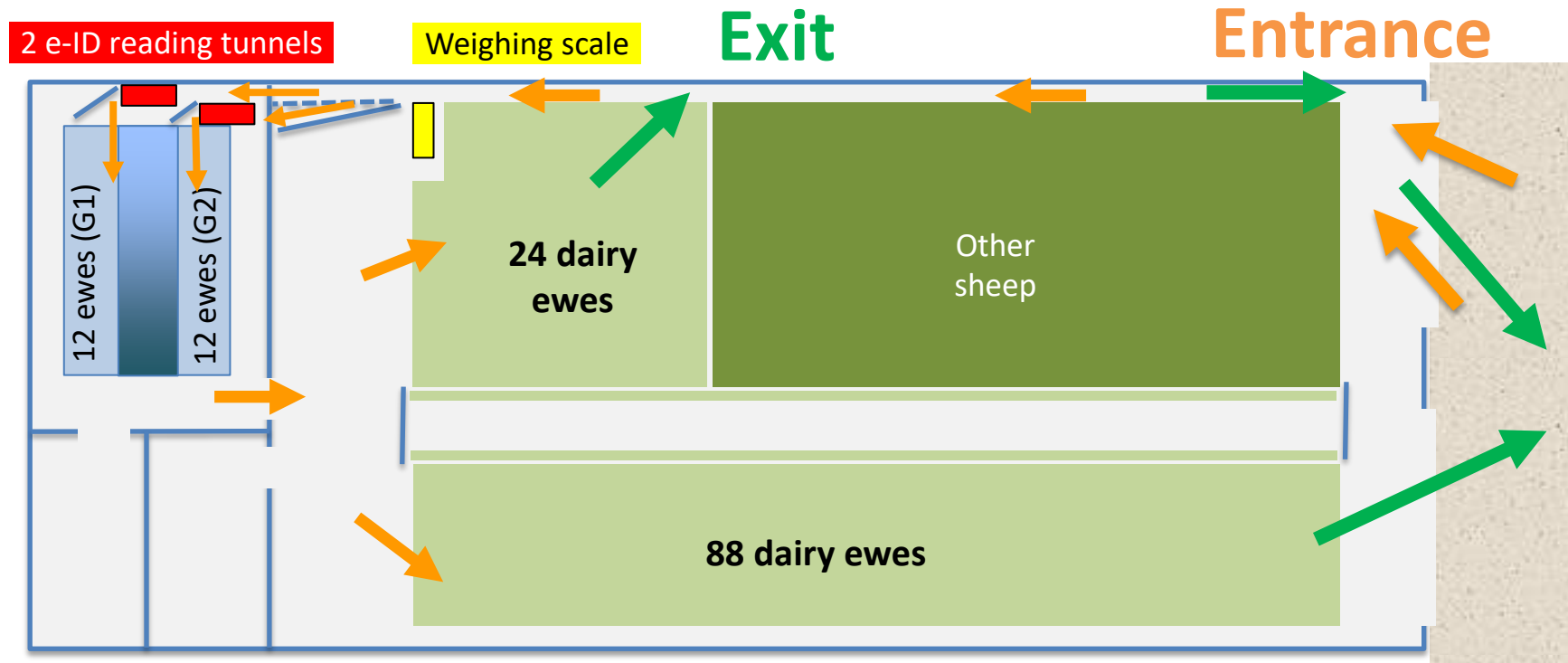
Materials & Methods: Sampling for bacterial culture

Milk sampling
for **bacterial**
culture
(**blood-agar**)
at 37°C (24
and 48 h)



Materials & Methods: Ewe's management

- Semi-intensive: 6 h/d grazing + TMR (F:C = 60:40) + 0.1 kg concentrate at each milking.
- **Joined as an only flock** for grazing (6 h/d) and milking (2x).
- Free entrance to milking and penned at random during the night.



Materials & Methods: Statistical analysis

- Use of **R Core Team (2021)** packages.
- Association of quantitative variables (milk yield, milk composition, SCC, BW) by Spearman correlations.
- Ordinal variables (milking order, IMI, parity, breed) medians compared by Wilcoxon Mann-Whitney test.
- Milking order consistency by intraclass correlations.
- Milking order SD assessed by non-linear segmented regression (“splines”)
- Significance declared at $P < 0.05$



Results: Reading efficiency and lactational data

- Dynamic reading efficiency (52 d): DRE = 11,403/11,648 = **97.9%**
- Milk yield and composition in mid lactation (n = 112 ewes):

Time (DIM)	Breed	Yield (L/d)	SCC (x10 ³)	Log ₁₀ SCC	Composition (%)			Urea (mg/L)	Mean order
					Fat	Protein	Lactose		
1 (d 110)	MN	0.50	389	4.90	9.44	6.84	4.53	327	70
	LC	0.89	1,622	5.73	7.16	5.92	4.57	353	58
	Mean	0.77	1,248	5.48	7.85	6.20	4.55	345	61
	±SE	±0.04	±210	±0.08	±0.14	±0.07	±0.04	±8	±7



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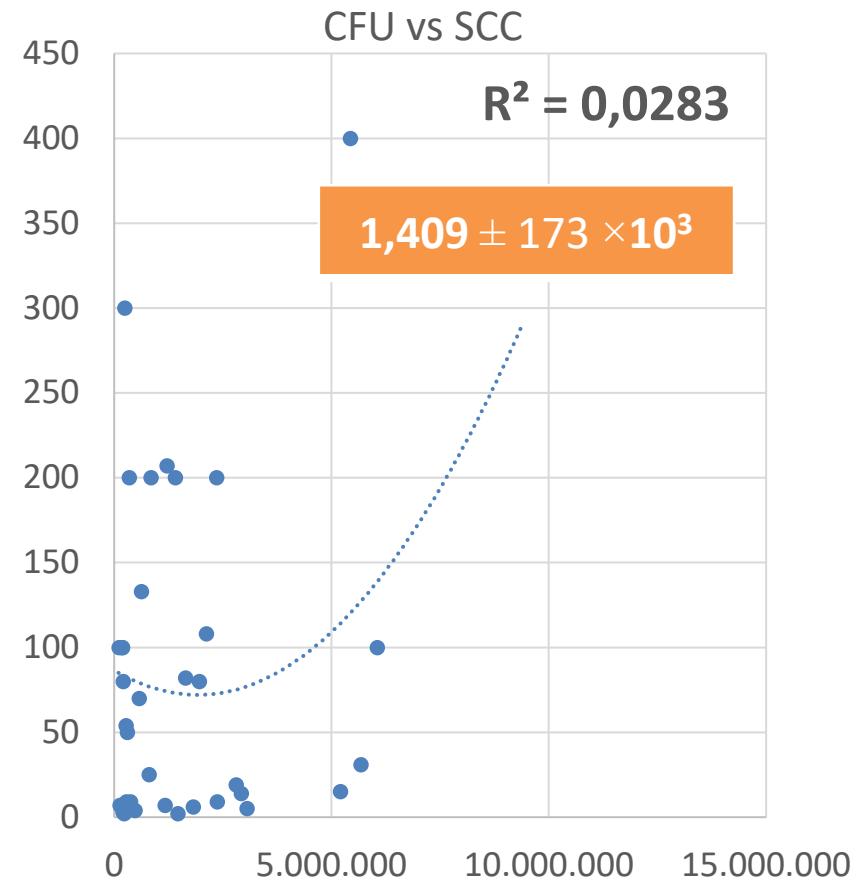
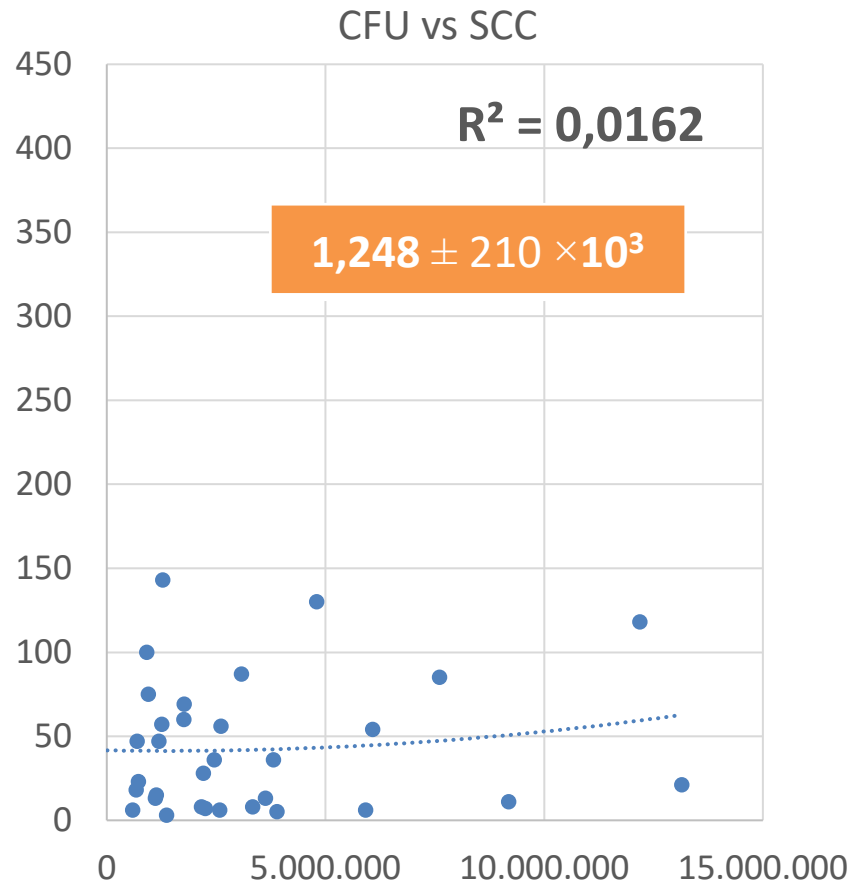
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	±SE	±0.04	±210	±0.08	±0.14	±0.07	±0.04	±8	±7
C2 (d 140)	MN	0.49	799	5.69	9.24	6.82	4.64	670	71
	LC	0.89	1,674	5.94	6.91	5.94	4.55	585	42
	Mean	0.74	1,409	5.87	7.62	6.21	4.58	611	58
	±SE	±0.13	±173	±0.05	±0.13	±0.07	±0.04	±9	±3



Results: Mammary infection and SCC value

IMI (Bacterial culture) and SCC (n = 112 ewes):

- Milk recording C1 (110 DIM):
- Milk recording C2 (140 DIM):

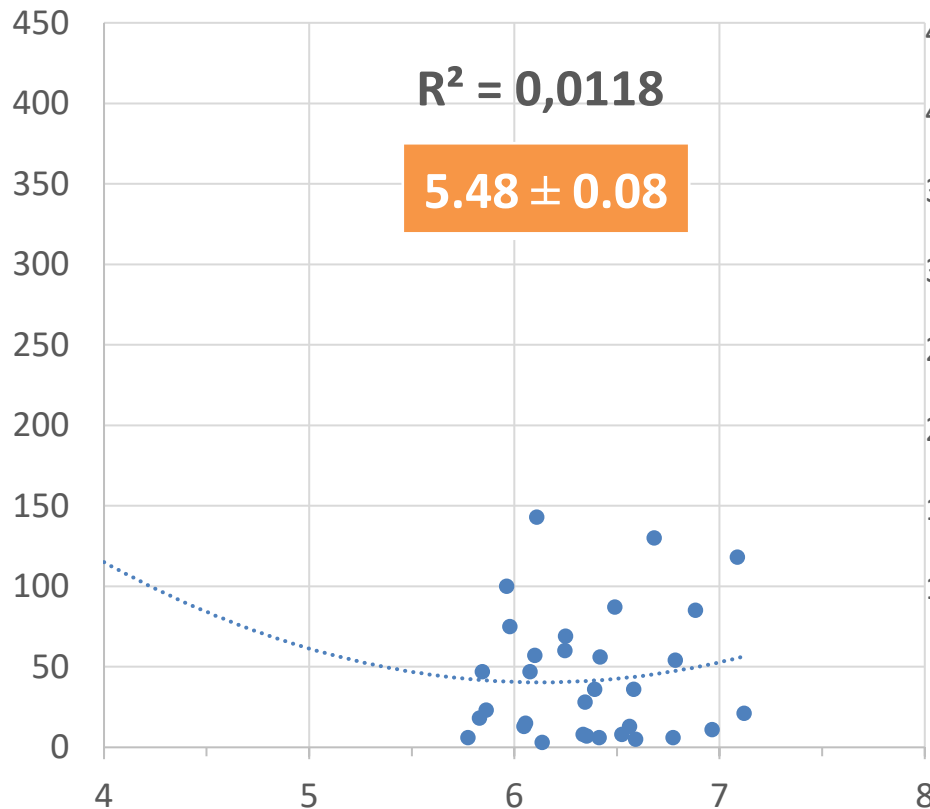


Results: Mammary infection and Log₁₀ SCC

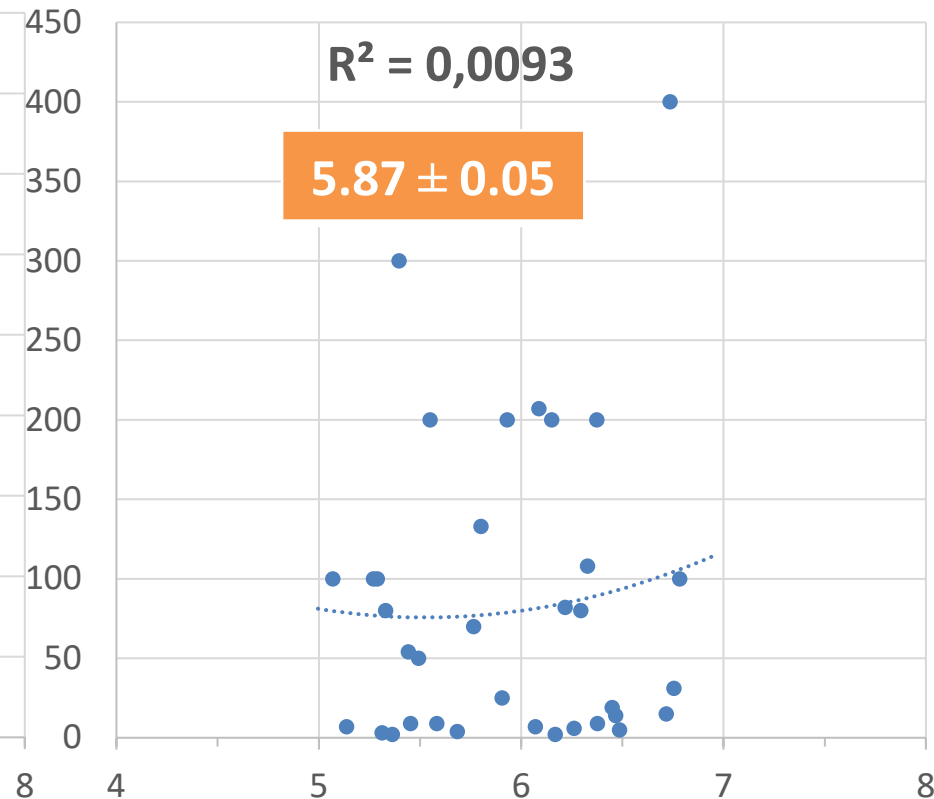
IMI (Bacterial culture) and Log SCC (n = 112 ewes):

- Milk recording C1 (110 DIM):
- Milk recording C2 (140 DIM):

CFU vs SCC



CFU vs Log SCC



Results: Mammary infected ewes (subclinically sick)

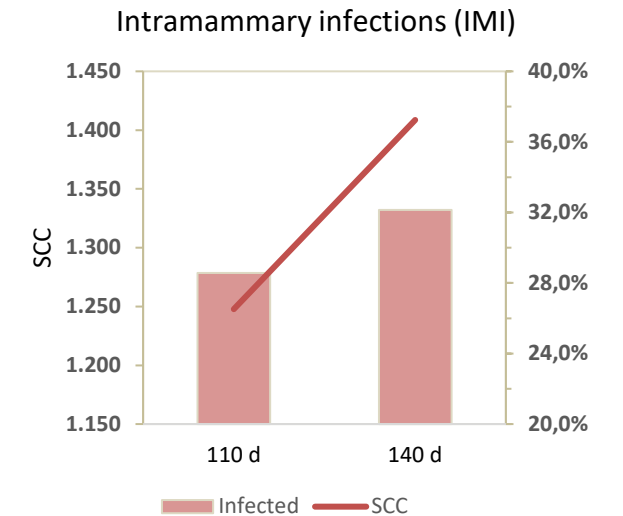
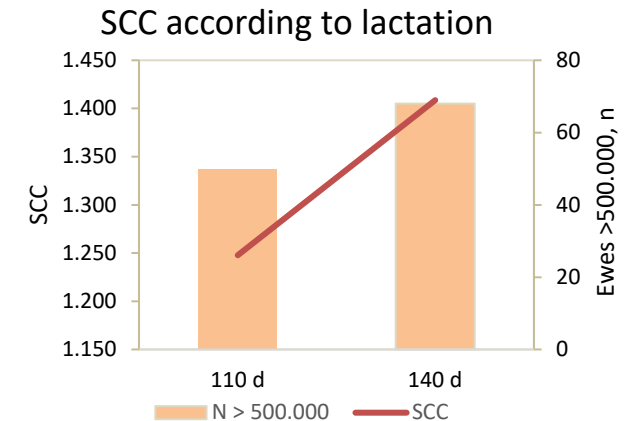
- Suspected mastitic ewes (subclinical): >500.000 cells/mL

Item	C1 (d 110)	C2 (d 140)	Change
Ewes	112	112	-
SCC, x10 ³ cells/mL	1,248	1,409	161
Log10 SCC	5.48	5.87	0.39
Ewes >500, n (%)	50 (44.6)	68 (60.7)	18 (16.1)
Infected ewes >5 CFU	32 (28.6)	36 (32.1)	4 (3.5)
Sensitivity ¹ , %	64.0	52.9	-11.1
Healed, n (%)		7 (21.9)	
New infections, n (%)		10 (27.8)	

¹Sensitivity, % = True positive/Sick individuals x100

Staphylococcus aureus

CNS (Coagulase negative staphylococcus)

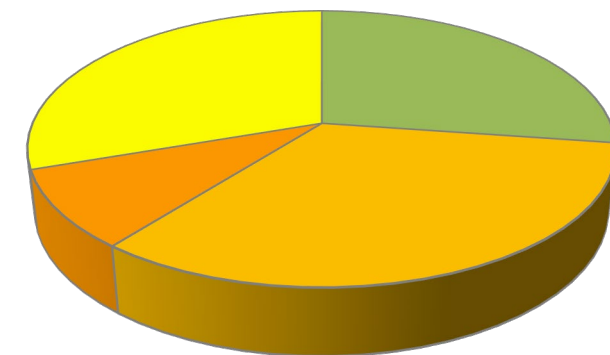


Results: Mammary infected udder halves

- Confirmed mastitic glands (subclinical): >499.000 cells/mL

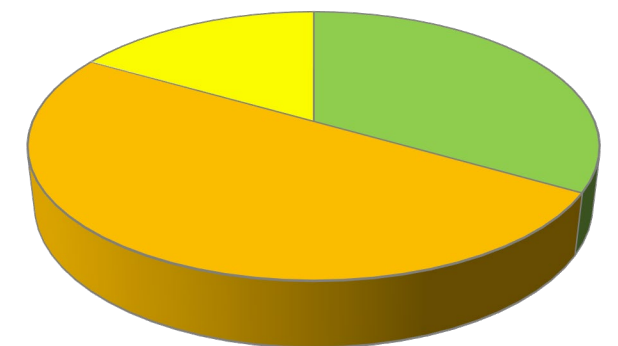
Item	C1 (d 110)	C2 (d 140)	Change
Udder halves, n	66	66	-
Healthy, n	18 (27%)	22 (33%)	4 (6)
CNS, n	22 (33%)	33 (50%)	11 (17)
CPS, n	6 (9%)	0 (0%)	-6 (-9)
<i>S. aureus</i>, 20	20 (30%)	11 (17%)	9 (-13)
IMI, n	48 (73%)	44 (67%)	-4 (-6)

C1 (d 110)



■ Healthy ■ CNS ■ CPS ■ *S. aureus*

C2 (d 140)



■ Healthy ■ CNS ■ *S. aureus* 14

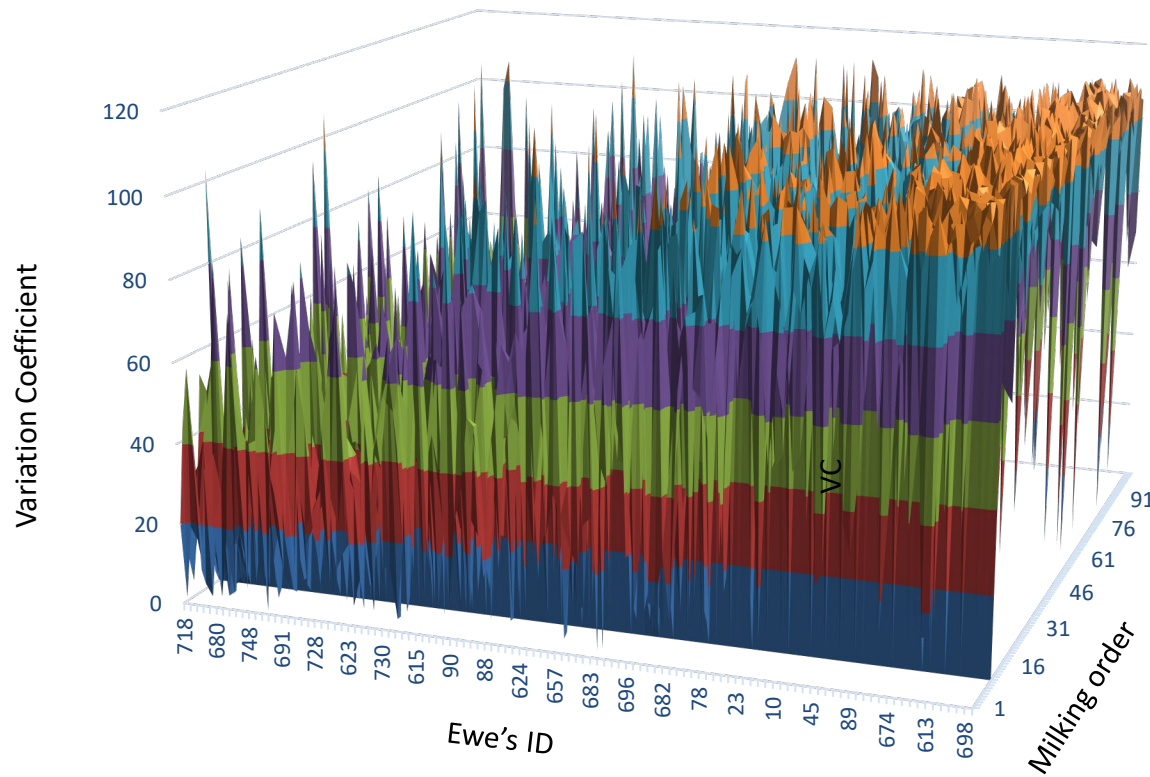
Staphylococcus aureus

CNS (*Coagulase negative staphylococcus*)



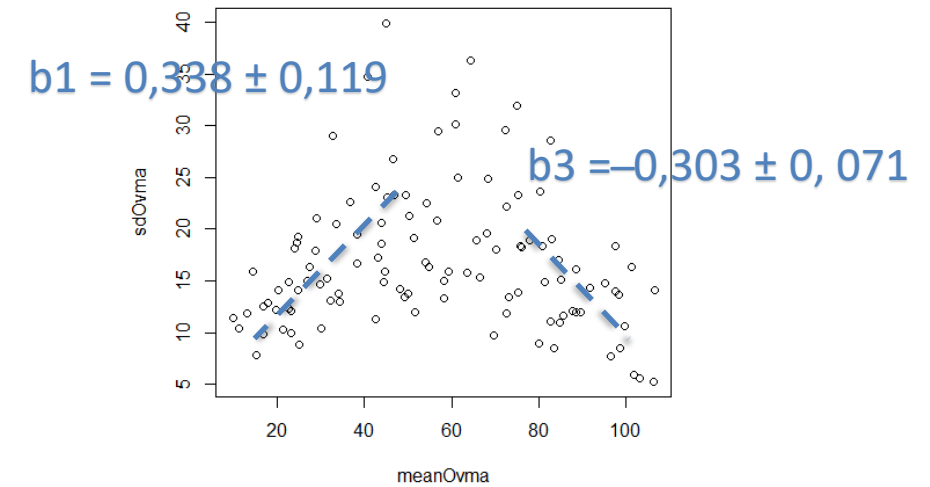
Results: Milking order consistency

VC and SD coefficients (%) of dairy ewes' milking order (n = 112)

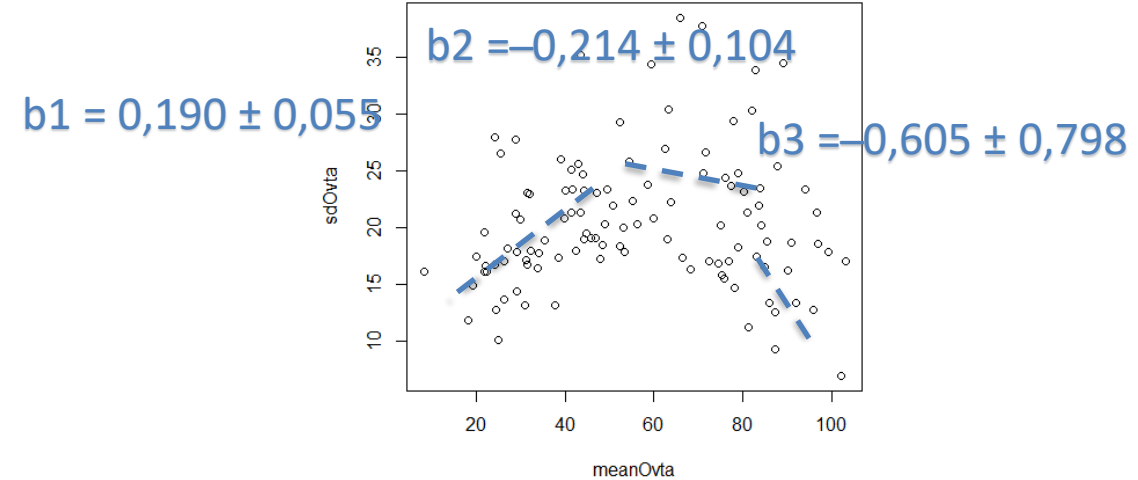


Mean milking order

Segmented regression (Spline)



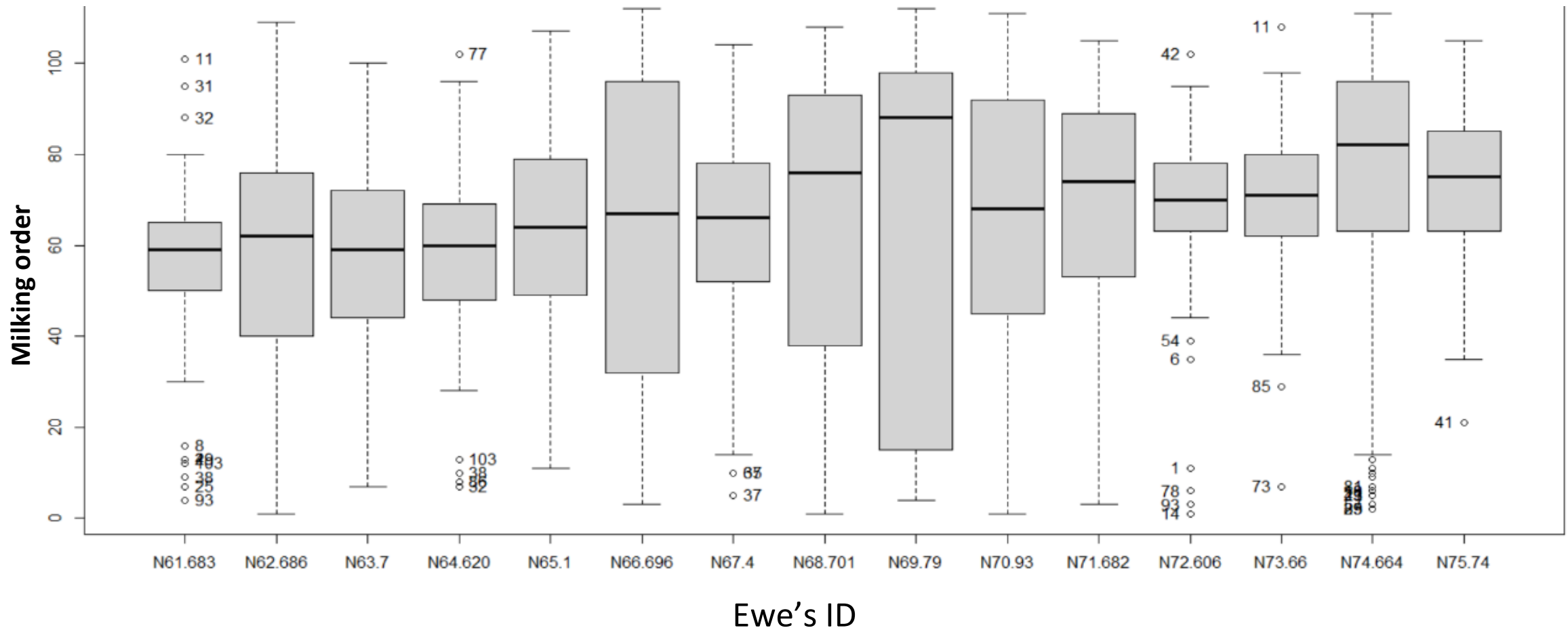
Morning



Afternoon

Results: Milking order consistency as EWS

- Consistency of milking order: Box-Plot outliers



Results: Milking order Spearman correlations

- Spearman correlations with milking order: 112 ewes

Item	C1 (d 110)		C2 (d 140)	
	Rho	<i>P</i> value	Rho	<i>P</i> value
Milk yield, L/d	– 0.07	0.44	– 0.17	0.07
Log ₁₀ SCC	– 0.01	0.91	0.15	0.10
Fat, %	0.25	0.007	0.26	0.005
Protein, %	0.21	0.03	0.24	0.01
Lactose, %	– 0.26	0.006	– 0.31	0.001
Urea, %	0.09	0.32	0.24	0.01
Body weight, kg	0.40	0.001	0.47	0.001



Results: Milking order Spearman correlations

- Spearman correlations with milking order by breed: **Manchega (n = 34)**

Item	C1 (d 110)		C2 (d 140)	
	Rho	<i>P</i> value	Rho	<i>P</i> value
Milk yield, L/d	0.19	0.28	– 0.03	0.85
Log ₁₀ SCC	– 0.06	0.71	0.05	0.79
Fat, %	0.20	0.26	0.09	0.43
Protein, %	0.10	0.59	0.31	0.08
Lactose, %	0.10	0.56	– 0.06	0.76
Urea, %	– 0.02	0.90	0.33	0.06
Body weight, kg	0.10	0.60	0.17	0.33



Results: Milking order Spearman correlations

- Spearman correlations with milking order by breed: **Lacaune (n = 78)**

Item	C1 (d 110)		C2 (d 140)	
	Rho	<i>P</i> value	Rho	<i>P</i> value
Milk yield, L/d	0.03	0.79	– 0.08	0.50
Log₁₀ SCC	0.24	0.04	0.31	0.006
Fat, %	0.11	0.36	0.18	0.31
Protein, %	0.15	0.20	0.14	0.22
Lactose, %	– 0.36	0.001	– 0.40	0.001
Urea, %	0.21	0.06	0.07	0.50
Body weight, kg	0.50	0.001	0.54	0.001



Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: 112 ewes

Item	W	P value	Median values	
C1 (d 110):				
Bacterial culture	254	0.49	IMI – = 56	IMI + = 55
Breed	927	0.016	MN = 71	LC = 50
Parturition nb.	1,666	0.001	Primp = 31	Multp = 64
C2 (d 140):				
Bacterial culture	306	0.021	IMI – = 44	IMI + = 69
Breed	936	0.014	MN = 70	LC = 50
Parturition nb.	1,745	0.001	Primp = 33	Multp = 65



Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: **Manchega (n= 34)**

Item	W	P value	Median values	
C1 (d 110):				
Bacterial culture	58	0.94	-	-
Parturition nb.	-	-	-	-
C2 (d 140):				
Bacterial culture	29	0.30	-	-
Parturition nb.	-	-	-	-



Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: **Lacaune (n = 78)**

Item	W	P value	Median values	
C1 (d 110):				
Bacterial culture	692	0.93	-	-
Parturition nb.	960	0.004	Primp = 31	Multp = 56
C2 (d 140):				
Bacterial culture	366	0.001	IMI – = 35	IMI + = 68
Parturition nb.	1,020	0.001	Primp = 33	Multp = 58



Conclusions: Is milking order feasible as EWS for mastitis in dairy ewes?

1) **SCC has not enough sensitivity** for subclinically infected sheep udders.

2) **Milking order:**

- Was efficiently recorded by **low RFID boluses and ISO readers** (134.2 kHz).
- Was **repeatable** with higher correlations for the earlier and later milking groups.
- **Does not discriminate subclinically IMI ewes.**
- Depends on **BW, breed** milkability and parity.
- **Last** entering ewes have **worst udder health.**

3) **Other indicators should be used as mastitis EWS.**



Thank you for attention!

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Integrating innovative TECHNOLOGIES along the value Chain
to improve small ruminant welfare management

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