Hannah Dairy Research Foundation Report

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Identifying critical control points for colostrum contamination and Mycoplasma prevalence in first milking colostrum from Scottish dairy herds.

TENSION FOR CHANGE FOR SCOTTISH FARMERS

- Farmers must improve attention to detail for hygiene of colostrum management from harvest to feeding
- Equipment must be easily cleanable
- Colostrum handling must be streamlined
- Colostrum should be stored appropriately and promptly
- Colostrum management is only one part of *M. bovis* prevention and airborne spread is likely more important

Background

Calves are born agammaglobunaemic due to the syndesmochorial anatomy of the bovine placenta. The calf's immunity in the first few weeks of life is reliant on the transfer of immunoglobulin G (IgG) from its dam's colostrum. Adequate colostral IgG absorption and subsequent adequate transfer of passive immunity has been associated with improved calf health as well as longer term improved growth and productivity. Adequate colostrum quality is defined as:

1. IgG (antibody) concentration (≥50g/L IgG or ≥22% Brix)
2. Bacterial contamination
    a. Total bacterial counts (TBC) <100,000 CFU/mL
    b. Total coliform counts (TCC) <10,000 CFU/mL

High bacterial counts in colostrum may impair absorption of antibodies from colostrum, even if colostrum antibody concentration is high (greater than 50g/L). In particular, coliform species (which are ubiquitous in farming environments) have been shown to impair IgG absorption, by a number of hypothesised mechanisms:

1. Bacteria physically bind to antibody within the gastrointestinal lumen, blocking uptake across enterocytes.
2. Pathogenic bacteria, such as *E.coli, Salmonella spp*, attach and damage intestinal cells, reducing gut permeability.
3. Pathogenic bacteria damage intestinal cells, accelerating neonatal calf gut closure.
4. Bacteria physically block antibody molecule absorption channels.

Previous published literature has shown colostrum to be highly contaminated. In Scottish samples approximately 30%-40% failed to meet bacterial industry thresholds.

Potential contamination sources include milking equipment, colostrum storage and colostrum feeding equipment. Bacterial contamination of colostrum could also include specific disease-causing calf pathogens such as *Mycoplasma*. *Mycoplasma bovis* (*M.bovis*) can cause respiratory disease, otitis and arthritis in neonatal calves. Transmission routes include: semen, milk/colostrum, direct nose-to-nose contact, aerosol spread and fomites. Recent UK work has focussed on isolating *Mycoplasma* from bulk milk samples using polymerase chain reaction (PCR) tests and concluded that contamination of UK bulk milk prevalence is low. Work from Belgium found only 1.9 % of colostrum samples tested positive for *M.bovis* on PCR..

Study objectives and hypotheses

The aim of this work was to investigate critical control points for colostrum contamination in Scotland. A secondary objective was to determine the prevalence of *M.bovis* in first milking colostrum in Scottish dairy herds.
Specific hypotheses included:

1. Bacterial contamination (TBC and TCC) would increase through the colostrum management process from harvesting to feeding.
2. There would be farm to farm variation in bacterial contamination of colostrum.
3. Specific colostrum management risk factors would be associated with highly contaminated colostrum.
4. *M.bovis* prevalence would be low in colostrum.

Materials and methods

This study was a prospective, observational study completed between March and November 2023 under University of Glasgow ethics license EA10/23.

Sample Size Calculations

Assuming repeated colostrum sampling with an autoregressive correlation structure, the estimated sample size required was 10 farms and 15 'sets' (teat, bucket, feeder) of colostrum samples per farm. To detect an actual prevalence of 5% *M.bovis* in colostrum with a desired precision of 0.05 and confidence of 95%, 75 colostrum samples were required.

- Eleven farms enrolled from the two veterinary practices in the Dumfries and Galloway region of Scotland.
- Farmers were asked to complete a questionnaire detailing their colostrum harvest, storage and feeding protocols.
- Farm staff were trained to use a standard operating procedure for colostrum sample collection:
    - Composite teat sample at time of first colostrum harvest post calving after standard farm protocol teat preparation for milking. (T=Teat sample)
    - Collection bucket for first milking colostrum (B= Bucket sample)
    - Storage bucket for first milking colostrum (if present) (B2, B3 = subsequent bucket sample)
    - Calf feeder at point of feeding to neonatal calf (F= Feeder sample)
- Samples were then stored at -20°C before being transported on ice for testing at the University of Glasgow internal laboratories.

Laboratory Analysis

- TBC was measured using Sheep Blood Agar (SBA), TCC was measured using MacConkey Agar (MAC)

- Colostrum IgG was estimated using a digital Brix refractometer. Each sample was run in duplicate.
- A subset of samples (n=79) were selected at random throughout the trial for testing for *M.bovis* PCR at the Scottish Rural College Laboratories.

Statistical Analysis

- Descriptive statistics were calculated for colostrum quality indicators: TBC, TCC and Brix %.
- Colostrum quality indicator outcomes were dichotomised using the industry thresholds of 100, 000 CFU/mL for TBC, 10, 000 CFU/mL for TCC and 22% Brix.
- One-way repeated measures ANOVA was conducted to determine if there was a difference in TBC, TCC and Brix % between each source (T, B, B2, B3, F).
- Multilevel linear regression models were constructed to explore risk factor variables

Results

The number of samples from each farm and from each source type is shown in Figure 1. Questionnaire data is shown in Table 1. Descriptive statistics for colostrum quality are detailed in Table 2. The proportion of samples which failed to meet industry thresholds for quality are detailed in Table 3.

The result of the one-way repeated measures ANOVA showed that there was a statistically significant difference in TBC and TCC counts between the source types, and the results are shown in Table 4. There was no statistically significant difference in the Brix % between the source types.

Final multilevel linear models for risk factors significantly associated ($p<0.05$) with colostrum TBC, TCC and Brix % are shown in Table 5.

Seventy-eight (n=78/79, 98.73 %) of the colostrum samples tested negative for *M.bovis*. One sample (n=1/79,1.27 %) was inconclusive, suggesting potentially low levels of *M.bovis*. The prevalence of *M. bovis* was determined to be 1.27% (95% CI = 0.03% – 6.85%) in this study population.

Figure 1 Bar chart showing number of colostrum samples collected from 11 Scottish dairy farms between March and November 2023 and the source type of each sample (T=teat, B=storage bucket, B2 = storage bucket 2, B3=storage bucket 3, F=feeder).

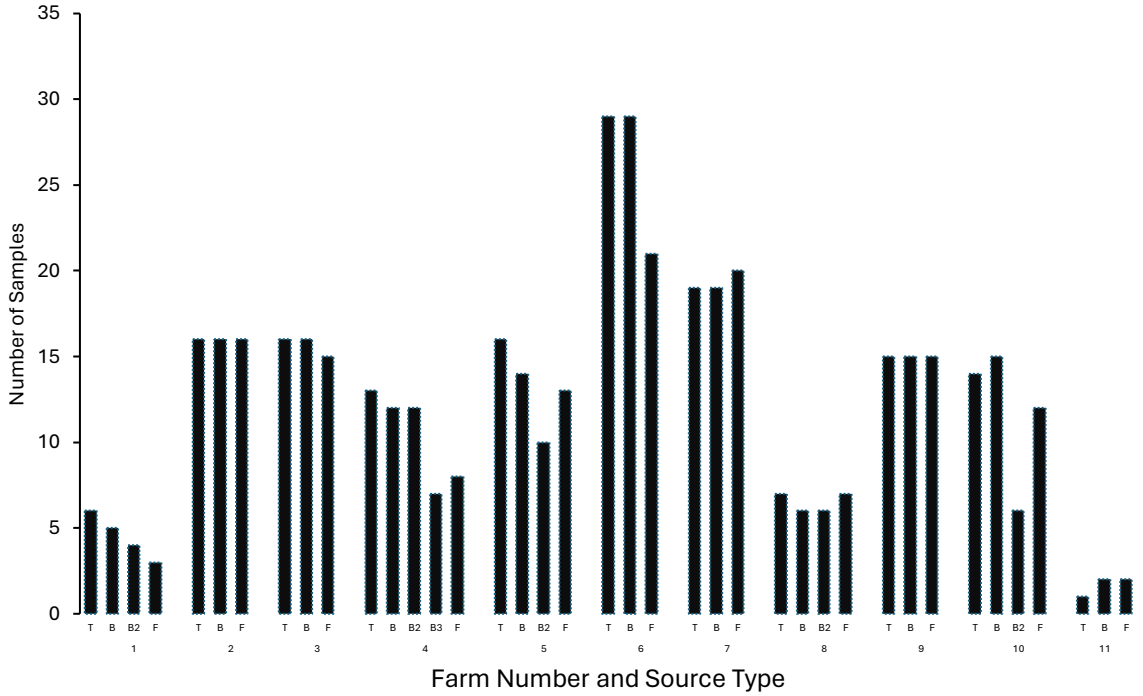

Table 1 : Questionnaire of on farm colostrum management protocols and responses from 11 Scottish dairy farms enrolled between March and November 2023 in Dumfries and Galloway, SW Scotland.

Question	Category	n farms	n samples
How many storage buckets are used between harvest and feeding colostrum?	One bucket	6	282
	Two buckets	4	144
	Three buckets	1	52
How are teats cleaned prior to harvesting first milking colostrum?	Dry Wipe	3	80
	Pre-spray and dry wipe	1	47
	Wet wipe	2	105
	Pre-spray and wet wipe	5	246
What is the average duration of time between calving and colostrum harvest?	Less than or equal to 3 hrs	2	43
	3.5 hrs to 6 hrs	6	294
	Greater than 6.5 hrs	3	120
What is the average duration of time to first feed of colostrum to calf for calving?	Less than or equal to 3 hrs	5	164
	3.5 to 6 hrs	3	175
	Greater than 6.5 hrs	3	139
What type of feeder do you use for you newborn calves first feed of colostrum?	Tube only	6	242
	Offer bottle, then tube	5	236
How often do you clean buckets and feeders?	Once a day	2	66
	After every use	9	412
Do you use a scrubbing brush to clean your buckets, feeders and other equipment	Yes	7	159
	No	4	167
If you store colostrum how is it stored?	Fridge	4	210
	Freezer	6	220
	Fridge and Freezer	1	48
What size of container is colostrum stored in?	1 - 2L	1	18
	2.5 - 3L	6	235
	Greater than 3.5L	4	225
How many people harvest colostrum for newborn calves on your farm?	Less than or equal to 3	9	420
	Greater than 3	2	58
How many people feed newborn calves colostrum on your farm?	Less than or equal to 3 hrs	6	268
	Greater than 3 hrs	5	210

Table 2 Descriptive statistics for colostrum samples collected from 11 Scottish dairy farms between March and November 2023 to measure colostrum contamination and Brix % at each stage of the colostrum harvest, storage and feeding process. Brix = %, TBC [1]and TCC[2] = CFU/ml.

Sample type	n samples	n farms	Min Brix	Max Brix	Mean Brix	STD Brix	Min TBC[1]	Max TBC[1]	Median TBC[1]	IQR TBC[1]	Min TCC[2]	Max TCC[2]	Median TCC[2]	IQR TCC[2]
Teat	152	11	10.80	34.70	23.05	4.70	0	1.07×10^7	4000	15000	0	2.75×10^7	1850	3775
Storage bucket	149	11	10.15	33.85	22.03	4.14	0	13.6×10^7	101000	930000	0	1.3×10^7	12000	108000
Storage bucket 2	38	4	9.15	28.35	20.71	4.82	1000	26.9×10^7	1.43×10^7	1.32×10^7	0	19.4×10^7	470000	0.54×10^7
Storage bucket 3	7	1	20.50	29.40	24.03	3.67	20000	1.22×10^7	0.15×10^7	0.46×10^7	10000	0.82×10^7	64000	585000
Feeder	132	11	10.80	34.70	23.07	4.68	0	29.5×10^7	410000	0.32×10^7	0	18.2×10^7	40000	375000

FOOTNOTE: Brix data was normally distributed and the min, max, mean and STD are reported. TBC and TCC data showed skewed distribution hence the min, max, median and interquartile (IQR) is reported.

Table 3 Colostrum quality indicators (total bacterial counts (TBC), total coliform counts (TCC) and Brix %) and the proportion of samples failing to meet the respective industry thresholds for 478 colostrum samples collected from 11 Scottish dairy farms between March and November 2023

Sample type	Proportion failing to meet TBC[3] quality threshold (%, 95% CI)	Proportion failing to meet TCC[4] quality threshold (%, 95% CI)	Proportion failing to meet Brix[5] quality threshold (%, 95% CI)
Teat	12/152 (7.89, 4.15-13.38)	27/152 (17.76, 12.04-24.78)	63/152 (41.45, 33.52-49.71)
Storage bucket	75/149 (50.34, 42.04-58.62)	81/149 (54.36, 46.01-62.54)	71/149 (47.65, 39.41-55.98)
Storage bucket 2	30/38 (78.95, 62.68-90.45)	29/38 (76.32, 59.76-88.56)	20/38 (52.63, 35.82-69.02)
Storage bucket 3	6/7 (85.71, 42.13-99.64)	7/7 (100.00, 59.04-100.00)	3/7 (42.67, 9.9-81.59)
Feeder	94/132 (71.21, 62.69-78.76)	101/132 (76.52, 68.35-83.45)	55/132 (41.67, 33.15-50.56)

[1] TBC = Total Bacterial Counts
[2] TCC = Total Coliform Counts
[3] Industry threshold for acceptable TBC = < 100,000 CFU/ml
[4] Industry threshold for acceptable TCC = < 10,000 CFU/ml
[5] Industry threshold for acceptable Brix % > 22%

Table 4. One-way repeated measures ANOVA for total bacterial counts and total coliform counts (CFU/ml) for colostrum samples collected from various stages of the colostrum management process (storage and feeding) from 11 Scottish dairy farms between March and November 2023.

Outcome (CFU/ml)	Source Type	Margins	P-value	95 % CI
Total bacteria count[1]	Storage bucket	4773490	0.07	-432040.8 - 9979022
	Storage bucket 2	2.26×10^7	<0.01	1.10×10^7 - 3.43×10^7
	Storage bucket 3	1.50×10^7	0.28	-1.23×10^7 – 4.23×10^7
	Feeder	1.59×10^7	<0.01	1.03×10^7 – 2.14×10^7
Total coliform count[2]	Storage bucket	1338623	0.46	-2239878 - 4917124
	Storage bucket 2	1.55×10^7	<0.01	7476766 – 2.35×10^7
	Storage bucket 3	8576868	0.368	-1.02×10^7 – 2.73×10^7
	Feeder	6925908	<0.01	3099053 – 1.08×10^7

[1] $F(3,168) = 4.08$, $p<0.05$
[2] $F(3,168) = 3.78$, $p<0.05$

Table 5. Final multilevel linear models for farm management risk factor variables (by questionaries) significantly associated (p<0.05) with colostrum quality from 478 colostrum samples collected from 11 dairy farms in SW Scotland sampled between March and November 2023

Outcome	Risk Factor	Category	Coefficient	95% CI	P-value
TBC[1] difference between each source type	What size of container is colostrum stored in?	1-2L	ref	ref	ref
		>3L	-3.77 x 10^7	-6.81 x10^7 - 7239893	0.01
TCC[2] difference between each source type	How are the teats cleaned prior to harvesting first milking colostrum?	Dry Wipe	ref	ref	ref
		Wet wipe	2.47 x10^7	7109763 – 4.22 x 10^7	< 0.01
Feeder TBC	What size of container is colostrum stored in?	1-2L	ref	ref	ref
		2-3L	-3.67	-5.93 - -1.41	<0.01
		>3L	-4.94	-7.17 - -2.72	<0.01
	How the is colostrum stored?	Fridge	ref	ref	ref
		Freezer	5.46	2.26 – 8.67	<0.01
	How are the teats cleaned prior to harvesting first milking colostrum?	Dry Wipe	ref	ref	ref
		Pre-spray & wet wipe	4.88	0.67 – 9.10	0.02
		Wet wipe	9.26	4.76 – 13.76	< 0.01
Feeder TCC	What size of container is colostrum stored in?	1-2L	ref	ref	ref
		2-3L	-4.84	-7.19 - -2.50	<0.01
		>3L	-6.14	-8.55 - -3.74	<0.01
	How is the colostrum stored?	Fridge	ref	ref	ref
		Fridge and freezer	-2.98	-5.66 - -0.31	0.03
	How are the teats cleaned prior to harvesting first milking colostrum?	Dry Wipe	ref	ref	ref
		Wet Wipe	4.5	2.25 -6.76	< 0.01
Teat Brix %	What is the average duration of time between calving and colostrum harvest?	≤3.5 hours	ref	ref	ref
		>6 hours	-3.50	-6.89 - -0.11	0.04

[1] TBC = total bacterial counts

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Dissemination of results and impact of the project

It is acknowledged that the Hannah Dairy Research Foundation's primary focus is the success and sustainability of Scottish dairying. Dissemination of the research outcomes from this project has had a Scottish focus, however some of the messaging is also applicable to vets and farmers in a wider UK and global context. As a result, some research dissemination activity has been expanded to include a UK and international audience. In all presentations and articles, the Hannah Dairy Research Foundation is clearly acknowledged as funders of the work.

Headline knowledge exchange messaging::

- Farmers must improve attention to detail for hygiene of colostrum management from harvest to feeding.
- Colostrum harvest, storage and feeding equipment must be easily cleanable.
- Colostrum handling must be streamlined (particularly, but not limited to, minimising the number of buckets used to store colostrum).
- Colostrum should be stored appropriately (refrigerated at 4°C or frozen at -20°C, or properly chemically preserved) and promptly.
- Colostrum management is only one part of *M. bovis* prevention and nose-to-nose, airborne or fomite spread is likely more important

Scottish focussed dissemination:

- In person farmer meeting (Castle Douglas, January 2024)
- In person vet meeting (Dumfries, January 2024)
- Scottish Farmer article in their February 2024 Dairy Focus
- Poster presentation at Glasgow University Inspire Students research conference (December 2023)
- SRUC Vet CPD Webinar
- SRUC 'On the Hoof' Podcast
- SRUC articles in Beef and Sheep News (March 2024) and Milk Manager (May 2024)

Wider (UK and international) dissemination

- Poster presentation at BCVA Congress (October 2023)
- CPD delivery to consultants at Kite Consulting
- World Buiatrics Congress – Oral presentation (May 2024)

Planned Future dissemination

- Farmer's Weekly article – completed to be published in due course
- Farming Connect (Wales) – On farm meeting, Pembrokeshire,July 2024
- BCVA: Webinar and article in their Cattle Quarterly to be published in due course.

Two peer reviewed publication have been prepared and are currently in the review process.

1. Identifying critical control points for colostrum contamination and *Mycoplasma* prevalence in first milking colostrum from Scottish dairy herds.

2. Validation of the 3M Petrifilm E. Coli/Coliform and Aerobic Count Plates to measure colostrum bacterial contamination on Scottish dairy farms.

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