QUALITY MILK
PRODUCTION SERVICES

Microbes, Machines, Management, and Mastitis

An approach from barn to bulk tank

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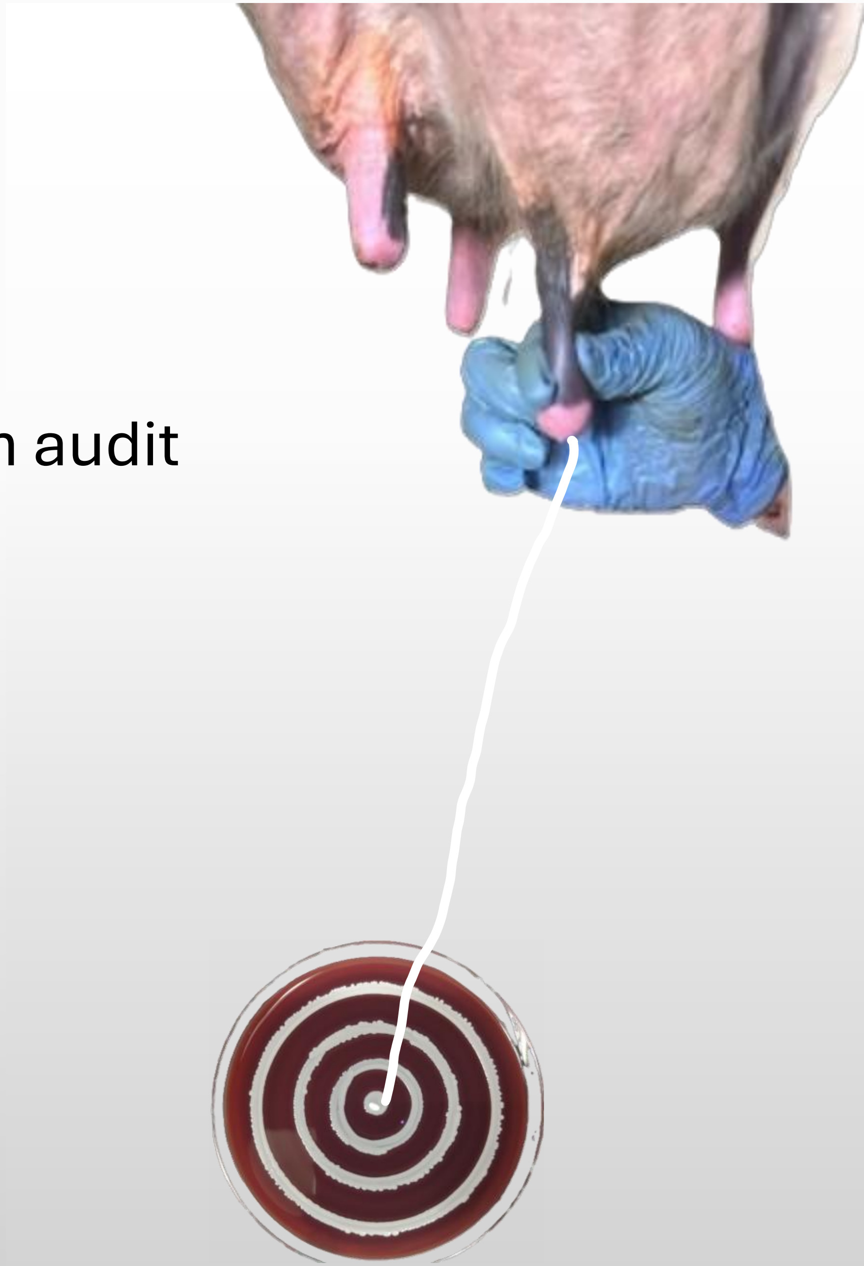
Objectives

- Overview and case study of milking center and farm audit
- How we evaluate equipment, people, and cows
- Case study on *Prototheca*
- Case study on *Strep uberis*



Strategies for Success

Milking Center and Farm Audit Program

- Identify the key players in your milk quality team:
  - Utilize each in their area of expertise
  - Encourage communication and data sharing between parties
  - Hold farm meetings of all parties to
    - Interpret all the data together
    - Identify specific opportunity areas for your farm

Goals of a Milking Center and Farm Audit Program

- Provide each individual herd and their milk quality team a robust set of data that can be used to identify opportunity areas to improve milk quality
- Clarify if these opportunity areas are at the:
  - Whole system level
  - Individual personnel management level
  - Combination of both
- After a change is made, re-assess all the relevant areas to document the effects of the change

Goals of a Milking Center and Farm Audit Program

- Repeat this data collection every 3-4 months to:
  - Develop a trend-line of the data
  - Prevent issues
  - Promote continuous improvement
  - Assess any changes that have been made

Why do we need scoring systems?

- Gives us a true picture of what is happening at the cow level that impacts the risk of mastitis
  - If these scoring systems are performed correctly and consistently, they can be an objective measurement

- Helps to identify opportunity areas
  - Is there a problem with the whole system or with an individual person in the system?

What do we measure?

- Average claw vacuum at peak flow
- Pulsation under load
- Milkline vacuum for 30 minutes
- Unit alignment scoring
- Milking routine timing
- Milk flow rate analysis
- Milking efficiency and throughput timing
- Strip yields
- Dry-off procedure audit
- Teat scoring
- Teat-end cleanliness
- Udder cleanliness
- Environmental assessment

Equipment/Machines

People/Management

Cows and Microbes

Perform full NMC equipment evaluation every 6 months

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Parlor and Farm Audit
March 3, 2026

- Katie Keller, QMPS-Cobleskill
- Kaitlyn McFarlan, The Ohio State University
- Mike Zurakowski, QMPS-Cobleskill

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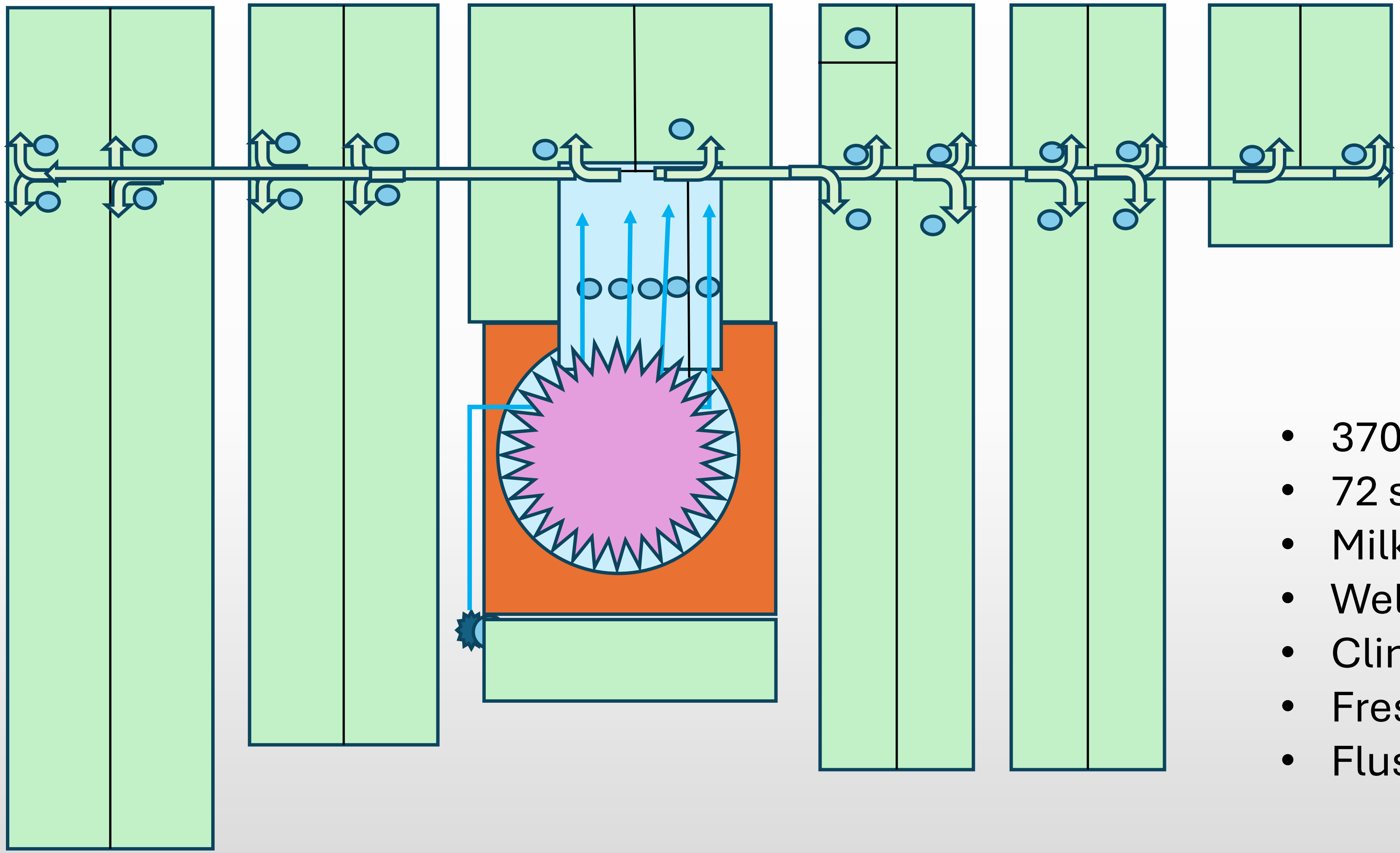
Case Report: Project Farm

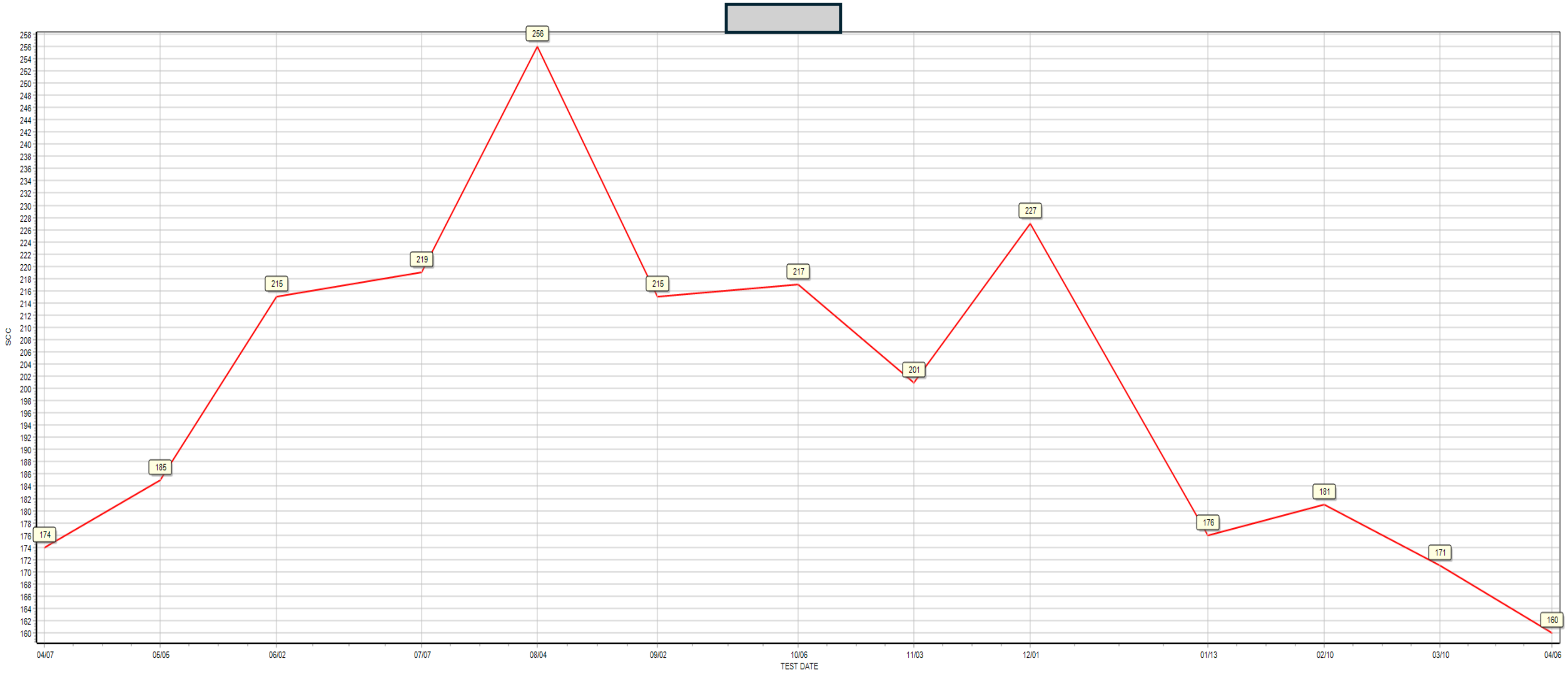


- 3700 cow dairy
- 72 stall rotary parlor
- Milking 3x
- Well managed
- Clinical mastitis cultures
- Fresh cow cultures
- Flush water system

General Summary

- Your records show that bulk tank SCC is trending downward. Percent chronic infections is still above goal. High fresh and dry cow new infection risk are meeting the goals.
- *Strep uberis* continues to be the dominant mastitis pathogen.
- Many teat ends are firm when the milking unit is removed but the goal of less than 20% is achieved. **Teat-end scores continue to not meet the goal with 35% of teats score slightly to very rough.**
- Lag time from stimulation to unit attachment continues to meet the goal.
- **Two minute milk is good but has declined and bimodal milk flow is still present. Consider having milkers strip for an additional second on the two teats they are stripping.**
- Strip yields are very good.
- **Teat-end cleanliness has improved with only 15% of teat-ends too dirty (25% last visit)**.
- Post-dip coverage of all four teats by the robot was successful 88% of the time.
- **Pulsators # 1, 30, 68, and 70 had shortened D phases.**
- Sand levels looked reasonable in the lactating cow pens with good stall cleanliness.

Dairy Herd Management Data



- This is the calculated test day bulk tank SCC which may not be the same as the SCC from the plant.

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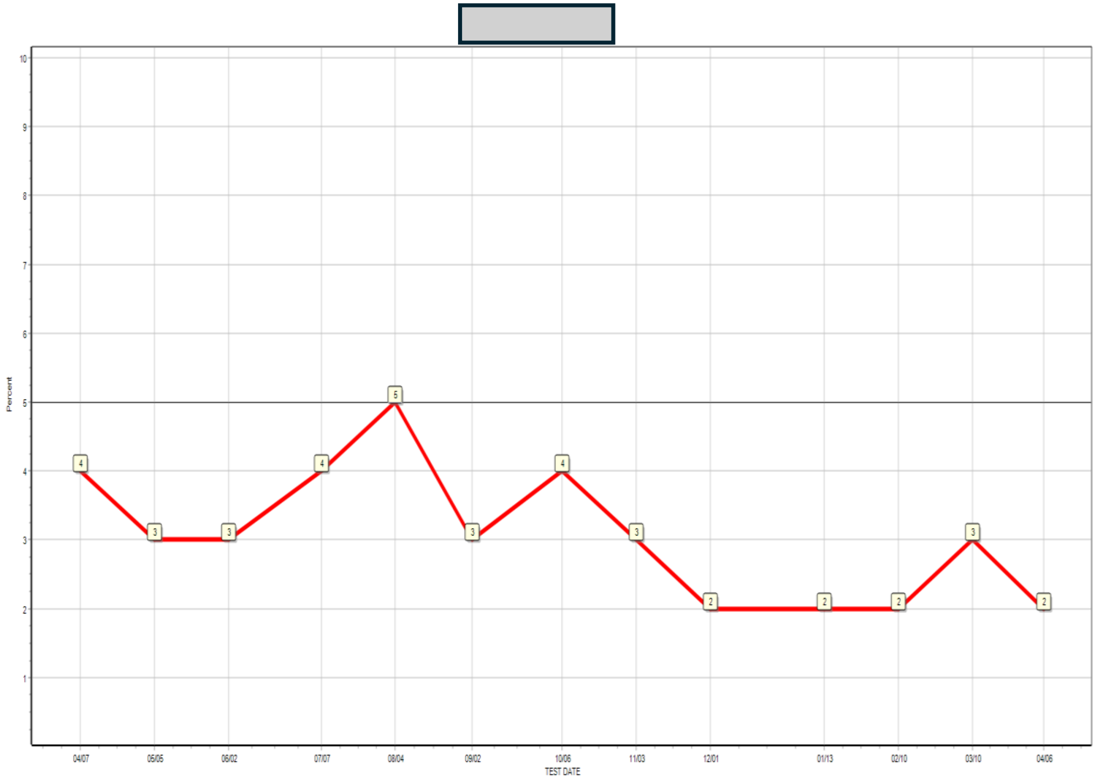
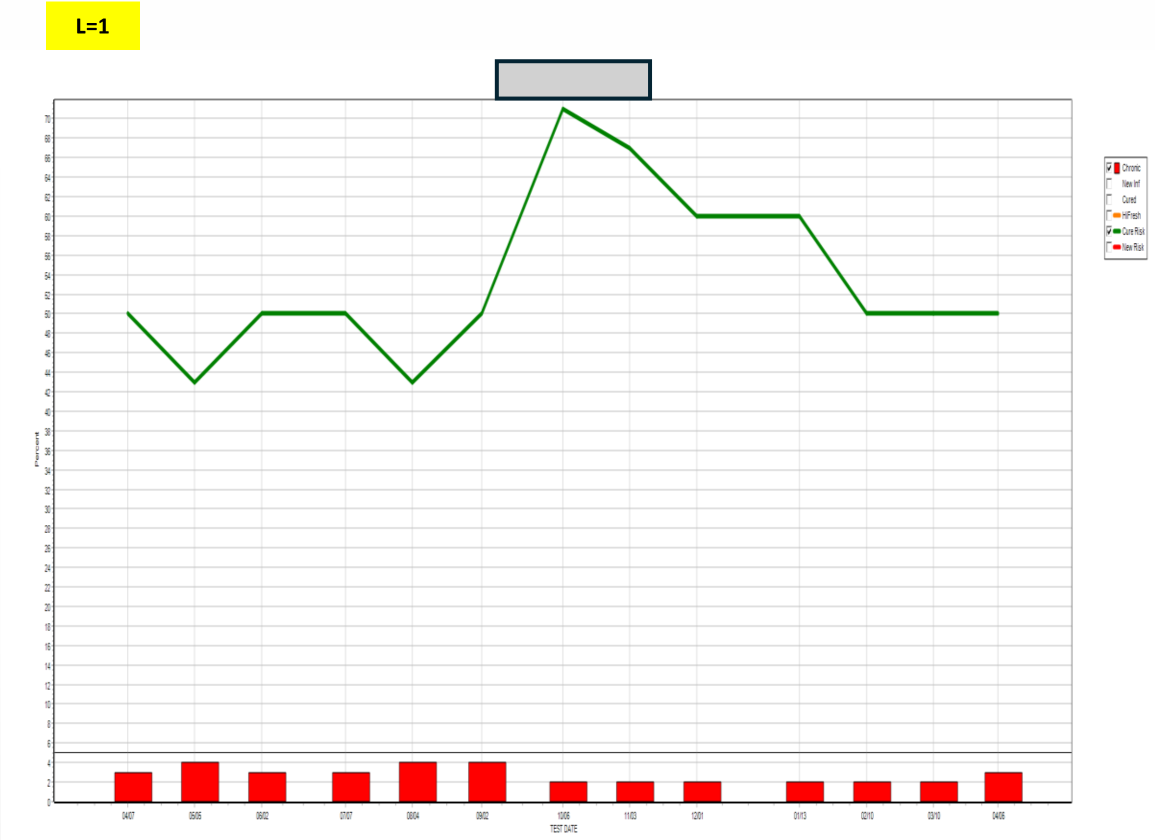
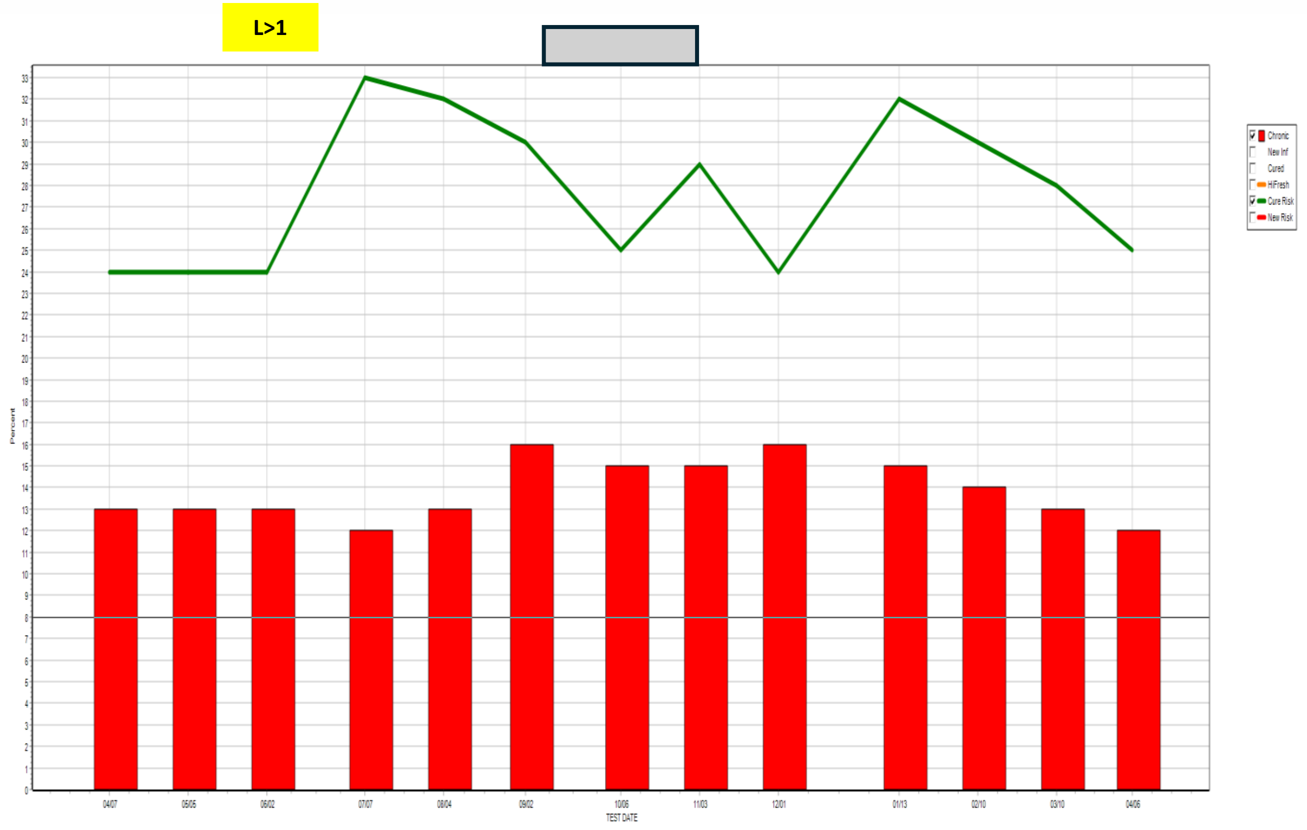


L>1

- This is a graph of the new infection risk over the last year by first lactation (left) and mature cows (right). The first lactation animals have been at or below the goal for past year. The mature cows are now below the goal of less than 8%.
- **The largest opportunity areas that we identified on this extension survey are to maintain teat end cleanliness, correct the abnormal pulsators, continue to work with the cow movers to make sure stall cleanliness stays good, and to continue to work on the positioning of cows in the stalls especially on the head to head rows in the big barns.**





- This is a graph of the percent chronic infections and cure risk for first lactation (left) and mature cows (right). The first lactation animals are meeting the goal of less than 5% chronic infections. The mature cows are trending down. The cure risk for mature cows has not met the goal of greater than 35% this year which means you continue to accumulate chronic cows. This is related to the number of environmental Streps and Strep Strep-like organisms identified in clinical milk samples.

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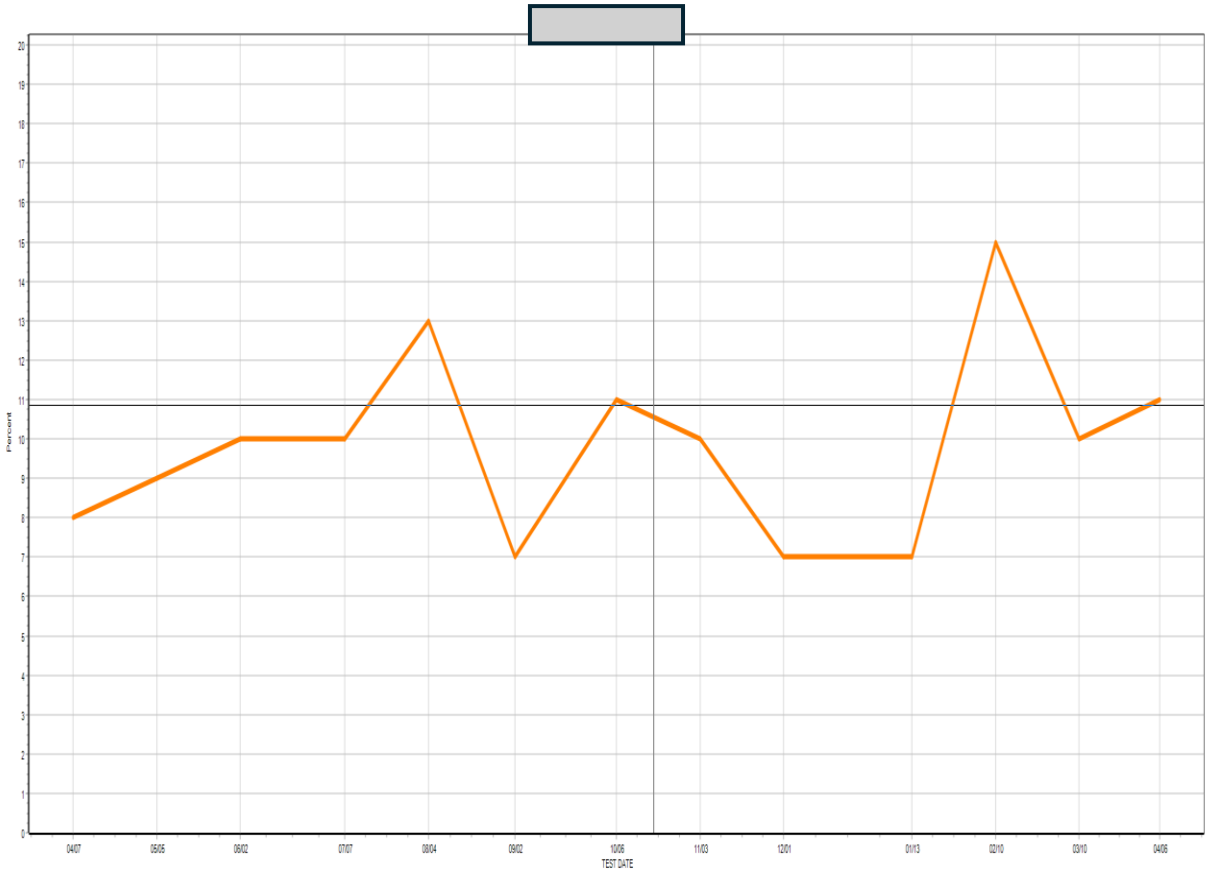

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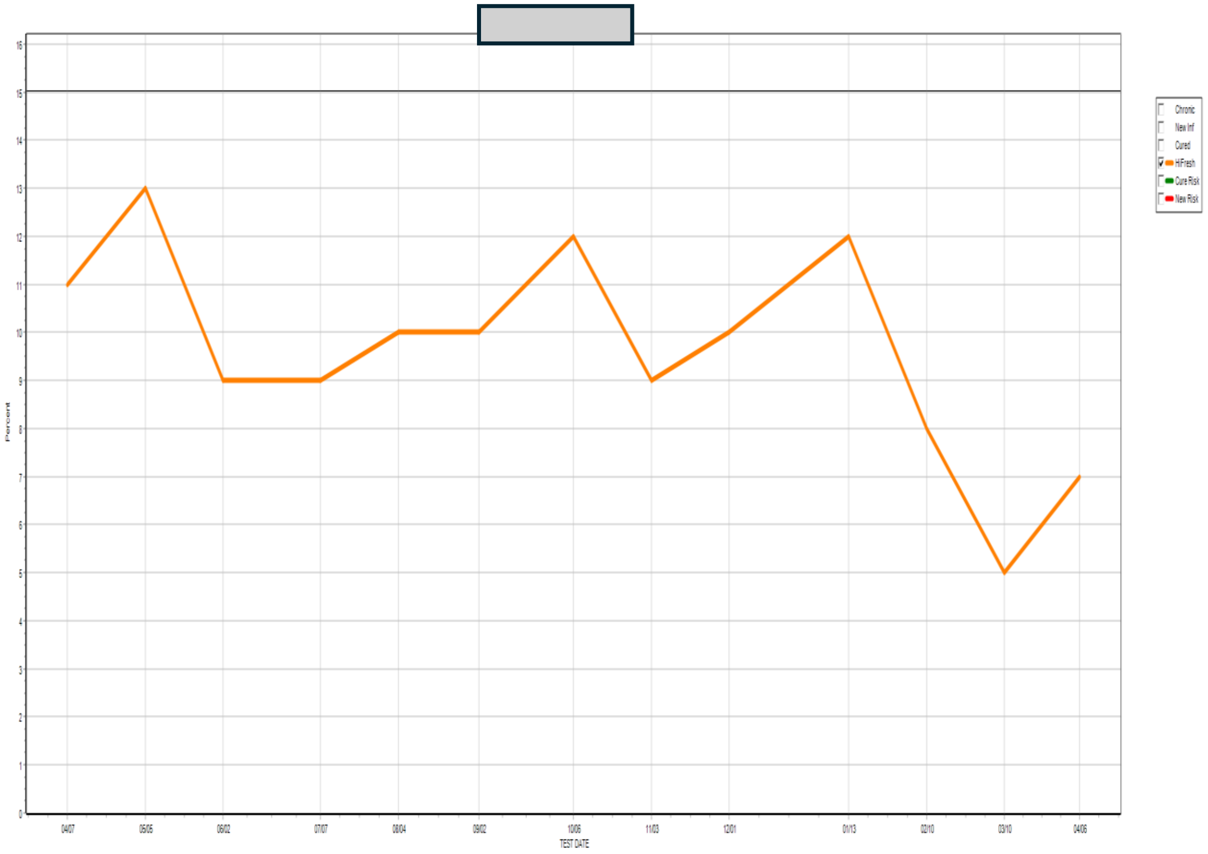
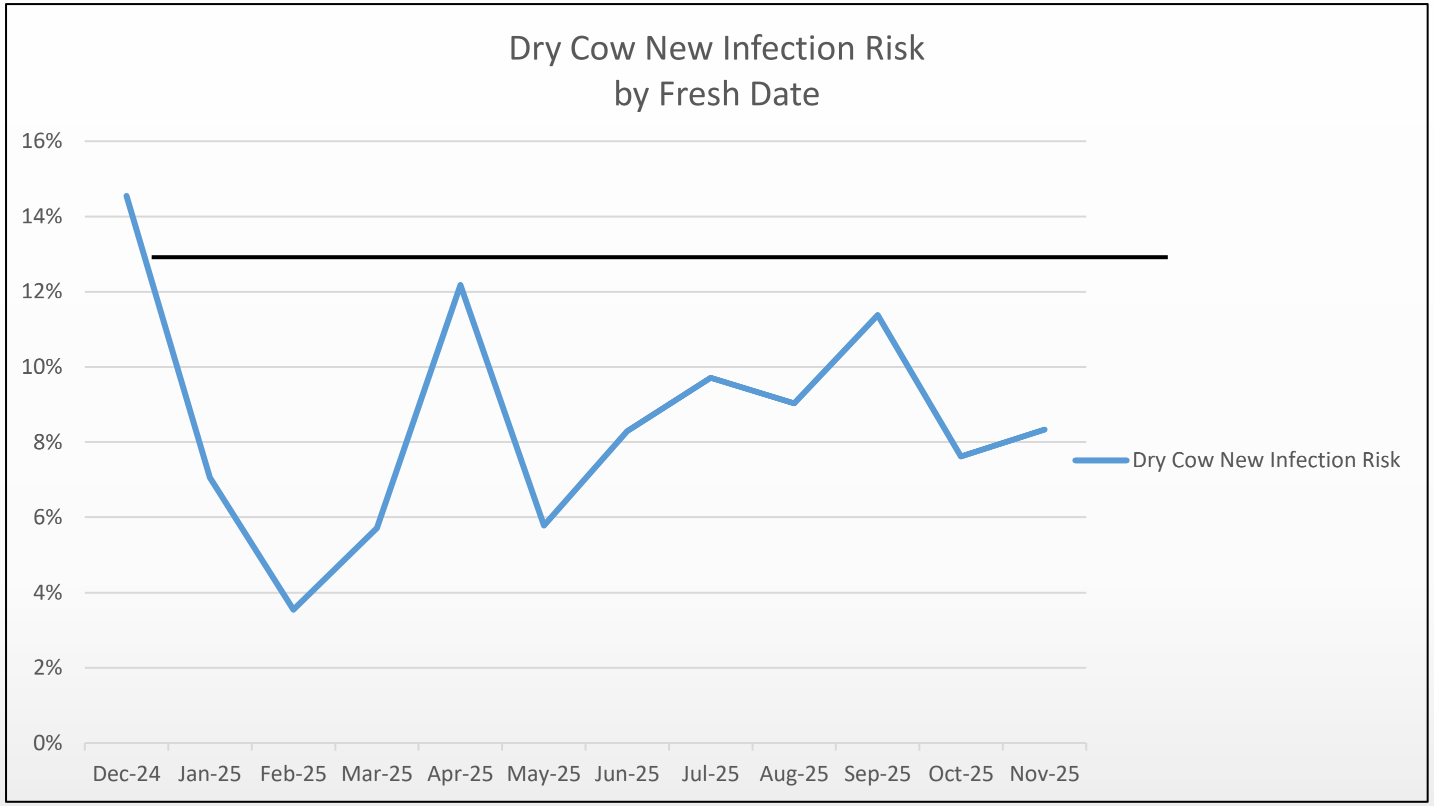
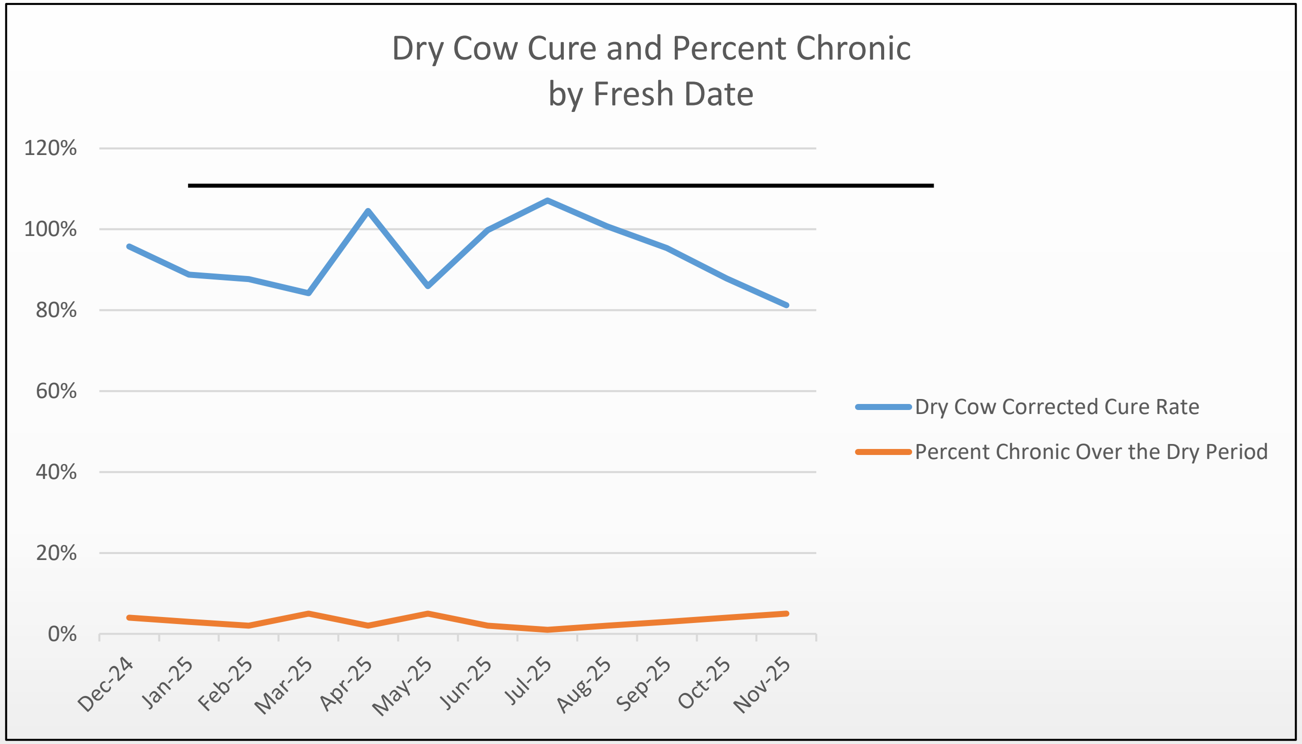


- This is a graph of the high fresh percent for first lactation animals (left) and mature cows (right). First lactation animals spiked in March 2026 and are currently at the goal of less than 15%. Mature cows have met the goal for the past year. Continue to monitor the dry off procedure and how the dry and pre-fresh stalls are managed.



- This is a graph of the dry cow new infection risk over the last year. Aside from spikes in April and September 2025, the dry cow new infection risk has been very good. Continue to regularly monitoring dry off procedures and pre-fresh pens (at least weekly). Continue to monitor calving areas in search of areas to improve.



- This is a graph of the dry cow corrected cure rate (blue line) and the percent chronic infections over the dry period (red line). The cure rate has been above the goal of 80% for the majority of the year which is good. The percent chronic is low and so is less of a factor.

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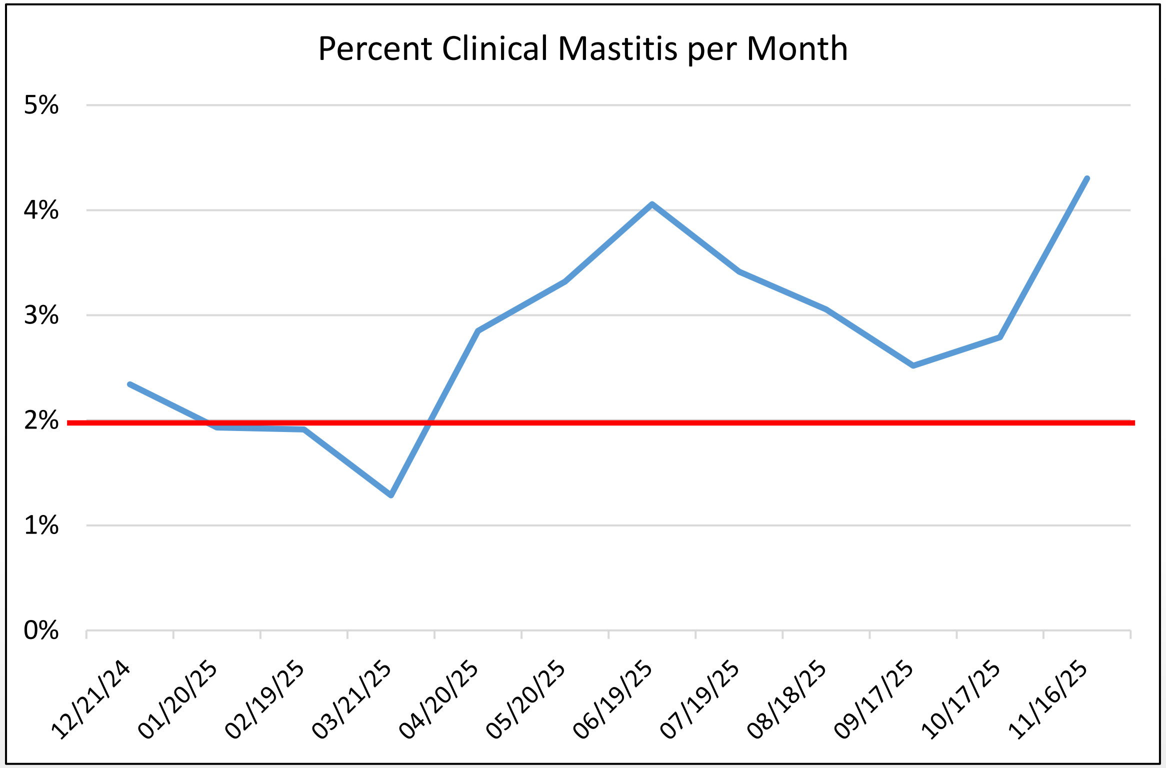
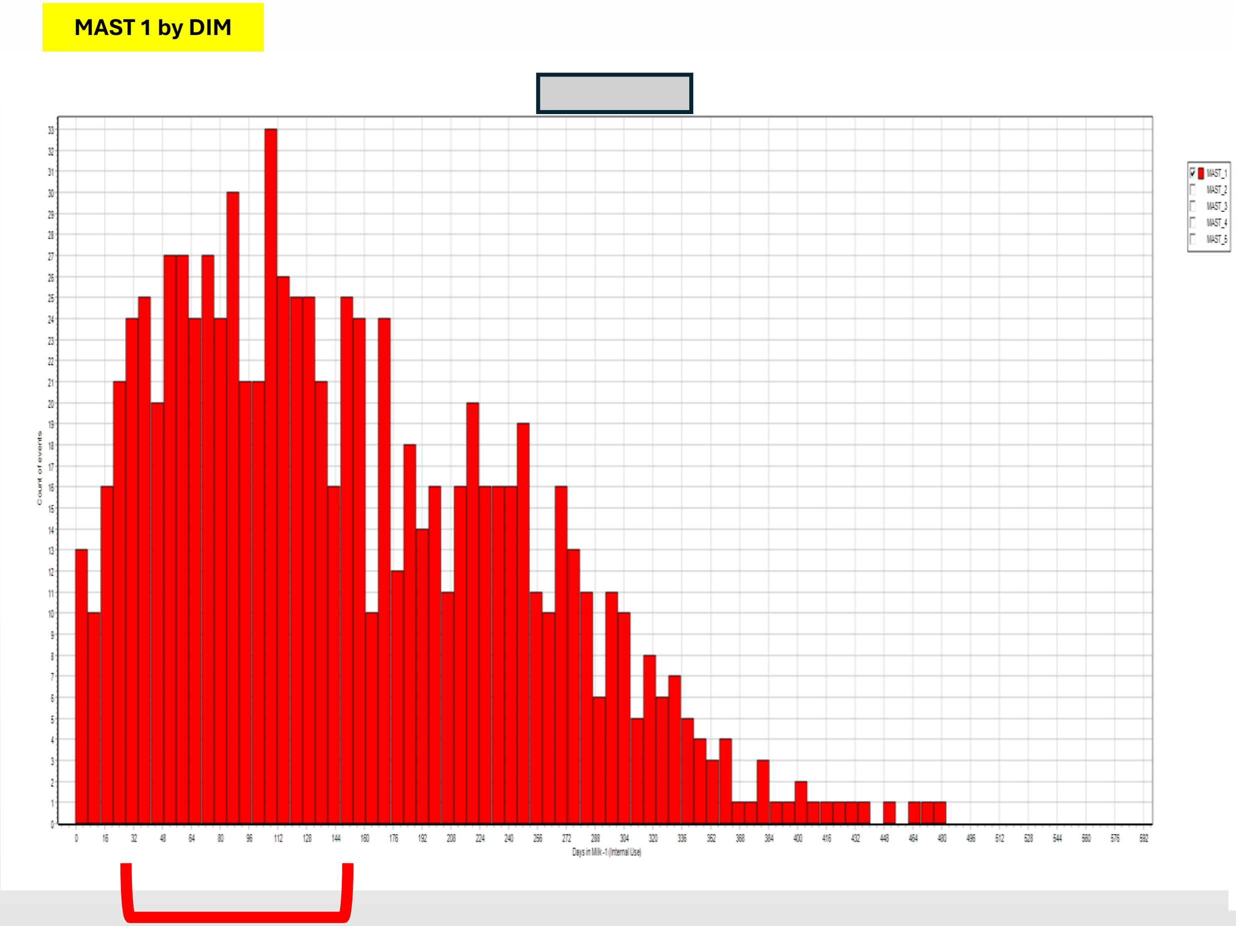
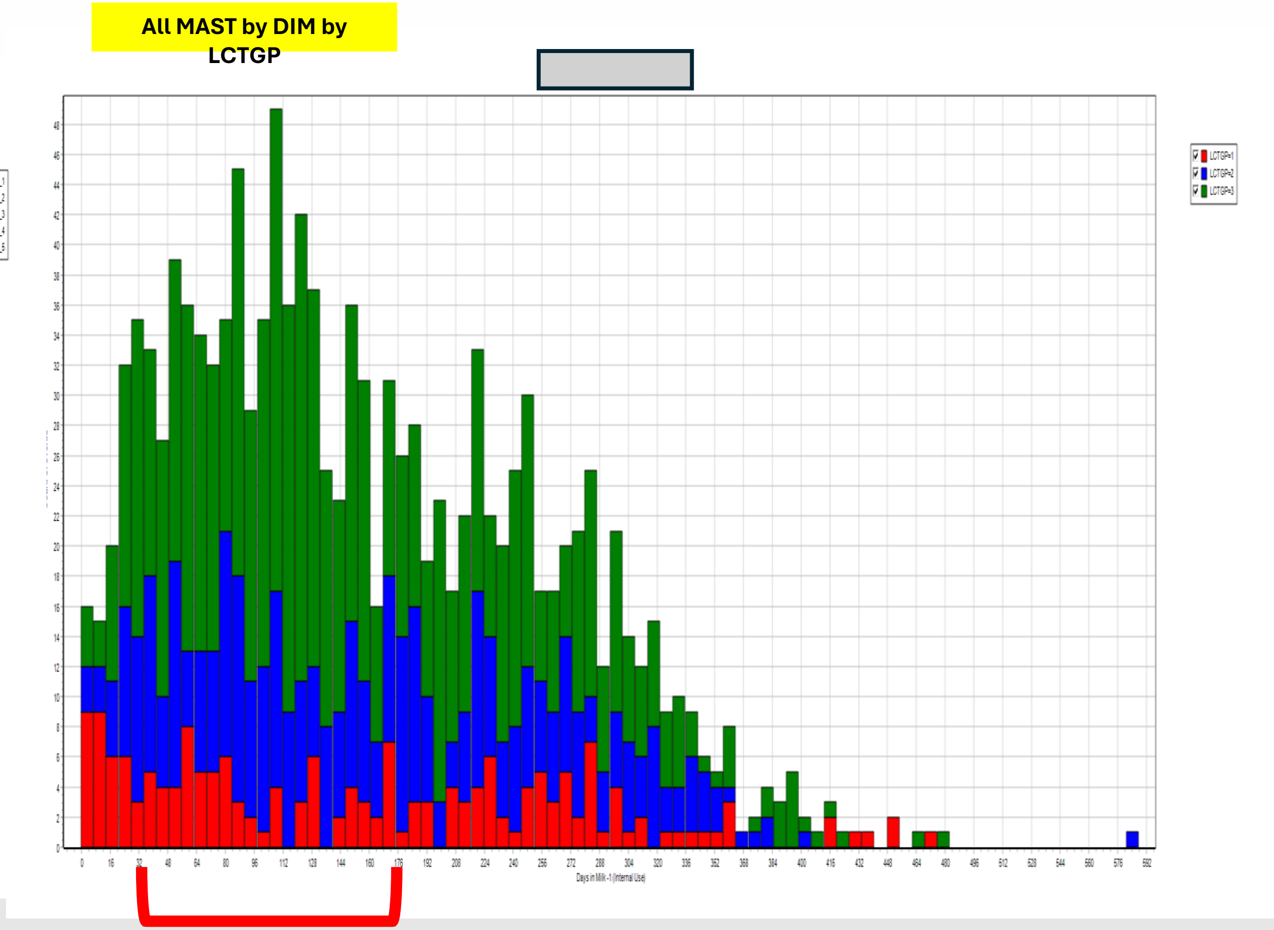


The percent clinical mastitis (based on entered MAST events) continues to be above the goal of less than 2%. Recurrent mastitis is 30%, which is within our goal of <50%.





- This is a graph of the MAST 1 events by DIM on the left and all mastitis by lactation group by DIM on the right. The highest risk period looks to be around 30-120 DIM. Parlor activities, movement of animals to and from the parlor and lactating stalls should be our focus. The trends by lactation do not stand out as abnormal this period.

Parlor Performance

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Two Minute Milk

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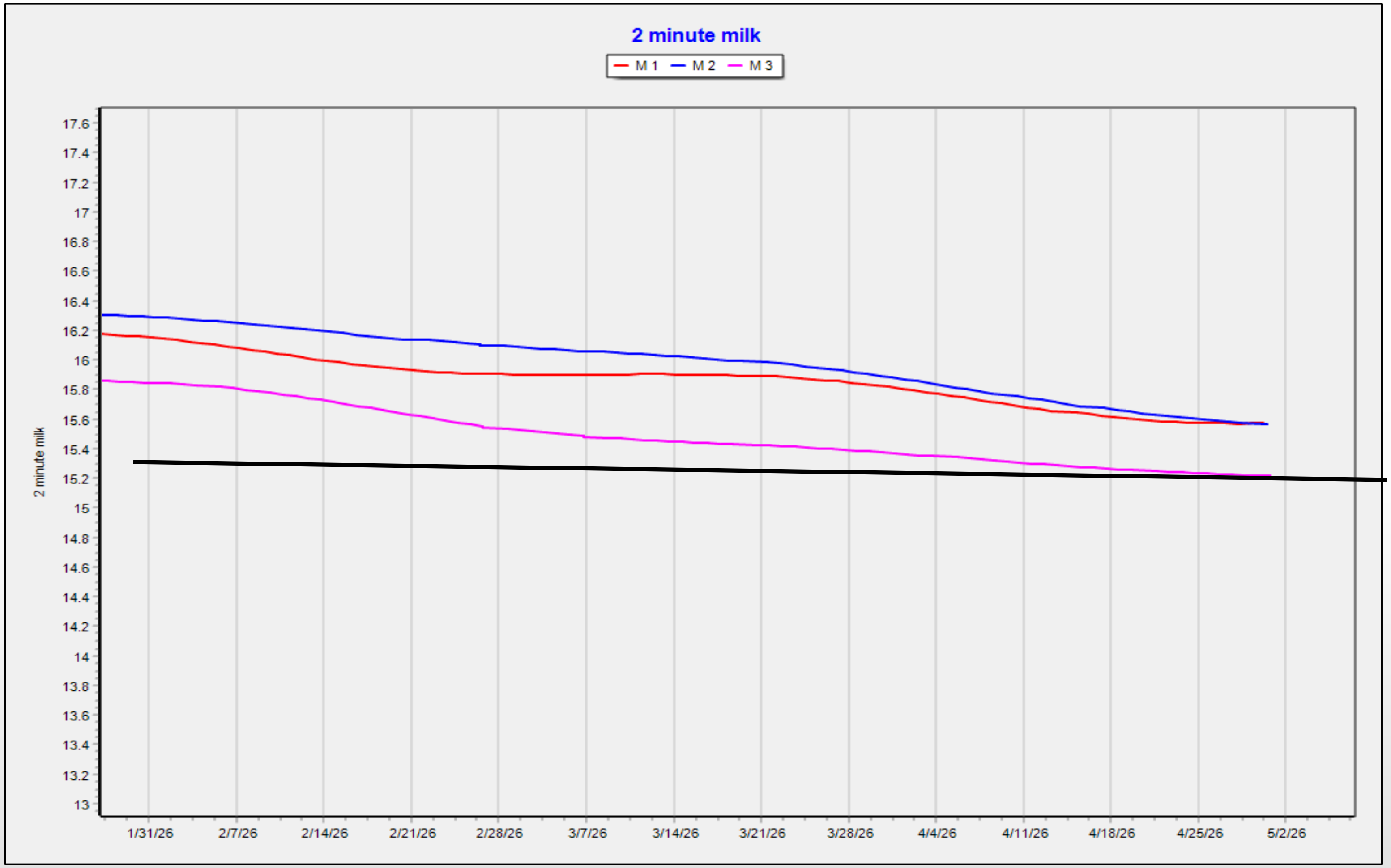
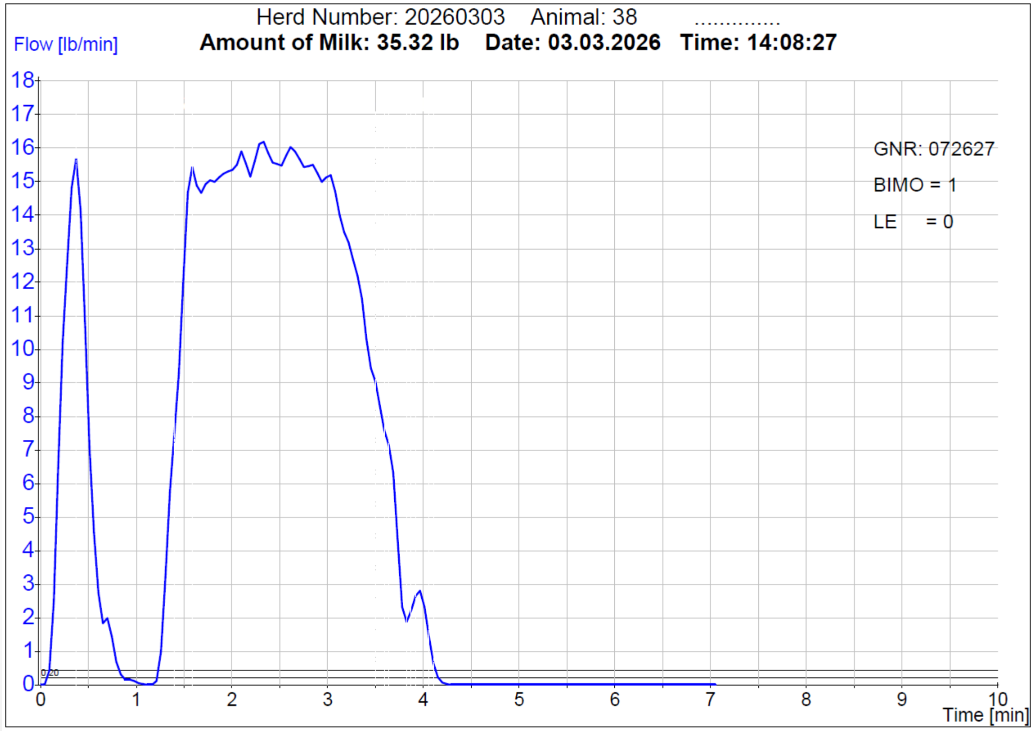
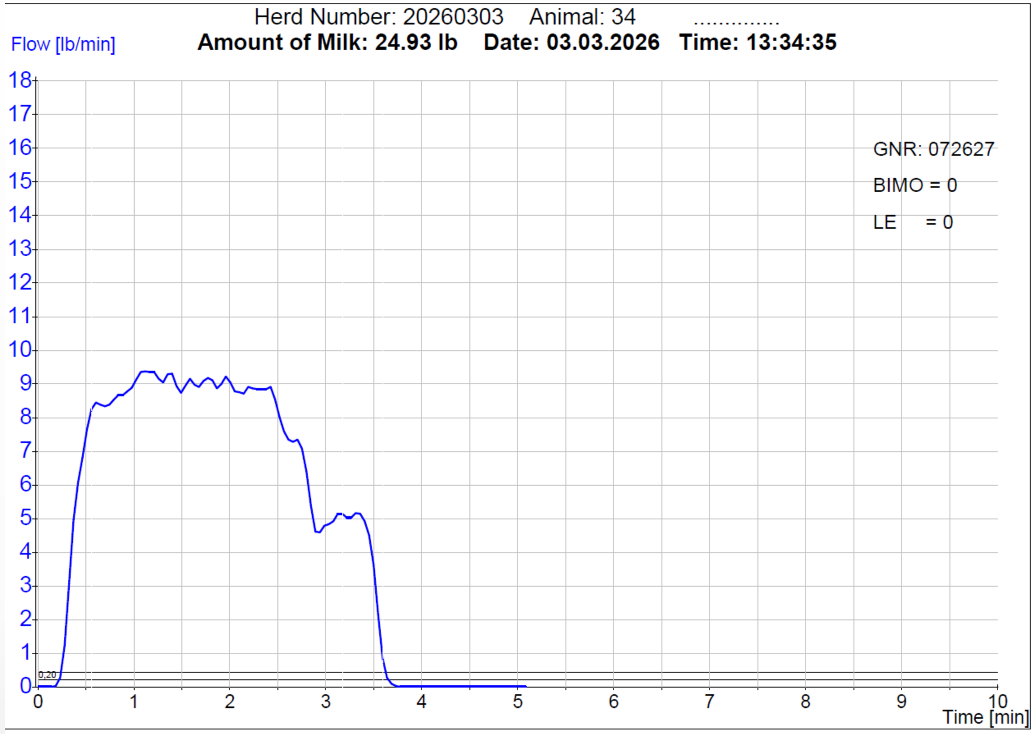


- This is a graph of 2 minute milk over the last 3 months by milking. The goal for this parameter is to be above 15 pounds for a 3X herd (black line). All three milkings continue to meet the goal.

LactoCorder® Analysis



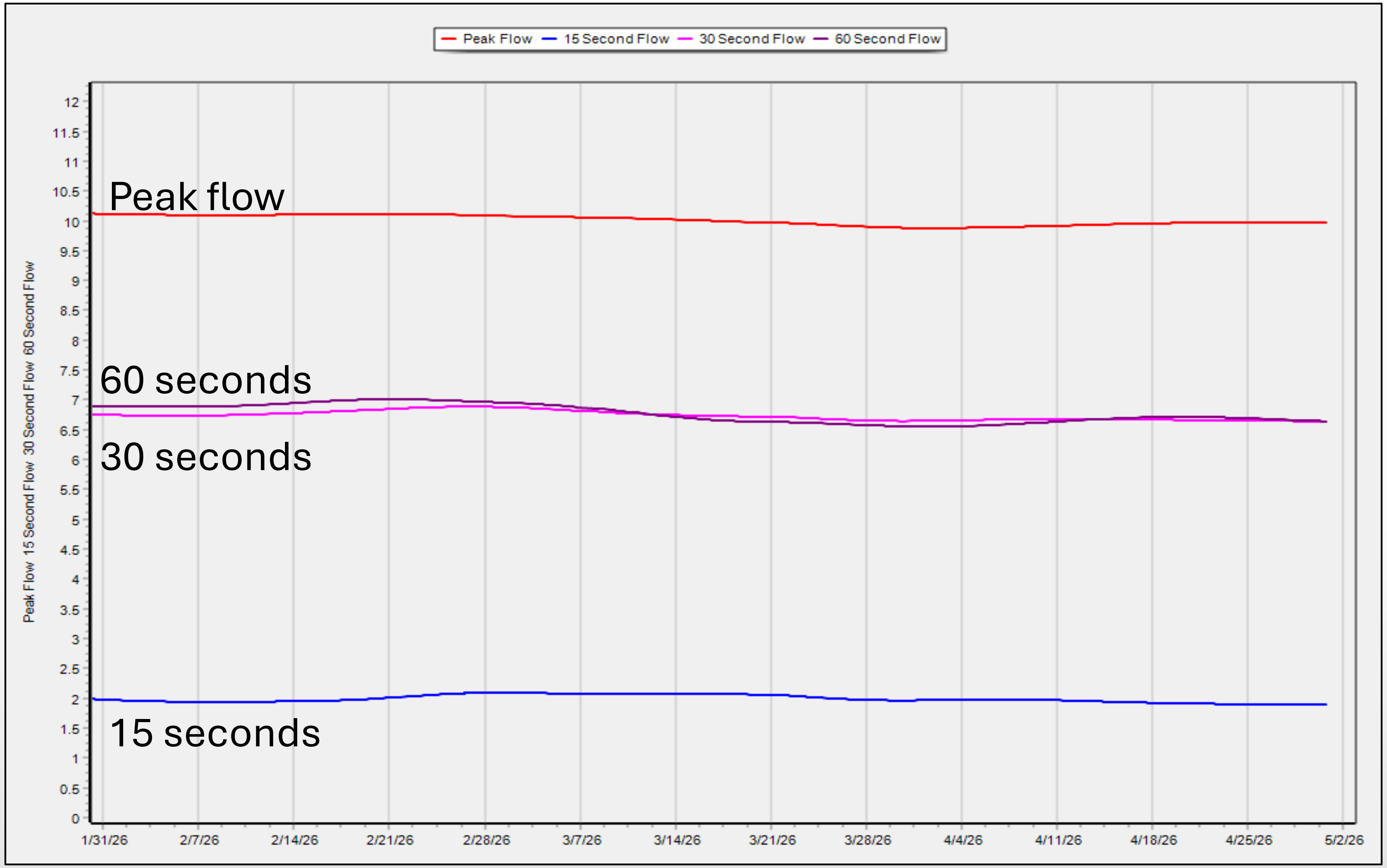
Bimodal Milk Curve



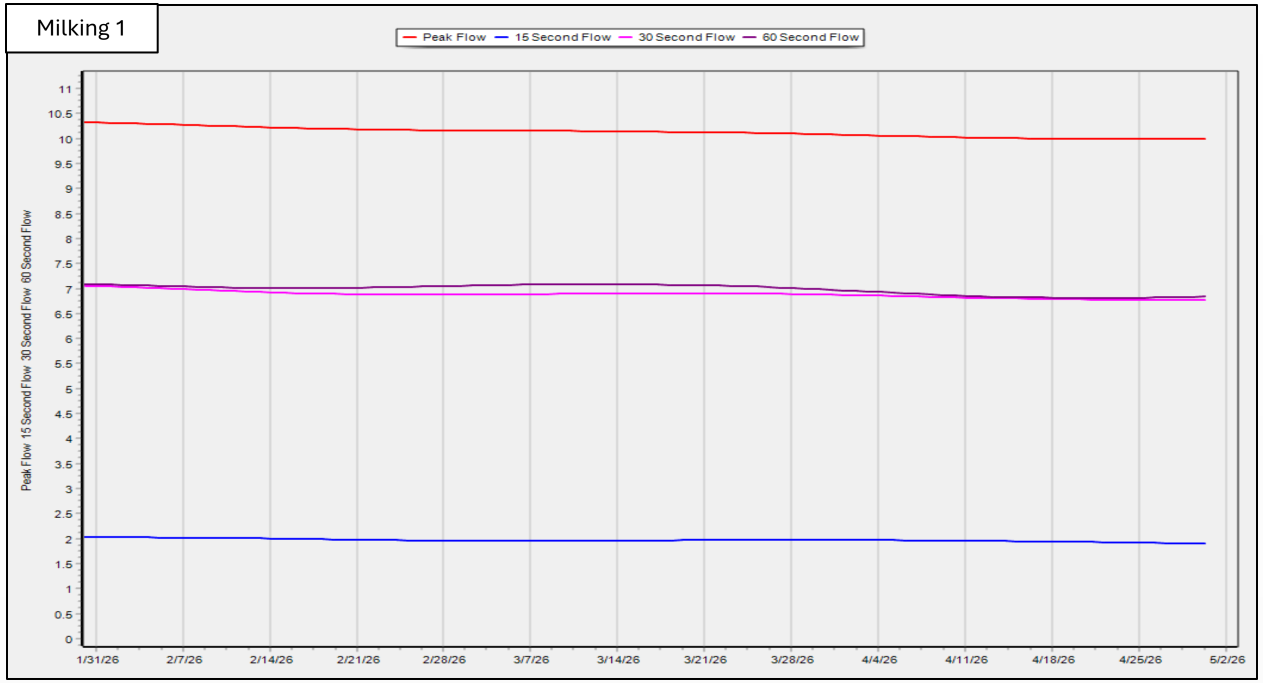
Good Let-Down Milk Curve

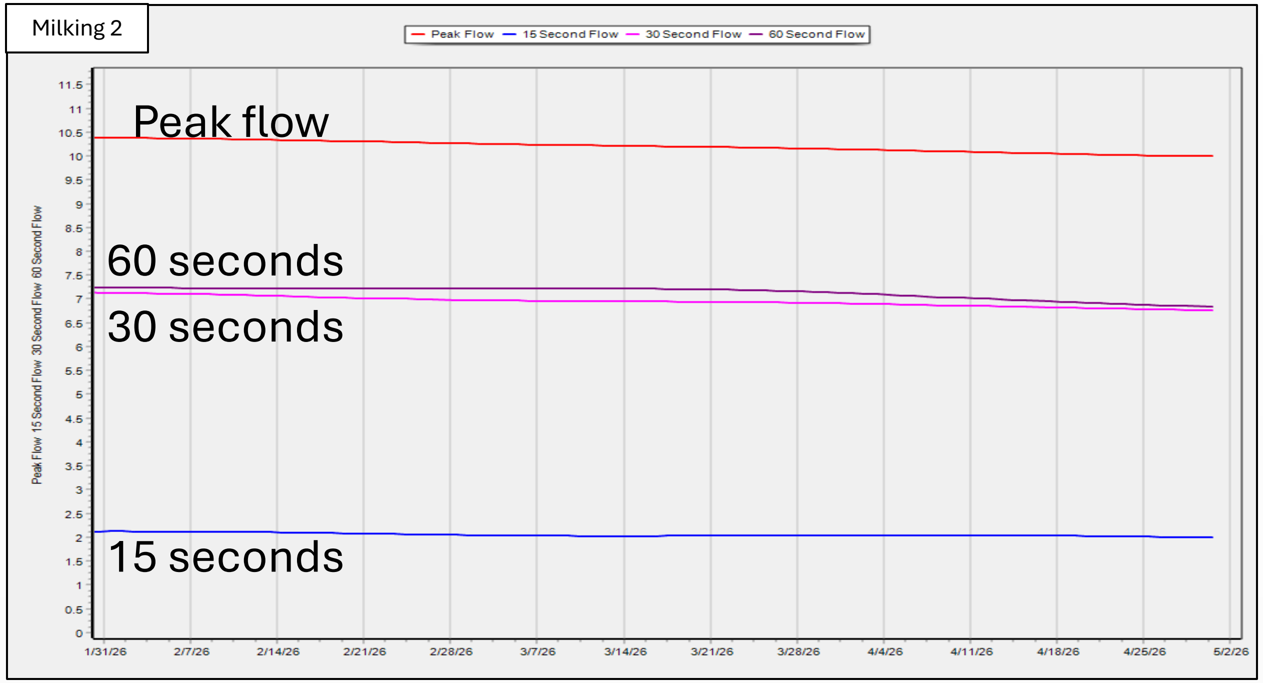
- During our visit, we ran 12 Lactographs. Lactographs record milk flow over time and generate milk flow curves (see above). 50% (6/12) showed bimodal milk curves. Our goals is less than 10% showing bimodal milk curves.
- The higher level of bimodal curves is most likely due to the shortened lag time.
- Stimulation time from pre-dip to unit attachment and pre-stripping to attachment tends to be short. All timings we performed showed appropriate lag times of greater than 90 seconds Perhaps improving stimulation will reduce the number of bimodal curves observed.

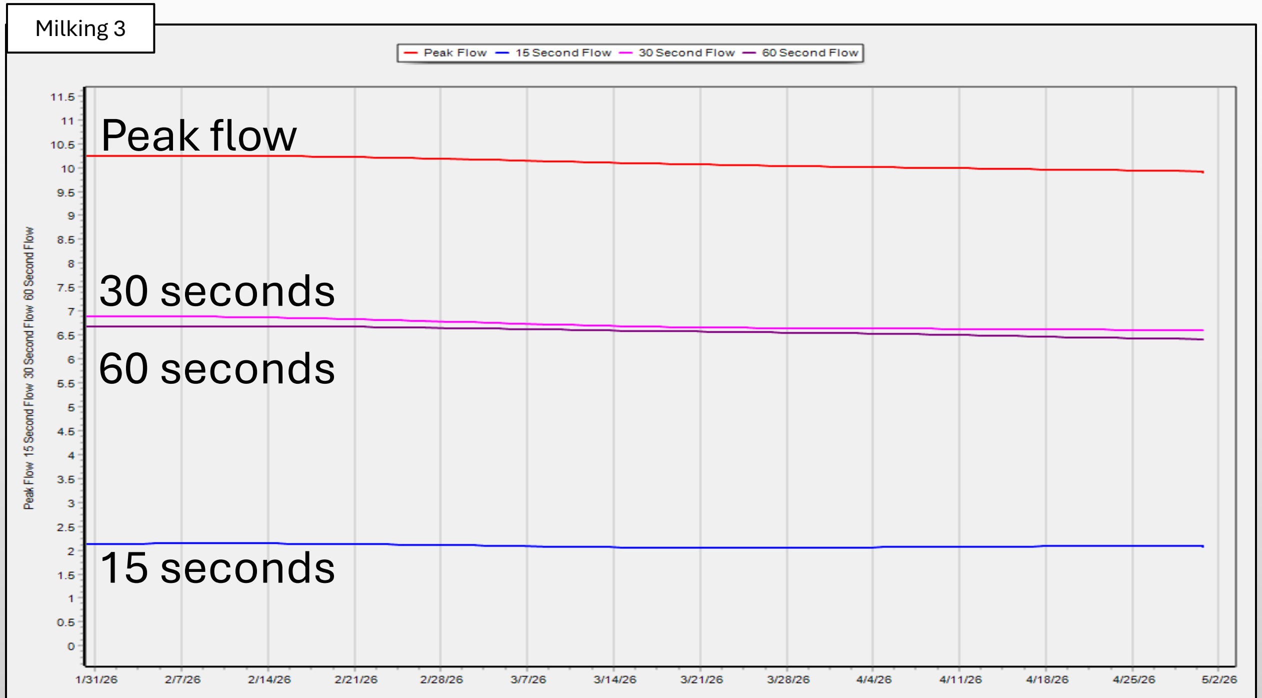
Flow Rate Intervals for 3 months



- This is a graph of the milk flow rate intervals over the last 3 months by milking. Based on this data, milk flows have gotten more bimodal since February 2026. The next slide shows this same data broken out by milking.

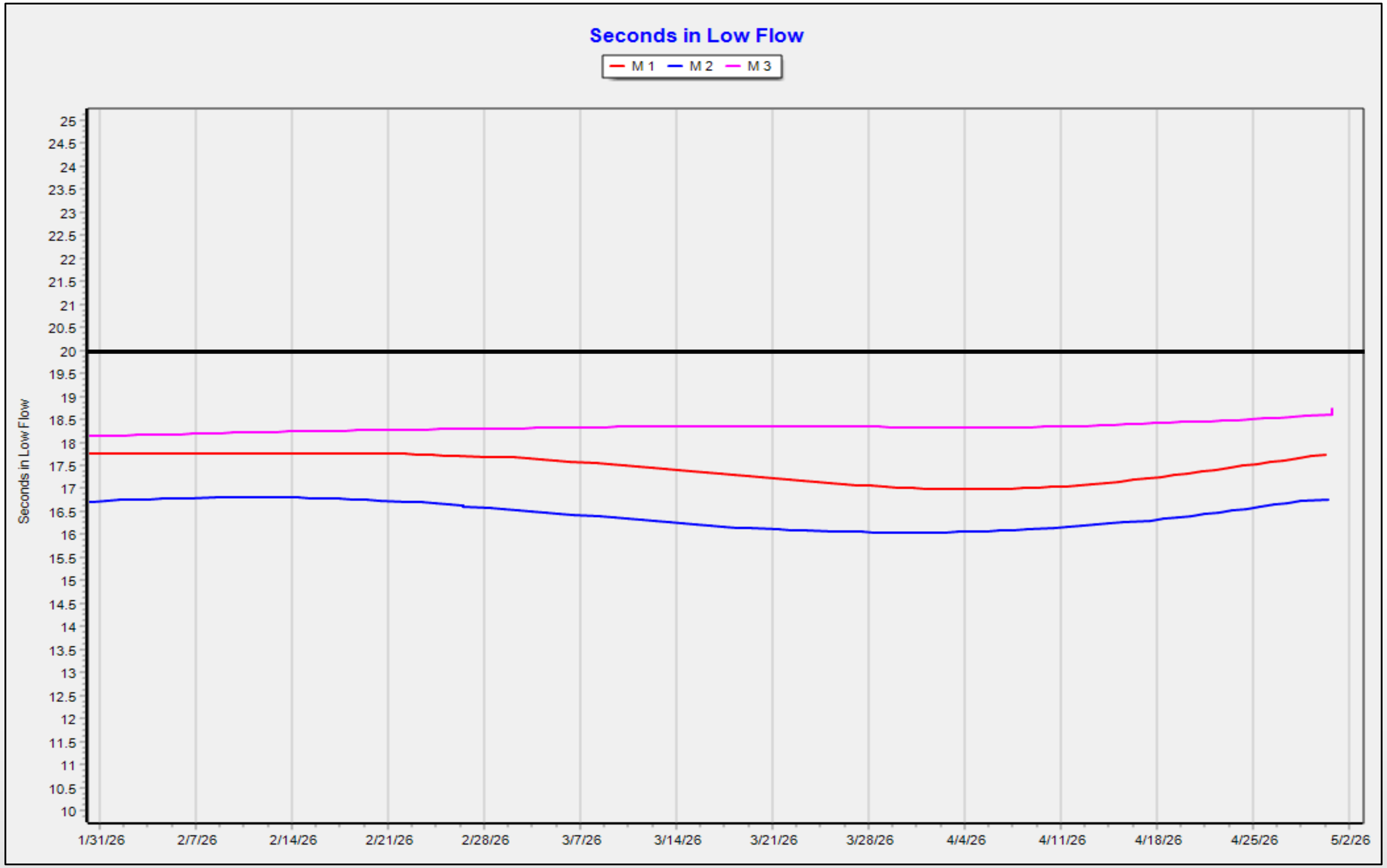






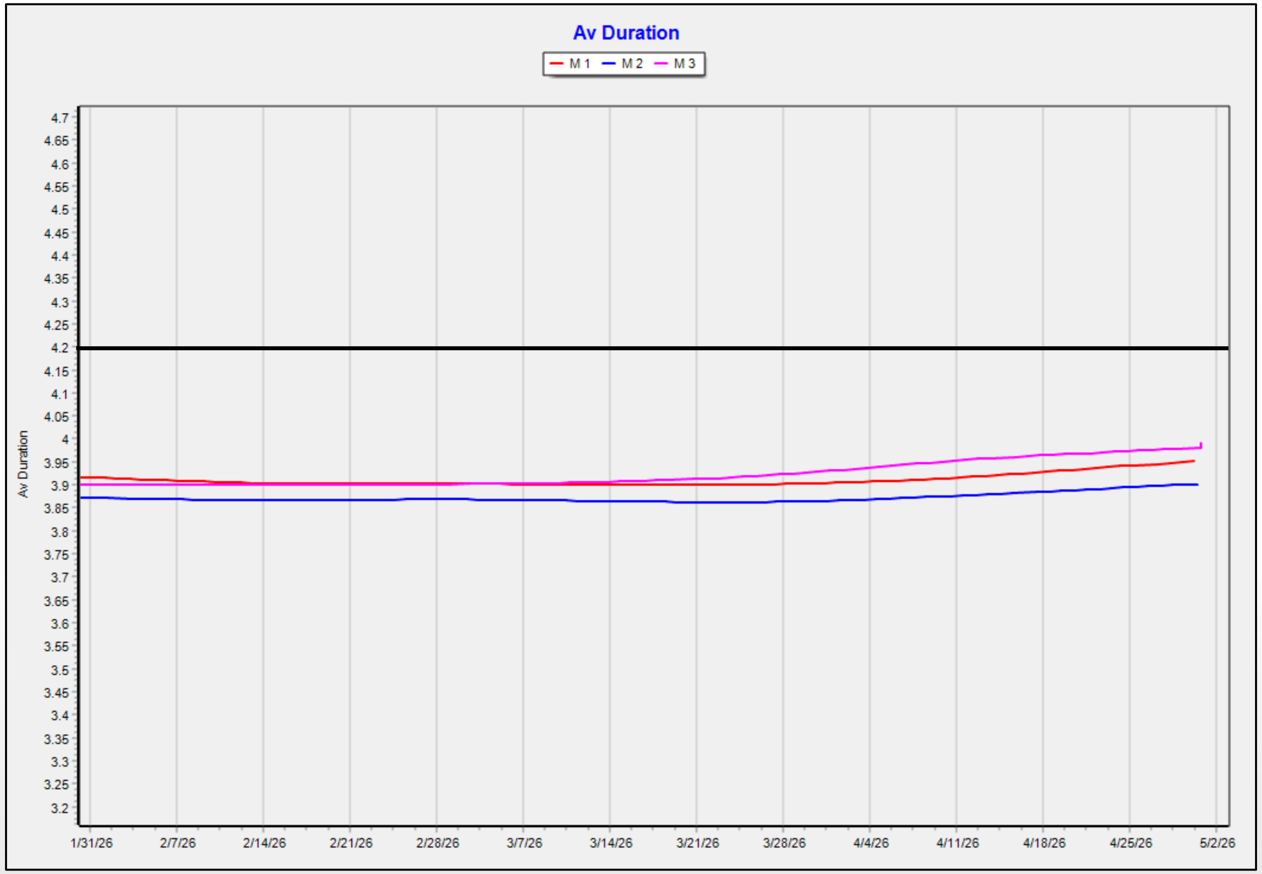
- Notice on milking 3 is consistently bimodal. Milking 1, the lines are on top of each other and 2 milking shows good milk flow.

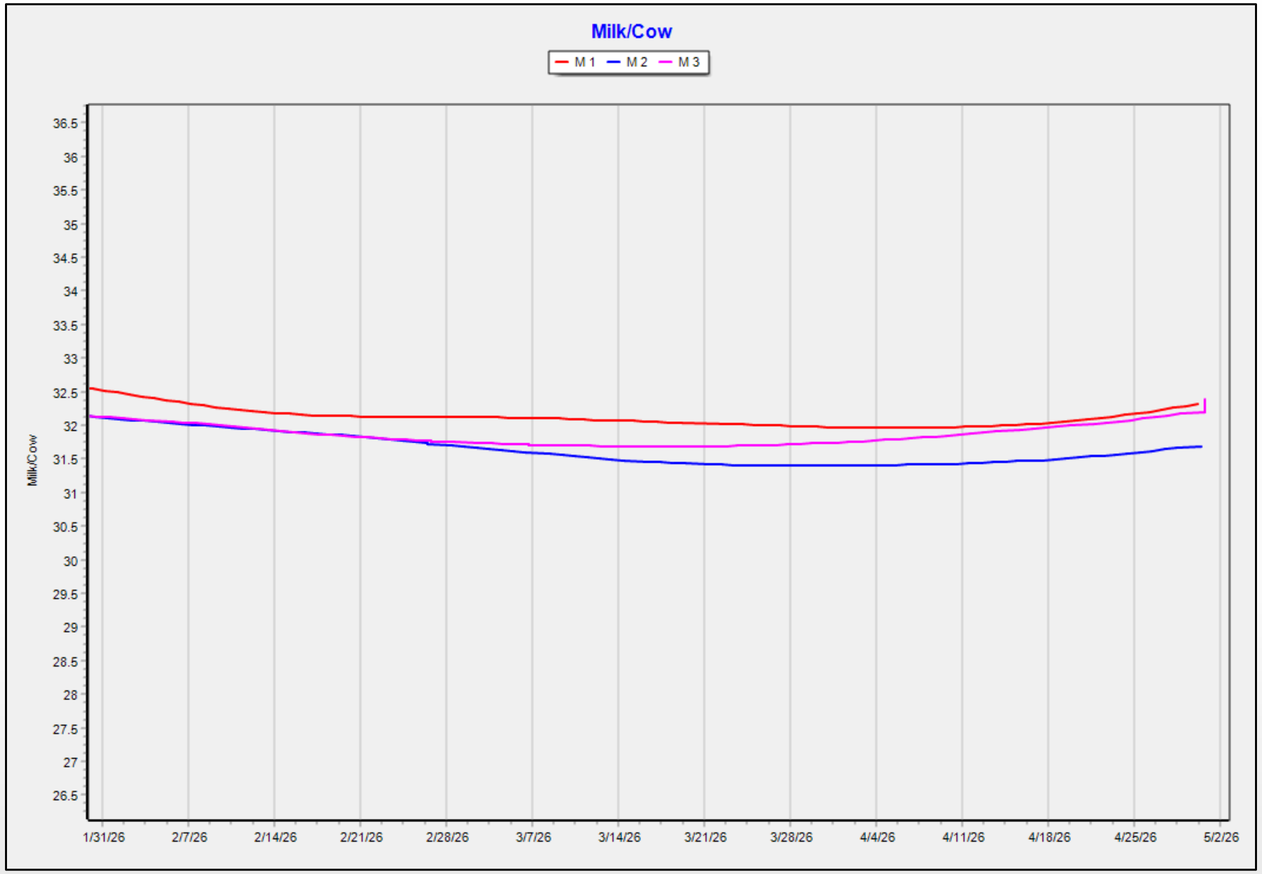
Seconds in Low Flow



- This is a graph of the seconds in low flow over the last 3 months by milking. The goal is less than 60 seconds per day or 20 seconds per milking on a 3X herd (black line). You are still meeting the goal on all shifts.

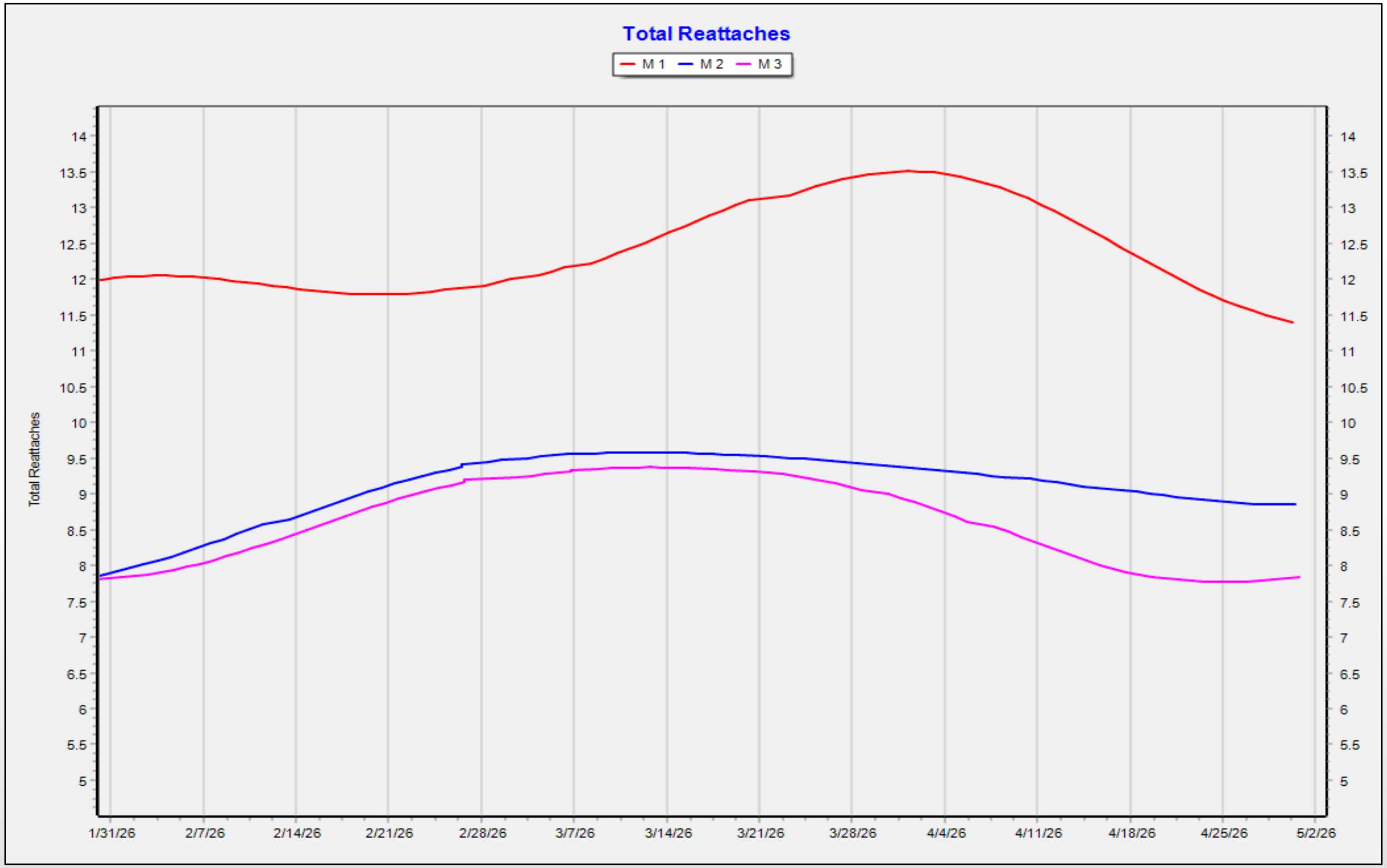
Unit on Time/Milk per Cow





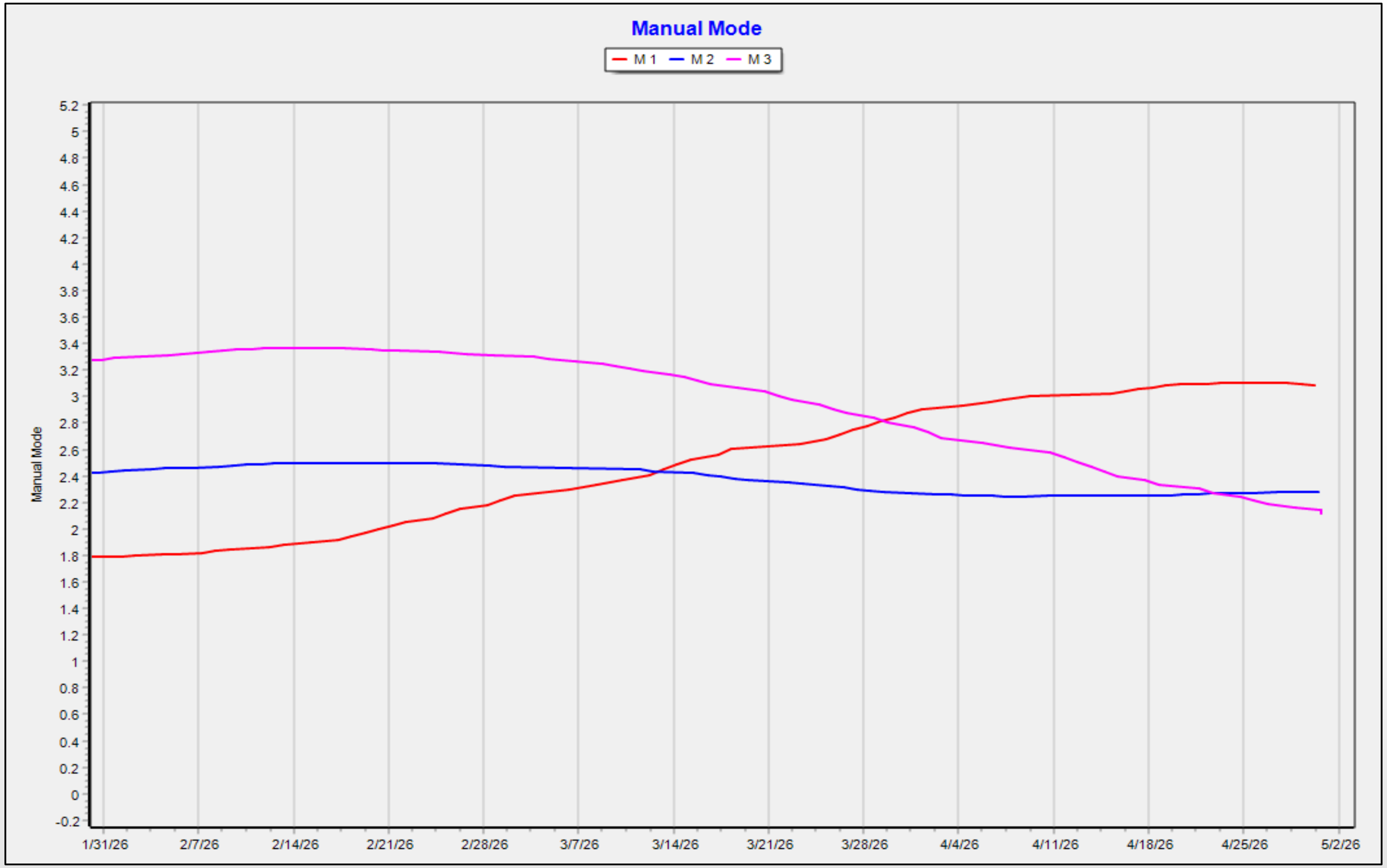
- This is a graph of the unit on time on the left and milk/cow on the right over the last 3 months by milking. The goal for unit on time based on your milk production would be less than 4.2 minutes per milking which is where the black line is placed. **Unit on time is meeting the goal on all 3 shifts.**

Reattaches



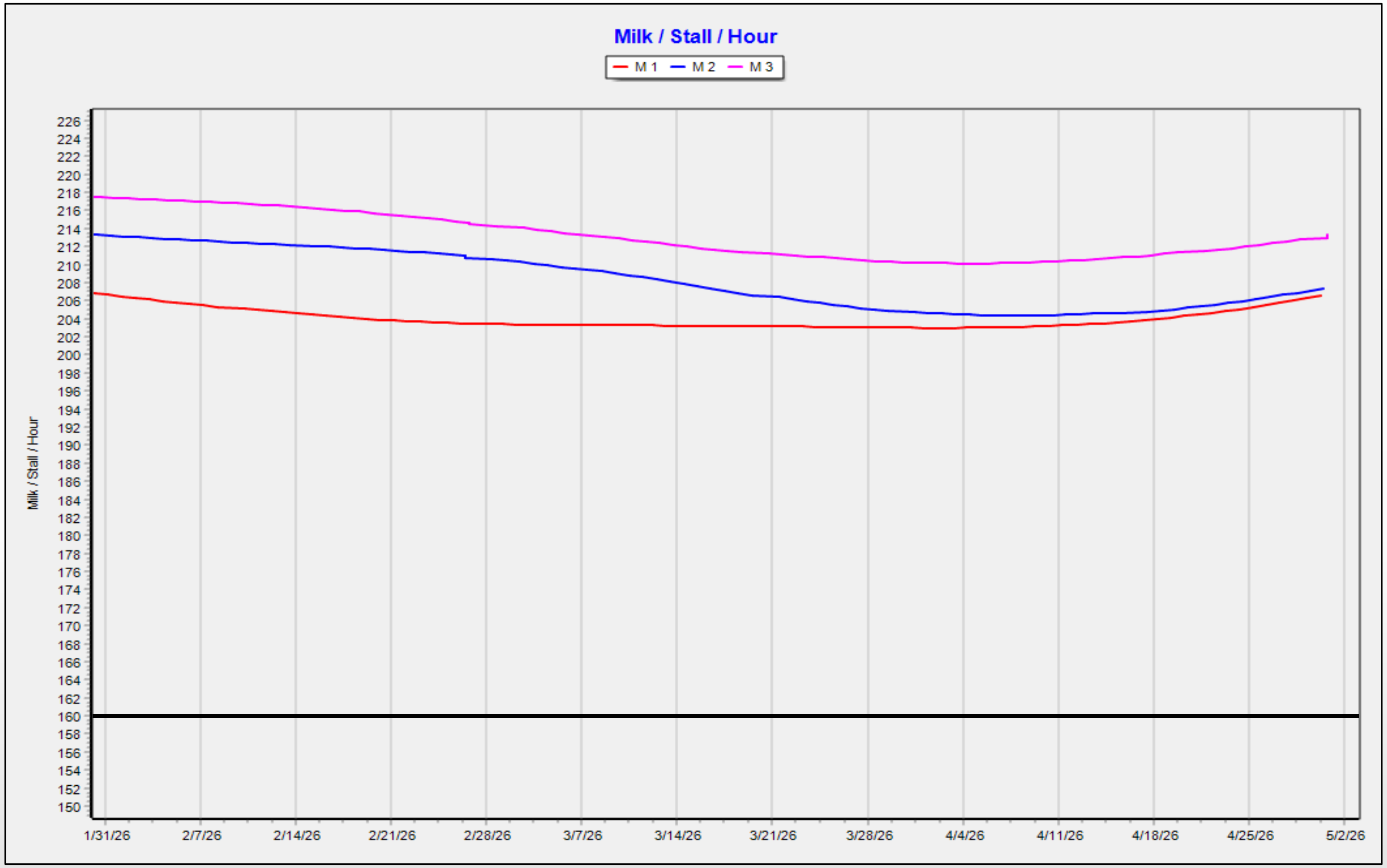
- This is a graph of the total reattaches over the last 3 months by milking. This does not look to be a significant issue on your farm.

Manual Mode



- This is a graph of the units on manual over the last 3 months by milking. This does not look to be a significant issue on your farm.

Milk/Stall/Hour



- This is a graph of the milk/stall/hour over the last 3 months by milking shift. The goal for a 3X rotary dairy is to be at 160 pounds of milk/stall/hour. **<u>You are well above this goal and climbing which is excellent</u>**!

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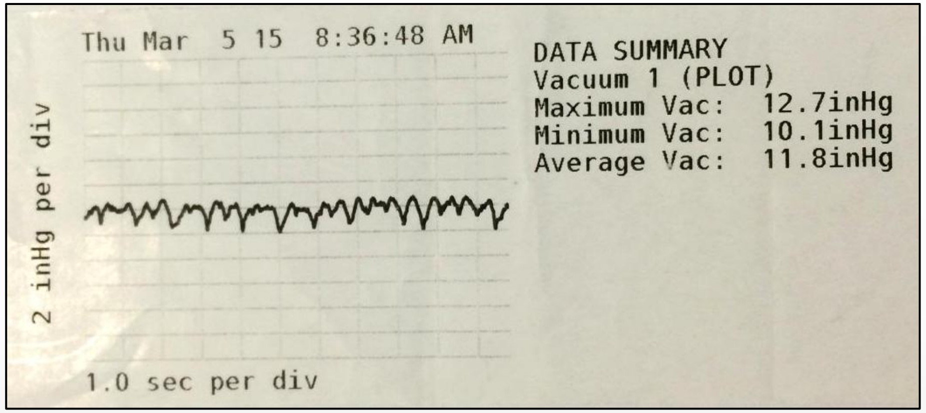
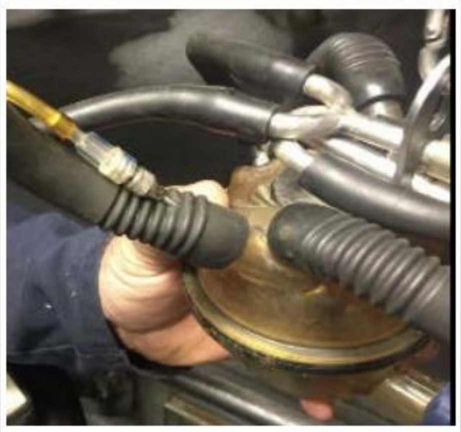
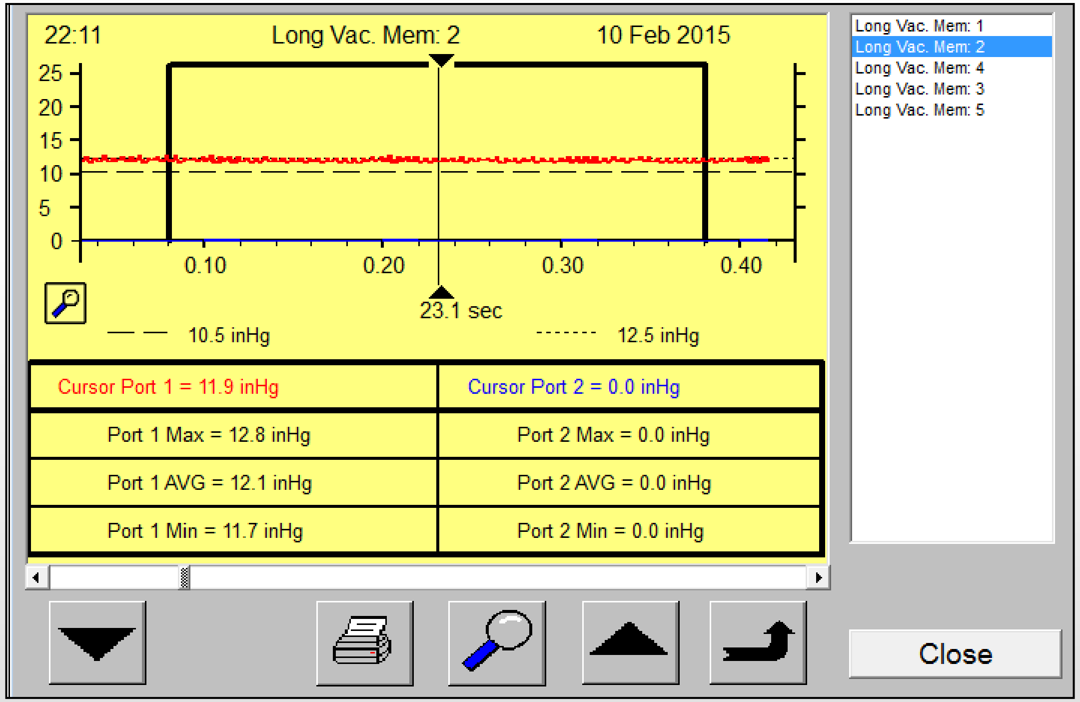
Average Claw Vacuum at Peak Flow for a 5 to 20 second interval

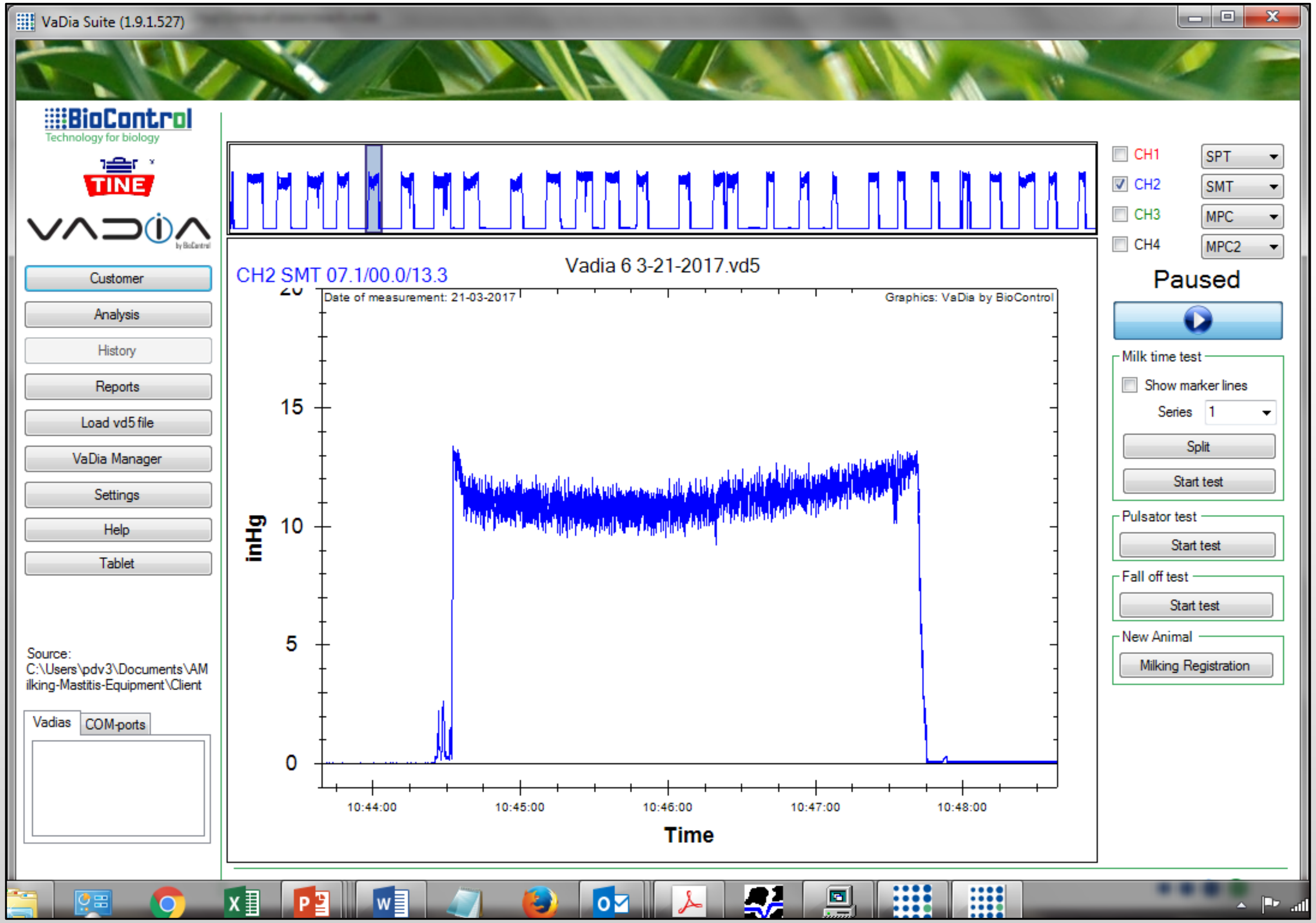






This is the vacuum reading that you use to set your system vacuum

Example: Claw Vacuum Graph



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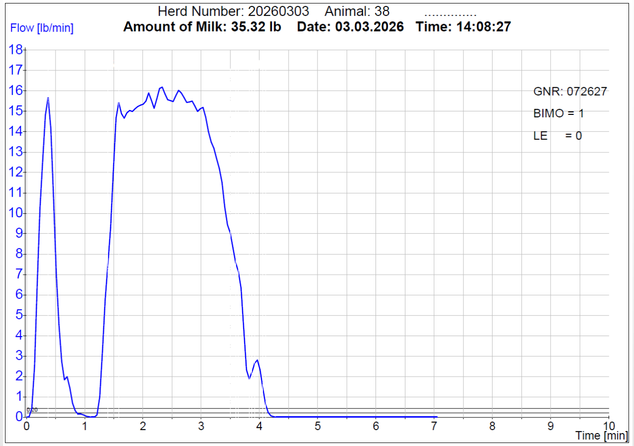
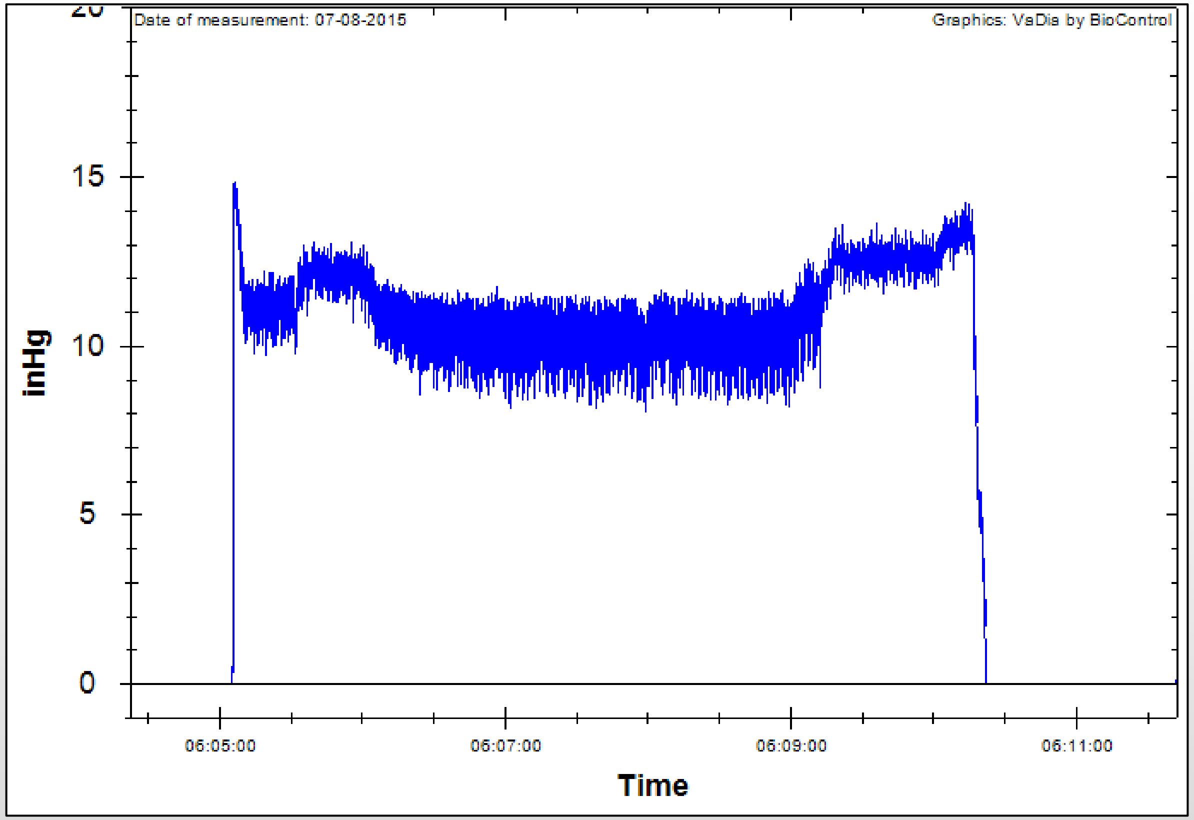
Example: Claw Vacuum Graph





What 2 things does this flow pattern suggest?

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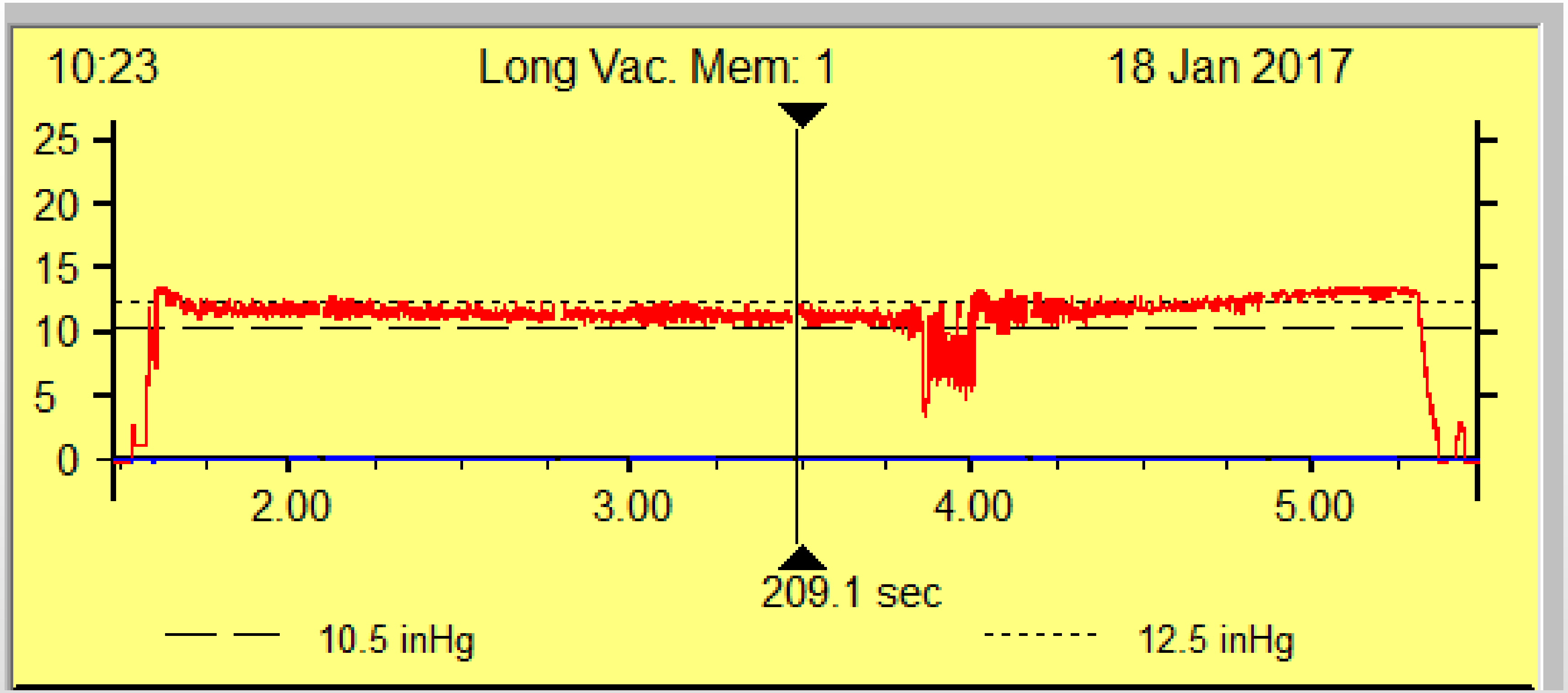
Example: Claw Vacuum Graph



Notice liner slip as vacuum starts to rise

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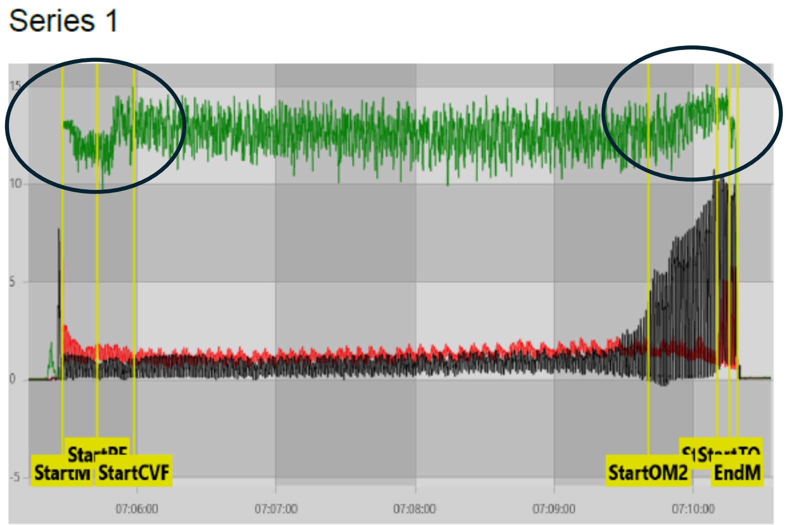
Average Claw Vacuum



Animal

Animal	8
Milk Yield	0.0
Av. Milk Flow	0.0

Time

Start Preparation	00:00:00
Preparation time	00:00
Attachment lag time	00:00
Attachment	07:05:27
MachineOn	04:51
Overmilking	00:05
General Overmilking	00:05

MPC vacuum

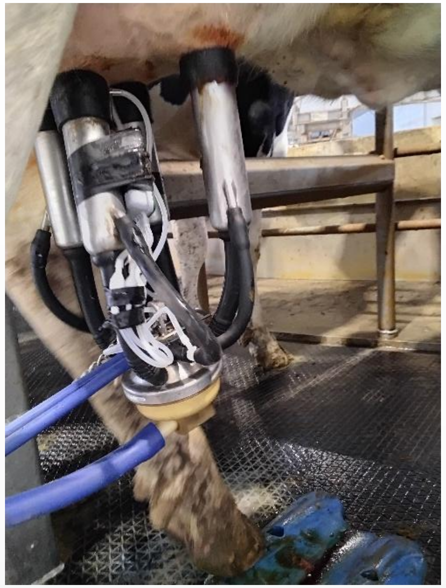
MPCVac. PFP	1.3
MPC2Vac. PFP	0.8
MPCVac. Total	1.4
MPC2Vac. Total	1.3
MPCVac. Overmilking	2.1
MPC2Vac. Overmilking	4.4
MPCVac. Favorable	0.0
MPC2Vac. Favorable	0.2

SMT vacuum

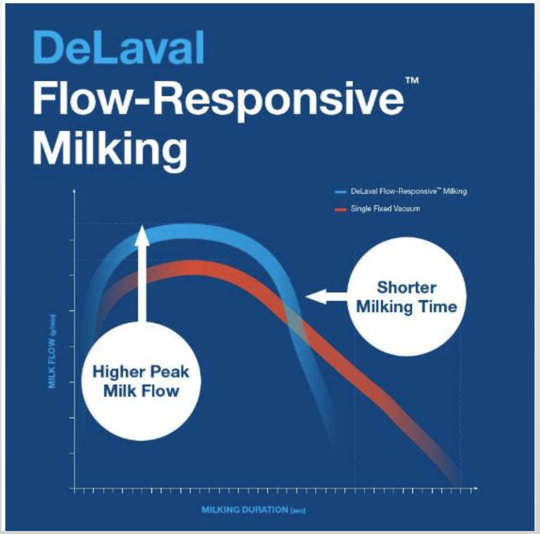
Average short milk tube vacuum	
- Total	12.6
- PF period	12.7
- Overmilking	14.0

Cyclic vacuum fluctuation

Cycl. Vac. Fl.	
- Minimum	12.0
- Average	13.0
- Maximum	14.1
Irr. Vac. Fl. Type1 (IVF1)	0



- The graph above depicts the last 30 seconds of milking before the systems lowers the system vacuum and during this time period the claw vacuum averaged 12.7" Hg and was as high as 14.0" Hg.



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Average Claw Vacuum

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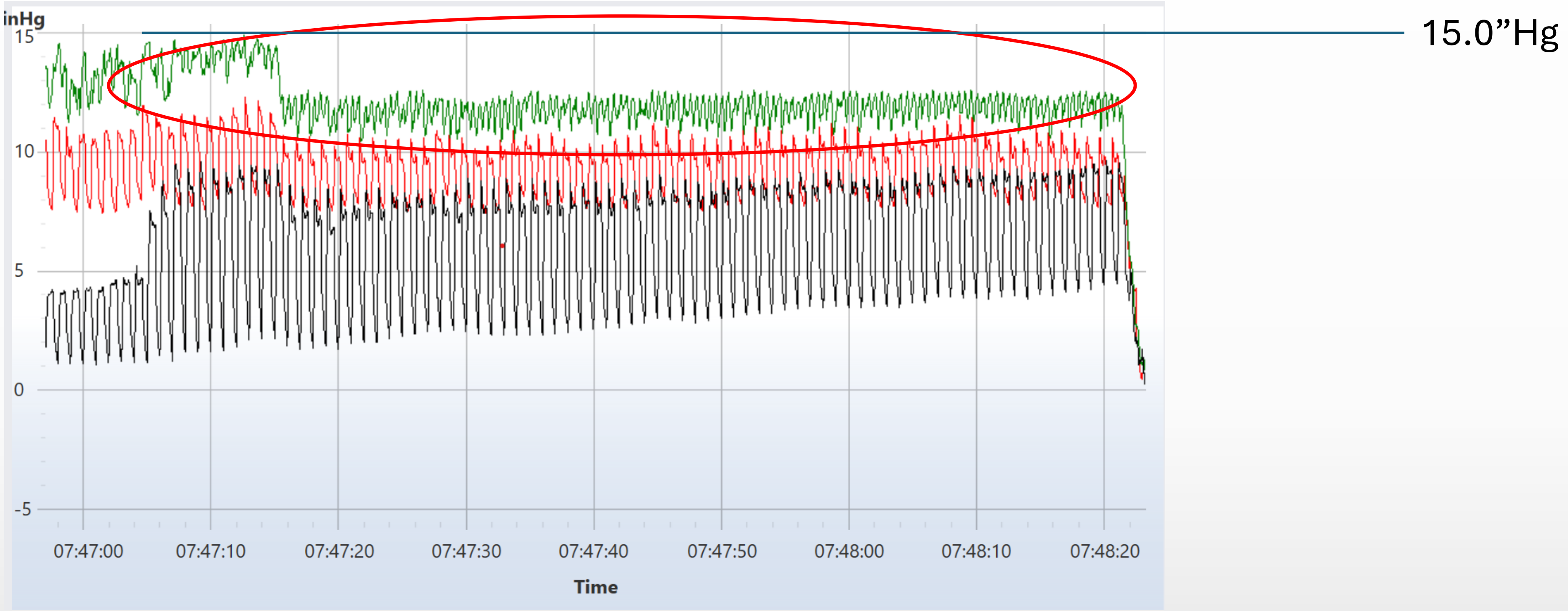


The graph above is of claw vacuum with the green line representing claw vacuum and the red and black lines representing mouthpiece vacuums. Circled is the vacuum drop during the last 60 seconds of milking.

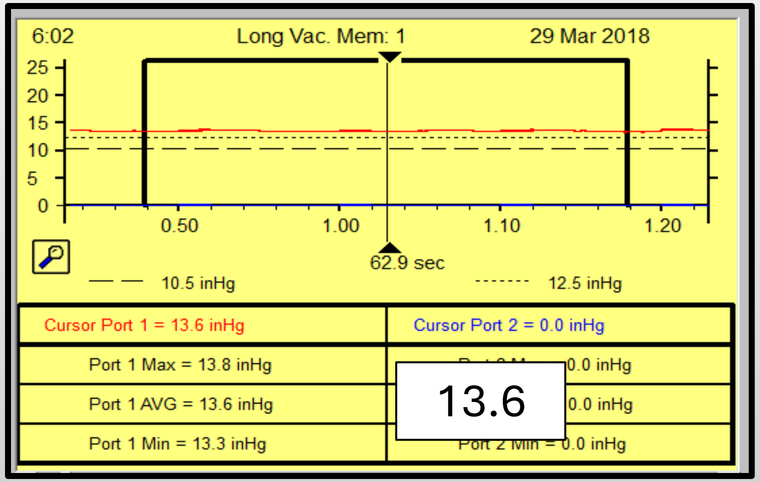
Average Claw Vacuum

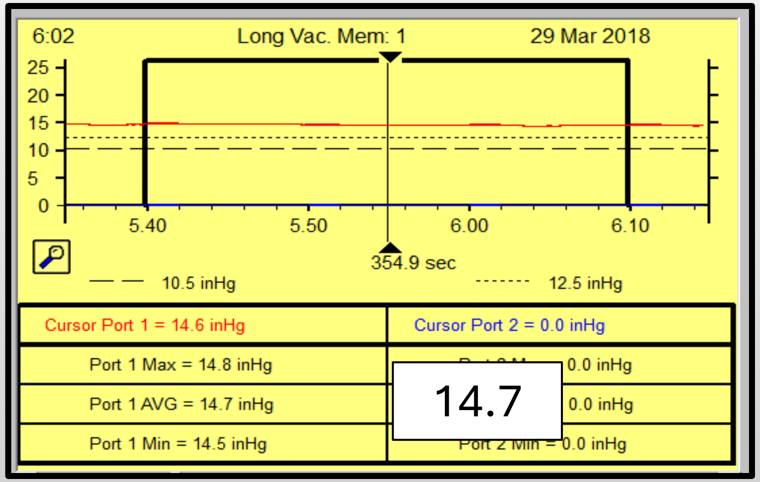
- Average Claw Vacuum at Peak Flow for 5 to 30 seconds
- Your average claw vacuum is: **12.2”** based on 23 cows
- 12/23 or 52% of claw vacuum recordings during peak milk flow exceeded 12.5" Hg
- 3/23 vacuum recordings ≥13.0" Hg.
- This is outside the NMC recommendation of 9.5-12.5”.

- **<u>There is concern for short-term teat end congesti0n after milking and long-term hyperkeratosis given the high claw vacuum recordings during peak milk flow.</u>**

Milkline Vacuum for 30 minutes

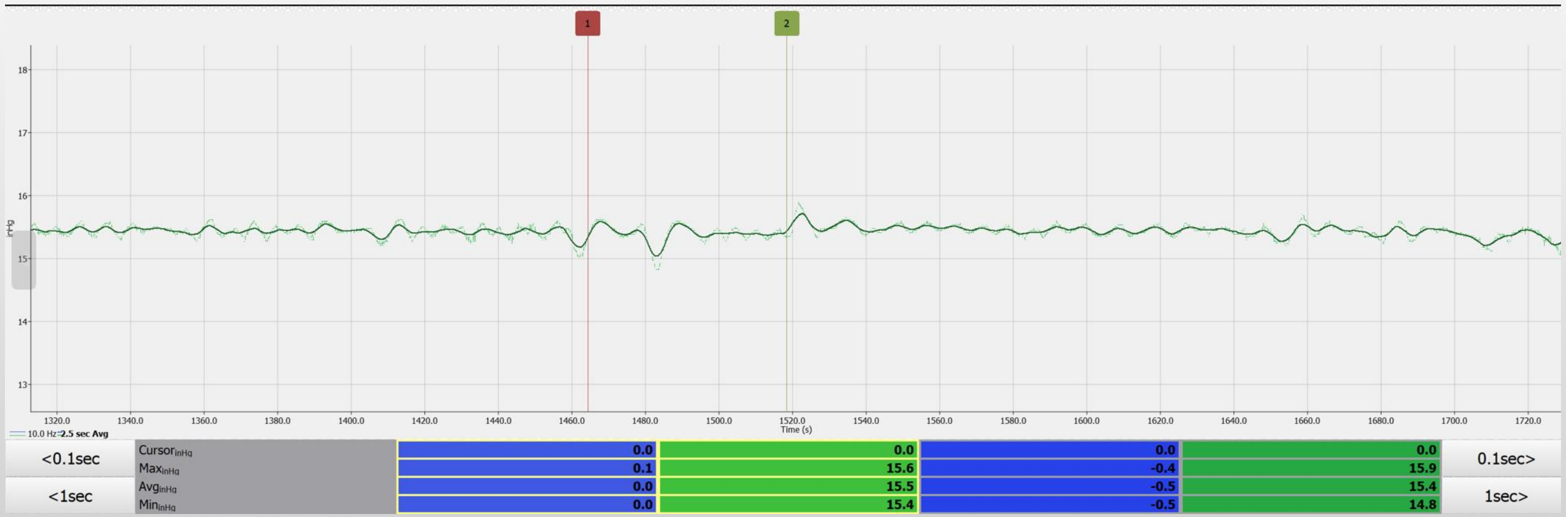
- Continuous recording of milkline vacuum for 30 minutes during normal milking.
- Allows a reasonable assessment of whether major problems with vacuum regulation are present.
- Dbl 24 parlor - Left graph with all units on one side and right graph is with only 12 units on





Milk-line Vacuum During Milking

- We ran a milkline vacuum for 28 minutes during normal milking to assess how well your system responds to normal events during milking.
- The milkline vacuum was within 0.1”Hg above and below the average of 15.5”Hg for the majority of time.
- We also performed a two unit fall off during this time and your system was able to maintain stable vacuum with a vacuum drop, undershoot, of 0.5”Hg and overshoot of 0.4”Hg.



Pulsators

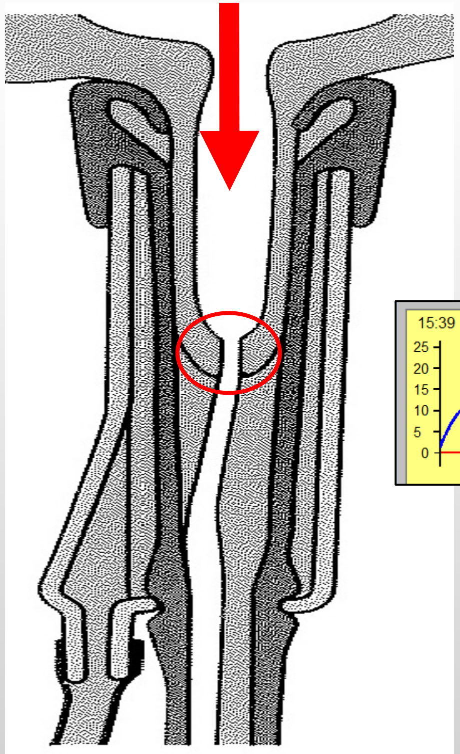
- Usually do 3 to 4 pulsators when the unit is on the cow and milk is flowing
- Save the pulsation graph at peak flow
- **Also do all pulsators in the parlor as a dry test**

Full Milking Load

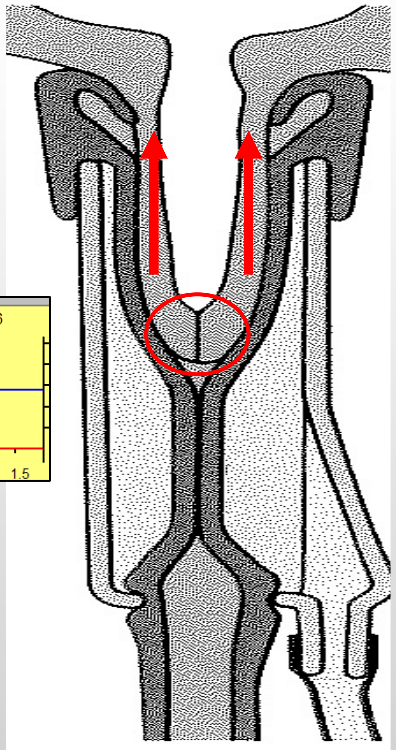
A = 16.0 %	A+B = 64.7 %
B = 48.7 %	Max Vac = 13.6 inHg
C = 11.4 %	Dsec = 236.0 ms
D = 23.5 %	Rate = 59.9 CPM
Limp = 0.0 %	B.Drp = 0.4 inHg

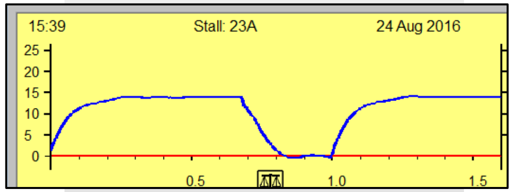
Forces on fluids within the teat during pulsation cycles

B phase (open phase)



D phase (closed phase)





Pulsator Evaluation

- Pulsator Rate= 60 ppm
- Pulsator Ratio = 65:35
- Pulsation under load:
  - B phase of pulsation = 501 ms
  - D phase of pulsation = 238ms

- **Pulsators #1, 30, 68, and 70, should be examined as they have a shortened D Phase.**

Shortened D phase reduces blood flow to the teat-end and may cause hardness/firmness causing discomfort and leading to roughened teat-ends. Often debris in the pulsator will cause pulsators to be out of specifications.

- All other pulsators conform to ASAE standards.
- Katie is planning on coming in every 2 months to graph pulsators.
- **No changes in pulsation parameters are recommended**.

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Strip Yields

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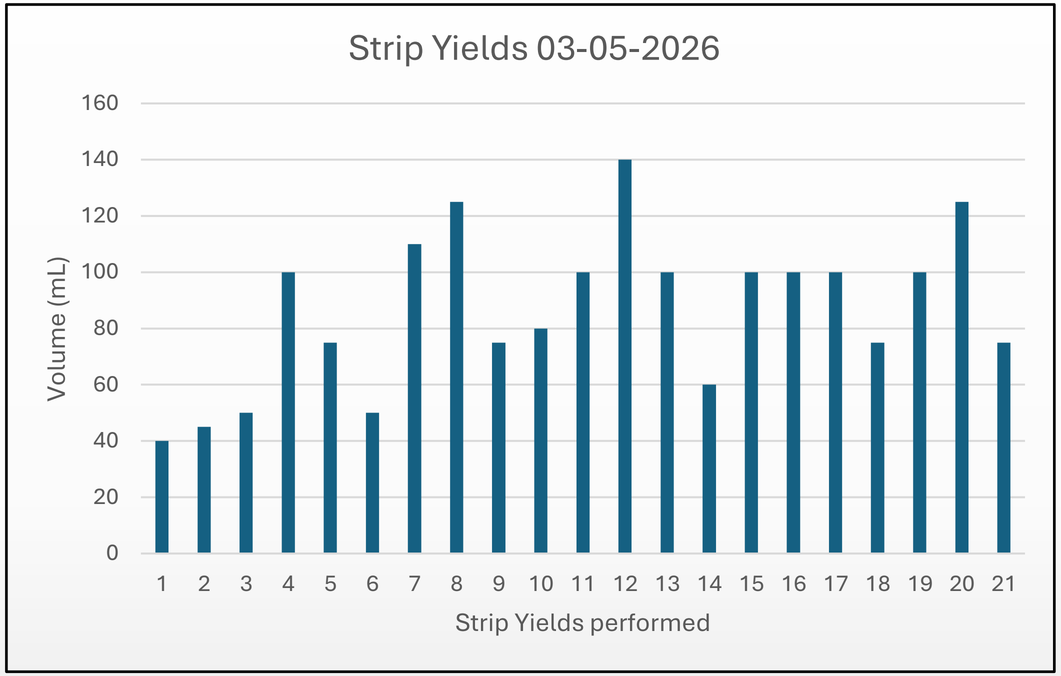
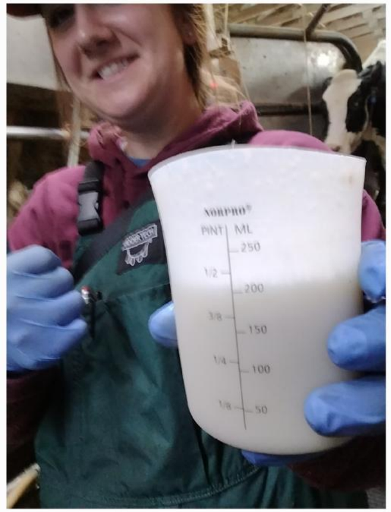
- Animals were stripped for 1 minute immediately after unit detachment. (15 seconds for each quarter)
- Strip yields on 21 animals has improved
  - All strip yields were less than 150 ml
  - No strip yields were between met the goal of 150 – 250 mL (1/2 to 1 cup)
  - Goal is 80% in this category
  - **Average strip yield was 87 ml**
  - Strip yields are too low.
- Consider adjusting take-off to come off earlier.





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Retraction Data

- This is the timing of the retraction time of each individual unit. We turn the unit on and then hit retract and start the stop watch when it starts moving and stop it at the point of full retraction.
- <u>**We identified any unit taking longer than 4 seconds to retract. Units #38 and 56 should be further evaluated.**</u>

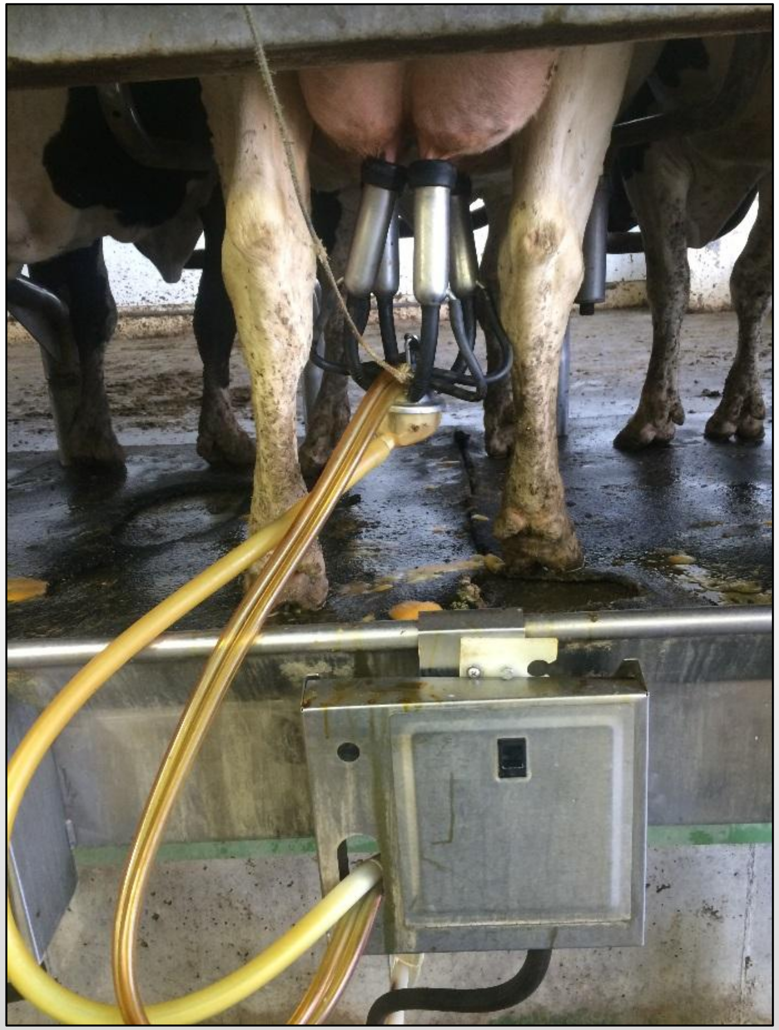
Unit	Time	Unit	Time
1	0.21	37	0.5
2	0.73	38	5.28
3	0.17	39	1.08
4	2.3	40	0.71
5	1.09	41	1.18
6	0.62	42	1.13
7	0.73	43	1.08
8	1.18	44	1.98
9	1.09	45	1
10	1.68	46	1.41
11	0.43	47	1.21
12	0.91	48	0.98
13	0.56	49	1.48
14	1.18	50	1.37
15	0.18	51	2.42
16	1.05	52	2.09
17	1.23	53	1.01
18	0.94	54	1.18
19	0.96	55	1.2
20	0.1	56	4.45
21	1.75	57	0.65
22	1.04	58	1.54
23	1.16	59	1.03
24	1.48	60	1.65
25	0.35	61	1.04
26	1.21	62	1.19
27	1.41	63	1.11
28	0.91	64	1.18
29	1.46	65	1.35
30	1.08	66	1.24
31	1.03	67	1
32	1.32	68	0.76
33	1.31	69	1.9
34	1.44	70	0.91
35	0.35	71	2.29
36	1.06	72	1.23

Unit Alignment Scoring

- 2 category scoring system
  - Proper
  - Improper
- Score within first 2 minutes of milking
- Do not score:
  - ¾ cows
  - Poor udder conformation

Unit Alignment Scoring

- Poor unit alignment
  - Increases the risk for liner slips
  - Influences milking speed of individual quarters
  - Increases the risk of teat damage
  - Decreases parlor efficiency
    - Prolonged unit on time
    - More chance of reattach

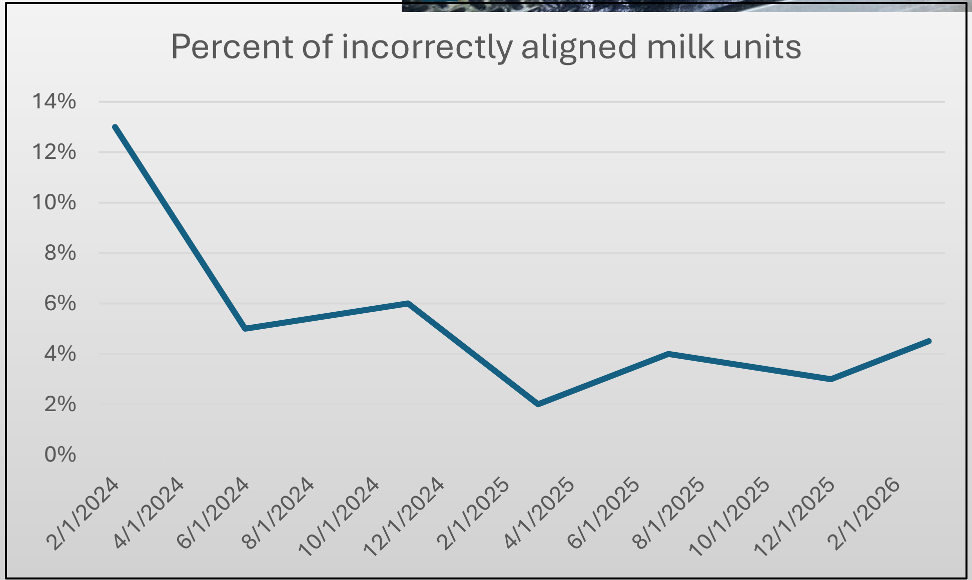


Unit Alignment

- **Unit alignment is very good.**
- **95.5% were properly aligned.**
- **Alignment is a function of adjusting the hose length in the coupling (red arrows)**

- Why does it matter?
  - Prolonged unit on time due to quarters milking at different speeds
    - Decreased parlor efficiency
    - Increased risk of teat damage due to prolonged time in low flow
      - Cows kicking at units leading to more manure on units and teats
  - Increased number of liner slips
    - Causing units to come off early as the detacher kicks in (major issue if bimodal letdown at front end of milking)
      - Either cow leaves not milked out

      or
      - Reattached which slows up the parlor and increases risk of teat damage due to prolonged time in low flow



People Performance

Milking Routine 72 Stall Rotary

• 1st Milker	1.) Pre-strips and then foams with a cup foamer (stall 1-2)
• 2nd Milker	2.) Wipes cow with cloth towel (stall ~7)
• 3rd Milker	3.) Attaches unit (see below as variable by group)

Turn time was approximately 6.5 seconds per stall. The 2 year olds and low production cows have a longer lag time with mat at approximately stall 20-21 and high production cows with a medium lag time with mat at stall 17-18.

Milking Routine Timing

	Dip contact time 1st cow	Dip contact time last cow	Lag time cow 1	Lag time cow 5
Average	0:48	2:26	1:23	0:53
	> 0:30	> 0:30	> 1:30	

- Example of timings from a recent farm visit where the lag time was too short.

Excellent	Needs attention	Poor values

Milking Routine 72 Stall Rotary

Dip contact time = 36 seconds = Good

Lag time from stimulation to unit attachment = 106 Seconds = Good

- Percent below 90 seconds = 0% = Excellent

Average Strip/stimulation time (2 teats left side) = 3 second = fair (1.5 seconds per teat)

Average unit on time: 03:46

Routine is meeting the goal of >30 seconds for dip contact time. The lag time from stimulation to unit attachment is within the goal of 90 to 180 seconds and is above 90 seconds for all cows we timed! 2 minute milk is good but has declined some.

Stripping stimulation is short. When stripping all four teats, hands-on stimulation lasted for approximately 1.5-2 seconds per teat (8 seconds total). Milkers are performing the same routine but now on only two teats.

<u>Is it possible for milkers to strip for an additional second on each teat?</u>

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Teat End Cleanliness

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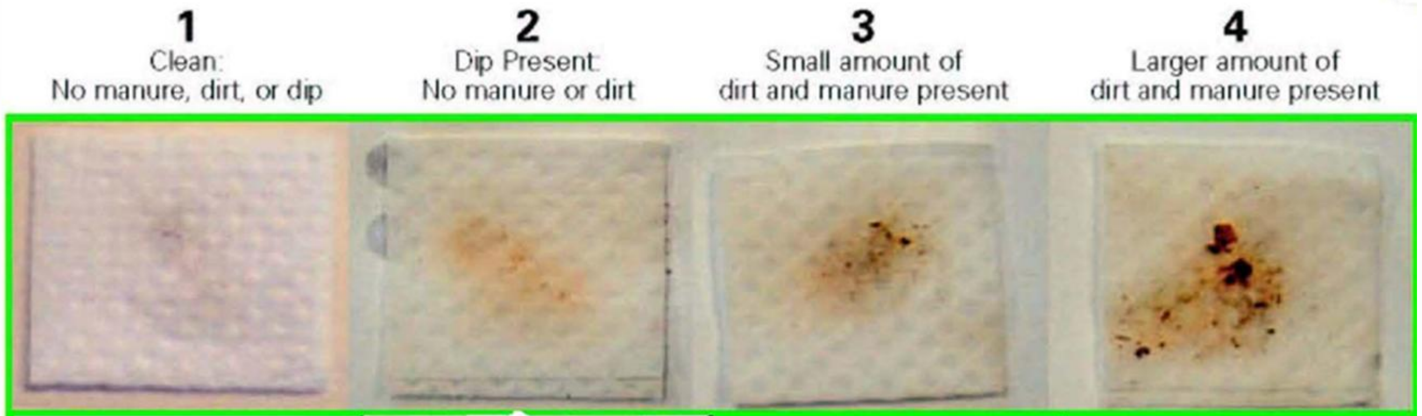
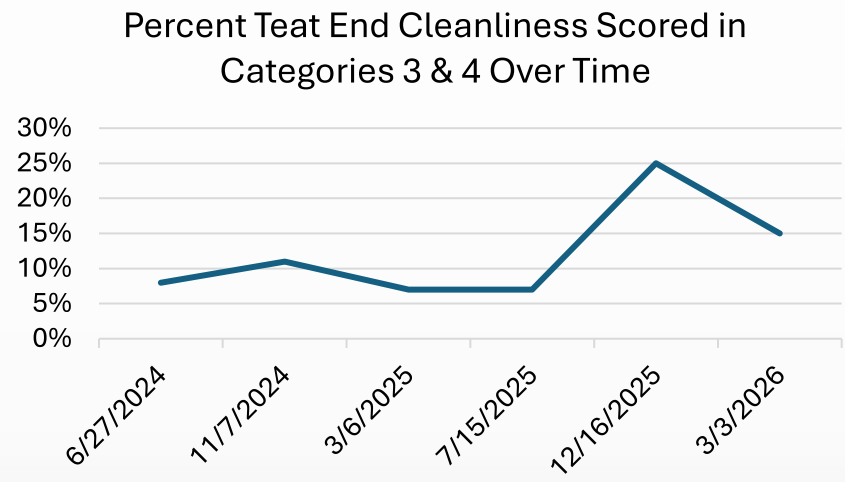
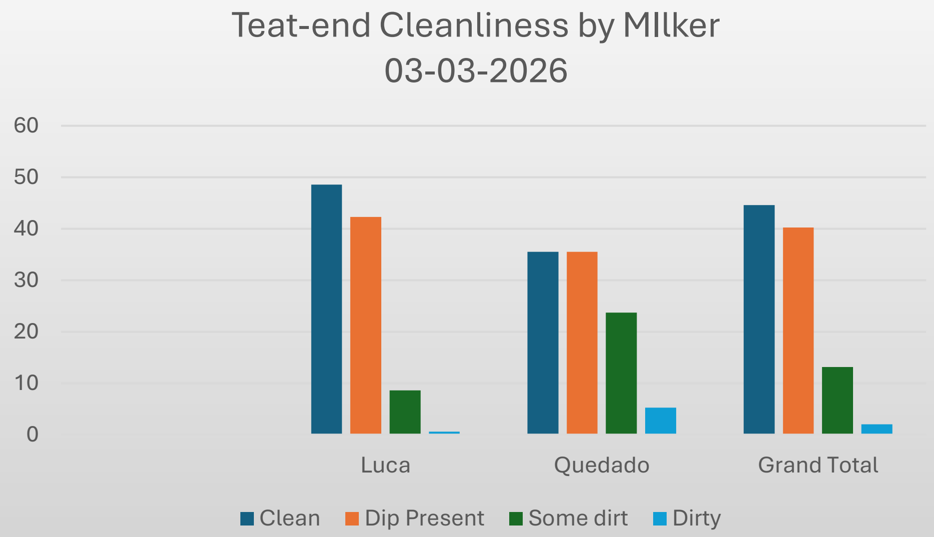
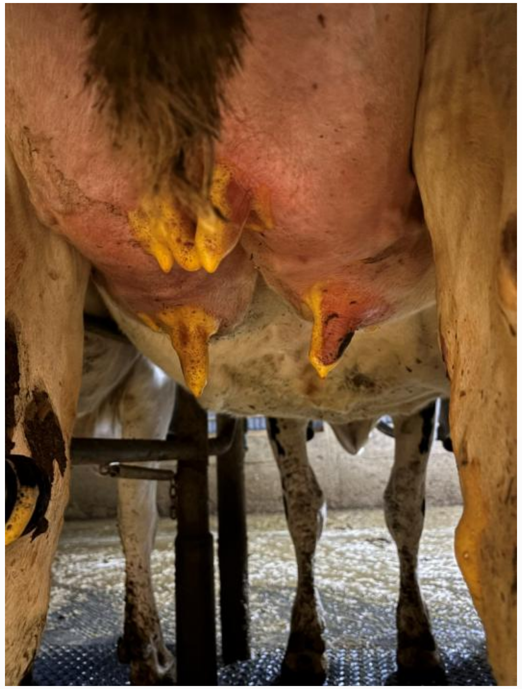


	1	2	3	4
All Groups	112 (40%)	101 (45%)	33 (13%)	5(2%)

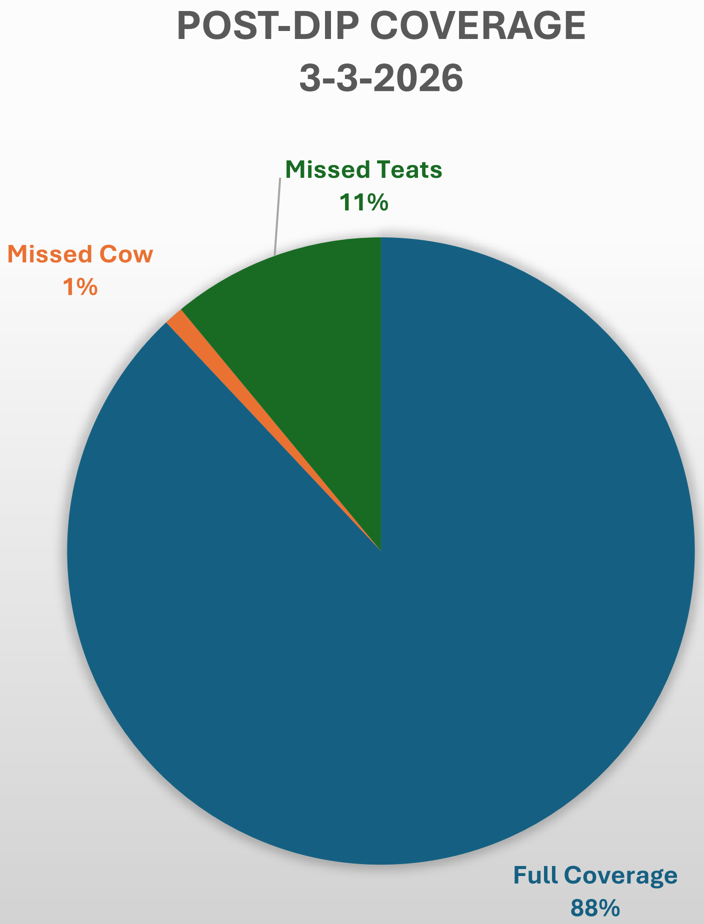


- 15% of the 251 teat-ends that we scored were too dirty.
- **<u>This has improved since December 2025 but is still higher than the goal of less than 10% in category 3 or 4</u>**.
- Continue to work on training and motivating milkers in this area.
- Continue to perform routine teat-end cleanliness scoring.



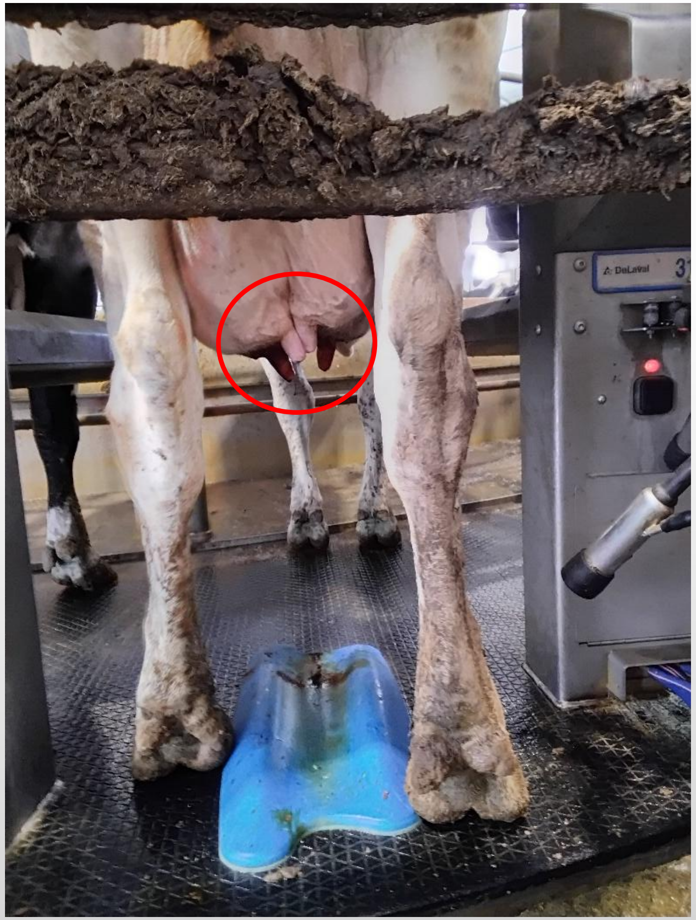


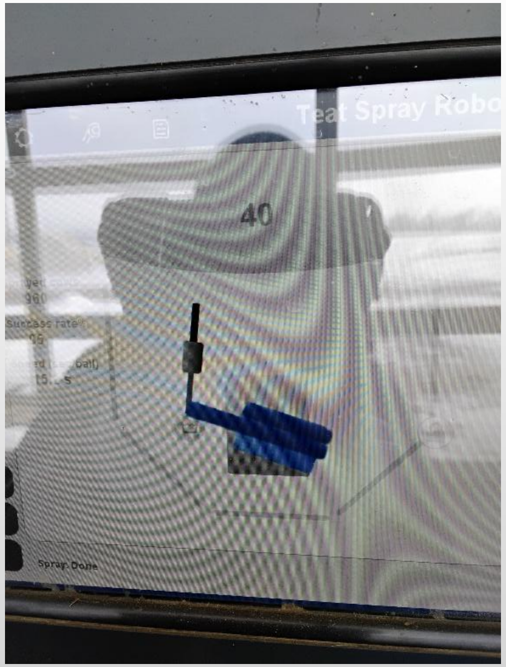
Teat-dip Coverage



- Pre-dip coverage looked reasonable on the day we visited. **<u>Just keep an eye on the consistency of the pre-dip to make sure it does not get too dry as you want it wet enough to flow to the teat-end.</u>**
- Post-dip is applied with a robot. See the chart to the right for the coverage data based at the cow level. **<u>88% of the 103 cows we observed showed full teat-dip coverage of all teats.</u>**

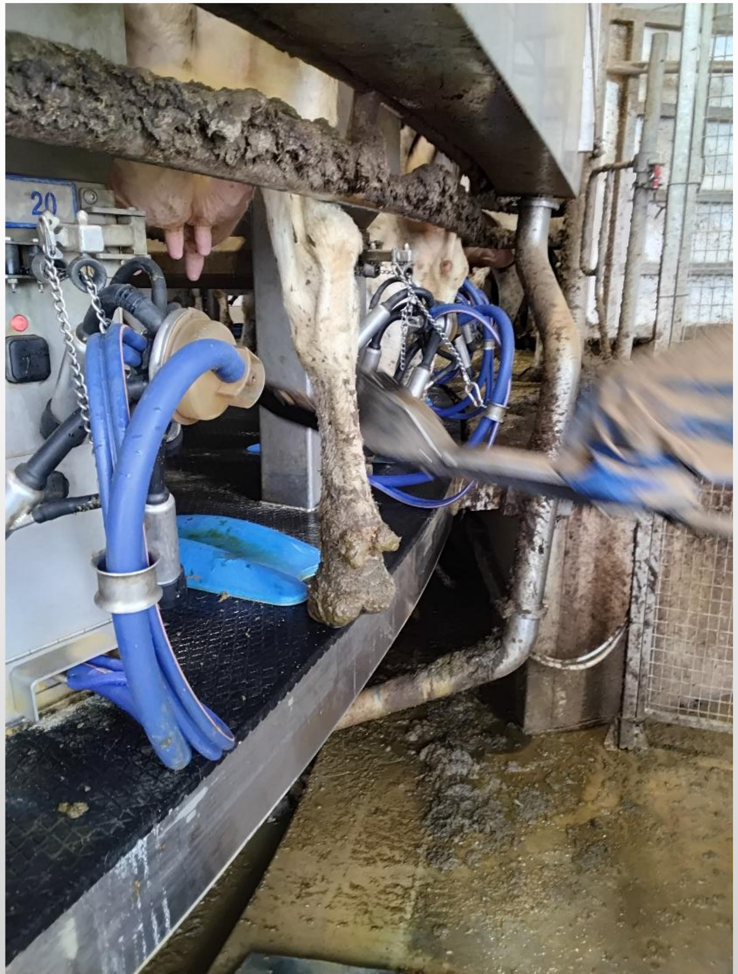
Post-dipping





- 100 animals were monitored during post-dipping
- 15% of the animals monitored had 1 or more teats missed.
- The robot reports that 95% success rate.

Post-dipping



- Cows tend to stand with their right hind leg back
- This prevents the robot from entering between their legs and spraying teats
- Consider moving leg separator back

Animal Assessments

Teat Scoring after Unit Detachment

- All teats are scored with 60 seconds of unit detachment using the Teat Club International guidelines

- Two main categories
  - Short/medium term effects
    - Is the cow happy with the way she was milked today?

  - Longer term effects
    - What changes are present that have happened over time?

Short and Medium-term Effects

- Teat color
- Swelling at the teat base
- Hardness at the teat end

Primarily associated with milking machine faults or poor milking management resulting in long periods of low flow (Teat Club International)

Firmness at Teat End

Post-Milking Teat Evaluation

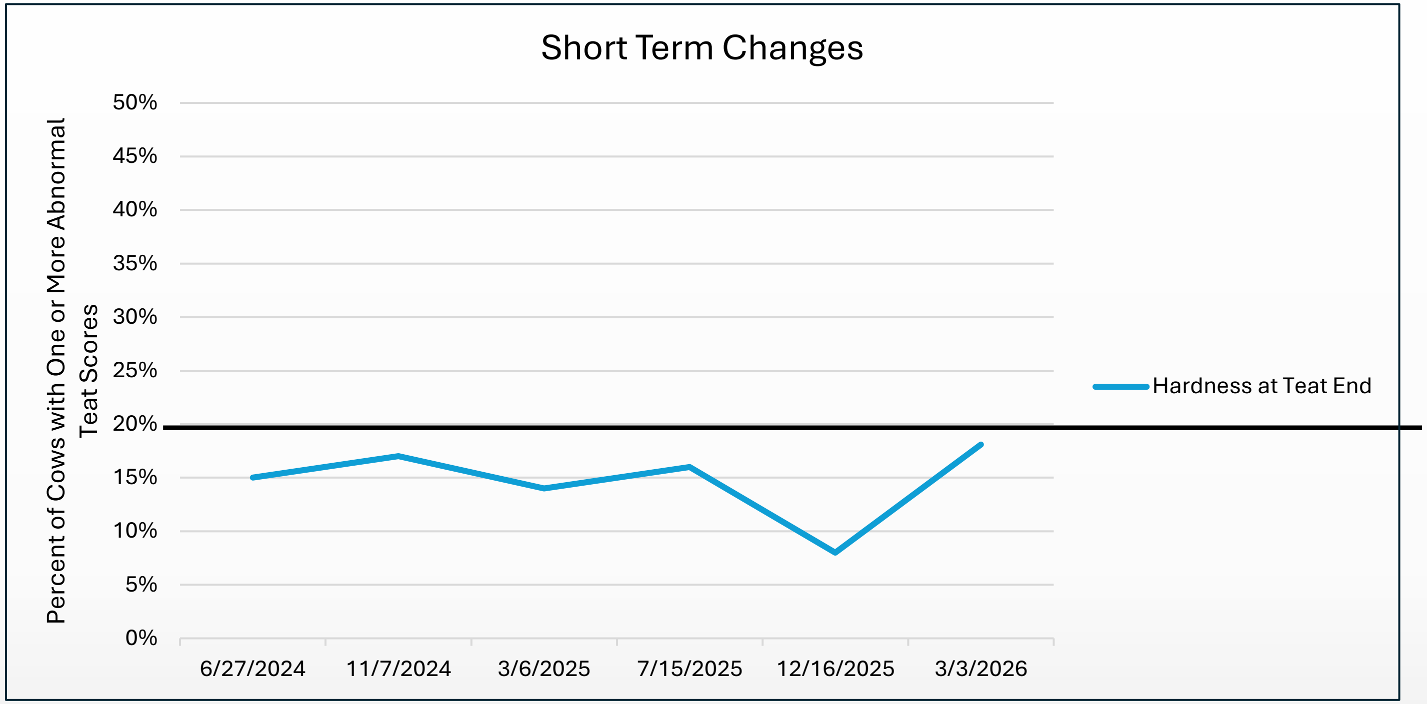
- 532 cows (2,128 teat-ends) were scored in Groups 6,8,9,10,11,12,and 13 to evaluate short-term and long-term teat-end conditions
- Teats were scored within 60 seconds after the milking unit detached
- Teat color, hardness at teat-end and swelling at teat base are short-term changes
- Skin condition and teat end scores are long-term changes

Post-Milking Teat Evaluation – Short Term

Categories	**GOAL** Percent of cows with one or more abnormal teats	**FARM DATA** Percent of cows with one or more abnormal teats
Teat color	<20%	3.4%
Hardness at teat-end	<20%	18.1%
Swelling near teat base	<20%	<1%
Visible mark	<20%	1.1%
Hemorrhage	<10%	Not noted
Total Cows Scored	>20% (of milking cows)	**532 cows**

- Short term teat-end condition is very good despite bimodal milk curves noted.

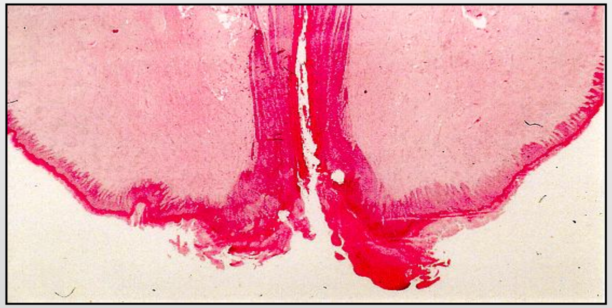
Post-Milking Teat Evaluation – Short Term



- The short-term teat scores are still good and meeting the goal of less than 20%.
- The 2-minute milk continues to improve which keeps the time in low flow at the beginning of milking lower.
- Based on the data collected on this survey, the opportunity areas to help keep the short-term teat scores in line are to correct the abnormal pulsators, and continue to actively monitor the milker performance.

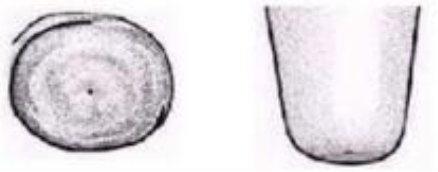
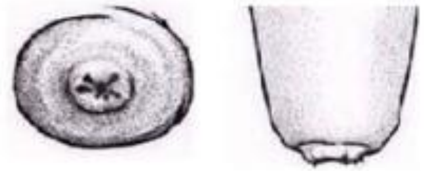
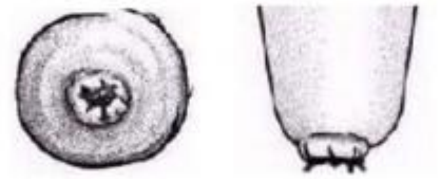
Longer Term Effects

- The changes that have happened over a slightly longer period of time
  - Teat Skin Condition
    - Normal, Dry, Open Lesion
  - Teat End Hyperkeratosis
    - Normal, Smooth, Slightly Rough, Rough, Very Rough



Shukken et al

Post-Milking Teat Evaluation – Long Term

Teat End Condition Score Card		
Score	**Description**	**Illustration**
Score 1 (N)	**No Ring.** The teat-end is smooth with a small, even orifice. This is a typical status for many teats soon after the start of lactation.	
Score 2 (S)	**Smooth or Slightly Rough Ring.** A raised ring encircles the teat orifice. The surface of the ring is smooth or it may feel slightly rough but no fragments of old keratin are evident.	
Score 3 (R)	**Rough Ring.** A raised, roughened ring with isolated fragments of old keratin extending a short distance from the teat orifice.	
Score 4 (VR)	**Very Rough Ring.** A raised ring with rough fragments of old keratin extending out from the teat orifice. The rim of the ring is rough and may be cracked, often giving the test-end a "flowered" appearance.	
Score 5	**Open Lesions or Scabs.**	*Not pictured.*

- 2128 teat-ends were evaluated.
- Teat-end condition has improved in each group evaluated. Now 35% of teats are slightly rough to very rough. This is down from 67% in March 2025,
- Approximately 80% of the animals evaluated had normal (76%) or smooth (4%) teat-ends.
- 9% of teat-ends were slightly roughened, 8% were rough, and 3% of animals evaluated had very rough teat ends.

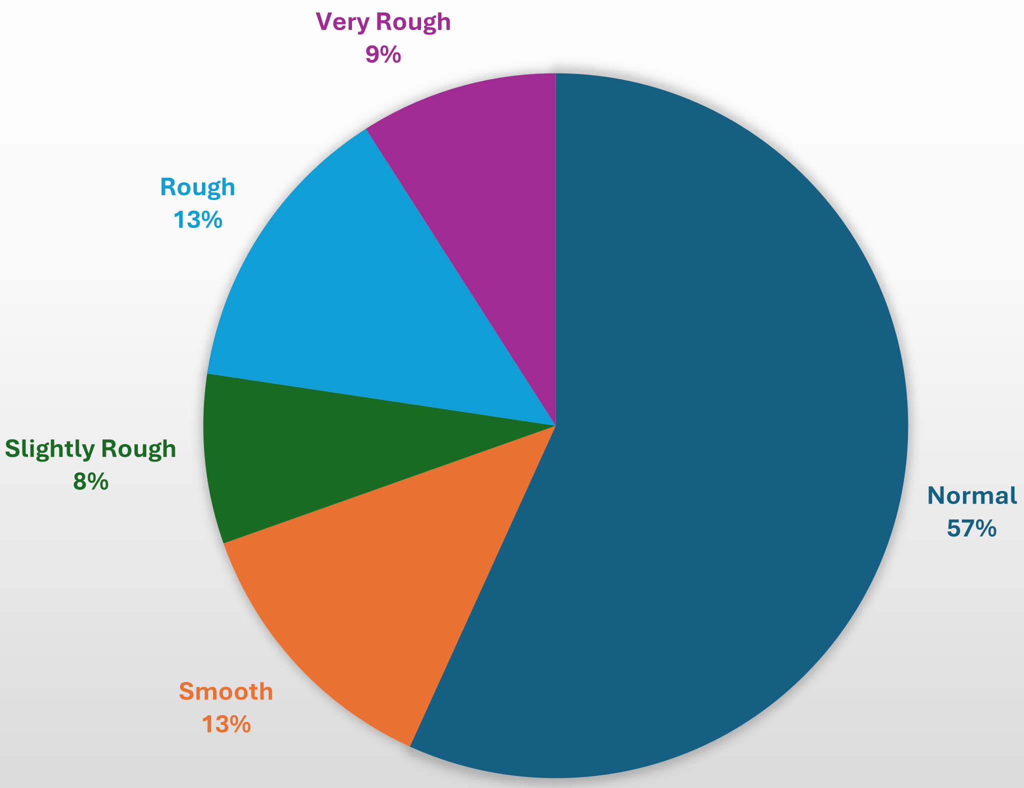
Post-Milking Teat Evaluation – Long Term

Categories	**GOAL** Percent of cows with one or more abnormal teats	**FARM DATA** Percent of cows with one or more abnormal teats
Teat-End Score	<20%	35%
Teat Skin Condition	<20%	2.7%
Open Lesions	<5%	<1%
Total Cows Scored	>20% (of milking cows)	532cows

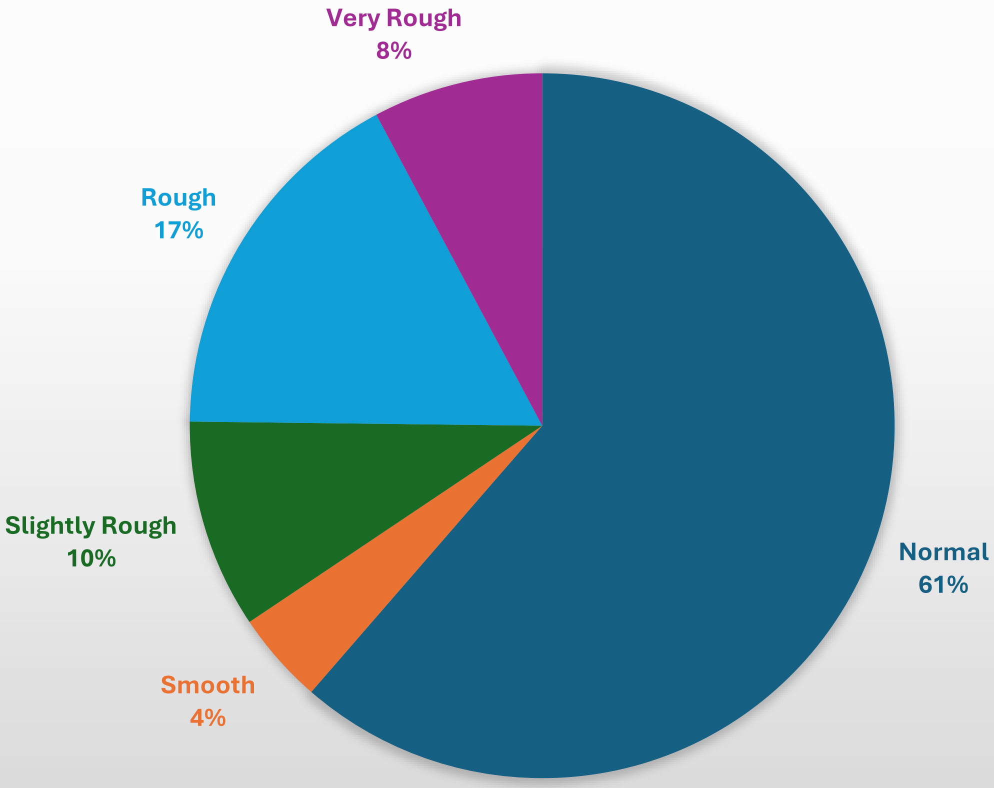
- 35% of animals were noted with abnormal slightly roughened to very roughened teat-ends. Herd goals are less than 20% of animals with abnormal teat-ends.
- Skin condition was very good with 2.7% dry teat abnormalities noted. Very few open lesions were observed.

Post-Milking Teat Evaluation – Long Term

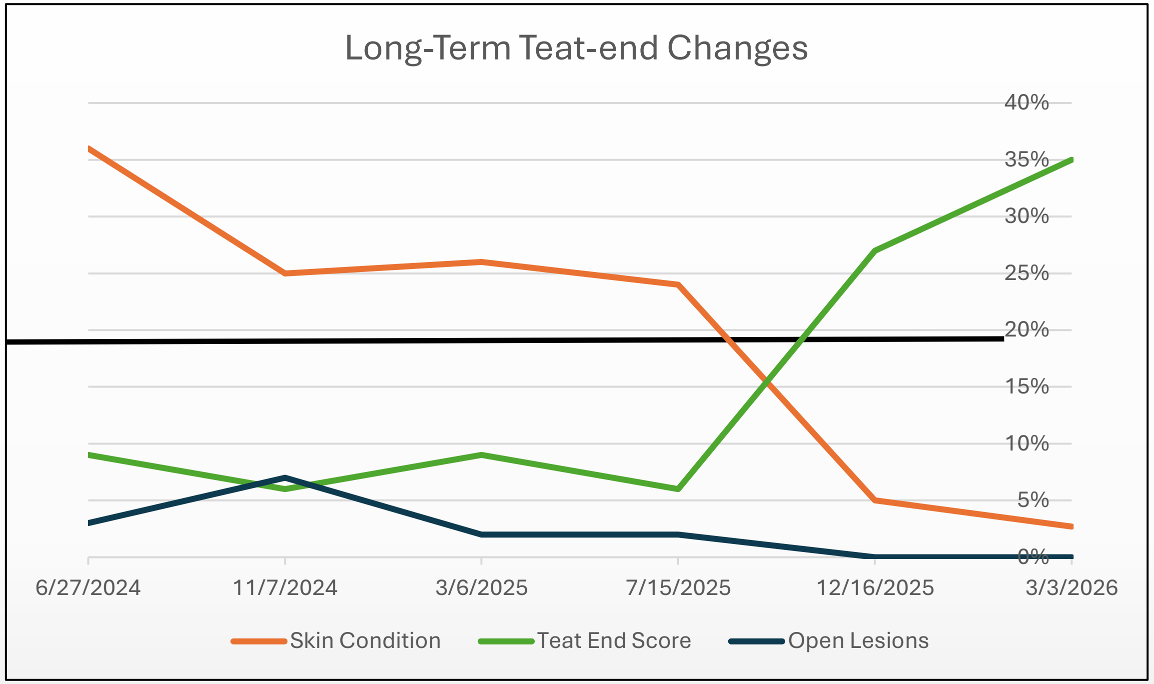
LONG-TERM TEAT END SCORES
DAIRY 12-16-2025



LONG TERM TEAT-END SCORES
DAIRY 03-03-2026



Post-Milking Teat Evaluation – Long Term

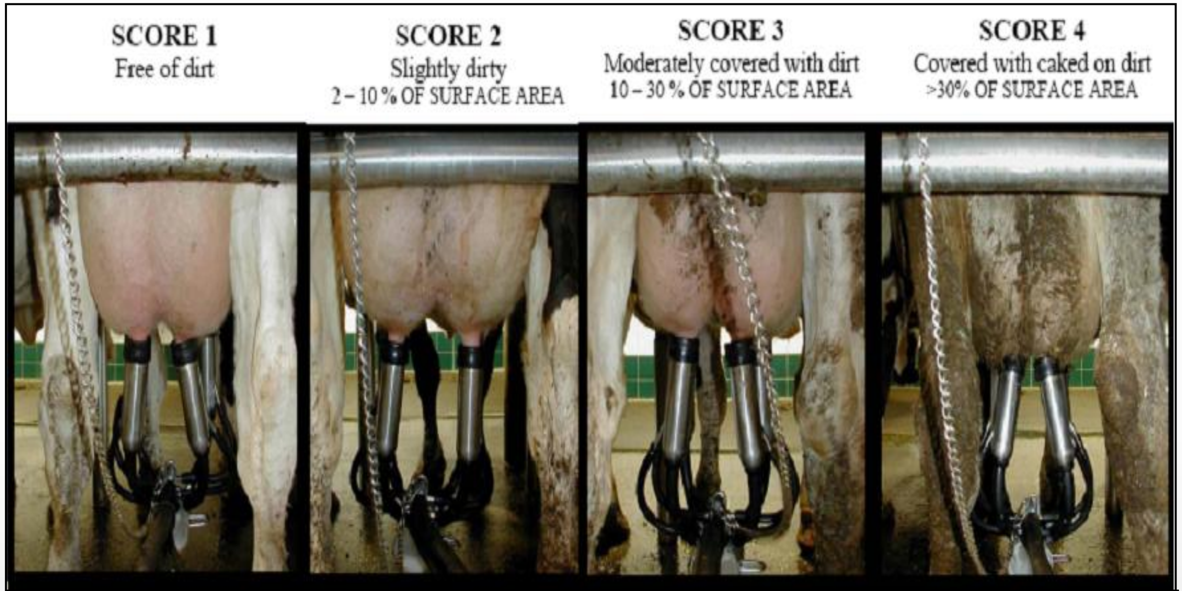


- Teat-end scores have improved some since out audit in December 2025.
  - Continue to focus on maintaining the short-term changes in order to maintain the teat-end scores.
- Skin condition – Skin condition has improved and open lesions are minimal.
  - **<u>Continue to use the same emollient package..</u>**
  - Continue to have the robot on when pre-fresh animals are brought through the rotary to get some dip on them and allow the emollients to work.

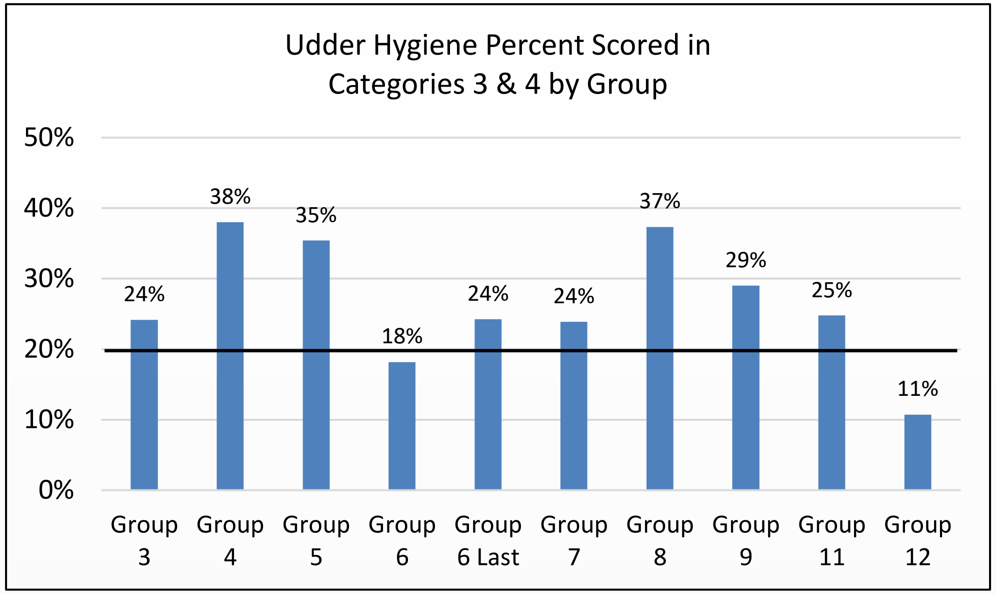
Udder Hygiene Scoring System

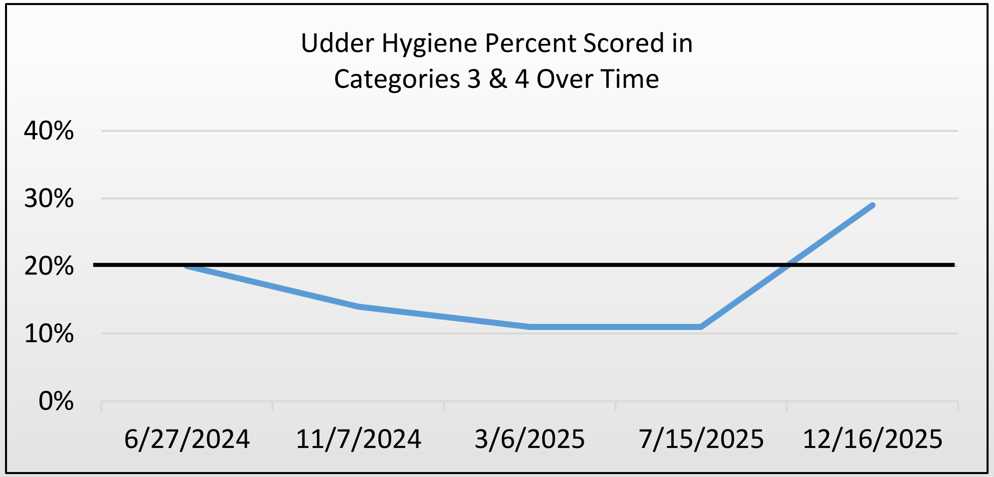
- Poor udder hygiene
  - Makes it harder to achieve clean and dry teats prior to unit attachment
  - Increases the risk for mastitis
  - Increases the milking time due to increased prep time

Udder Hygiene



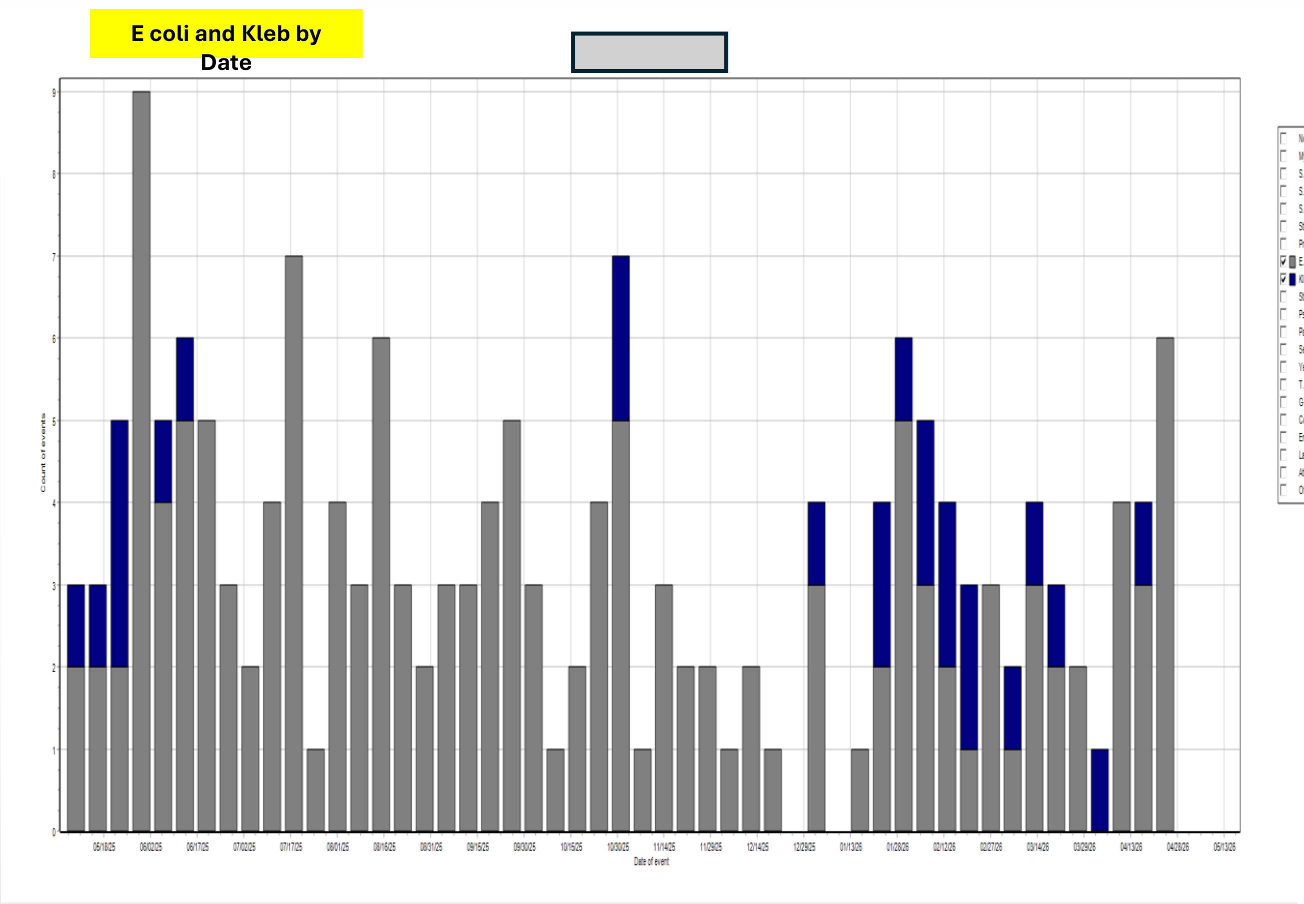
816 (71%)	**332 (29%)**

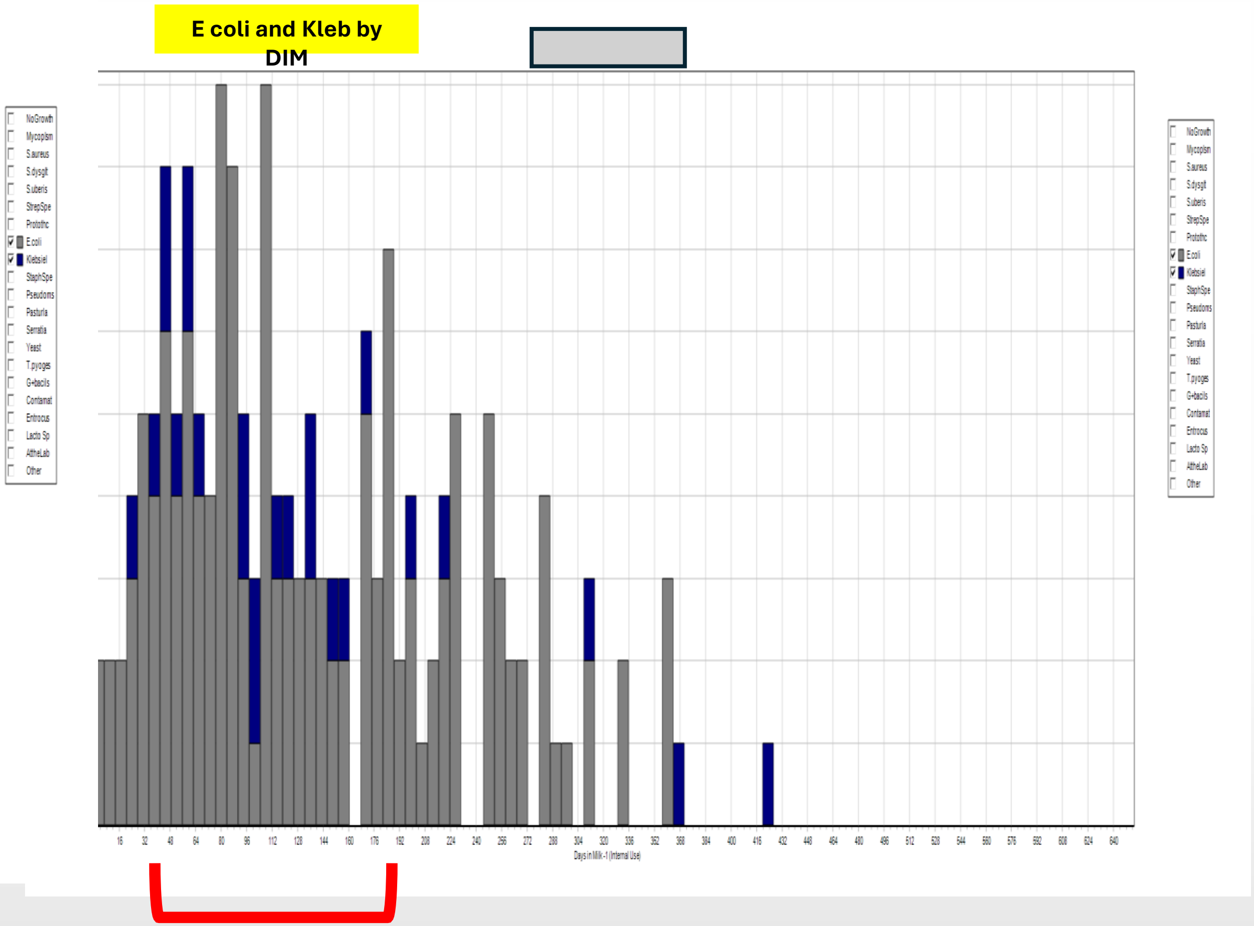




- Cows were more dirty than usual
- 29% of the cows that we scored were in category 3 or 4. **This is not meeting the goal of less than 20% in category 3 and 4**.
- As shown on the next few slides there is still room for improvement as the back of the stall beds are being contaminated with manure due to poor cow positioning in some groups.

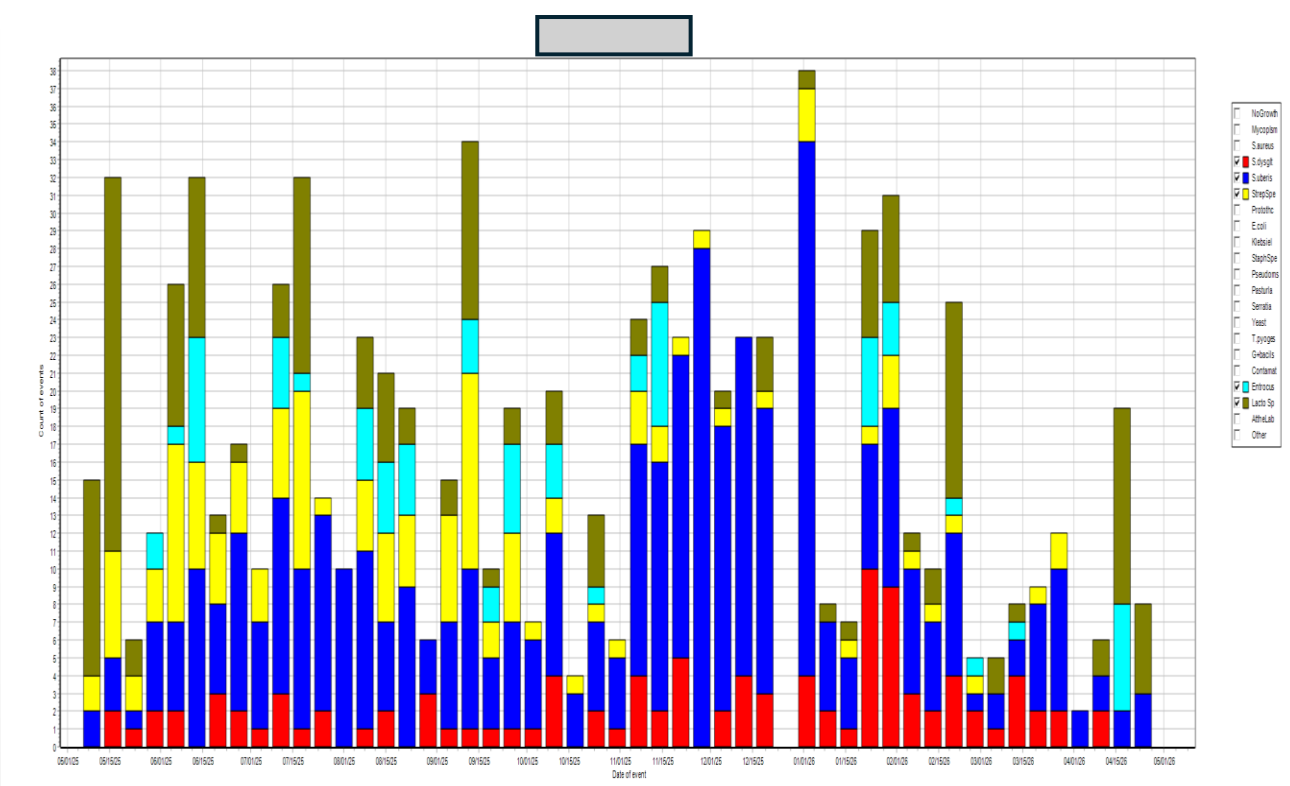
Culture Data



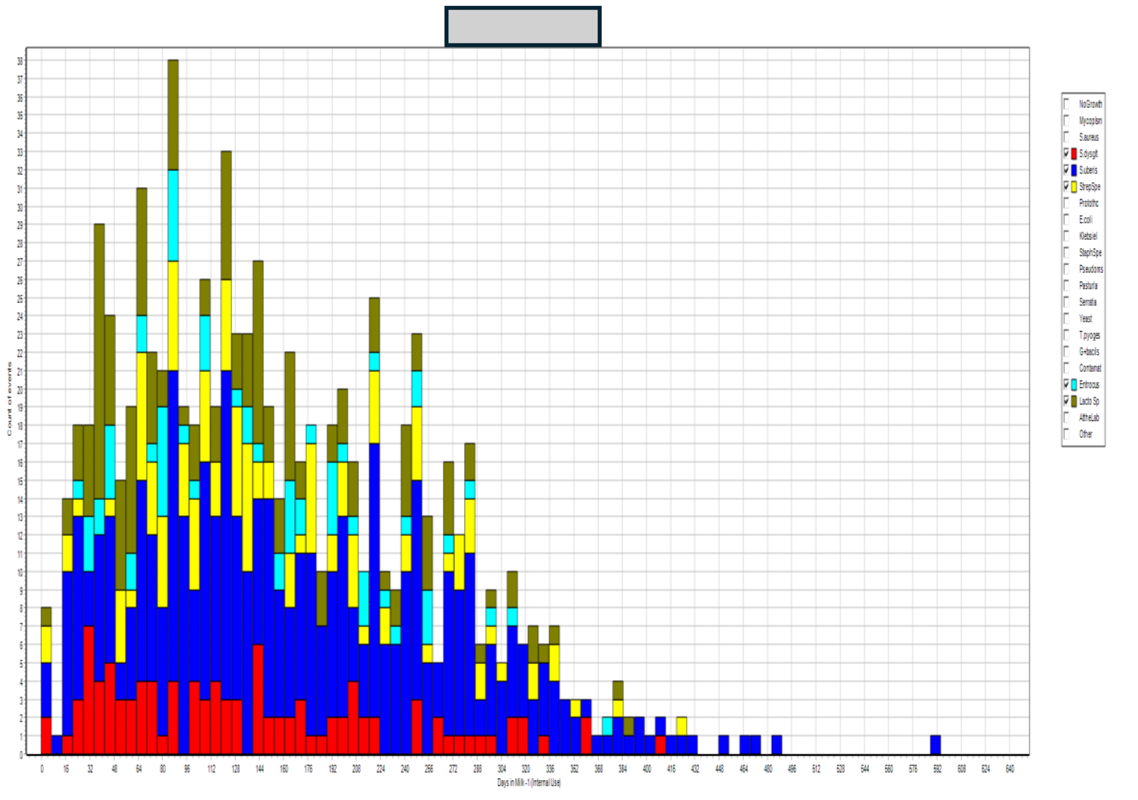


- This is a graph of just the *E coli* and *Klebsiella* cultures by date on the left and by DIM on the right. The highest risk period for E.coli infection is also 30-120 days in milk. Parlor activities, movement of animals to and from the parlor and lactating stalls should be our focus.

Streps by Date



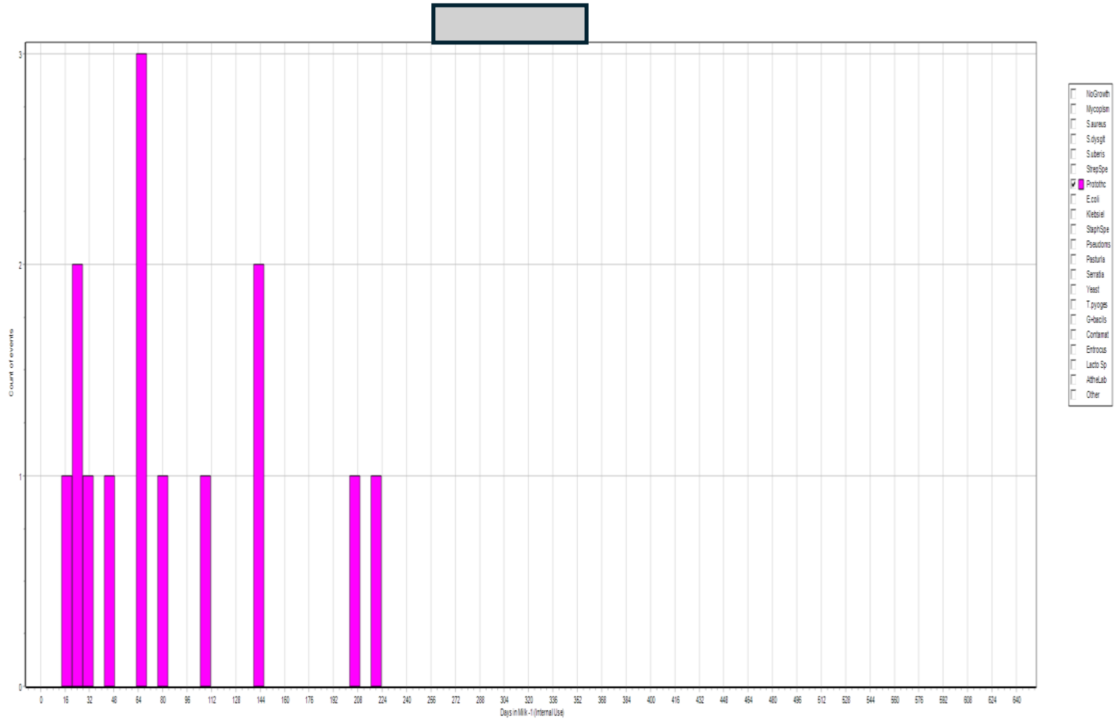
Streps by DIM



- This is a graph of just the *Strep* and *Strep*-like cultures results by date on the left and by DIM on the right over the last year from DC 305. *Strep uberis* was a predominant organism during the fall 2025. Many of these infections occur in early to mid lactation.

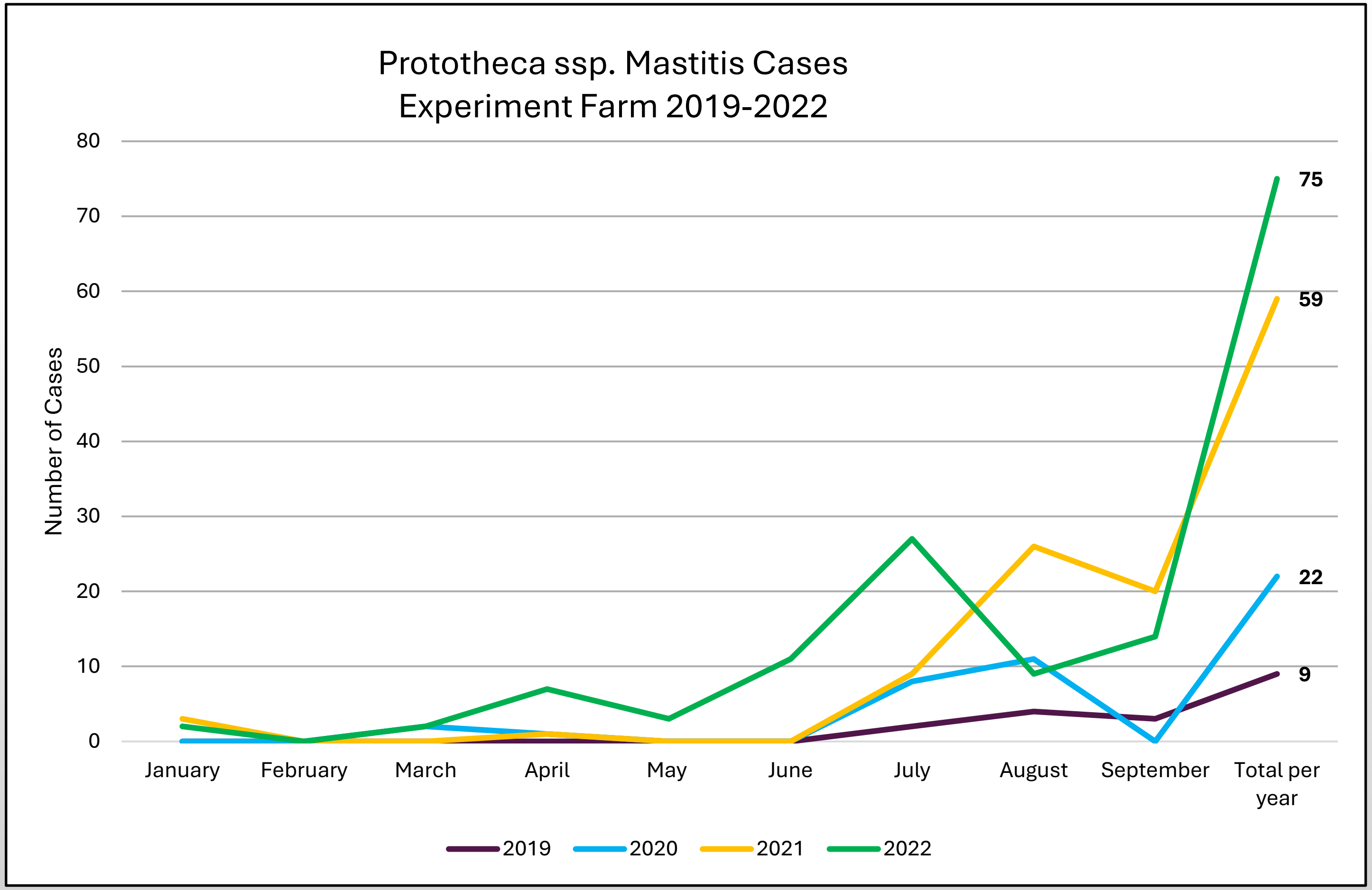
Prototheca by Date

Prototheca by DIM



- This is a graph of just the *Prototheca* cultures by date on the left and by DIM on the right over the last year from DC 305. There does continue to be some found although it looks lower recently.

Please fix this...



Case Report: Reducing *Prototheca* Biofilms with Ultra-Pure Chlorine Dioxide

Michael J. Zurakowski[1], Jeniece L. Schroeter[2], Katelyn M. Keller[1], and Scott M. Sloan[2]

[1]Quality Milk Production Services, Cornell University
Cobleskill, New York, USA
[2]Virus Shield Biosciences, Melbourne, Florida, USA

Cornell University College of Veterinary Medicine
Animal Health Diagnostic Center

virus SHIELD BIOSCIENCES

Objectives

- *Prototheca* characteristics
- *Prototheca* mastitis
- *Prototheca* diagnostics
- Case Report
- Future Plans

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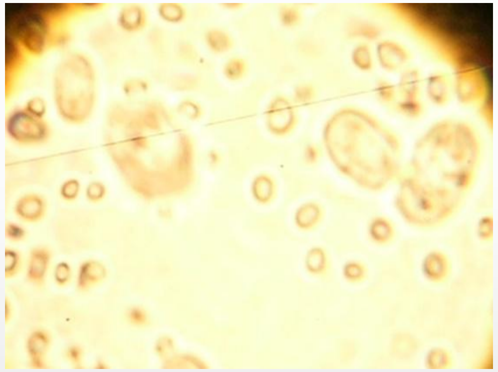
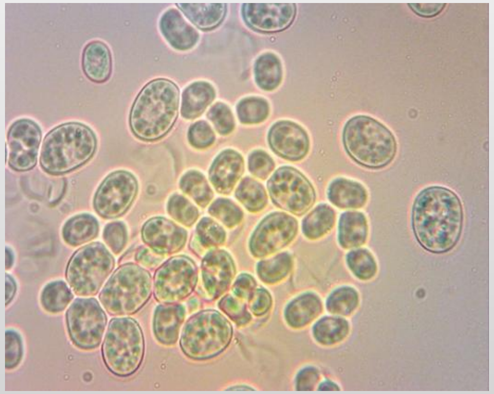
Prototheca characteristics

- First identified in 1894 by Kruger from slime flux of trees
- First identified as a mastitis pathogen in 1952 by Lerche
- Unicellular
- Colorless
- Chlorophyll-lacking
- Algae
- Often placed in yeast or yeast-like group
- Linked to disease in mice, rats, dogs, pigs, deer, cattle, and man
- Zoonotic





Prototheca characteristics

Prototheca bovis
April 2020

Pathogenic

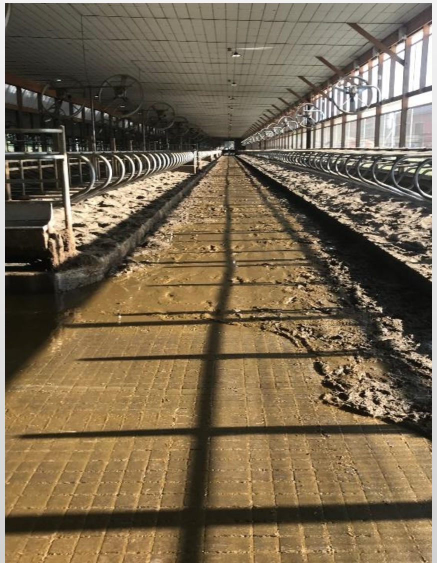
- *P. zopfii* genotype 1 & 2
- *P. blaschkeae*
- *P. wickerhamii*

Environmental

- *P. ulmea*
- *P. stagnora*
- *P. moriformis*

Prototheca characteristics
Environmental isolation sites

- Moist, humid environments
- Ample decaying organic matter
- Dairy farm sites include: feces, drinking toughs, manure piles, sewage ponds, standing water puddles, bedding and silage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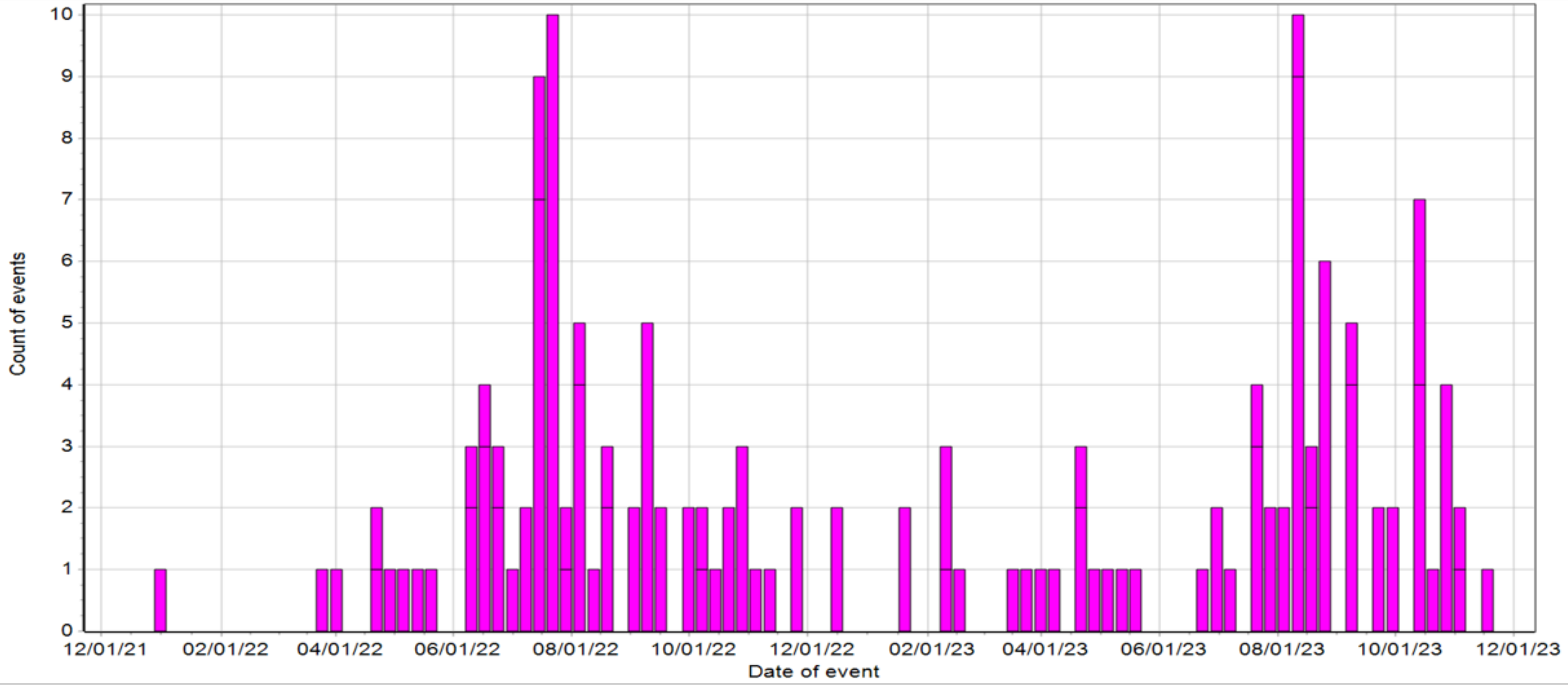
Prototheca mastitis

- Environmental pathogen capable of contagious transmission
- Chronic, non-responsive, subclinical, and clinical mastitis
- Intermittently shed
- Seasonal effect



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Prototheca Diagnostics

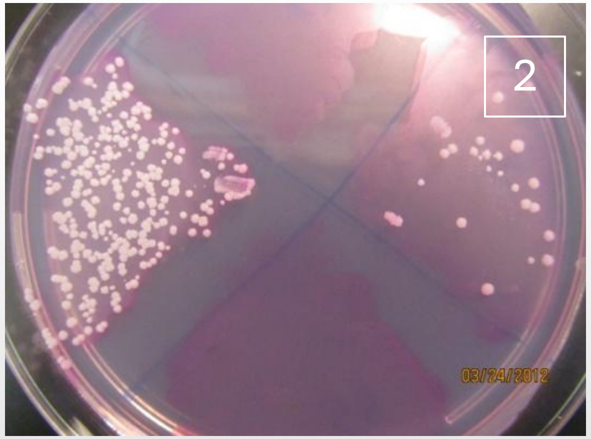
Colonial Morphology Trypticase Soy Agar with 5% Sheep Blood and Prototheca Isolation Media (PIM)

Colonies on blood agar, after 48 h of incubation are small white-grayish, dull, granular and pasty (1). The colonies are small at 24 hrs. and may be overlooked. They are much larger at 48 hrs. On PIM are small and whitish (2).

Blood Agar media

Prototheca bovis

48 hour growth



Prototheca Isolation Media (PIM)

Applied Microbiology, Oct. 1973, p. 648-649 Vol. 26, No. 4
Printed in U.S.A.

Selective Medium for the Isolation of *Prototheca*

R. SCOTT PORE
Department of Microbiology, West Virginia University School of Medicine, Morgantown, West Virginia 26506

Received for publication 21 May 1973

A medium was devised which permitted the selective isolation of *Prototheca* spp. from nature.

Table 1. *Chemical constituents of Prototheca isolation medium (PIM)*

Constituent	Grams/liter
Distilled water	
Potassium hydrogen phthalate	10.0
Sodium hydroxide	0.9
Magnesium sulfate[a]	0.1
Potassium hydrogen phosphate[a]	0.2
Ammonium chloride[a]	0.3
Glucose[a]	10.0
Thiamine hydrochloride[a]	0.001
Agar	20.0
5-fluorocytosine (5-FC),	0.25
pH 5-5.2	

[a] Essential nutrient.

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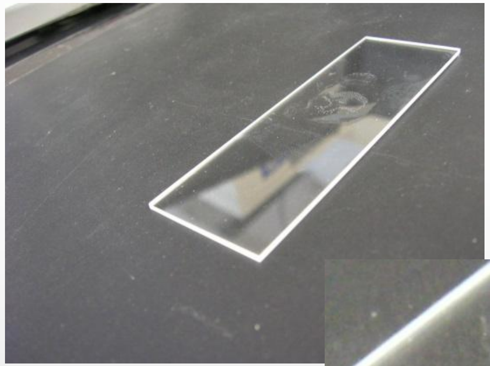
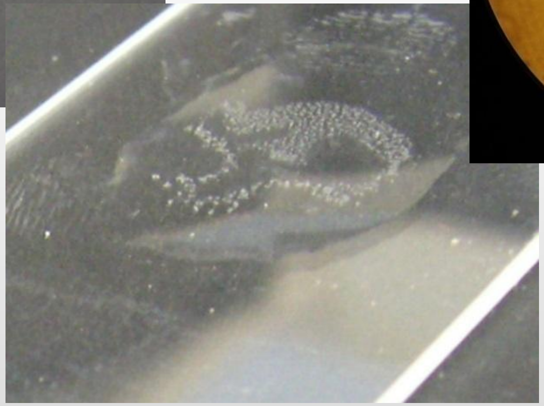
Prototheca diagnostics

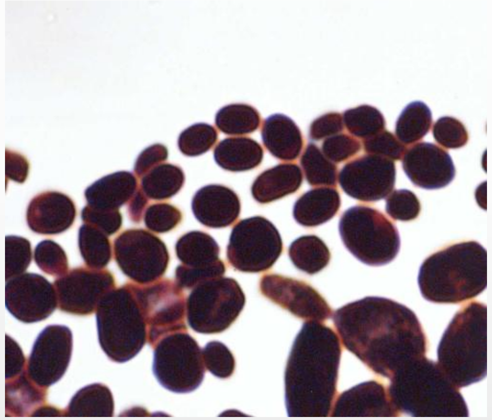
Microscopic
Confirmation





Wet Mount

Wet Mount



Gram stain

Crystal violet

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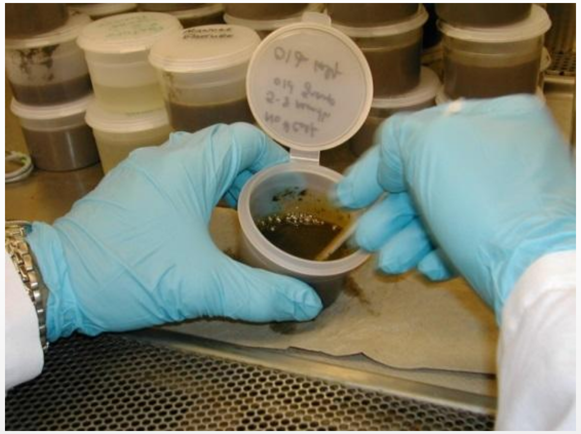
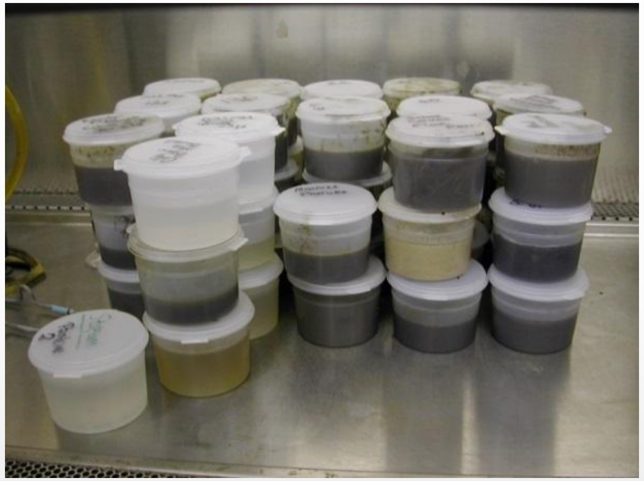
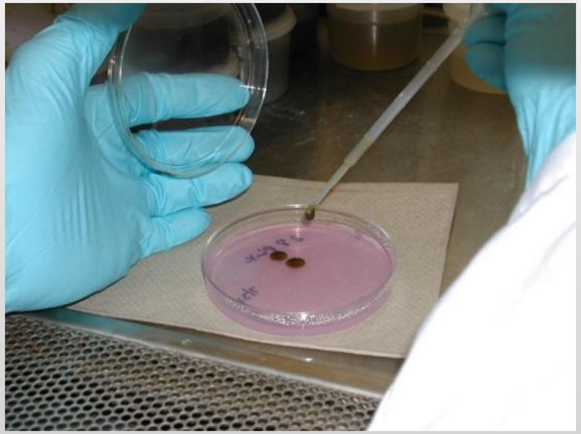
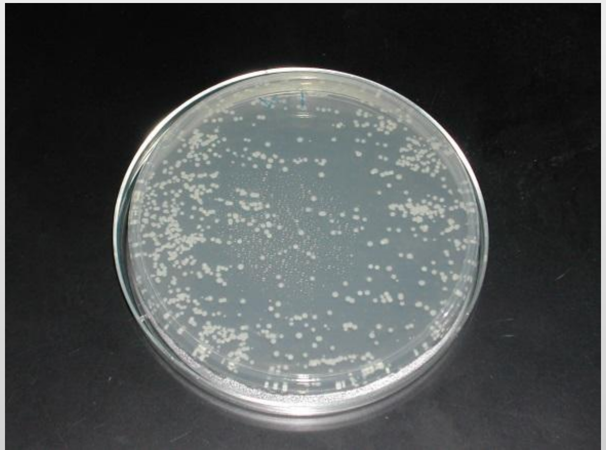
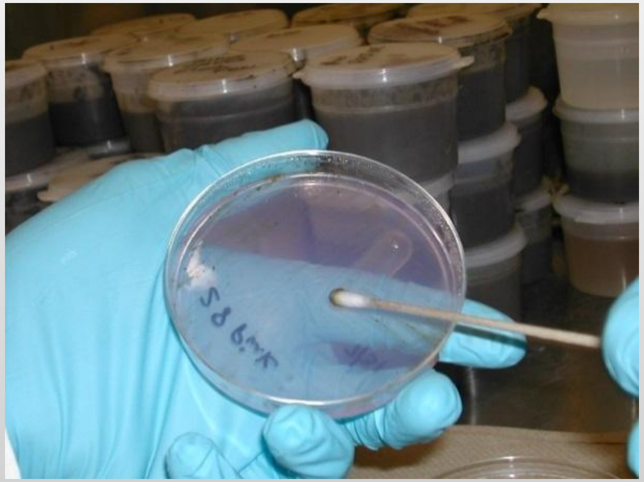
Prototheca diagnostics

Environmental Cultures



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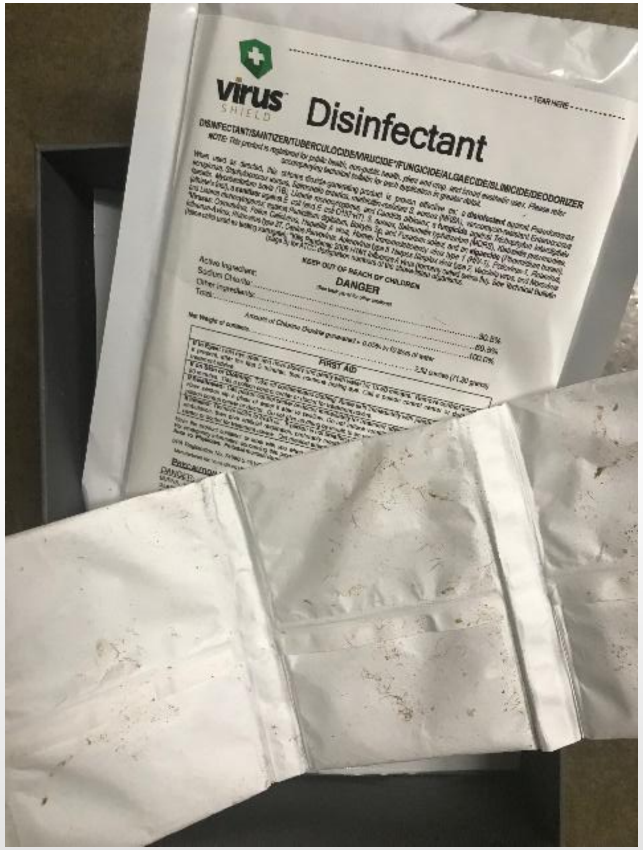
Case Report: Ultra-Pure Chlorine Dioxide

Date Finalized	Organism and ATCC	SPC (cfu/ml)	Run	DIP: Virus Shield Chlorine Dioxide 5ppm seconds	% reduction	DIP: Virus Shield Chlorine Dioxide 20ppm seconds	% reduction	DIP: Virus Shield Chlorine Dioxide 50ppm seconds	% reduction
3/30/2023	Streptococcus uberis ATCC 700407	5.0×10^5	1	15	>99.99	15	>99.99	15	>99.99
				30	>99.99	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99
			2	15	>99.99	15	>99.99	15	>99.99
				30	>99.99	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99
3/30/2023	Staphylococcus aureus ATCC 25923	3.4×10^6	1	15	**97.79**	15	>99.99	15	>99.99
				30	**98.82**	30	>99.99	30	>99.99
				60	**99.68**	60	>99.99	60	>99.99
			2	15	**98.21**	15	>99.99	15	>99.99
				30	**99.38**	30	>99.99	30	>99.99
				60	**99.91**	60	>99.99	60	>99.99
3/30/2023	Escherichia coli ATCC 25922	9.3×10^6	1	15	>99.99	15	>99.99	15	>99.99
				30	>99.99	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99
			2	15	>99.99	15	>99.99	15	>99.99
				30	>99.99	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99
3/30/2023	Prototheca zopfii field strain	8.5×10^3	1	15	**89.41**	15	**95.29**	15	>99.99
				30	**98.82**	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99
			2	15	**85.88**	15	**97.65**	15	>99.99
				30	**88.24**	30	>99.99	30	>99.99
				60	>99.99	60	>99.99	60	>99.99

Ultra-Pure ClO_2 is produced by mixing sodium chlorite (NaClO2) with a powdered acid. This chemical reaction is controlled via a patented semi-permeable membrane that allows only water and ClO_2 to pass through. This results is ClO_2 gas in the solution. The gas remains in the solution until it is applied when the gas is released and acts as a disinfectant by penetrating and destroying the cell membranes of pathogens and removing biofilms.



virus™
SHIELD
BIOSCIENCES

info@vsbiosciences.com

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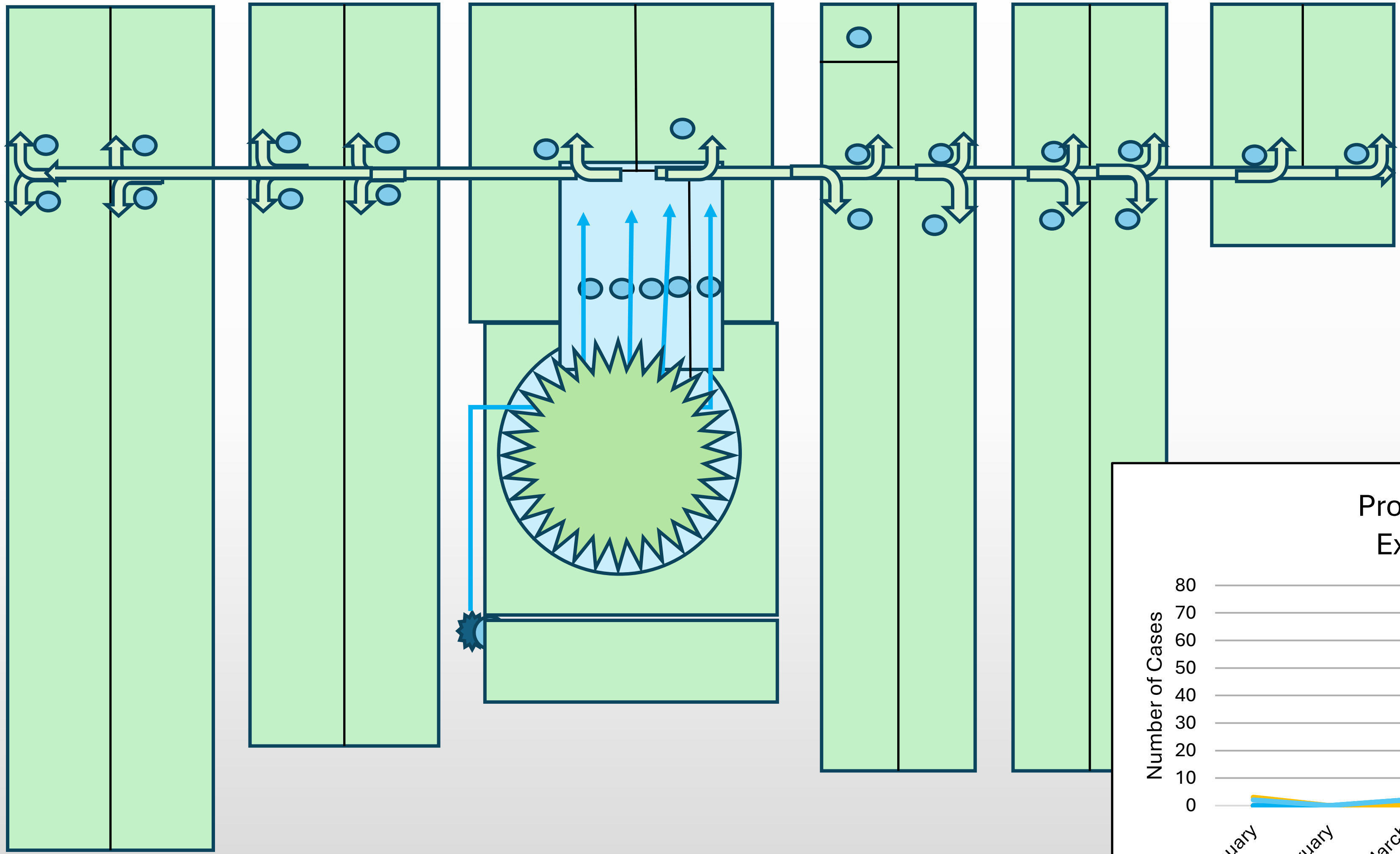
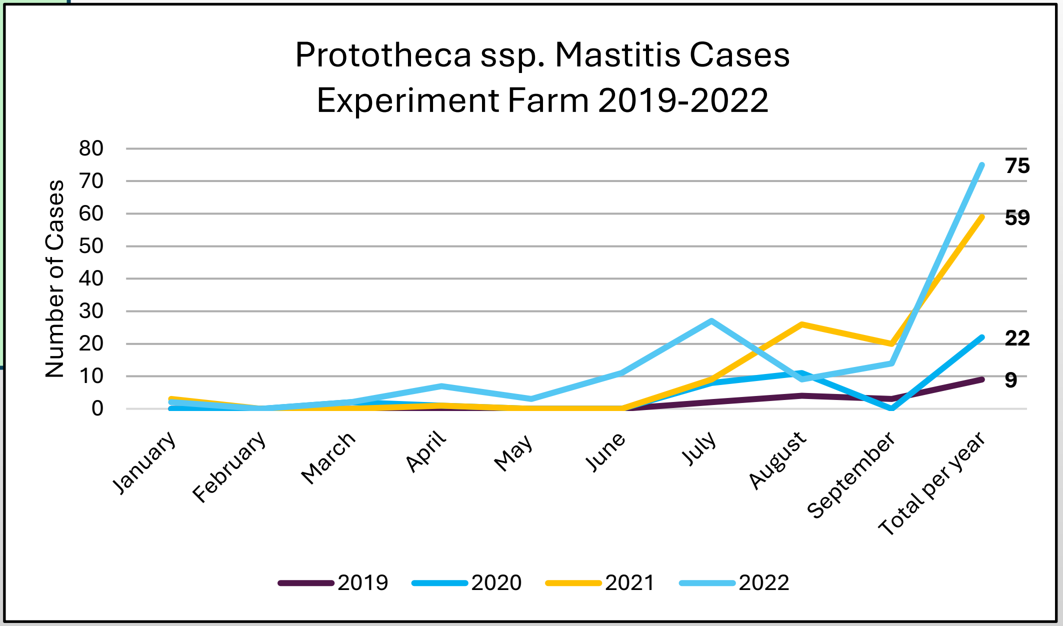
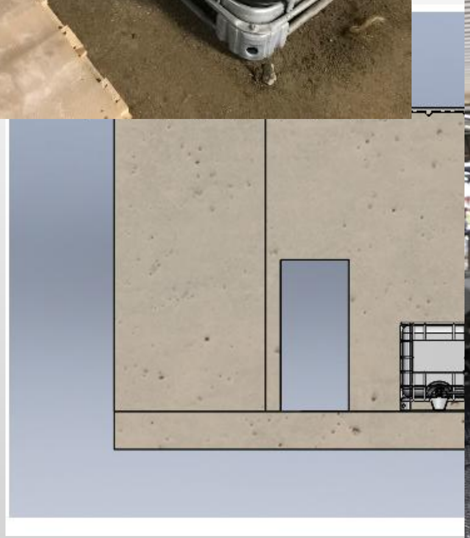
Case Report: Project Farm



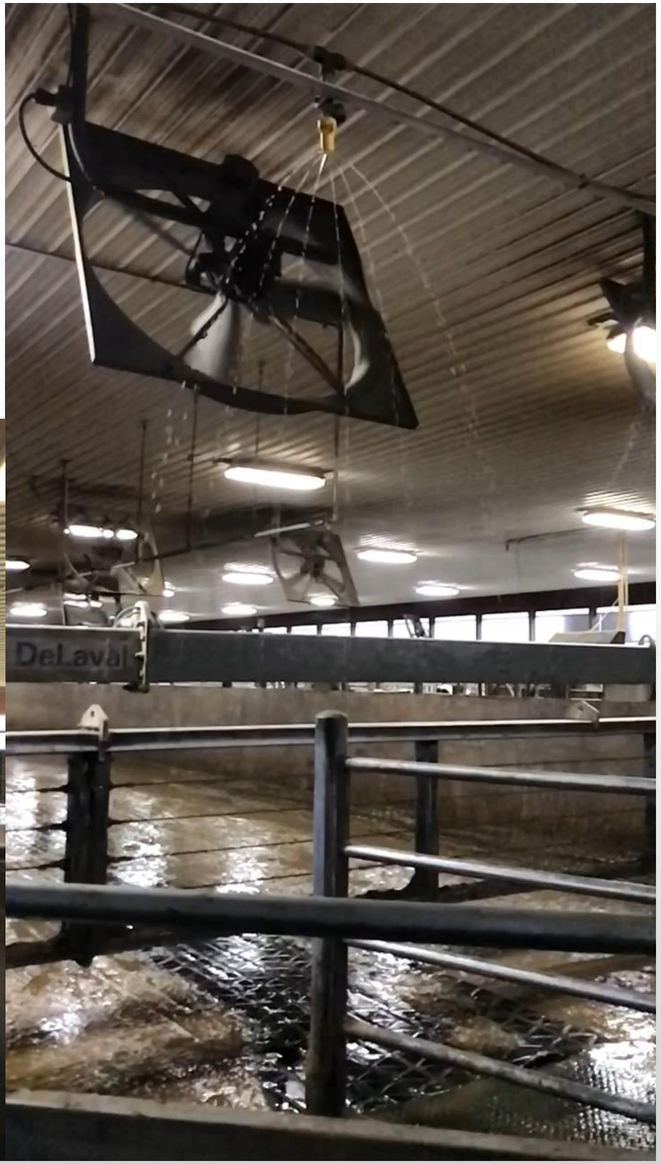
- 3400 cow dairy
- 72 stall rotary parlor
- Milking 3x
- Well managed
- Clinical mastitis cultures
- Fresh cow cultures
- Flush water system



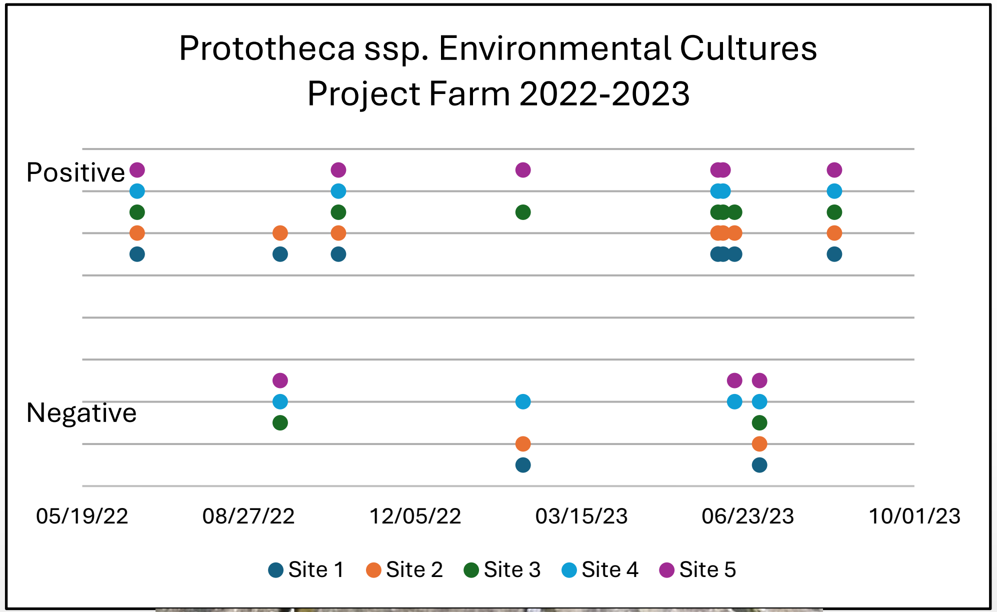
Case Report: Experiment 1- CLO_2 Spray System

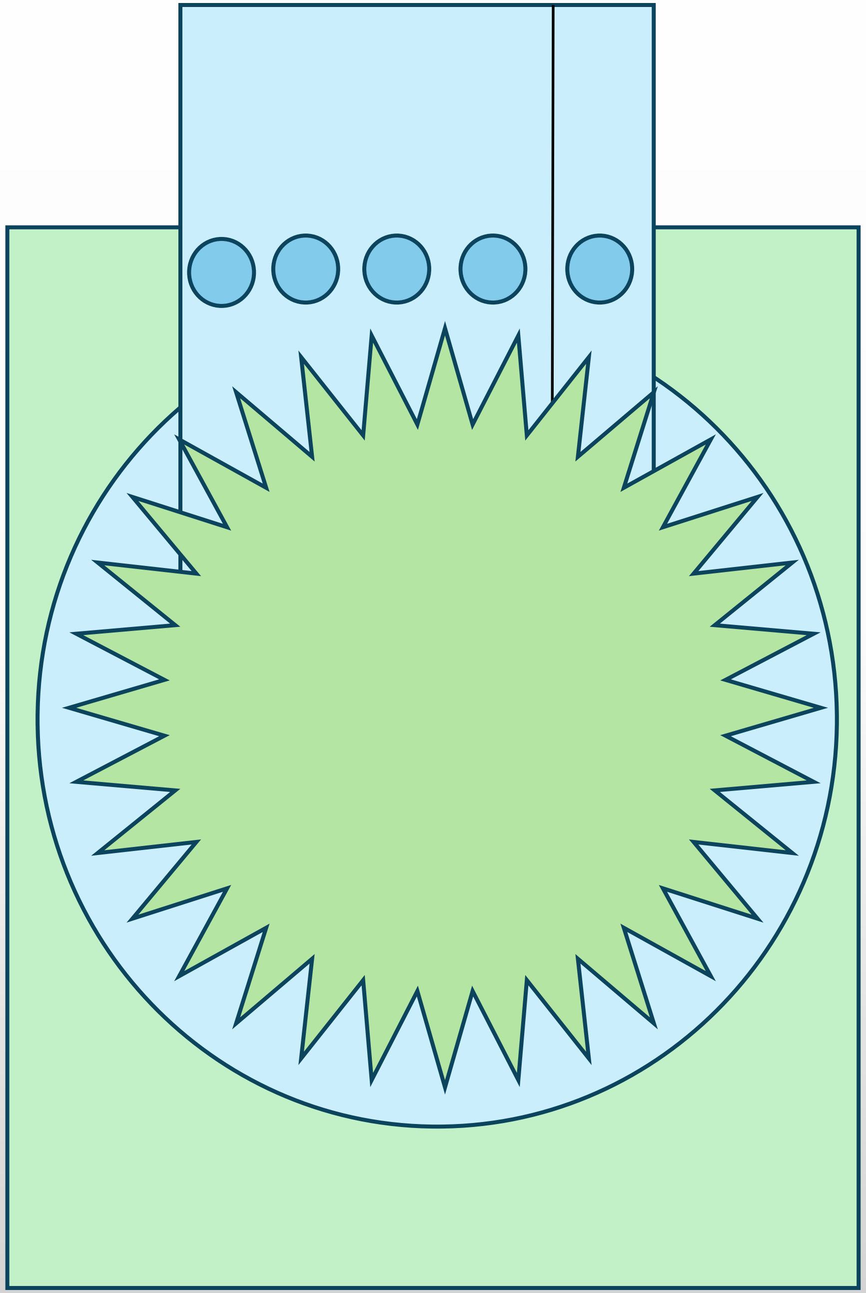


- 30 second delay after completion of the flush water system.
- Activated for 30 seconds.
- Nozzle size and spray duration dispersed 2.1L (0.5 gal) of chlorine dioxide (100 ppm) in zone 1 and 0.89L (0.24 gal) in zone 2.

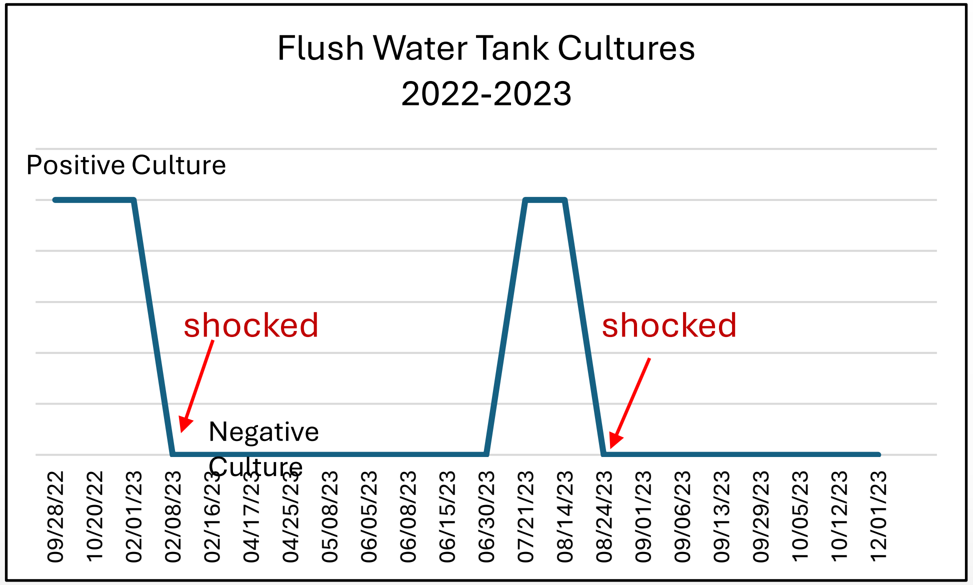


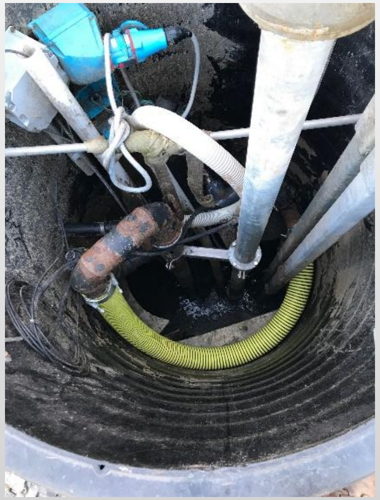
Case Report: Experiment 1: CLO_2 Spray System

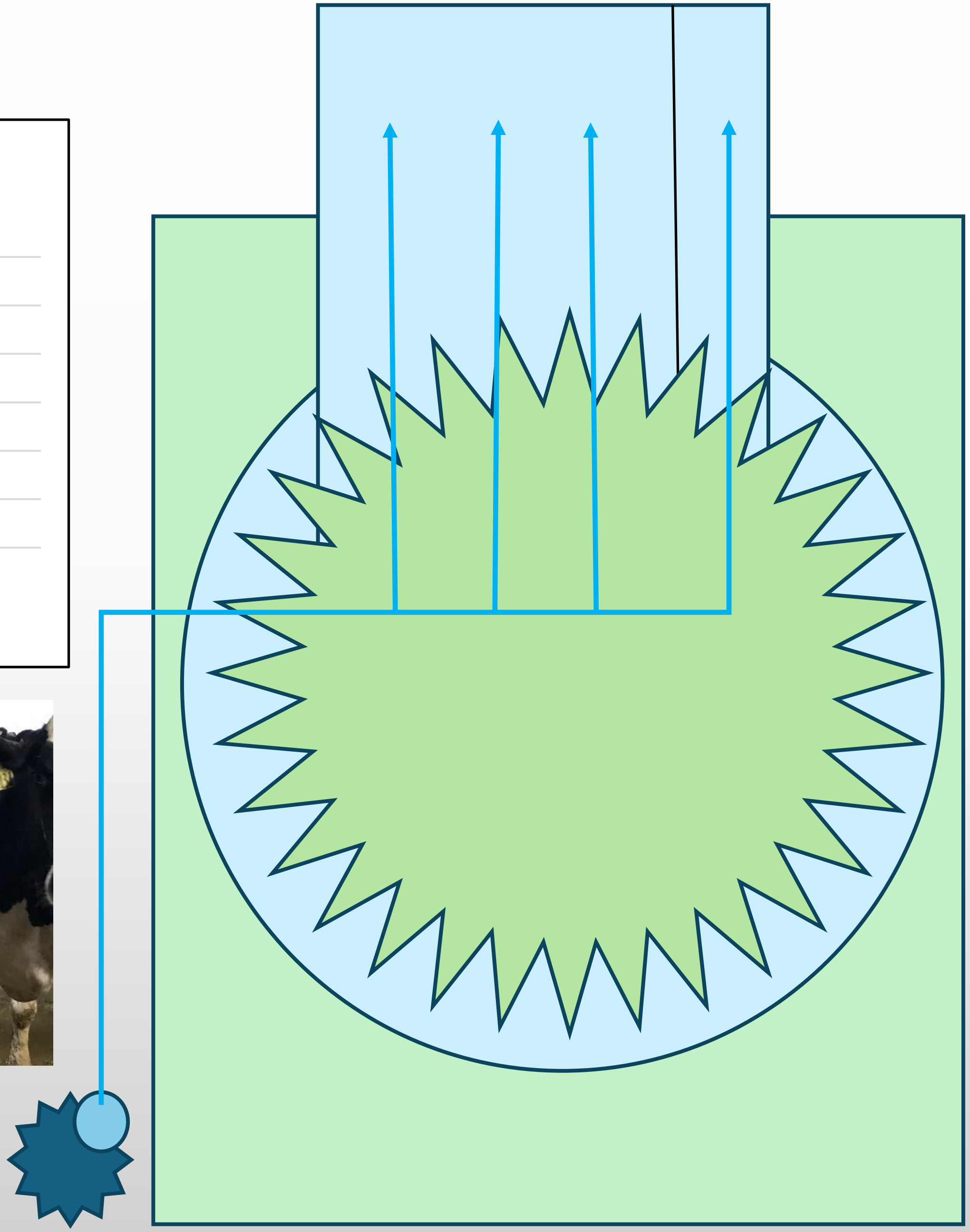




Case Report: Experiment 2- Flush Water Tank CLO_2 Shocking

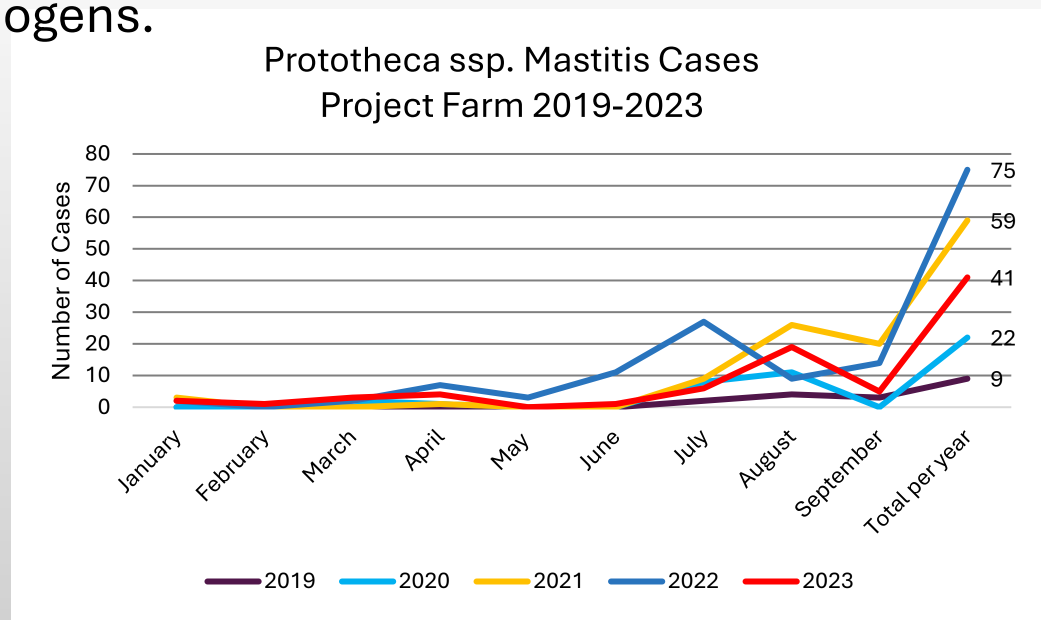






Case Report: Results and Conclusions

- We observed a 66% reduction in the presence of *Prototheca* ssp. in the flush water after shocking the flush water tank. The tank was negative for *Prototheca* for five months after initial shock.
- A minimal change (1% reduction) in *Prototheca* positive environmental samples was observed.
- Slime layer/biofilm presence in the less trafficked areas and flush water tank was noticeably reduced.
- During March to July 2023 Prototheca mastitis cases dropped from 50 cases in 2022 to 14 cases in 2023 (70% reduction).
- Routine CLO_2 shocking (weekly) of the flush water tank is necessary during the warmer months May-September.
- Treating the environment with disinfectants such as chlorine dioxide along with traditional, good dairy environment management practices can reduce mastitis cases of *Prototheca* and other environmental pathogens.



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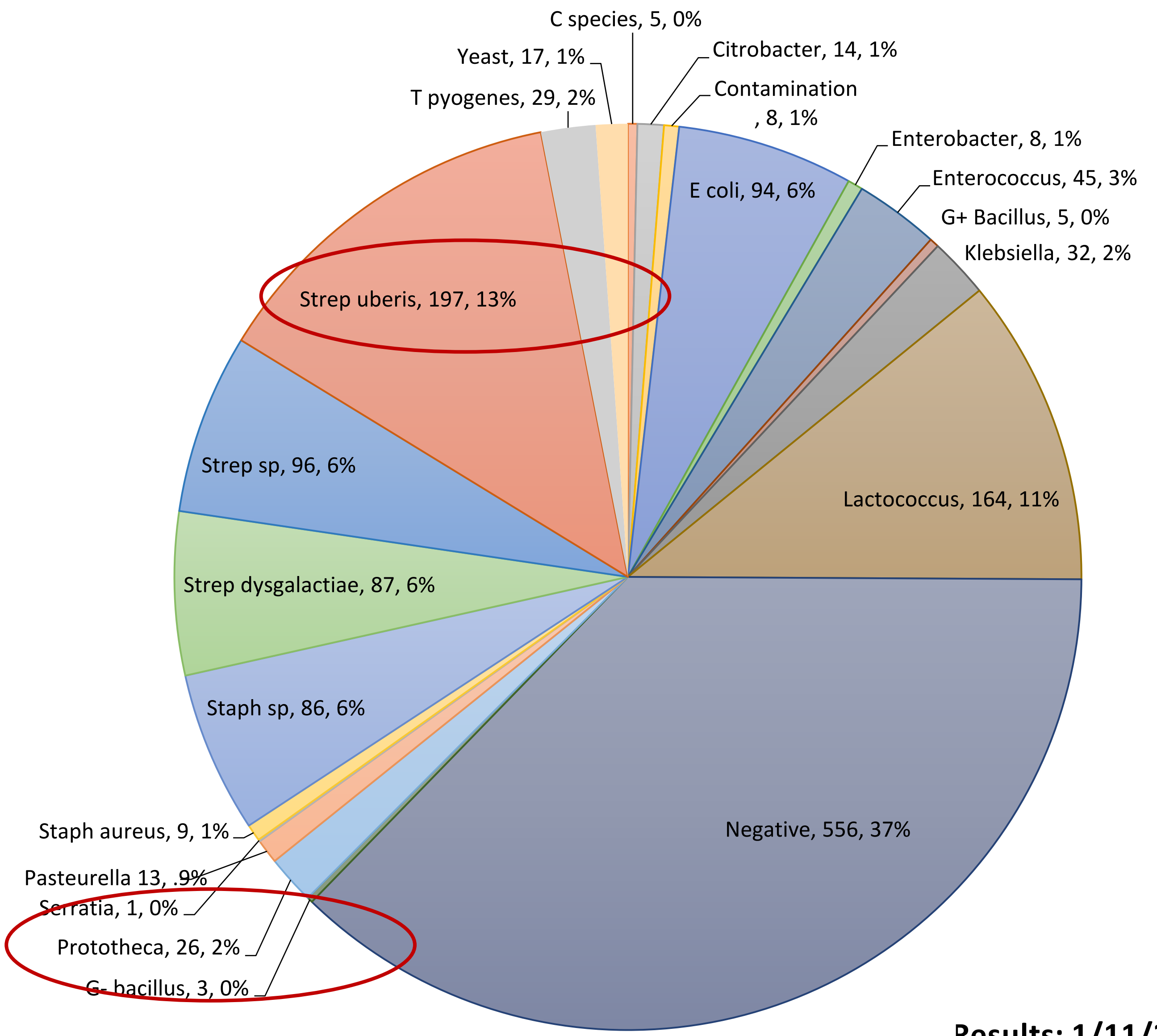
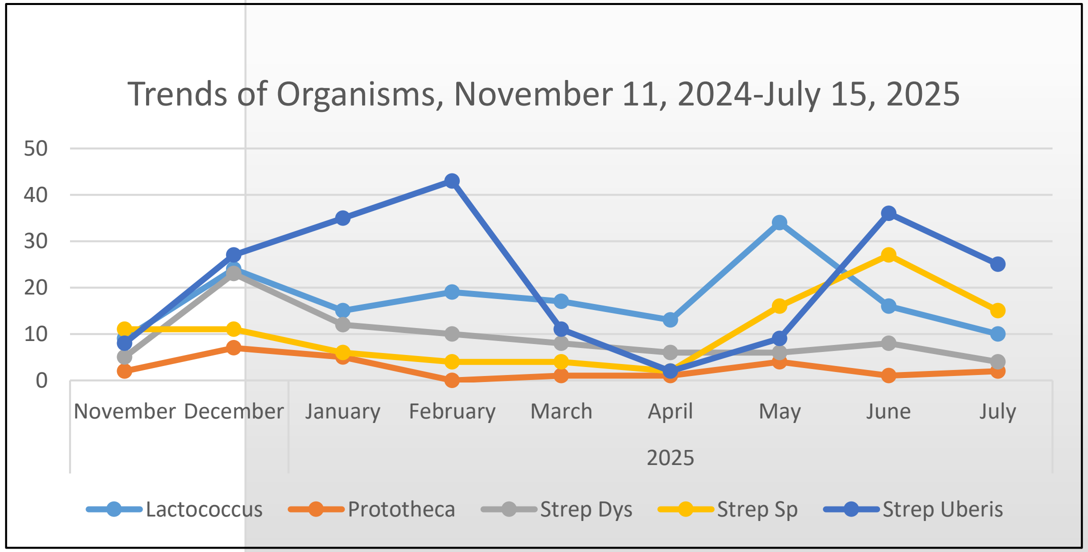


Please fix this...



Results: 1/11/2024-7/15/2025

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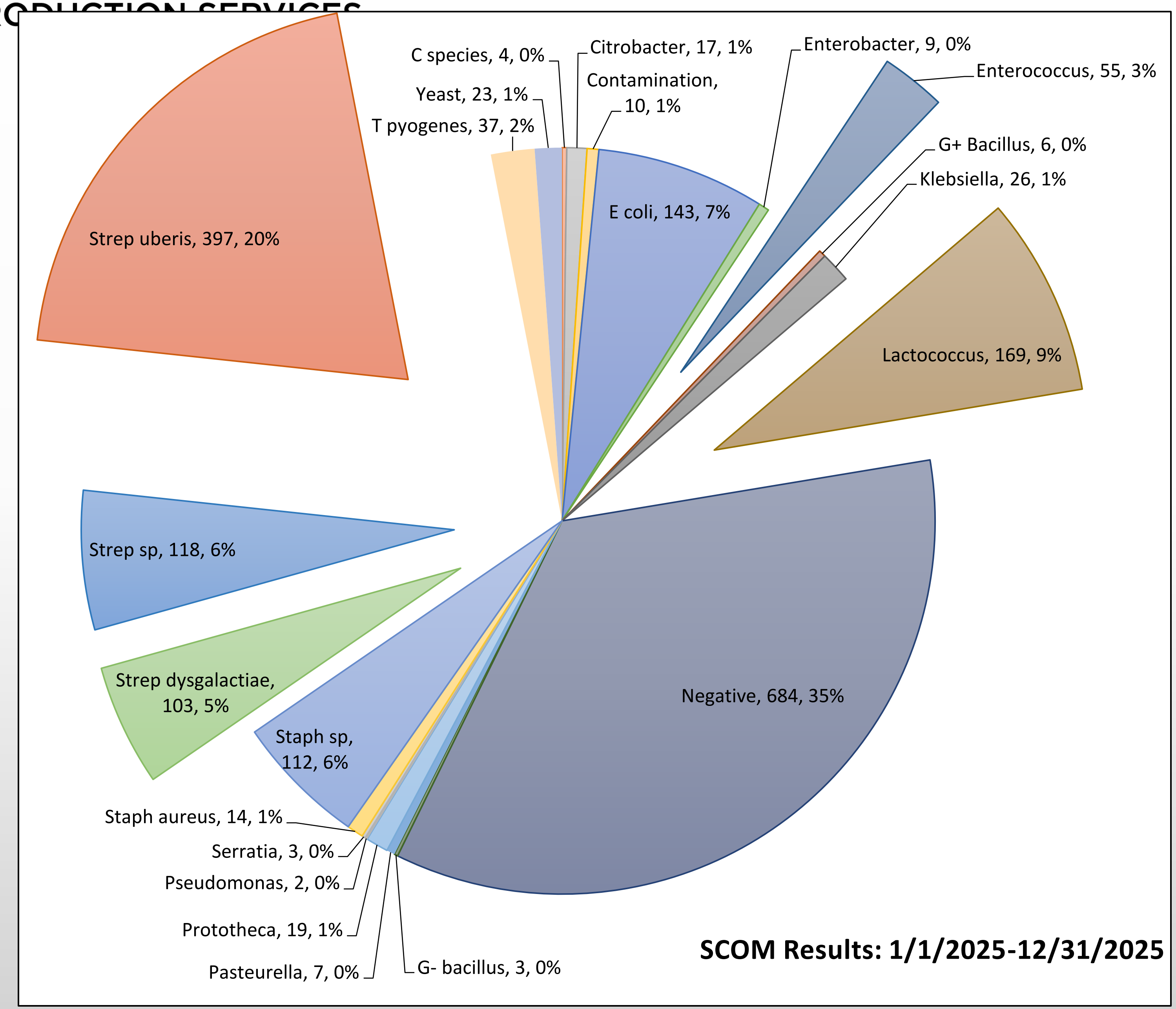
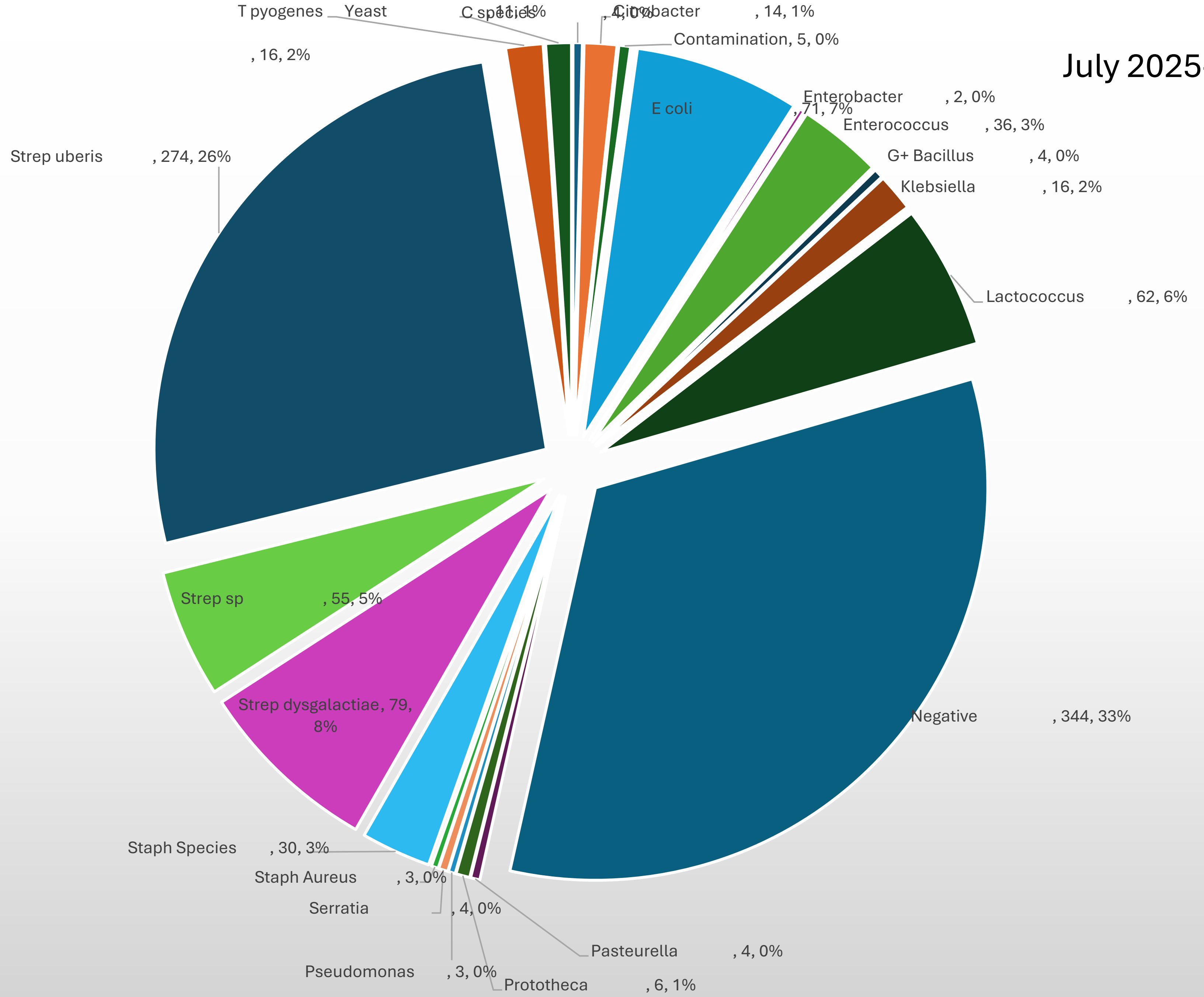


Strep and Strep-like pathogens=43%

July 2025-March 2026



Strep and Strep-like pathogens=48%

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Case Report: Chasing *Streptococcus uberis*

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Quality Milk Production Services
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Mjz6@cornell.edu

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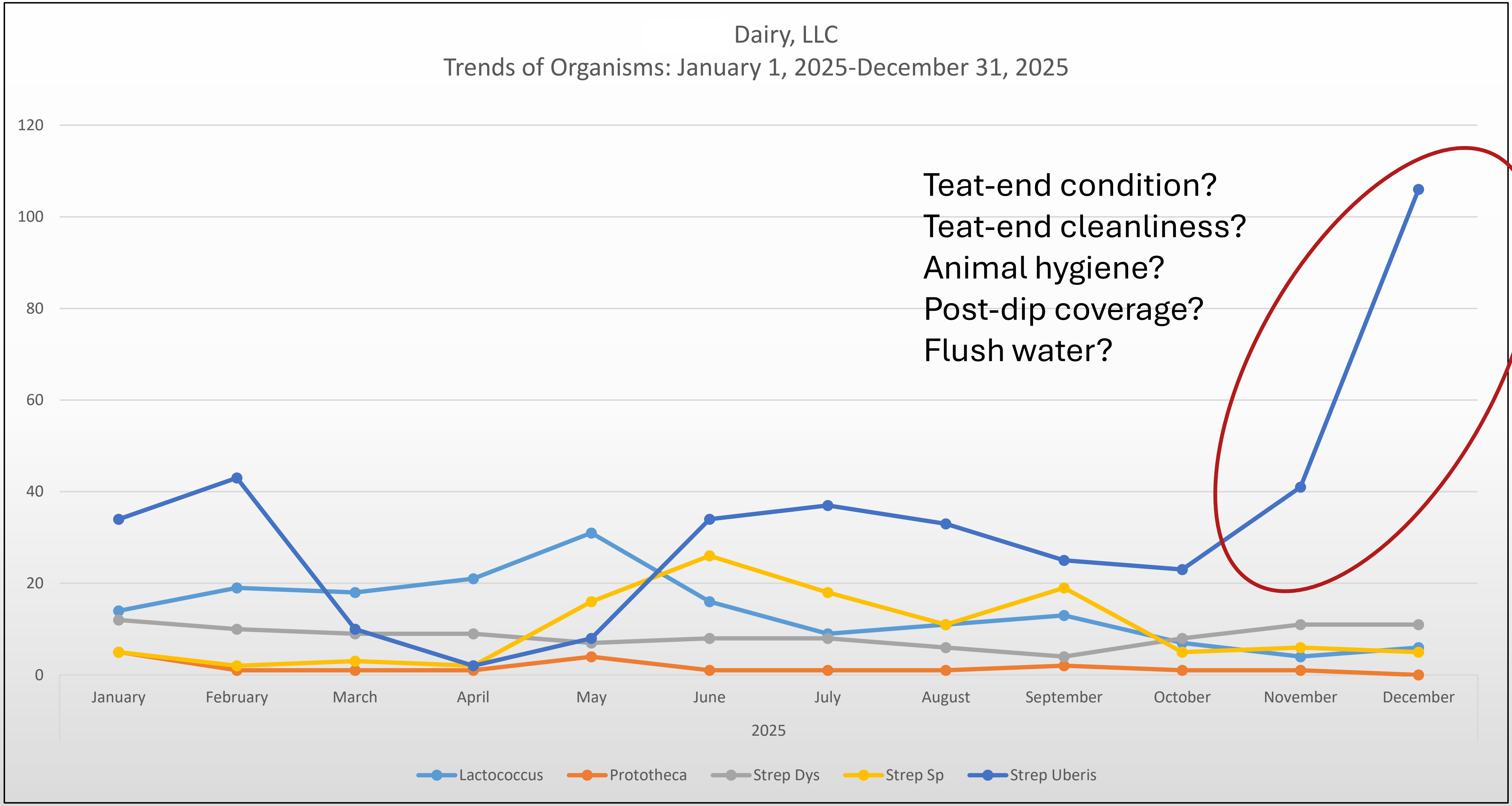

Chasing *Streptococcus uberis*

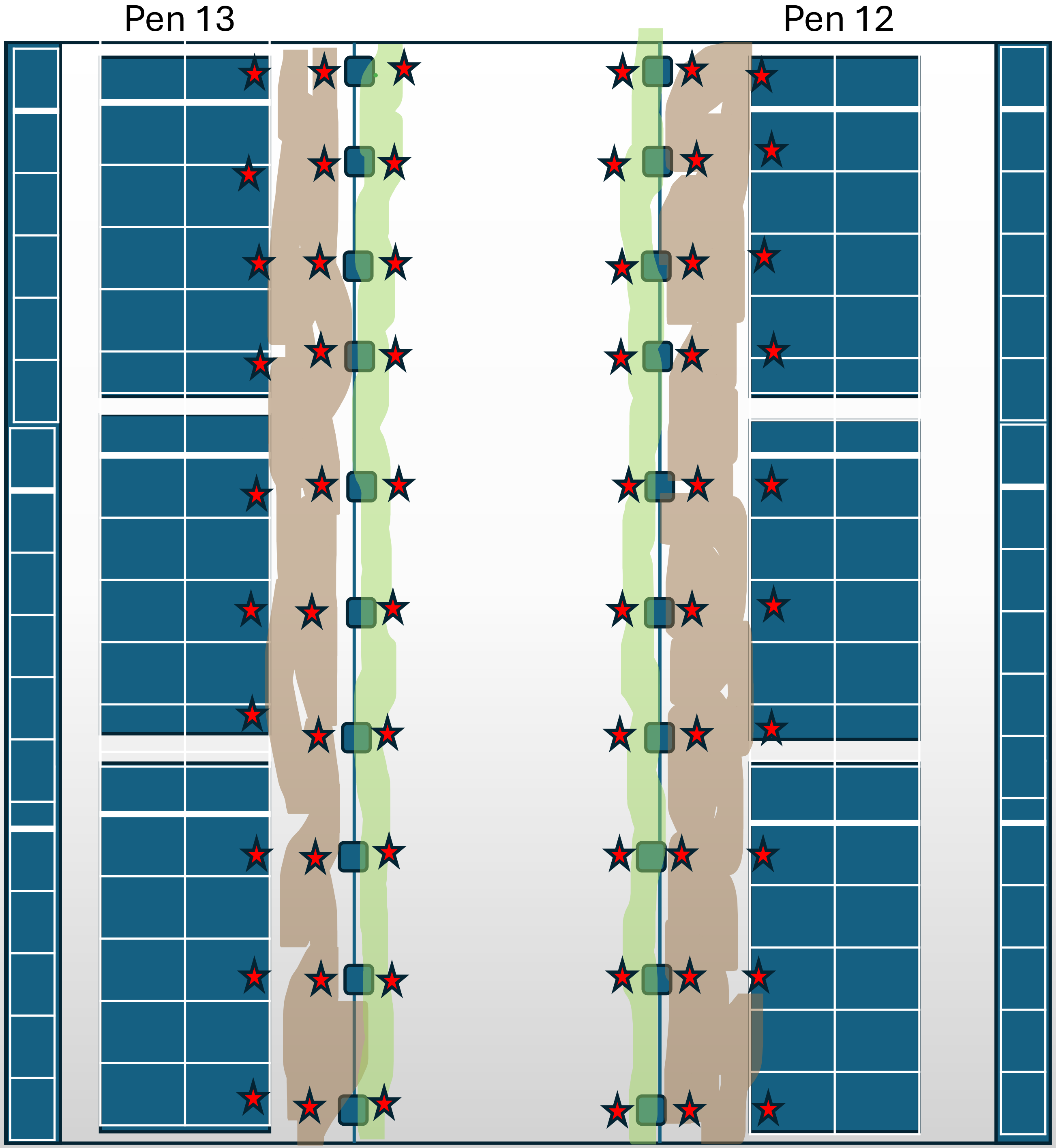

plate code	date collected	pen	type	total strep	TOTAL CFU/G	MALDI	CFU	MALDI		
G	7/16/2025	na	clean towel		78,875	Micrococcus luteus 9.638		Macrococcus caseolyticus 9.014		
C	5/15/2025	13	feed composite	1,000,000	6,380,952	Lactococcus lactis sibsp cremoris				
F	5/15/2025	12	feed composite	20,000,000	4,476,190	Enterococcus faecalis	30 cfu	Lactococcus lactis		
C	6/2/2025	13	feed composite	29,703	74,257	Aerococcus viridans	1	Enterococcus faecalis		
F	6/2/2025	12	feed composite	1,425	332,542	Enterococcus casseliflavus				
C	6/23/2025	13	feed composite	1,173,594	161,369	Enterococcus faecalis	2	Enterococcus faecium		
F	6/23/2025	12	feed composite	203,310	2,836,879	Lactococcus garvieae	13	Enterococcus faecium		
C	7/16/2025	13	feed composite	171,838	2,100,239	Enterococcus faecalis	13	Enterococcus faecium		
F	7/16/2025	12	feed composite	239,024	2,634,146	Enterococcus saccharolyticus	10	Lactobacillus farciminis		
C	8/4/2025	13	feed composite	753	211,765	Enterococcus gallinarum	10	Enterococcus faecium	3	Enterococcus faecalis
F	8/4/2025	12	feed composite	5,140	364,486	Enterococcus faecium	5	Enterococcus casseliflavus		Aerococcus viridans
C	8/27/2025	13	feed composite	261,905	6,619,048	Enterococcus faecalis	3	Enterococcus hirae	15	Enterococcus faecium
F	8/27/2025	12	feed composite	27,273	1,244,019	Enterococcus faecalis		Enterococcus gallinarum		Enterococcus faecium
B	5/15/2025	13	manure composite	77,272,727	139,393,939	Enterococcus aquimarinus	4 cfu	Enterococcus italicus		
E	5/15/2025	12	manure composite	28,930,818	110,691,824	Aerococcus sp	2 cfu	Enterococcus saccharolyticus		
B	6/2/2025	13	manure composite	4,739,336	53,080,569	Aerococcus viridans	1	Lactococcus garvieae		
E	6/2/2025	12	manure composite	2,264,151	47,798,742	Lactococcus garvieae				
B	6/23/2025	13	manure composite	28,402,367	137,278,107	Lactococcus garvieae	10	Enterococcus italicus		
E	6/23/2025	12	manure composite	13,669,065	176,978,417	Lactococcus garvieae	25	Enterococcus aquimarinus		
B	7/16/2025	13	manure composite	29,787,234	127,659,574	Globicatella sanguinis	7	Lactococcus garvieae		
E	7/16/2025	12	manure composite	47,852,761	101,840,491	Streptococcus suis				
B	8/4/2025	13	manure composite	7,142,857	31,746,032	Aerococcus viridans				
E	8/4/2025	12	manure composite	8,227,848	30,379,747	Aerococcus viridans	1	Lactococcus garvieae	6	Streptococcus lutetiensis
B	8/27/2025	13	manure composite	35,211,268	76,056,338	Aerococcus viridans		Lactococcus garvieae		
E	8/27/2025	12	manure composite	44,585,987	90,445,860	Aerococcus viridans				
A	5/15/2025	13	sand bedding composite	5,197,505	118,087,318	Lactococcus lactis	10 cfu	Enterococcus sacchararolyticus		
D	5/15/2025	12	sand bedding composite	6,458,333	77,500,000	Lactococcus garvieae	9 cfu	Strep lutetiensis		
A	6/2/2025	13	sand bedding composite	3,340,292	78,826,722	Lactococcus garvieae	4	Enterococcus italicus		
D	6/2/2025	12	sand bedding composite	4,338,843	56,198,347	Lactococcus garvieae	6	Enterococcus italicus		
A	6/23/2025	13	sand bedding composite	8,351,178	53,533,191	Lactococcus garvieae	13	Enterococcus saccharolyticus		
D	6/23/2025	12	sand bedding composite	9,583,333	52,083,333	Lactococcus garvieae	16	Enterococcus saccharolyticus	2	Streptococcus lutetiensis
A	7/16/2025	13	sand bedding composite	10,147,992	52,854,123	Lactococcus garvieae	6	Enterococcus saccharolyticus	10	Streptococcus lutetiensis
D	7/16/2025	12	sand bedding composite	7,073,955	53,590,568	Lactococcus garvieae	5	Streptococcus lutetiensis		
A	8/4/2025	13	sand bedding composite	1,851,852	42,181,070	Lactococcus garvieae	5	Aerococcus viridans		Enterococcus casseliflavus
D	8/4/2025	12	sand bedding composite	6,073,298	52,356,021	Aerococcus viridans		Streptococcus suis		Lactococcus garvieae
A	8/27/2025	13	sand bedding composite	6,584,362	51,440,329	Lactococcus garvieae	2	Aerococcus viridans		Streptococcus fallolyticus
D	8/27/2025	12	sand bedding composite	4,123,711	32,164,948	Aerococcus viridans	3	Lactococcus garvieae		

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Chasing *Streptococcus uberis* in flush water

Organism	Plate Type	Score	Date	Source
Strep Parauberis	Edwards	9.3	3/6/2026	Flush
Strep Parauberis	Edwards	9.419	3/6/2026	Flush
Strep Parauberis	Edwards	9.458	3/6/2026	Flush
Strep Parauberis	Edwards	7.27	3/6/2026	Flush
Strep Parauberis	Unknown	9.162	Feb-26	Flush
Strep Parauberis	Unknown	5.842	Feb-26	Flush
Strep Parauberis	Uknown	9.244	Feb-26	Flush
Strep Parauberis	Inulin	6.539	Feb-26	Flush
Strep Parauberis	Inulin	8.849	Feb-26	Flush
Strep Parauberis	Edwards	9.245	3/23/2026	Dump
Strep Parauberis	Inulin	7.692	3/23/2026	Dump
Strep Parauberis	Edwards	9.022	3/23/2026	Dump
Strep Parauberis	Inulin	9.423	3/6/2026	Flush
Strep Parauberis	Inulin	9.377	3/6/2026	Flush
Strep Parauberis	Inulin	9.047	3/6/2026	Flush

Date	Source	Organism	Plate Type
3/6/26	Flush	Strep Parauberis	Edwards
3/6/26	Flush	Strep Parauberis	Edwards
3/6/26	Flush	Strep Parauberis	Edwards
3/6/26	Flush	Strep Parauberis	Edwards
2/1/26	Flush	Strep Parauberis	Unknown
2/1/26	Flush	Strep Parauberis	Unknown
2/1/26	Flush	Strep Parauberis	Uknown
2/1/26	Flush	Strep Parauberis	Inulin
2/1/26	Flush	Strep Parauberis	Inulin
3/23/26	Dump	Strep Parauberis	Edwards
3/23/26	Dump	Strep Parauberis	Inulin
3/23/26	Dump	Strep Parauberis	Edwards
3/6/26	Flush	Strep Parauberis	Inulin
3/6/26	Flush	Strep Parauberis	Inulin
3/6/26	Flush	Strep Parauberis	Inulin

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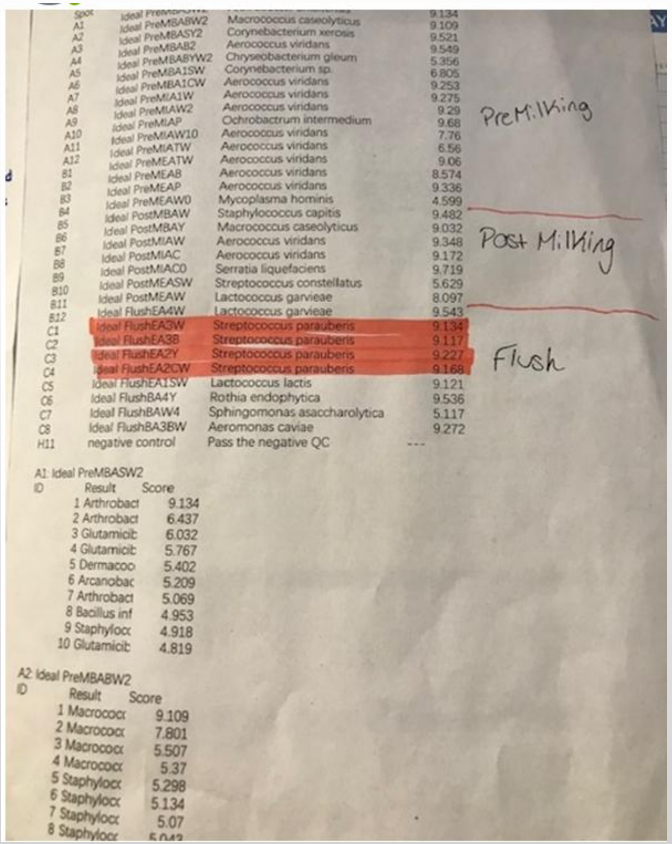
Chasing *Streptococcus uberis* on teat-ends



Spot			
A1	Ideal PreMBASW2		9.134
A2	Ideal PreMBABW2	Macrococcus caseolyticus	9.109
A3	Ideal PreMBASY2	Corynebacterium xerosis	9.521
A4	Ideal PreMBAB2	Aerococcus viridans	9.549
A5	Ideal PreMBABYW2	Chryseobacterium gleum	5.356
A6	Ideal PreMBA1SW	Corynebacterium sp.	6.805
A7	Ideal PreMBA1CW	Aerococcus viridans	9.253
A8	Ideal PreMIA1W	Aerococcus viridans	9.275
A9	Ideal PreMIAW2	Aerococcus viridans	9.29
A10	Ideal PreMIAP	Ochrobactrum intermedium	9.68
A11	Ideal PreMIAW10	Aerococcus viridans	7.76
A12	Ideal PreMIATW	Aerococcus viridans	6.56
B1	Ideal PreMEATW	Aerococcus viridans	9.06
B2	Ideal PreMEAB	Aerococcus viridans	8.574
B3	Ideal PreMEAP	Aerococcus viridans	9.336
B4	Ideal PreMEAW0	Mycoplasma hominis	4.599
B5	Ideal PostMBAW	Staphylococcus capitis	9.482
B6	Ideal PostMBAY	Macrococcus caseolyticus	9.032
B7	Ideal PostMIAW	Aerococcus viridans	9.348
B8	Ideal PostMIAC	Aerococcus viridans	9.172
B9	Ideal PostMIAC0	Serratia liquefaciens	9.719
B10	Ideal PostMEASW	Streptococcus constellatus	5.629
B11	Ideal PostMEAW	Lactococcus garvieae	8.097
B12	Ideal FlushEA4W	Lactococcus garvieae	9.543
C1	Ideal FlushEA3W	Streptococcus parauberis	9.134
C2	Ideal FlushEA3B	Streptococcus parauberis	9.117
C3	Ideal FlushEA2Y	Streptococcus parauberis	9.227
C4	Ideal FlushEA2CW	Streptococcus parauberis	9.168
C5	Ideal FlushEA1SW	Lactococcus lactis	9.121
C6	Ideal FlushBA4Y	Rothia endophytica	9.536
C7	Ideal FlushBAW4	Sphingomonas asaccharolytica	5.117
C8	Ideal FlushBA3BW	Aeromonas caviae	9.272
H11	negative control	Pass the negative QC	---

PreMilking

Post Milking

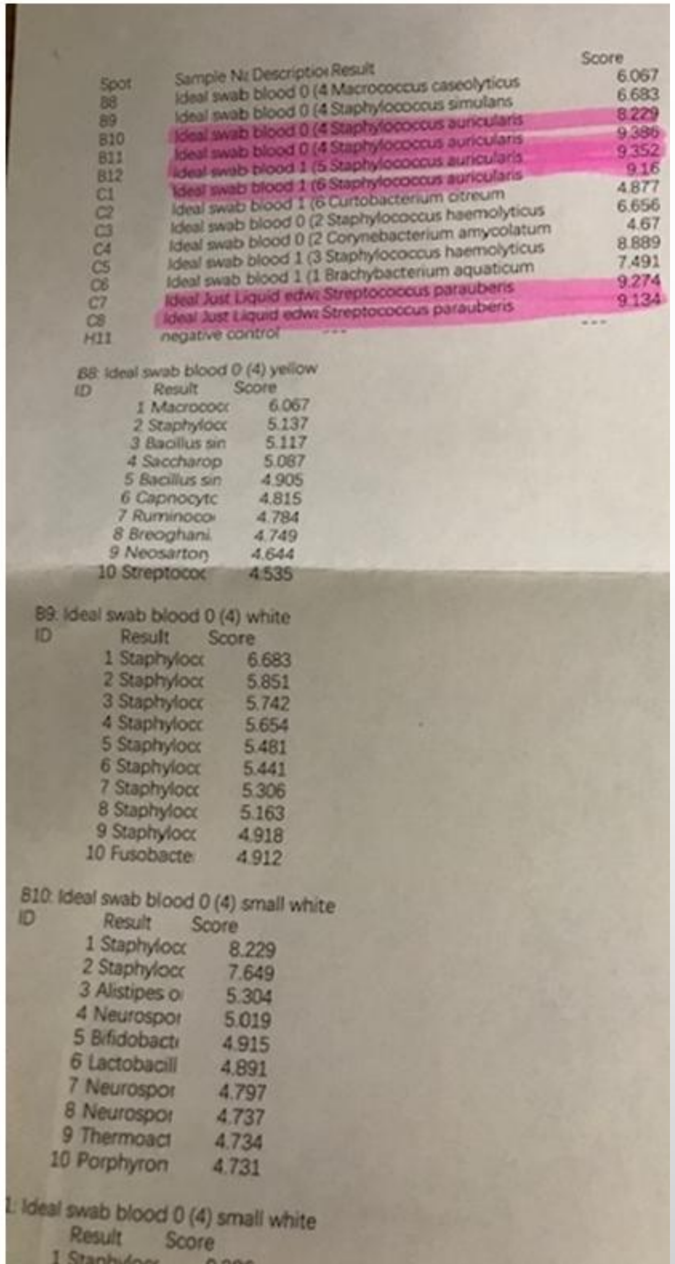
Flush

A1: Ideal PreMBASW2

ID	Result	Score
	1 Arthrobact	9.134
	2 Arthrobact	6.437
	3 Glutamicib	6.032
	4 Glutamicib	5.767
	5 Dermacoo	5.402
	6 Arcanobac	5.209
	7 Arthrobact	5.069
	8 Bacillus inf	4.953
	9 Staphylocc	4.918
	10 Glutamicib	4.819

A2: Ideal PreMBABW2

ID	Result	Score
	1 Macrococc	9.109
	2 Macrococc	7.801
	3 Macrococc	5.507
	4 Macrococc	5.37
	5 Staphylocc	5.298
	6 Staphylocc	5.134
	7 Staphylocc	5.07
	8 Staphylocc	



Spot	Sample N Descriptio Result	Score
B8	Ideal swab blood 0 (4 Macrococcus caseolyticus	6.067
B9	Ideal swab blood 0 (4 Staphylococcus simulans	6.683
B10	Ideal swab blood 0 (4 Staphylococcus auricularis	8.229
B11	Ideal swab blood 0 (4 Staphylococcus auricularis	9.386
B12	Ideal swab blood 1 (5 Staphylococcus auricularis	9.352
C1	Ideal swab blood 1 (6 Staphylococcus auricularis	9.16
C2	Ideal swab blood 1 (6 Curtobacterium citreum	4.877
C3	Ideal swab blood 0 (2 Staphylococcus haemolyticus	6.656
C4	Ideal swab blood 0 (2 Corynebacterium amycolatum	4.67
C5	Ideal swab blood 1 (3 Staphylococcus haemolyticus	8.889
C6	Ideal swab blood 1 (1 Brachybacterium aquaticum	7.491
C7	Ideal Just Liquid edwi Streptococcus parauberis	9.274
C8	Ideal Just Liquid edwi Streptococcus parauberis	9.134
H11	negative control ---	---

B8: Ideal swab blood 0 (4) yellow

ID	Result	Score
	1 Macrococc	6.067
	2 Staphylocc	5.137
	3 Bacillus sin	5.117
	4 Saccharop	5.087
	5 Bacillus sin	4.905
	6 Capnocytc	4.815
	7 Ruminococ	4.784
	8 Breoghani	4.749
	9 Neosarton	4.644
	10 Streptococ	4.535

B9: Ideal swab blood 0 (4) white

ID	Result	Score
	1 Staphylocc	6.683
	2 Staphylocc	5.851
	3 Staphylocc	5.742
	4 Staphylocc	5.654
	5 Staphylocc	5.481
	6 Staphylocc	5.441
	7 Staphylocc	5.306
	8 Staphylocc	5.163
	9 Staphylocc	4.918
	10 Fusobacte	4.912

B10: Ideal swab blood 0 (4) small white

ID	Result	Score
	1 Staphylocc	8.229
	2 Staphylocc	7.649
	3 Alistipes o	5.304
	4 Neurospor	5.019
	5 Bifidobact	4.915
	6 Lactobacill	4.891
	7 Neurospor	4.797
	8 Neurospor	4.737
	9 Thermoact	4.734
	10 Porphyron	4.731

1: Ideal swab blood 0 (4) small white

ID	Result	Score
	1 Staphylocc	

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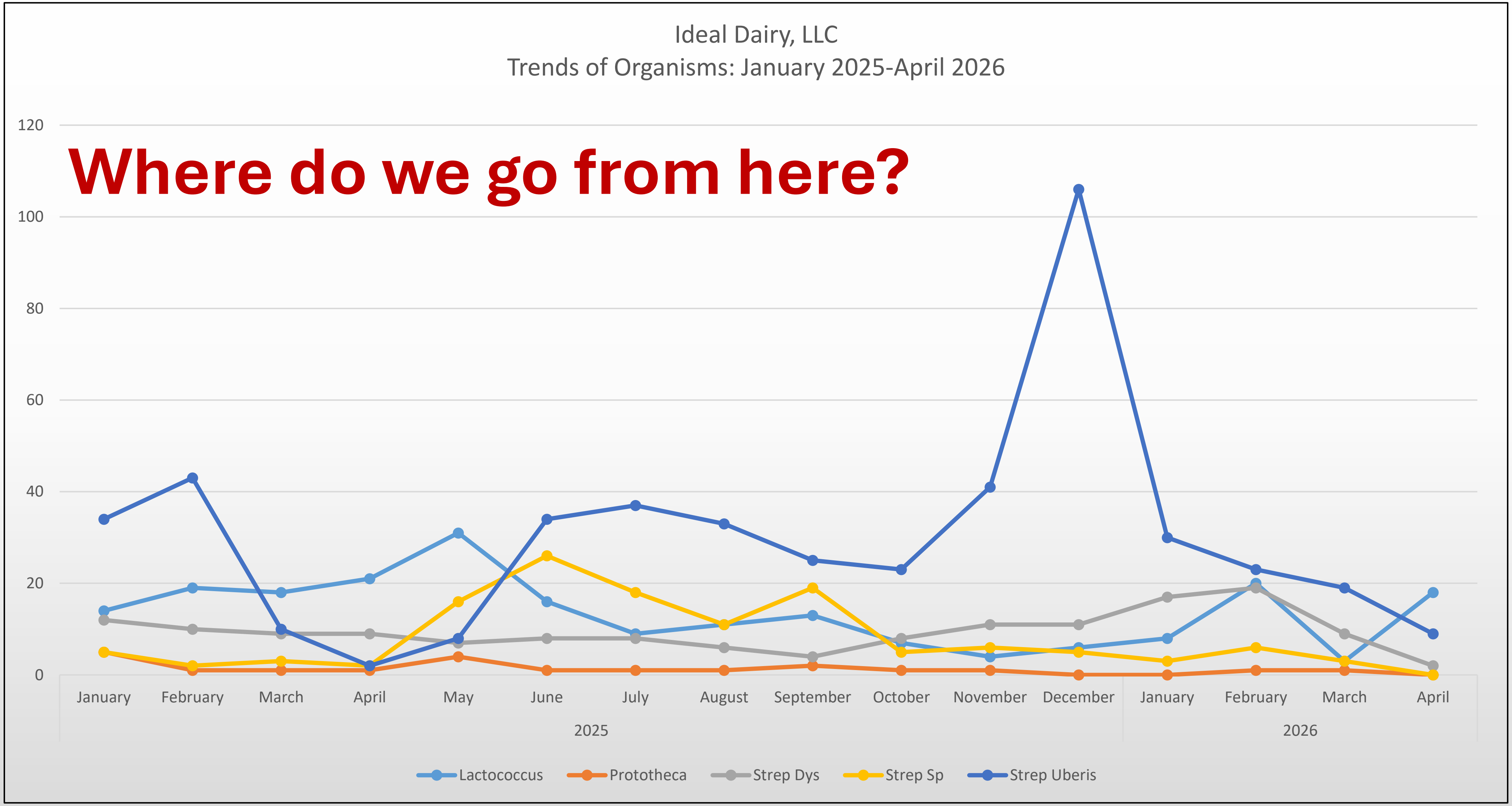
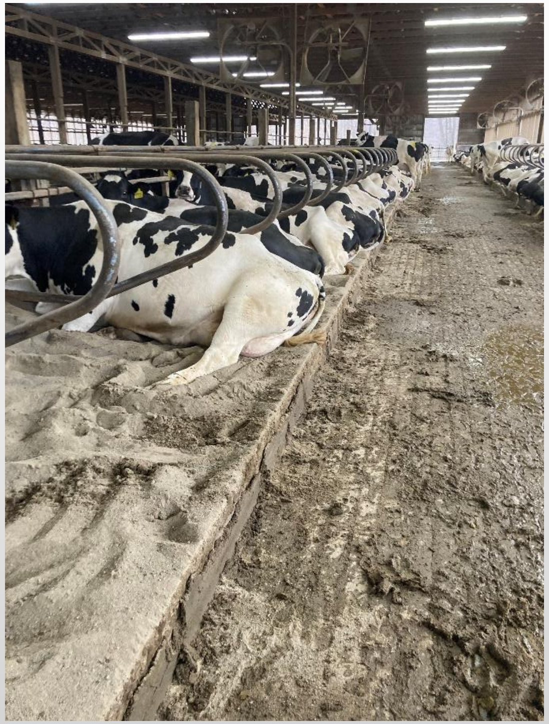
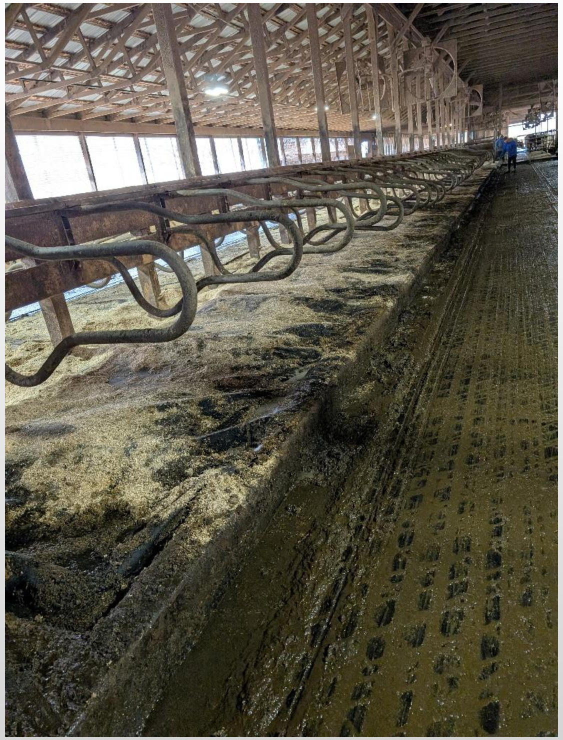


Barn Evaluation

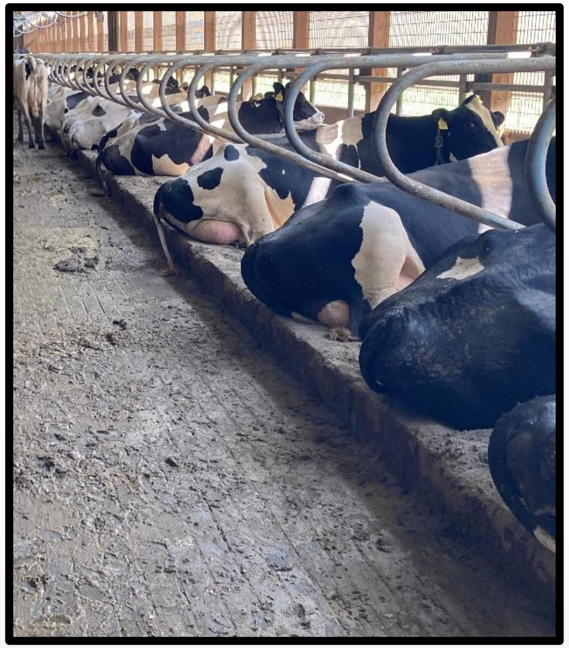
Environmental Assessment

- Walk through of all pens including prefresh and dry
- Record the following:
  - Stall cleanliness
  - Bedding level
  - Cow positioning in stall
  - Areas of manure accumulation





Lactating Cow Environment



- Cow positioning was good with 89% of the 786 cows that we scored positioned correctly although there is variation by pen and within pen by row of stalls.
- Stall cleanliness is good with 96% scored as clean.
- Bedding levels showed 100% of the stalls having adequate bedding.

Dry-Off Procedure Assessment

- Watch all employees who perform the dry-off procedure
- Score with an OSCE type score sheet
- Observe how cows are sorted, where dried off, and when they were milked last

Future Plans

- We will keep you on the schedule for approximately every 4 months to perform this type of analysis. This provides you and all your advisors an excellent set of data that can be used to evaluate changes that are made and also assess the progress toward the goal of better milk quality.

Discussion topics

- Calf hygiene audits?
- Flush water culture?
- Dry teat swabs and culture?

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Ruby-2023

Thank you

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