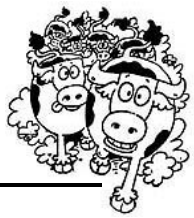

Solving Calf Morbidity and Mortality Problems

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Investigation Steps

- Identify the problem
  - In most calf investigations, the problem will be identified as one of morbidity and/or mortality. When is there too much? Treatment of more than 25% of the preweaned calves and heifer calf death losses > 5% warrant an investigation.
- Locate the problem source(s)
- Characterized the important risk factors that create susceptibility

Problem Identification

Get real numbers. At minimum, get recent 3 months of individual calf treatment and losses. If possible, get mortality rates for the past 12 months so that seasonal effects can be factored into the investigation. From the data, try to establish the at-risk age group, time, or season. Finally, examine calves that are considered to be typical of the problem so that the problem identification is current and accurate. In a representative group of at-risk calves, sample a minimum of 6 calves or 10% of the group. For a diarrhea work-up, feces are submitted for *Salmonella sp.* culture, *Cryptosporidium parvum* detection by acid-fast stain performed on a fecal smear, and electron microscopy for rota- and coronavirus detection. For *Salmonella* culture, 1-2 gm (pea-size portion) is placed in iodine-supplemented tetrathionate enrichment and selenite enrichment broth while at the farm. Fecal smears are made fresh upon return to the hospital. In a calf pneumonia work-up, 2 nasal swabs from each calf are submitted – 1 for bacterial and the other for *Mycoplasma* culture. Sample fecal results follow from a group of 7-10 day old calves in a herd where 90% of the calves develop diarrhea at 7 days of age.

Animal ID	Age or date of birth	Fecal Consistency	EM for Virus	Smear for *C. parvum*	*Salmonella* culture
740	7/18	2	None	+	Negative
742	7/19	0	None	++	Negative
743	7/19	2	Coronavirus	+++	Negative
744	7/19	2	None	+++	Negative

747	7/20	1	None	++	Negative
749	7/21	3	Rotavirus	+++	Negative
750	7/21	1	None	++	Negative

If more than 20% of the calves sampled are shedding any one of the potential pathogens listed above, an infection source should be identified and addressed.

Nasal swabs taken from at-risk calves are used, not so much for pathogen identification but as a tool to identify the antibiotic susceptibility pattern and abnormal nasal flora. If more than 20% of the calves grow *Mycoplasma bovis* from the nose, a source should be identified and addressed.

Where is the Problem or Infection Source(s)?

Where are the calves exposed to the source of the problem? For a diarrhea outbreak, the source of the problem is usually manure (fecal-oral transmission), but oral secretions and aerosols can be sources of infection. In a herd with calf respiratory disease, the source of infection can be contaminated aerosol, other calves or adult cattle, waterers, feeding utensils or feed.

- "Manure meals" provided to calves
  - Calving pen bedding
  - Calving cows – manure on the udder and legs
  - Manure-contaminated colostrum - when fresh cow preparation, milking equipment sanitation, milking equipment function and/or colostrum storage is not optimum.
  - Manure in communal warming area for calves
  - Manure in calf transport vehicles – wheelbarrows, carts, trucks or trailers
  - Calf pen bedding – when there is manure retention in the bedding between calf occupants (inadequate cleaning or disinfection, hutches in same location, or inadequate time between successive occupants), when there is < 3" of dry bedding between the calf and manure, when there is calf to calf contact or continuous bedding base, milk, water or feed refusals are dumped in the calf pen, and/or calf barns are warm and damp
  - Contamination of liquid or dry feed – when milk or milk replacer storage is inadequate, when feed preparation or the area where feed is prepared is not clean, or when feeding equipment is contaminated

- Contact animals – when there are non-immune shedders (FPT), crowding, commingled stressed (weaned calves, calving cows), sick or lame adult cows

- Aerosolized source of infection for calves
  - Commingled adults or weaned heifers
  - Calf housing – when ventilation, humidity, temperature, dampness, animal density or air quality are issues or when shedding animals are present in a shared air space. Shedding animals are FPT calves, stressed calves, chronically sick or poor doing calves.

- Calf treatments or medications used inappropriately (dose, route, frequency, timing, storage, wrong condition) can be the problem source in a calf morbidity or mortality problem.

Risk Factor Assessment

The risk factors that could play a significant role in a calf disease problem are listed. With each investigation, particularly those associated with chronic diarrhea or pneumonia problems and from which no consistent agent has been isolated, each of these risks should be evaluated

- **Failure of passive transfer (FPT)**. Non-immune shedders contaminate the environment to a much greater extent than calves that have adequate immunoglobulin absorption from colostrum. An FPT problem will increase the number of pathogens in the environment. *Goal: 0% FPT (100% of calves with adequate absorption of colostral immunoglobulins)*.
- **Bedding Management.** Calves that are in close contact with manure or other liquid runoff will have continuous exposure to pathogens in the environment. Warm, damp, humid calf housing will compound bedding contamination problems, especially when there is calf to calf contact, inadequate sanitation between successive occupants of an individual pen, accumulation of waste in porous stall base, or dumping feed refusals into calf pens. Pneumonia or diarrhea pathogens from the bedding can be aerosolized. *Goal: At all times, calves have 3" of clean, dry bedding between them and a clean stall base or pack. Feed refusals and contaminated bedding are removed from calf housing.*
- **Spatial Density.** Calf to calf contact increases the number of pathogens in the environment. This is rarely the most important risk factor but *distancing calves or*

creating barriers that prevent cross suckling, licking or manure contact can reduce the rate of exposure.

- **Temporal Density.** Between successive occupants of an individual calf pen, there should be adequate time for removal of all bedding (to the level of the ground or stall base), removing organic material from stall walls, cleaning and disinfection of feeding utensils, drying and addition of fresh bedding. Rapid succession of calf occupants increases the survival time of pathogens in the environment. *Goal: have 15% more calf pens than required at maximum occupancy to allow a minimum of 7 days between successive occupants of the same pen.*
- **Commingled Age Groups.** Pre-weaned calves that share the housing facility with adult cows, sick cows or recently weaned calves have a much greater risk of exposure to pneumonia and fecal pathogens. Stressed and calving cows shed bacteria at a much higher level than their unstressed peers. *Goal: Move dairy calves to an individual pen before they stand (30 min) and suckle (90 min).*
- **Air Hygiene.** Inadequate ventilation, humidity, dampness and high animal density create conditions conducive to a high number of aerosolized organisms, noxious gases and other contaminants that may compromise calf health. Power washing may enhance aerosolization of organisms for contact calves. *Goal: Evaluate ventilation in calf barns associated with endemic calf pneumonia problems and be aware of potential seasonal limitations.*
- **Other stressors**
  - Feeding
  - Water availability
  - Medications
  - Vaccinations

Herd Based Testing for Passive Transfer in Calves

Investigation of calf morbidity or mortality requires an accurate assessment of the colostrum-feeding program. Herd-based testing to assess colostral immunity is quite different than testing individual calves. Frequently, conclusions regarding colostrum feeding are made based on assurances of spoken word, rather than observation of colostrum feeding practices or testing calves. Conclusions that incriminate or overlook problems can occur without substantive data. Accurate conclusions require appropriate sample size, a discriminating test and an appropriate population of calves to test. In our herd investigations, we use a total protein concentration of 5.5

g/dl as the cut point and we are interested in the proportion of calves that fall below the cutpoint. We set an alarm level of 20%. That is, greater than 20% of calves falling below the cutpoint is indicative of a herd problem of failure of passive transfer. Using a proportional outcome based test, a minimum of 12 calves should be sampled to yield a 75% confidence interval. For smaller herds, accumulate test results until 12 have been run. If the results in any herd are close to the cut-point, more tests should be done. Herd test results are analyzed as follows:

Outcome (< 5.5 g/dl)	Percentage	Interpretation
0/12	0%	No colostrum feeding problem
1/12	8.3%	No colostrum feeding problem
2/12	16.7%	Borderline concern
3/12	25%	Borderline concern
4/12	33.3%	Failure of passive transfer in this herd
5/12	41.7%	Failure of passive transfer in this herd
6/12	50%	Failure of passive transfer in this herd

There should be a minimum 6-hour time lapse between feeding colostrum and testing a calf for adequate passive transfer of immunity. Any calf < 7 days of age is eligible for testing until a sample size of 12 calves is desired. Regular testing of problem herds is encouraged. Three herd results follow with all protein results derived from refractometer and reported as g/dl:

Calves	Herd 1	Herd 2	Herd 3
1	7.6	4.2	5.1
2	7.6	5.2	4.9
3	8.0	5.3	6.1
4	7.2	5.6	5.9
5	5.9	5.4	5.9
6	8.0	5.6	6.0
7	7.6	5.3	4.0
8	6.4	5.7	
9	6.5	5.5	
10	6.8	5.0	
11	7.5		
12			

Analysis of a Herd FPT Problem

- Unobserved calvings occur on a regular basis, e.g. night time
- Calves remain with dam for $\geq$ 90 minutes
- Colostrum administration occurs $\geq$ 4 hours after calving
- Fresh cows are milked $\geq$ 6 hours after calving
- Calves do not routinely receive either 4 quarts (3 quarts for Jerseys, Ayrshires, Guernseys) of first milk colostrum or 1 package of colostrum replacer within 4 hours of **birth**
- Colostrum replacement or supplement are mixed in with colostrum
- There is a shortage of colostrum from appropriate donors without a back-up supply of colostrum replacement product or frozen colostrum readily available
- There is more than a 2-hour lapse between colostrum milking and either feeding or refrigeration of colostrum
- Refrigerated colostrum is > 7 days, frozen colostrum is > 1 year or has been through more than 1 freeze-thaw cycle
- Bacterial contamination of colostrum is excessive (total bacterial count > 1,000,000 cfu/ml and/or fecal coliform count > 10,000 cfu/ml)
- Colostrum is routinely pooled
- Fresh cow health is poor
- Transition cow management (nutrition, group changes, bedding, density, vaccinations, medications) is a concern

Colostrum Bacterial Contamination

We have found that colostrum bacterial contamination is a significant problem on farms with calf morbidity and mortality problems. Total colostrum bacterial counts > 100,000 cfu/ml are associated with FPT and many of the contaminated samples have fecal coliform counts in excess of 10,000 cfu/ml. While colostrum bacteria establish the normal gastrointestinal flora of the newborn calf, the presence of fecal coliforms suggests that colostrum may also be a source of infection (fecal oral transmission) of such important pathogens as *Mycobacterium paratuberculosis, Salmonella Dublin, Cryptosporidium parvum* and other enteric pathogens. To my knowledge, colostrum cultures are not routinely performed in laboratories doing bulk tank

milk cultures because of the technical difficulty of the process. Due to the high bacterial counts in colostrum, multiple dilutions are routinely required.

- In our laboratory, initial plating is done on blood agar at 1:50, 1:500, 1:5,000, and 1:50,000 dilutions.
- EMB plates are used to distinguish fecal coliforms (potential pathogens) from other environmental gram negative bacteria like *Proteus, Pseudomonas spp.*
- 1:500 and 1: 5,000 dilutions are plated onto TKT agar to differentiate the different species of *Streptococcus*.
- Blood agar plates distinguish amongst *Staphylococcus sp.*

Any other bacteria with a dominant population is subcultured onto appropriate agar and identified. Colostrum is not the ideal sample to identify *Mycoplasma bovis* infected cows.

Colostrum Culture Goals

- Total bacterial count < 100,000 cfu/ml
  - Fecal coliforms (lactose positive) < 10,000
  - Other gram negative bacteria < 50,000
  - *Strept. ag.* 0
  - *Strept.* non-ag < 50,000
  - Coag positive *Staph.* 0
  - Coag negative *Staph.* < 50,000
  - Others 0 (*Mycoplasma bovis, Salmonella*)

Colostrum Sample Results

	1	2	3	4	5
Total Count (cfu/ml)	220,000	3,750,000	>15,000,000	8,650,000	8,700,000
Fecal coliforms	4,000	0	>15,000,000	0	8,700,000
Other gram neg	210,000	3,700,000	0	8,650,000	0
Strep ag	0	0	0	0	0
Coag pos *Staph*	0	0	0	0	0
Coag neg *Staph*	10,000	200,000	0	0	0

Source of Colostrum Bacterial Contamination

Depending on the bacterial types and numbers, the source of contamination may be any of the following:

- Inadequate cow preparation can be blamed when fecal coliform counts are high
- Improper sanitation or malfunction of fresh cow milking equipment is incriminated when other gram-negative bacteria or environmental *Staph.* and *Strep.* species are present
- The cleanliness of the milk collection bucket or containers is suspect when other gram-negative bacteria or environmental *Staph.* and *Strep.* species are present
- Inadequate cooling and storage of colostrum will result in amplification of any bacterial populations that are present after collection. Fecal coliform populations will increase by a log every 30 minutes if storage and cooling is not appropriate.
- Mastitis – *Strep ag, Staph aureus,* or other environmental mastitis can increase the level of bacterial contamination of colostrum
- Dirty calf bottles and nipples represent the final source of bacterial contamination of colostrum with other gram-negative bacteria or environmental *Staph.* and *Strep.* species

The Role of Colostrum Replacement and Supplement Products in Solving FPT

The abstract reproduced below is excerpted from a paper in preparation reporting on a clinical trial run in 8 Wisconsin dairy herds.

ABSTRACT

Field Evaluation of a Commercial Bovine Colostrum Replacement Product

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The objective of this study was to compare the immunoglobulin absorption and health of calves fed either colostrum or a colostrum replacement product containing concentrated bovine immunoglobulin derived from plasma. Two hundred eighty nine newborn calves from 8 dairies were fed 4 quarts of colostrum (n=142) or 2 quarts of a colostrum replacer, followed by 2 quarts of a colostrum supplement (n=147) within 12 hours of birth. The colostrum replacer (CR) and colostrum supplement (CS) contained 21.2 and 11.1% of dry matter as the immunoglobulin fraction of bovine plasma. The CR and CS provided 125 and 45 g globulin, respectively. Significant differences between groups were detected in mean serum total protein levels and immunoglobulin G (IgG)

concentrations measured by radial immunodiffusion (RID) test. Mean serum total protein concentration measured within 7 days of birth was 5.58 and 5.26 g/dl for colostrum and CR/CS calves, respectively. Colostrum fed calves had 1,974 mg/dl IgG compared to 1,460 mg/dl for the CS/CR calves. Despite the differences in absolute serum total protein and IgG concentrations between groups, the number of calves that failed to reach a minimum serum total protein concentration of 5.2 g/dl (n=32 and 61 for colostrum and CR/CS groups, respectively) or reach an IgG of 1,000 mg/dl (n=18 and 42 for colostrum and CR/CS groups, respectively) was not significantly different between groups. A lateral flow immunoassay test performed on calves in both groups with total serum protein concentrations below 5.2 g/dl provided further evidence that a critical level of 1,000 mg/dl immunoglobulin had been successfully reached in both groups (n=20 and 42 for colostrum and CR/CS groups, respectively). A scoring system designed to monitor the health of calves for 14 days showed no differences between groups. These data suggest that feeding a CR/CS combination to newborn dairy calves is a safe and effective alternative to feeding colostrum. For those dairies that may not have an adequate supply of colostrum on hand, effective results are anticipated with sequential administration of CR followed by CS to newborn calves.

Advantages of feeding colostrum replacement product

- Readily available and conveniently packaged
- Ease of mixing in 2-qt of water
- Above 2 reasons frequently resulted in administration at a younger age than colostrum
- No bacterial contamination or transfer of contagious disease
- Adequate immunoglobulin levels can be acquired
- Calf health is not compromised

Disadvantages of feeding colostrum replacement product

- Immunoglobulin levels are lower than colostrum
- Though the product has nutritional supplements added, it is not like colostrum
- Non-specific immune factors and immune cells are not present
- High carbohydrate content could result in rapid gastric emptying, especially when mixed with colostrum. This may enhance the risk for enterotoxemia.

Bedding Assessment

To evaluate the relative risk of infection from bedding in the maternity pen, calf transport vehicles or calf housing, bedding material can be submitted for *Salmonella* culture and quantitative bacterial counts. For *Salmonella* culture, 1 to 2 gm of a mixture of bedding material (from 4 quadrants and the center of the pen) is placed into Buffered Peptone Water Pre-Enrichment Media (BPW) at the farm and submitted to the laboratory upon return to the hospital. For quantitative bacterial assessment of the bedding, a similar bedding mixture is submitted in whirl packs to the University of Minnesota Laboratory for Udder Health (St. Paul, MN 55108). The presence of *Salmonella* in calf bedding poses a significant risk of infection since calves spend a significant amount of time in recumbency where fecal-oral contact is likely. The standards for the level bacterial contamination in calf bedding has not been established but some farm results follow along with goals that are consistent with the level of risk cited for environmental mastitis. In a clean environment that is ready to accept a newborn calf, the total bacterial count should be < 5,000 colonies/ml. During occupancy, the count should remain < 2,000,000 colonies/ml.

SAMPLE LOCATION	COLIFORMS	ENVIRON-MENTAL *STREP*	*STAPH* SPECIES	TOTAL (COLONIES /ML)
Maternity Pen	15,000,000	20,000,000	9,215,000	44,215,000
Communal Hutch	2,000	775,000	275,000	1,052,000
Transport vehicle	6,900,000	21,500	250	6,921,750
New hutch	750	6,250	4,500	11,500
Hutch - 5 days	1,500	575,000	1,000	577,500
Calf barn – 5 days	775,000	1,300,000	225,000	2,300,000
Calf barn – 7 days	41,000,000	25,000,000	450,000	66,450,000
Goal for clean pen	**< 1,000**			**< 5,000**
Goal for occupied pen	**< 500,000**			**< 2,000,000**

Assessment of Feeds and Feeding Practices

Bacterial contamination of liquid feeds – milk or milk replacer – may be a source of infection for calves. In most herds, the concern is fecal coliform contamination but milk or milk replacer refusals may also contain an abundant concentration of respiratory pathogens that have accumulated as calves hold their head in the bucket for prolonged periods during illness, expelling oral and nasal secretions. Some sample farm findings along with goals are included in the following table.

	MILK REPLACER 1	MILK REPLACER 2	MR – NEW BUCKET	WASTE MILK	GOALS
Total bacteria (cfu/ml)	10,500,000	430,000	13,000	14,960,000	< 10,000
Fecal coliforms	7,900,000	50,000	0	12,900,000	0
Other gram neg	2,600,000	305,000	9,500	0	< 5,000
Strep non-*ag*	0	50,000	0	650,000	< 5,000
Coag neg *Staph*	0	25,000	3,500	0	< 5,000
Other	*Bacillus sp*	0	0	*Mycoplasma bovis*	0

Milk replacer and oral electrolyte solution sodium concentration and osmolality measurements are made if there are concerns about calf illness associated with ileus, bloat, enterotoxemia, and/or medication failure. When colostrum replacement or supplement products are added to colostrum and when oral electrolyte solution (OES) is added to milk or milk replacer, samples are also obtained for analysis. Our samples are submitted in red-top tubes and are run by serum chemistry analyzer and freezing point depression osmometer, respectively.

Osmolality of Milk Replacers

Definition: Osmolality refers to the number of osmoles per kg of water. One osmole is one gm molecular weight (1 mol) of any nondissociable substance such as glucose or lactose. The freezing-point depression method of measuring osmolality measures all solutes in relation to their concentration, even though they may be ineffective osmoles (solutes that are able to cross

membranes). Osmolality measurements of the liquid feed given to calves give some idea of the carbohydrate content of the diet. The number of osmoles in solution will reflect lactose or other sugars, proteins and sodium concentration.

Some background for interpreting milk replacer and oral electrolyte solution osmolality data:

- Normal serum osmolality is 280-290 mOsm/kg
- Milk as fed is isosmotic
- As milk is digested, the osmolality increases in the duodenum and then declines as it moves down the gut.
- As the osmolality of milk, milk replacers or oral electrolyte solutions increase (up to 600 mOsm/L), gastric emptying is progressively more rapid and complete.
- Delayed gastric emptying may enhance absorption of some substances like glucose in OES fed to calves.

Sodium concentration is not routinely monitored in milk replacers**.** Depending on protein source and processing, sodium concentration could be high in some milk replacers. Cheese plant whey products may have enough brine to increase sodium content.
To alkalinize acidified liquid whey, NaOH or $NaHCO_3$ are added and may elevate sodium levels. Osmolality data is very variable and is not directly proportional to sodium content of the milk replacer. We suspect that osmolality is more reflective of sugars and protein in milk replacers than it is of sodium concentration. From more than 50 farm samples, we have measured values that range from 278 to 1210 mOsm/L. Median and mean values are approximately 500 and 525 mOsm/L, respectively with deviations > 220 mOsm/L. In general, the high protein milk replacers tend to have higher values than the traditional (22-20) powders. Mixed as directed on the label, we have found no milk replacer or oral electrolyte solution osmolality or sodium concentration that exceeds the goals shown in the table below. Mixing errors, concentrating powder, feeding for cold weather, electrolyte powder in milk replacer, colostrum supplement mixed in colostrum have all been reasons for some sample results shown in the same table.

	MILK REPLACER	MR FOR COLD	OES IN MR	OES 1	OES 2	GOALS
Sodium (mEq/L)	63	147	189	137	314	< 120 mEq/L
Osmolality (mOsm/L)	595	897	1108	726	1539	< 600 mOsm/L

Although benchmarks are not established, we believe that fluids with sodium concentrations > 120 mEq/L or osmolalities > 600 mOsm/L should be fed with caution and should **NEVER** be fed if fresh water is not made available to calves twice daily. Relating these data back to herds where there is concern for *Clostridium perfringens* enteritis, it is useful to remember that for the syndrome to occur, there are at least 3 requirements:

- The organism is present in the normal intestinal tract of calves. While *Clostridium perfringens* are normal inhabitants of the intestinal tract, they rarely grow in a fecal culture. Once the population is proliferating, the generation time in the intestines is short at 8.8 minutes.
- Carbohydrates are presented to the intestinal tract in amounts and/or types that can cause rapid proliferation, sporulation and toxin production. In the calf diet, a high carbohydrate load is likely to come from milk replacer, oral electrolyte solutions and/or other additives in the liquid feed. When osmolalities in excess of 600 mOsm/L have been found, there is a greater risk for enterotoxemia.
- When intestinal stasis occurs for any reason, toxin accumulation and absorption is more likely to occur. Calves that develop diarrhea from any pathogenic cause frequently have associated motility disturbances that increase the risk for enterotoxemia.