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Evaluation of Colostrum Quality, Passive Immunity, and Dairy Calf Management Practices in Alberta

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Evaluation of Colostrum Quality, Passive Immunity, and Dairy Calf Management Practices in Alberta

by

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Abstract

Failure of passive transfer of immunity (FPT) in dairy calves is associated with increased risk of morbidity and mortality. In this study, which involved 14 dairy farms in central Alberta, 44.2% of calves had FPT when serum total protein was used as a measure while 27.8% had FPT when serum immunoglobulin G (IgG) was used. Risk of FPT was associated with amount of colostral IgG consumed. Low risk of FPT was associated with feeding a combination of waste, sale, and milk replacer as the second feeding after colostrum. Higher risk of FPT was associated with feeding a colostrum supplement compared to frozen-thawed or colostrum replacer. Tube feeding of colostrum was associated with reduced growth and increased risk of death. Both the Brix refractometer and colostrometer are suitable tools to indirectly measure colostral IgG when used at cut points of 23% and 80 mg/mL, respectively.

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Dedication

To my parents, for encouraging me to pursue what I really love, and to Brad, for making me want to be a better person while pursuing it.

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List of Abbreviations

ADG	Average daily gain
AEA	Apparent efficiency of absorption
BW	Body weight
CR	Colostrum replacer
CS	Colostrum supplement
FPT	Failure of passive transfer of immunity
Ig	Immunoglobulin
IgG	Immunoglobulin G
MC	Maternal colostrum
MR	Milk replacer
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
RID	Radial immunodiffusion
RR	Relative risk
SCC	Somatic cell count
STP	Serum total protein
TPC	Total plate count

Chapter One: Review of the Literature

1.1 Introduction

In the gestating dairy cow, maternal and fetal blood supplies are completely separate (Godden, 2008), therefore, there is no transfer of immunoglobulins (Ig) across the placenta from the dam to the fetus. Subsequently at birth, neonatal calves have minimal immunological defences against environmental pathogens. They must acquire immunity passively through the consumption of colostrum, the first milk that a cow produces immediately following parturition. If a calf does not receive an adequate amount of colostrum, or if the colostrum is of poor quality, the calf may suffer from failure of passive transfer of immunity (FPT). Failure of passive transfer of immunity is defined as calf serum immunoglobulin G (IgG) levels less than 10 mg/mL or serum total protein (STP) less than 5.2 g/dL (Tyler et al., 1996; Calloway et al., 2002). While FPT is not in itself a disease, it does predispose calves to dehydration, diarrhea, and dullness (Weaver et al., 2000) and can lead to an increased risk of morbidity, decreased growth rate and reduced lifetime milk production (Chigerwe et al., 2009). By far the greatest factor contributing to mortality of preweaned calves is FPT, associated with 39 to 50% of preweaned calf mortality (Margerison and Downey, 2005). Failure of passive transfer of immunity is widespread in farms across the US and Canada; 19.2% of calves on US farms had FPT (Beam et al., 2009) and in Ontario the estimated prevalence was 37.1% (Trotz-Williams et al., 2008). McGuirk and Collins (2004) state that FPT can be the result of improper formation, ingestion, or absorption of colostrum. Certain preweaned calf management practices are associated with FPT; therefore, proper colostrum and calf management practices are crucial in preventing FPT.

1.2 Measures of Failure of Passive Transfer of Immunity

Failure of passive transfer of immunity in calves can be measured using many different methods. An ELISA or radial immunodiffusion (RID) analysis measures serum IgG directly; RID is considered to be the gold standard (Gay, 1983; Bielmann et al., 2010). Indirect methods of determining FPT include measurement of STP concentrations with a refractometer, using the zinc sulfide turbidity and sodium sulphite turbidity tests, g-glutamyl transferase activity, and whole-blood glutaraldehyde coagulation tests. Out of these indirect methods, measuring STP with a refractometer is the easiest method, is reliable and has good correlation with RID-measured serum IgG levels (Tyler et al., 1996; Weaver et al., 2000).

1.2.1 Serum IgG

Immunoglobulins are globular proteins secreted by B-lymphocytes, which function to identify and inactivate antigens found in the bloodstream. There are five classes of Ig, three of which are present in colostrum. These include Ig A, G, and M. Each are composed of two identical light peptide chains and two identical heavy peptide chains. Each class is identified by a characteristic type of heavy peptide chain. The largest class, in terms of prevalence in serum, consists of the γ-type heavy chains and is therefore named γ-globulin, or IgG. This is the most abundant antibody found in both bovine colostrum and serum. In Holstein-Friesian calf serum, 82 ± 4.9% of total immunoglobulins are IgG (Kaske et al., 2005). IgM is present at only 3 to 12 mg/mL, and IgA at 3 to 4 mg/mL (Kehoe et al., 2007). IgG is useful for providing long-term immunity, whereas IgM is present at the time of initial infection and provides short-term protection. Immunoglobulin A provides immunity through mucosal secretions whereas IgG and IgM are present throughout the blood and lymph.

Many studies suggest that passive transfer of immunity is adequate when serum IgG concentrations, measured by RID, are a minimum of 10 mg/mL. Virtala et al. (1999) estimated that serum IgG levels between 8.9 to 13 mg/mL were associated with increased risk of calf death. Similarly calves with serum IgG > 10 mg/mL had significantly reduced risk of death compared to calves with lower serum IgG (Chelack et al., 1993) Other studies, however, have demonstrated that a lower serum IgG value (5 mg/mL) is associated with higher relative risk of death (Rea et al., 1996; Donovan et al., 2008). Gay (1983) defines FPT as serum IgG levels of < 10 mg/mL at 48 hours of life. Tyler et al. (1996) subsequently used this value in a study to determine appropriate cut-points for indirect methods of measuring IgG. Similarly, Calloway et al. (2002) described FPT as 10 mg/mL measured by RID when using this cut-point as test positive or negative in comparison to refractometer measurements. This value has since been widely accepted as the cut-point for FPT.

1.2.2 Serum Total Protein

The direct measure of serum IgG levels by RID is time-consuming and cannot be performed by producers on-farm or by veterinarians in a farm setting. An alternative is to use STP measured by refractometry as an indirect measure of IgG concentration. Calloway et al. (2002) measured different models of refractometers to determine diagnostic test characteristics compared to RID measurement of IgG, and found the highest combined sensitivity and specificity occurring at 5.2 g/dL. The authors suggested a threshold where risk of death increases between 5.0 and 5.5 g/dL, so adequate passive transfer likely occurs above 5.0 but below 5.5. Tyler et al. (1998) found that calves with STP < 4.0 g/dL had a relative risk (RR) of dying of 4.6 compared to calves with STP > 6.0 g/dL. Rea et al. (1996) found that calves with STP < 4.5 had

a RR for dying of 2.6, and those with STP of < 5.0 had RR of 1.7 compared to calves with STP < 5.5 g/dL (RR=1.24). Both studies suggest that STP is not an infallible predictor of pre-or postweaned mortality.

Calloway et al. (2002) suggested that a STP cut-point range of 5.0 to 5.4 g/dL equates to serum IgG levels of 8.9 and 13.4 mg/mL. Similarly, Tyler et al. (1996) found that high sensitivity and specificity occurred at 5.2 g/dL, and suggested that a range of 5.0 to 5.5 g/dL is appropriate for determining adequate passive transfer. Although this range is generally useful, hydration status of the calf should be considered when using refractometry, as clinically ill dehydrated calves have elevated STP (5.5 – 6.0 g/dL) and can be misclassified having adequate passive transfer of immunity (Tyler et al., 1999). When compared to RID analysis, refractometers have a correlation of up to 0.84 (Tyler et al., 1996; Quigley et al., 2002).

1.3 Colostrum Quality

Colostrum contains a high percentage of proteins such as lactoferrin, transferrin, albumin, and α-and β-lactoglobulin, as well as different classes of Ig (IgG, IgA, IgM, and IgE) (Kehoe and Heinrichs, 2007). Approximately 85-90% of Ig in colostrum is IgG, with 80-90% of this being IgG_1 (Larson et al., 1980). During colostrogenesis, high concentrations of IgG are transported from the blood into the mammary glands through receptors on the alveolar epithelial cells (Godden, 2009).

The concentration of serum IgG in the calf is dependent on the efficiency of colostral IgG absorption across the gut, mass of IgG ingested, and the calf's blood volume. The mass of IgG, rather than the mass of colostrum consumed after birth, is the biggest factor influencing serum IgG, 68% of the variation in serum IgG can be explained by the amount of colostral IgG

consumed (Bush and Staley, 1980). In dairy cows, good quality colostrum is widely considered to contain at least 50 mg of IgG per mL (Godden et al., 2009a). Data that supported this value originated from a study by Kruse (1970), which stated that 2 kg of maternal colostrum should be consumed and have an Ig content of at least 5%, equating to 50 mg/mL. Cows in their third or higher lactation produce colostrum with significantly higher IgG levels than cows in first or second lactations (Tyler et al., 1999; Chigerwe et al., 2009).

Colostral quality can vary widely within and between farms. Quigley et al. (2013) reported a range of 7.1 to 159 mg/mL on a single farm, and similar ranges have been found in other studies (Godden, 2009; Fidler et al., 2011). The proportion of colostrum containing < 50 mg/mL IgG ranges from 16 to 32% (Chigerwe et al., 2008a; Morrill et al., 2012; Quigley et al., 2013). Due to this range in colostral IgG levels, it is important to measure colostral IgG prior to feeding in order to ensure calves will receive an adequate amount of IgG. Numerous tools are available to measure colostral IgG directly and indirectly.

1.3.1 Radial Immunodiffusion

Radial immunodiffusion has been used successfully to directly and quantitatively measure colostral IgG. This assay uses an anti-IgG antibody dissolved in an agarose gel. The sample is pipetted into small wells punched in the gel and left to precipitate. The diameter of the rings formed by the precipitation of the antibody complex is measured and compared to the size of rings formed by known concentrations of IgG. This procedure has been used successfully for bovine colostral IgG (Chelack et al., 1993). To obtain the most accurate results, whole maternal colostrum (MC) should be used rather than fat-free colostrum or whey (Fleenor and Stott, 1981).

This procedure, commonly used to analyze colostral IgG, is also used for bovine serum IgG (Chelack et al., 1993).

1.3.2 Colostrometer

Fleenor and Stott (1980) first developed a hydrometer calibrated specifically to the IgG content in colostrum. They found a strong relationship between colostrum total solids and colostral specific gravity (r^2=0.76) and a stronger relationship between total protein and specific gravity (r^2=0.90). They suggested that IgG accounted for the largest amount of variation related to specific gravity. Colostral specific gravity itself may be influenced by season of calving, dam parity, and temperature of the colostrum (Mechor et al., 1992; Morin et al., 2001). According to the calibration scale on the colostrometer, the cut-point between "excellent" and "moderate" colostrum quality occurs between 49.8 mg/mL and 52.4 mg/mL (Fleenor and Stott, 1980). Later studies tested the colostrometer against RID-measured IgG levels in order to determine diagnostic test characteristics such as sensitivity and specificity at different cut-points. Pritchett et al. (1994) found that the colostrometer overestimated colostrum quality, and recommended using a cut-point range of 60 to 85 mg/mL in order to reduce the number of false negative misclassifications. Chigerwe et al. (2008a) found that when compared to 50 mg/mL IgG as measured by RID, the colostrometer had an optimal cut-point of 70 mg/mL with sensitivity and specificity of 0.75 and 0.78, respectively.

McGuirk and Collins (2004) describe the colostrometer as being the most common tool used on-farm to assess colostrum quality, but suggest that it is not sensitive or specific and works only within a small temperature range. As previously stated, the colostrometer measures specific gravity which is correlated with IgG content; however, there are also other molecules such as

non-IgG protein and fat in colostrum that can affect specific gravity (Morin et al., 2001). Though dairy producers are familiar with the colostrometer, utilization is low, as 4% of farms in Quebec were reported to use the colostrometer consistently (Vasseur et al., 2010). The reason for such low utilization could be due to the fragility of the tool (Bielmann et al., 2010) or the fact that it is very sensitive to temperature (Mechor et al., 1992).

1.3.3 Brix Refractometer

The Brix refractometer was developed to measure the amount of sugar dissolved in water as a percentage of weight (i.e. 1% Brix = 1 g sugar in 99 g water). Distilled water has a Brix reading of 0%. This tool has been used to indirectly measure equine colostrum IgG content for over a decade with successful results (Cash, 1999). Brix refractometers have successfully estimated total solids dissolved in bovine waste milk (Moore et al. 2009), and have recently been recognized as a tool to determine bovine colostrum IgG content (Bielmann et al., 2010). Brix values for colostrum are reported to range from 13.6 to 37% with a mean of 26.3% for frozen-thawed samples (Bielmann et al., 2010) and from 12 to 32% with a mean of 23.8% (Quigley et al., 2013).

A range of Brix values associated with good colostrum quality has been described. A Brix score of 22% was equated to an IgG concentration of 50 mg/mL (Heinrichs and Jones 2011). Bielmann et al. (2010) also reported that 22% is an appropriate cut-point for both digital and optical refractometers based on sensitivity and specificity when compared to 50 mg/mL through RID analysis. Chigerwe et al. (2008a) suggested using a range of 20% to 23%, in agreement with the 21% suggested by Quigley et al. (2013) but higher than the 18% suggested

by Morill et al. (2012b). The correlation between Brix values and RID-measured IgG levels ranges from 0.64 to 0.94 (Quigley et al., 2013).

Unlike the colostrometer, the Brix refractometer is not sensitive to temperature, as demonstrated by a study comparing Brix refractometer results with colostrum samples ranging from 5°C to 38°C with no significant difference in readings (Bielmann et al., 2010). This gives the Brix refractometer an advantage over the colostrometer for on-farm use as it could be used for refrigerated or just-harvested colostrum.

1.4 Factors Influencing Failure of Passive Transfer of Immunity

1.4.1 Absorption of Immunoglobulins

Cells in the immature small intestine non-selectively absorb macromolecules via pinocytosis. The molecules move through the epithelium, into the lymphatic system and then the bloodstream (Quigley et al., 2005). Gut cell maturation and other changes such as a decrease in the pH of the abomasum and the initiation of intestinal digestive enzyme secretion prevent absorption of intact IgG starting at birth, with complete cessation of non-selective absorption occurring by 24 hours of life (Quigley, 2004; Quigley et al., 2005). As the gut non-selectively absorbs molecules, it will also absorb harmful molecules such as pathogenic bacteria. It is therefore crucial to ensure the calf receives as much clean, good quality colostrum as soon as possible after birth so that IgG, rather than pathogens, is absorbed and enters the bloodstream .

1.4.2 Timing of Colostrum Intake

In a herd-level survey of American dairy farmers, those who fed colostrum more than 4 hours after birth were 2.7 times more likely to have calves with FPT than farms who fed the first

colostrum within 4 hours (Beam et al., 2009). As optimal absorption occurs within 4 hours of birth and declines rapidly after 6 hours, calves should ideally be fed within the first 4 hours of birth, with the first feeding no later than 6 hours after calving (Davis and Drackley, 1998; Godden, 2009). A Finnish study indicated that for every 30 minutes that consumption of first colostrum was delayed, serum IgG decreased by 2 mg/mL (Rajalan and Castrén, 1995).

1.4.3 Volume of Colostrum Fed

Inadequate colostrum intake by the calf (not enough colostrum to reach a minimum of 10 mg/mL IgG in the serum) has been associated with short and long term effects, such as increased calf morbidity and decreased growth rate (Robison et al., 1988; Kaske et al., 2004) and lower average milk production (deNise et al., 1989).

Davis and Drackley (1998) advise that the minimum amount of IgG that should be consumed is 100 grams, though some literature suggests that 150 to 200 grams is more appropriate (Chigerwe et al., 2008b). Calves fed more than 100 g of IgG in the first feeding had a low prevalence of FPT (Besser et al., 1991, 1993). Depending on the concentration of IgG in the colostrum, this may be given in one or multiple feedings. Feeding the entire volume of colostrum necessary to achieve ingestion of 100 to 200 g of IgG in a single feeding may be beneficial as this reduces labour associated with a second feeding, provided that the calf can comfortably consume the required volume.

Producers in Ontario reported feeding a median volume of 2.5 L in the first 6 hours of life, with a range of 0 to 6 L (Trotz-Williams et al., 2008). Several studies show that calves fed a larger volume of colostrum (4 L versus 2 L in the study of Morin et al., 1997, and 3 L versus 1.5 L in the study of Godden et al., 2009a) had significantly higher STP levels when fed by the same

method and at the same time after birth. The risk of FPT was shown to decrease as the volume of colostrum fed in the first 6 hours of life increased (Trotz-Williams et al., 2008). Between 2 and 4 L of colostrum is appropriate to ensure adequate mass of IgG is consumed, depending on body weight (BW) of the calf and colostral IgG concentration (McGuirk and Collins, 2004; Kaske et al., 2005). Calves may consume 4 or more litres of colostrum at a single feeding, and they can also consume an additional 3 L at 12 hours of age, even if a large volume was fed at the initial feeding (Chigerwe et al., 2009). This suggests calves can consume a high volume of colostrum if it is offered, and allowing calves to consume as much colostrum as possible in two feedings up to 12 hours of age will significantly reduce the risk of developing FPT (Chigerwe et al., 2009). This study suggests that calves that do not consume at least 3 L of colostrum in the first 4 hours of life should be tube-fed 2 L by 12 hours to ensure an adequate amount of colostrum is consumed, and that calves who do not consume at least 2 L in the first feeding should be tube-fed to reach a minimum amount of 3 L total.

1.4.4 Method of Administration

Leaving the calf with the dam to suckle exclusively may result in inadequate voluntary consumption of colostrum within the critical 4-hour window and so contributes to the development of FPT (McGuirk and Collins, 2004). Indeed, a study by Edwards and Broom (1979) indicated that 46% of calves from 2nd parity cows left with the dam did not suckle in the first 6 hours of life. Trotz-Williams et al. (2008) reported that farms that left calves with the dam had a significantly higher risk of calves with FPT compared to farms that reported not leaving the calf to suckle. A Finnish study showed that calves left with the dam for 5 days had a higher prevalence of FPT compared to calves separated immediately (Rajala and Castrén, 1995). This

was attributed to the inability of the calf to suckle within the critical 6-hour time frame due to poor calf vigor, poor mothering, or abnormal udder conformation. The authors suggest that calves left with the dam should be assisted in order to suckle as soon as possible, or else be hand-fed colostrum.

In Ontario, producers reported that 50% of calves were left with the dam for three hours or less while 33% were left for 3 to 12 hours (Trotz-Williams et al., 2008). Beam et al. (2009) reported that 25% of farms in the US allow calves to obtain colostrum via suckling and 75% of calves are hand-fed with 89% of those calves being bottle-fed and the remaining fed via esophageal tube. American data indicate that 61.4% of calves that received colostrum via suckling developed FPT compared to 19.3% that were bottle-fed and 10.8% that were tube-fed (Beam et al., 2009). Bottle-feeding is considered to increase passive transfer due to closure of the esophageal groove, compared to tube-feeding which does not allow this closure (Godden et al., 2009a). Esophageal groove closure allows the colostrum to bypass the reticulum and rumen and deposit directly into the omasum and abomasum. This direct route shortens the time that the colostrum takes to reach the small intestine where absorption occurs. As well, the abomasum allows the colostrum to clot, and clotting is necessary as it allows whey proteins such as IgG to travel to the small intestine (Longenbach and Heinrichs, 1998). Calves may not voluntarily consume more than 2.5 L of colostrum at a single meal, necessitating the need to tube-feed to ensure adequate volumes of colostrum are ingested. A study by Kaske et al. (2005) showed that calves fed 4 L via esophageal tube had higher STP concentrations compared to bottle-fed calves (25.2 mg/mL and 14.1 mg/mL respectively). However, the calves in this study were not fed the same volume; tube-fed calves received 4 L and bottle-fed calves received 2 L. The authors also reported that the rate of increase in STP in bottle-fed calves was greater than that in tube-fed

calves and suggested this was related to esophageal closure in the bottle-fed calves. In tube-fed calves, there is a 2 to 3 hour delay of colostrum movement from the reticulum to small intestine compared to movement of colostrum in bottle fed calves. Godden et al. (2009a) demonstrated that calves fed the same volume of colostrum had higher STP levels when fed by bottle than by tube.

1.4.5 Source and Storage of Colostrum

Volume of colostrum produced is one factor that affects the concentration of IgG. Morin et al. (2010) reported that for every litre of colostrum produced, IgG concentration decreases by 3.7%, suggesting that colostrum should be harvested as soon as possible after calving. The transport of IgG into the mammary glands stops abruptly at calving although milk production continues. This results in a dilution of IgG concentration with increased time to colostrum harvesting.

A study by Holloway et al. (2001) indicated that both fresh and frozen colostrum can provide adequate amounts of IgG. In that study, 26 calves were fed either fresh or frozen-thawed colostrum from the same pool, and serum IgG was determined at 48 hours. There was no significant difference in either serum or colostral IgG between the treatment groups. In another study, no difference was found in IgG content between fresh, frozen, or refrigerated colostrum, however refrigerated colostrum had significantly higher total coliform plate count (TPC) compared to fresh or frozen samples (Morrill et al., 2012). Colostrum containing > 50 mg/mL IgG, and containing < 100,000 cfu/mL of bacteria is considered good quality colostrum and should be fed to calves (Morrill et al., 2012). Bacterial contamination of colostrum is a concern because free IgG in the colostrum could bind to bacteria in the gut thus preventing absorption, or

bacteria could block absorption by binding directly to enterocytes (Godden, 2008). Bacterial contamination can be avoided through hygienic preparation of the dam's teats and maintaining clean and well-functioning milking and storage equipment (McGuirk and Collins, 2004). Colostrum stored in a refrigerator had higher coliform counts compared to fresh or frozen colostrum, however refrigerated colostrum had the lowest somatic cell count (SCC) compared to fresh or frozen colostrum (Morrill et al, 2012). Freezing high quality colostrum is an appropriate storage method (Klobasa et al., 1998; Quigley et al., 2005).

Pooling colostrum from several cows is a practice used on some dairy farms. A review by Weaver et al. (2000) indicates that pooling colostrum increases the volume, but decreases the concentration of IgG thereby decreasing the overall quality. Pooling can also increase disease exposure as calves are receiving colostrum from many different cows (McGuirk and Collins 2004). Beam et al. (2009) indicated that approximately 20% of dairy farms in the USA use pooled colostrum. They found that farms that pooled colostrum were 2.2 times more likely to have calves with FPT compared with those that did not pool. Samples of colostrum from individual dams had significantly greater IgG concentrations and lower SCC and TPC compared to pooled samples in a survey of American farms (Morrill et al., 2012).

1.4.5.1 Pasteurization

One management practice shown to reduce bacterial contamination of colostrum is pasteurization; however, there is evidence to suggest that certain methods of pasteurization can destroy Ig. Godden et al. (2003) looked at the effects of batch pasteurization at 63°C and found that colostrum pasteurized in large batches (95 L) had to be heated for 2.5 to 3 hours in order to reach a temperature of 63°C, and had a 58.5% reduction in IgG. Colostrum pasteurized at 63°C

for 1 hour in small batches (57 L) had a 23.6% reduction in IgG. Pasteurizing colostrum for 60 minutes at 60°C was shown to be very effective at reducing total bacterial count while preserving IgG levels (Godden et al., 2006). This indicates that it is possible to effectively pasteurize colostrum, but care must be taken to ensure the appropriate method is used to preserve IgG while reducing the pathogen load. Another study showed calves fed pasteurized colostrum had significantly higher serum IgG than calves fed non-pasteurized colostrum with timing, method, and volume of colostrum the same for both groups (Johnson et al., 2007). Other studies found that heating at 60°C for 30 minutes was sufficient to reduce bacterial counts, and that calves fed heat-treated pooled colostrum had significantly higher serum IgG at 24 hours compared to calves fed unpasteurized colostrum from the same pool (Godden et al., 2006; Elizondo-Salazar and Heinrichs 2009). Contrary to these results, Beam et al. (2009) did not find an association between pasteurization (unspecified temperature or duration) and prevalence of FPT.

1.4.5.2 Colostrum Replacer and Supplements

Due to variation in colostrum management, non-compliance of determining colostrum quality, shortages of maternal colostrum (MC), and concerns about controlling infectious pathogens, colostrum replacers (CR) and colostrum supplements (CS) have been developed to help support calf immunity. Depending on the source of IgG (colostrum, milk, eggs, or bovine blood), the product can be very expensive, or prohibited where laws control the use of blood-derived IgG products. Colostrum replacers are designed to provide ≥ 100 g of IgG per dose, while CS are designed to provide < 100 g of IgG per dose (Quigley et al., 2002). Colostrum replacer is designed to take the place of a colostrum meal, whereas a supplement is designed to boost poor quality colostrum used in initial feedings. There are mixed results in terms of the

efficacy of these products (Godden et al., 2009b). A recent study by Poulsen et al. (2010) indicated that there was no significant difference in the risk of FPT when calves were fed 1 dose of replacer containing 125 g IgG within 2 hours of birth followed by another dose of supplement containing 45 g IgG within 12 hours of birth, compared to calves fed MC. Though the average STP and serum IgG of both groups were above the cut-off for FPT, calves fed MC had significantly higher STP and serum IgG (5.59 g/dL and 18.68 mg/mL) compared to the other group (5.27 g/dL and 13.48 mg/mL). Godden et al. (2009b) determined serum IgG, STP, and risk of FPT (serum IgG < 10 mg/mL) of calves fed CR and MC. They found that 46% of calves ingesting the manufacturer recommended dosage of CR (1 dose=100 g IgG) had FPT compared to 9% of calves fed MC or 0% of calves fed two doses of CR. Calves ingesting either MC or 2 doses of CR had significantly higher STP and serum IgG levels at 24 hours compared to calves receiving 1 dose of CR. Similarly, in a separate study, calves fed MC had significantly higher serum IgG (17.6 mg/mL) and STP (5.4 g/dL) compared to calves fed 100 g of IgG in CR (7.5 mg/mL and 4.4 g/dL) or calves fed 150 g IgG in CR (9.1 mg/mL and 4.7 g/dL). Only 5% of calves fed MC had FPT compared to 95% of calves fed 100 g IgG from CR and 76% of calves fed 150 g IgG from CR (Smith and Foster, 2007). Calves fed an additional 185 g of IgG via CS mixed into 2L of colostrum, fed at 0 and 12 hours, had similar STP levels compared to calves fed colostrum by itself (Morin et al., 1997). There are numerous other studies providing similar results, whereby calves have higher serum IgG and STP levels when fed MC rather than CR (Swan et al., 2007; Priestly et al., 2013). This indicates that feeding MC over CR can reduce the risk of FPT and that producers should have knowledge about the proper use of replacers and supplements

There are several possible explanations for the inadequacy of replacer and supplement products. One is that these products do not contain bioactive components that are present in MC, such as colostral leukocytes, growth factors, and cytokines that may provide unknown benefit to the calf or assist in passive transfer of immunity (Godden, 2008). Other explanations are that the quality control or manufacturing protocols used to create these products are not adequate, or that the generally accepted assumption that consuming 100 g of IgG is sufficient to prevent FPT is incorrect and a higher mass of IgG needs to be consumed (Chigerwe et al., 2008a; Godden et al., 2009b). It has also been suggested that casein in colostrum supplements is the cause of reduced apparent efficiency of absorption (AEA) of IgG in calves when it is added to MC (Smith and Foster, 2007).

1.4.6 Factors Unrelated to Colostrum that Affect Failure of Passive Transfer of Immunity

Proper management of dairy calves can increase the productivity, health, and welfare of the entire dairy herd. Poor management often leads to increased morbidity and mortality, decreased lifetime productivity, and economic losses due to increased veterinary care and poor growth and reproductive performance (Quigley et al., 2005). In the USA the cost of reduced productivity and increased veterinary care is estimated to be ≥ $200 million per year (Quigley et al., 2005).

Donovan et al. (1986) reported that dystotic calves have a significantly lower STP compared to calves with an easy birth. Similarly, another study found the main causes of FPT were low volume of colostrum intake and poor vigor associated with dystocia (Furman-Fratczak et al., 2011). This same study described that calves from primiparous cows were more at risk of FPT than those from multiparous cows, with all management practices being equal.

There is a gap in the current literature regarding associations between calf management practices unrelated to colostrum in the first days of life with measures of FPT. Much of the current literature describes management practices used throughout the preweaning period, whereas the current study is interested in how management practices in the first days of life affect calf health.

1.5 Factors Affecting Preweaned Growth

There is conflicting data regarding the relationship between FPT and calf growth rate. One study found that serum IgG at < 60 hours was not significantly associated with growth rate up to 6 months (Furman-Fratczak et al., 2011), however calves with average serum IgG >15 mg/mL had the highest growth rates from 12 to 14 months of age. In calves with FPT (defined as IgG ≤ 8 mg/mL at 48 hours) average daily gain (ADG) was reduced by 48 g in the first month of life, and BW at three months of age was reduced by 2.1 kg compared to calves without FPT (Virtala et al., 1996). A meta-analysis by Bateman et al. (2012) indicated that STP was not an important predictor of preweaned growth. Calf information in that analysis was taken from 20 studies and combined into 1 dataset. The authors found calves with higher BW at birth had the lowest ADG, possibly due to having greater maintenance requirements than smaller calves, which were not met due to a constant volume of MR fed to all calves regardless of size. They also found that the greatest predictors of preweaned growth were MR and starter intake. As well, calves fed 4 L at the first feeding had on average 0.23 kg higher ADG in the first 4 months of life compared to calves fed 2 L (Faber et al., 2005).

1.6 Factors Affecting Preweaned Mortality and Morbidity

Calves left with the dam for more than 24 hours after birth were 3.2 times more likely to die compared to calves removed prior to 24 hours (Wells et al., 1996). Another study reported that calves that were immediately separated from the dam experienced diarrhea for 3 times as many days compared to calves left with the dam for 5 days post calving (Rajala and Castrén, 1995). Wells et al. (1996) reported that calvings involving mechanical force resulted in calves 4.2 times more likely to die compared to unassisted calvings. Another study reported calves that were assisted were more likely to be stillborn or die within the first 30 days of life (Lombard et al., 2007). Waltner-Toews et al. (1986) reported a lower rate of mortality for farms where the farm owner cares for the calves, compared to farms where personnel other than the owner are in charge of calf management.

The timing, volume, and method of administration of the first colostrum feeding are significantly associated with death before 21 days of age. Calves fed no colostrum in the first 6 hours of life were 74 times more likely to die by 21 days of age compared to calves fed at least 1.9 L in the first 6 hours (Wells et al., 1996). The odds of mortality for calves left to nurse for less than 6 hours was higher than for any other time frame, and the authors stated that 31% of calf mortality could be prevented by bucket or bottle feeding > 1.9 L of colostrum in the first 6 hours of life (Wells et al., 1996). Calves fed similar volumes of colostrum by esophageal feeder had higher odds of death compared to bucket-fed or nursed calves (Wells et al., 1996).

Calves born in individual calving pens compared to group pens are at a lower risk of diarrhea, respiratory issues, and bacterial infections (Vasseur et al., 2010). Similarly, calves born in single cow calving pens rather than open housing or stanchions had a lower incidence of diarrhea and respiratory diseases (Pithua et al., 2009). Those authors suggested that the removal

of soiled bedding between calvings in the individual calving pens may have been the reason for the decrease in incidence of morbidity and mortality when compared to the multiple cow calving pens. As the calf gut is immature, it will absorb macromolecules non-specifically, including any environmental pathogens to which the calf may be exposed; therefore it is critical to maintain a sanitary environment during the first few days of life (Quigley et al., 2005). All in-all out management is the best recommended practice for group pens to reduce pathogen presence (Margerison and Downey, 2005). Calves born in the summer season that are housed in individual pens are 3.9 times less likely to die within the first 28 days of life compared to group-housed calves (Waltner-Toews et al., 1986).

1.7 Research Question

Many of the calf and colostrum management practices discussed above are known to have a direct association with FPT in dairy calves. It is useful for producers to be aware of the practices that are associated with increased passive transfer of IgG, and that encourage optimal growth and performance of replacement heifers. It is currently unknown which practices are being implemented on dairy farms in Alberta, and which practices being used may be associated with FPT. This study aims to identify current calf and colostrum management practices in Alberta, and to determine which practices are associated with FPT and preweaned growth rate.

1.8 Objectives

This study aims to determine:

1) The type of calf and colostrum management practices used on Alberta dairy farms;

2) The quality of colostrum being used on Alberta dairy farms;

3) The relationship between calf management practices and colostrum quality with calf growth rate, serum IgG, and STP concentrations; and
4) The relationship between Brix refractometer measurements, colostrometer measurements, and RID-measured IgG concentrations in colostrum.

1.9 Hypotheses

The hypotheses for this study are:

1) Both the Brix refractometer and the colostrometer will be adequate tools to use on-farm,
2) Prevalence of FPT in Alberta dairy calves will be similar to other Canadian data;
3) Management practices concerning colostrum consumption will have a significant effect on risk of FPT, morbidity, mortality, and growth.

Chapter Two: The Effectiveness of On-Farm Tools for Measuring Colostrum Quality

2.1 Introduction

Dairy calves are born without any acquired immunity as there is no transfer of Ig across the placenta from the dam to the fetus. This means that newborn calves must acquire immunity passively through the consumption of colostral IgG (Baumrucker et al., 2010). Prevention of FPT is achieved by feeding adequate quantities of good quality colostrum as soon as possible after birth. The current recommendation is that good quality colostrum should contain a minimum of 50 mg IgG/mL as measured by RID (McGuirk and Collins, 2004; Godden, 2008; Beam et al., 2009). Immunoglobulin G concentrations in colostrum vary considerably; in a recent US study, the reported range was 7.1 to 159 mg/mL (Quigley et al., 2013). Due to this variability, dairy producers should measure colostral IgG prior to feeding it to calves. Colostral IgG measuring tools must be reliable, accurate, and easy to use. Traditionally, a colostrometer has been recommended to determine quality at the farm level (Fleenor and Stott, 1980). The specific gravity of colostrum, as measured by the colostrometer, has been shown to have a high correlation with IgG levels determined by RID (Fleenor and Stott, 1980). Although the colostrometer has been shown to be a relatively good predictor of colostrum quality, it is made of glass and is therefore very fragile. It also needs to be thoroughly cleaned prior to each use. Additionally, the colostrum must be at room temperature to obtain a reliable reading and 250 ml of colostrum is needed. Recently, Bielmann et al. (2010) and Quigley et al. (2013) indicated that the Brix refractometer may be a reliable tool for determining colostrum quality. The Brix refractometer measures the total solids content of milk (Moore et al., 2009) and has successfully been used to determine quality of equine (Cash, 1999) and bovine colostrum (Bielmann et al., 2010). Various studies suggest that a range of 18% to 23% Brix is an appropriate cut-point for

good quality colostrum (Chigerwe et al., 2008a; Bielmann et al., 2010; Morrill et al., 2012b). A study comparing RID-measured IgG against the Brix refractometer and colostrometer was performed by Chigerwe et al. (2008a), however, no such study has been performed analysing the quality of colostrum on Alberta dairies, or the effectiveness of these tools in the Alberta dairy population. The objectives of this study were to determine the IgG content of colostrum used on Alberta dairy farms, and to determine whether the colostrometer or Brix refractometer is better able to accurately determine colostrum quality compared to RID.

2.2 Materials and Methods

2.2.1 Colostrum Sample Collection and Analysis

Fourteen farms from central Alberta participated in the study between February and June of 2012. These farms were representative of most herds in Alberta in terms of breed (Holstein), housing type (free stall), and herd size (60 to 300 lactating cows). The producers collected 250 ml samples of MC and/or CR representing what the calf was given at its initial feeding. Samples were labeled with the cow identification number and date of collection. Samples were frozen at the farm at -20^{o}C until they were transported to the University of Calgary, where they were stored at -80°C until analysis. One farm did not submit any MC or CR samples so results are shown for the remaining 13 farms.

The MC and CR samples were thawed at 4°C and then warmed to room temperature. When the samples were between 20 and 22°C, they were analysed for specific gravity using a colostrometer (JorVet Bovine Colostrometer CO, USA) and total solids with a digital Brix refractometer (PAL-1, Atago Co. Ltd. WA, USA). The procedures used were as described by Fleenor and Stott (1980) for the colostrometer and Bielmann et al. (2010) for the refractometer.

For the RID analysis, the colostrum was warmed to 42°C to ensure homogeneity of fat and other particles, and an aliquot was frozen at -80°C. The quantitative RID analysis was performed at Prairie Diagnostic Services (University of Saskatchewan, Saskatoon, SK) using rabbit anti-bovine IgG (heavy and light chains) as previously described (Chelack et al., 1993). The IgG values reported refer to the sum of all IgG isotypes.

2.2.2 Statistical Analysis

In total, 569 colostrum and 3 CR samples were collected. For the colostrometer analysis, 53 samples were outside of the measurement range of 0 to 140 mg/mL, thus 519 samples were used in the statistical analysis. The Brix refractometer had a range of 0-53% Brix and all samples were within this range. The lower detection limit for RID analysis was 0.7 mg/mL. The samples that were analyzed were all above this lower limit, however, not all samples collected were analyzed. This study was part of a larger study in which serum IgG levels in newborn calves were measured, and so only colostrum samples that were paired with a calf serum sample were analyzed; in total, 462 samples were analyzed for IgG by RID.

Assumptions of normality were tested using PROC UNIVARIATE of SAS (version 9.3; SAS Institute Inc., Cary, NC) and the Kolmogorov-Smirnoff, Anderson-Darling, Shapiro-Wilk and Cramervon Mises tests. Correlation analysis to compare RID and Brix refractometer data, and RID and colostrometer data was conducted using PROC CORR. The colostrometer data were not normally distributed, therefore, the Spearman Correlation coefficient was used to compare colostrometer and RID data. The Pearson correlation coefficient was used to compare the Brix refractometer and RID data. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated to determine the appropriate cut-point for

good quality colostrum (≥ 50 mg/mL of IgG) for the Brix refractometer and colostrometer. These diagnostic test characteristics of the colostrometer and Brix refractometer were measured against RID using 50 mg/mL on the RID as the cut-point for good quality colostrum. A test positive sample contained < 50 mg/mL and a test negative sample contained ≥ 50 mg/mL. Five cut-points at intervals of 10 mg/ml from 50 to 100 mg/mL were considered for the colostrometer. For the Brix refractometer, 8 different cut-points at 2% intervals from 18 to 26% Brix were considered. The association between colostral IgG as measured directly by RID and indirectly by the colostrometer and Brix refractometer was analyzed using the MIXED procedure of SAS with a statistical model that included parity and month of colostrum production as fixed effects and farm as the random effect. Regression analysis comparing Brix and colostrum data to RID-measured IgG was performed using PROC REG.

2.3 Results

Descriptive analyses of the colostrum samples analyzed by each of the three methods are shown in Table 2-1. For the colostrometer, 73 out of 519 (14.1%) were samples below 50 mg/mL (Figure 2-1). The RID-measured IgG data had 136 out of 462 samples (29.4%) below 50 mg/mL (Figure 2-1). The normally distributed Brix refractometer data ranged from 6.8 to 52.6% Brix with a mean of 24.3% (Figure 2-2).

Table 2-1. Descriptive statistics for maternal colostrum and colostrum replacer samples collected from 13 farms in central Alberta and analysed by radial immunodiffusion, a Brix refractometer, and a colostrometer.

	n	Mean	SEM	Minimum	Maximum
Brix, %	572	24.3	0.18	6.8	52.6
IgG by colostrometer, mg/mL	519	82.3	1.23	0.0	140.0
IgG by RID, mg/mL	462	63.7	1.11	8.3	128.6

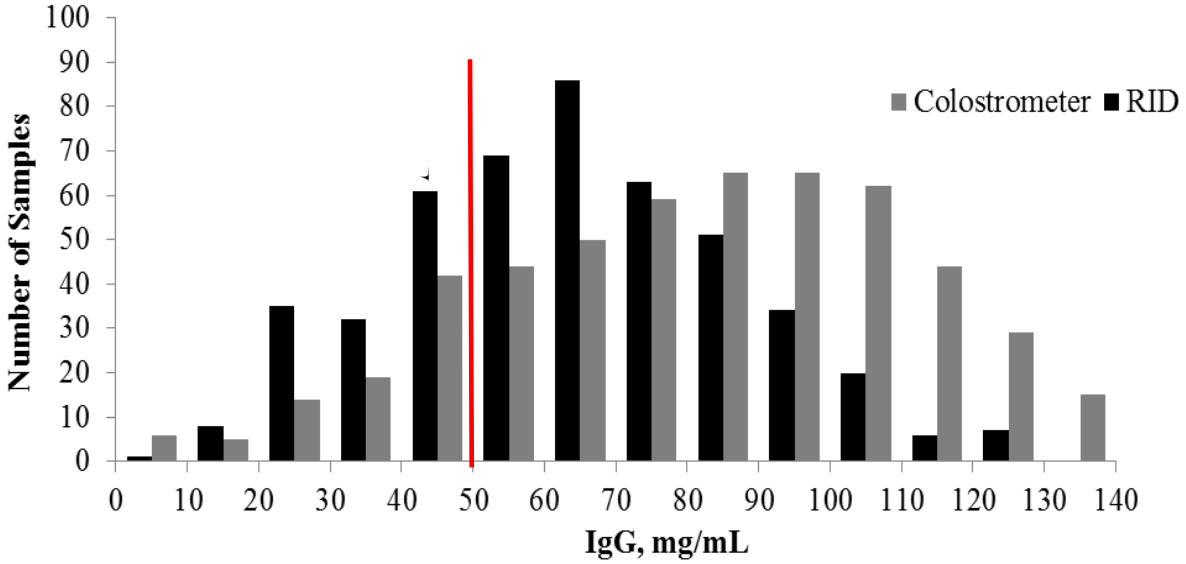

Figure 2-1. Distribution of radial immunodiffusion and colostrometer determined IgG concentrations of maternal colostrum and colostrum replacer samples (n = 519 colostrometer analysis, n = 462 RID analysis). Samples were collected from 13 farms around central Alberta. The line indicates the cut-point between good and poor quality colostrum.

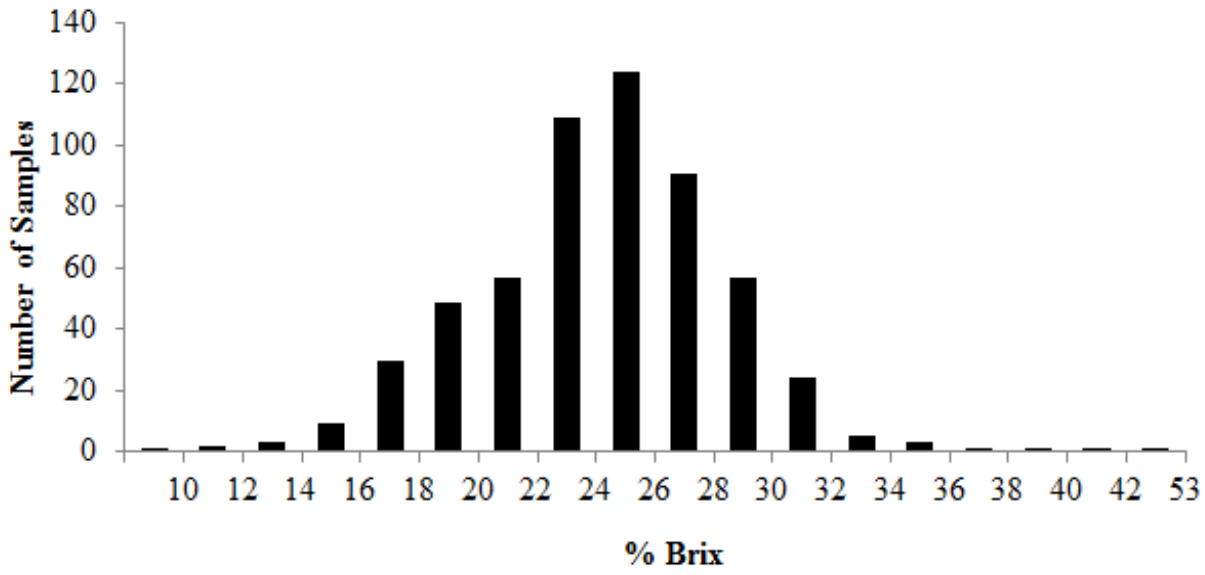

Figure 2-2. Distribution of colostrum samples analysed with a digital Brix refractometer (n = 572). Samples were collected from 13 farms around central Alberta.

Overall, there was not a significant effect of month on colostral IgG ($p = 0.2$). There was a significant effect of parity on colostral IgG ($p < 0.02$). Cows in their 3rd or higher lactation had significantly higher colostral IgG (69.5 ± 1.98 mg/mL, $p < 0.01$) compared to 2nd parity (59.80 ± 2.06 mg/mL) and 1st parity dams (62.2 ± 1.73 mg/mL).

Table 2-2. Sensitivity, specificity, positive and negative predictive values for different measurement cut-points on the Brix refractometer compared to 50 mg/mL immunoglobulin G as measured by radial immunodiffusion. Data presented as means with 95% confidence intervals in the parenthesis.

Cut-point (% Brix)	Sensitivity (%)	Specificity (%)	Se + Sp[†]	PPV (%)	NPV (%)
18	22.1(15.2-29.7)	98.2(96.0-99.3)	1.20	83.3 (67.2-93.6)	75.3 (70.3-78.8)
19	29.4 (21.6-37.3)	98.2 (96.0-99.3)	1.28	87.0 (73.7-95.1)	77.1 (72.2-80.5)
20	41.9 (33.0-50.0)	97.0 (95.1-98.8)	1.39	85.1 (76.5-93.6)	80.2 (75.2-83.55)
21	48.5 (39.3-56.5)	95.1 (92.2-97.2)	1.44	80.5 (70.3-88.4)	81.7 (76.9-85.0)
22	55.2 (45.7-62.9)	91.5 (88.2-94.5)	1.47	72.8 (63.9-81.8)	83.2 (78.3-86.4)
23	66.9 (57.4-73.8)	82.4 (78.0-86.5)	1.49	61.1 (53.1-69.4)	85.8 (80.7-88.9)
24	75.7 (66.5-81.7)	72.0 (67.2-77.2)	1.48	52.8 (46.1-60.6)	87.8 (82.5-90.8)
25	83.1 (74.4-87.9)	60.5 (54.3-66.7)	1.44	46.5 (40.3-53.2)	89.6 (83.8-92.6)

PPV= positive predictive value; NPV= negative predictive value

[†]Sensitivity and specificity percentage was converted into a unit value

Table 2-3. Sensitivity, specificity, positive and negative predictive values for different measurement cut-points on the colostrometer compared to 50 mg/mL immunoglobulin G as measured by radial imunodiffusion. Data presented as means with 95% confidence intervals in the parenthesis.

Cut-point (mg/mL)	Sensitivity%	Specificity %	Se + Sp[†]	PPV%	NPV%
50	41.3 (32.2-50.0)	98.3 (96.6-99.6)	1.40	91.2 (82.7-98.0)	80.0 (75.1-83.6)
60	61.1 (51.6-69.2)	94.0 (91.0-96.6)	1.55	81.1 (72.6-89.1)	85.2 (80.5-88.5)
70	70.6 (61.3-77.9)	88.0 (84.0-91.7)	1.59	71.2 (63.0-79.5)	87.7 (83.0-90.9)
80	84.1 (76.7-90.1)	77.0 (69.6-84.4)	1.61	60.6 (53.2-68.1)	92.0 (87.8-95.0)
90	92.8 (87.0-98.7)	62.0 (53.2-68.1)	1.55	50.7 (44.7-58.0)	95.4 (91.4-97.9)
100	96.0 (91.1-98.7)	49.3 (44.7-56.3)	1.45	44.3 (38.8-51.0)	96.7 (92.5-98.9)

PPV= positive predictive value; NPV= negative predictive value

[†]Sensitivity and specificity percentage was converted into a unit value

The highest combined sensitivity and specificity occurred at 23% Brix (Table 2-2), and 80 mg/mL for the colostrometer (Table 2-3). Radial immnodiffusion-measured IgG concentrations had a Pearson correlation coefficient of 0.62 compared to Brix refractometer data and a Spearman correlation coefficient of 0.77 compared to colostrometer data. Regression correlations for Brix refractometer versus RID-measured IgG, and colostrometer versus RID-measured IgG are shown in Figures 2-3 and 2-4. The colostrometer had a higher r^2 (0.60) than the Brix refractometer (r^2=0.43) compared to RID-measured IgG. Based on the regression equation 50 mg/mL IgG (RID) is equivalent to 19.7% Brix and 60.7 mg/mL on the colostrometer.

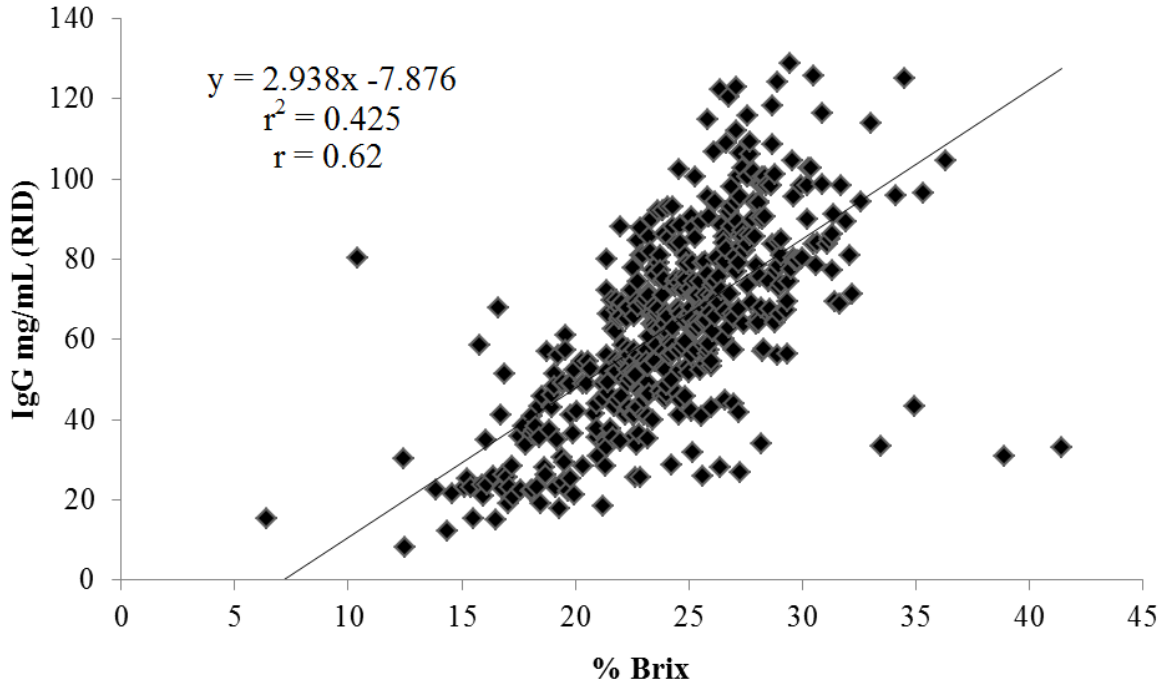

Figure 2-3. Scatter plot of percent Brix and radial immunodiffusion-measured immunoglobulin G values (n=462)

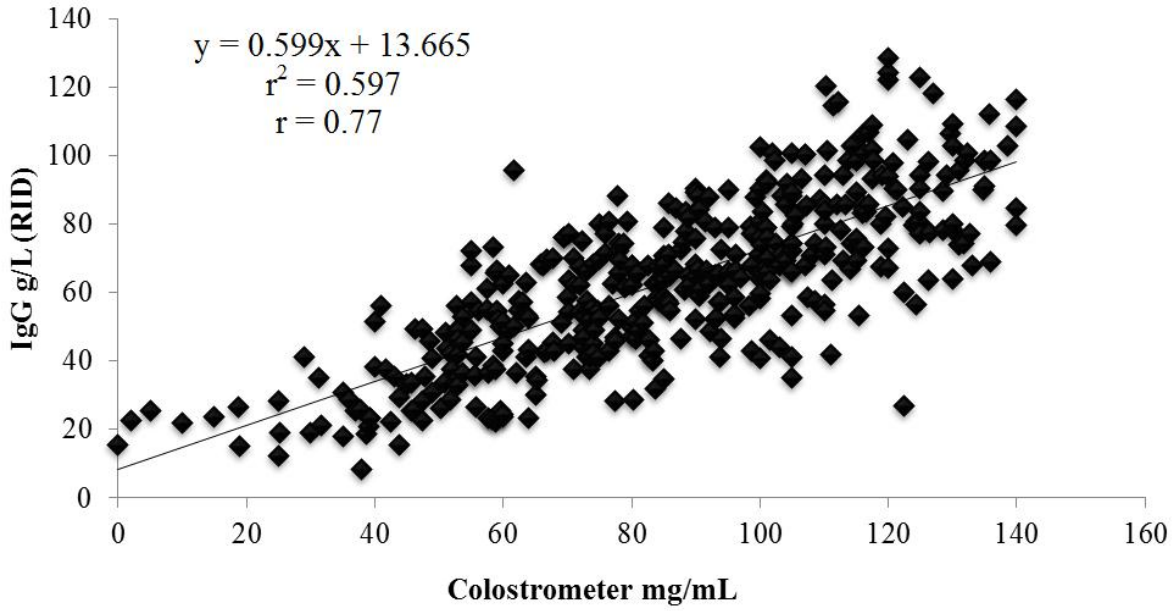

Figure 2-4. Scatter plot of the colostrometer and radial immunodiffusion-determined immunoglobulin G levels (n=462)

2.4 Discussion

The range in RID-measured colostrum IgG concentrations in this study was smaller than that reported in previous studies. Bielmannn et al. (2010) reported values from 22.4 to 196.9 mg/mL with a mean of 94.4 mg/mL for 273 samples taken from three herds. Similarly, Morrill et al. (2012a) in a nationwide American survey, reported colostrum IgG values ranging from 1.8 to 200.2 mg/mL with a mean of 68.8 mg/mL IgG. Quigley et al. (2013) reported a range of 7.1 to 159.0 mg/mL with a mean of 73.4 mg/mL. The studies also varied in the percentage of samples that were below 50 mg/mL from 7.7% (Bielmann et al., 2010) to 32.0% (Chigerwe et al., 2008a). In the current study, the mean % Brix was similar to previously reported means of 26.1% Brix (Bielmannn et al., 2010) and 23.8% (Quigley et al., 2013). The colostrometer values are similar

to data from Pritchett et al. (1994) who reported a mean of 82.1 mg/mL IgG with 13.2% of the samples measuring > 50 mg/mL.

There are many possible explanations for the difference between the distribution of IgG concentration reported in the present study and previous studies. Thirteen different commercial farms were involved in this study while 60 farms were used in the Morrill et al. (2012a) study, which likely represented a greater range of environmental conditions, management, and nutritional practices that are known to affect colostrum quality. The reported range in our study may be smaller as the herd sizes in our samples ranged from 60 to 300 milking cows; whereas the Morrill study had herd sizes ranging from 70 to 5000 milking cows.

Variation in time to colostrum collection may have affected the colostral IgG levels among samples. Previous studies show that colostrum collected more than 2 hours after parturition is significantly lower in IgG than colostrum collected within a shorter time frame (Hostetler et al., 2003; Moore et al., 2005; Chigerwe et al., 2008a). There is also evidence to suggest that bacteria in colostrum can bind to IgG (Godden, 2008). If colostrum was stored at room temperature for a length of time prior to freezing, this would allow bacteria to grow and potentially bind with free IgG. This could affect the IgG levels if both antigen binding sites were bound to bacteria or other pathogens, or if the conformation of IgG changed after being bound by bacteria such that anti-IgG could not bind to IgG during the RID assays.

Colostrum samples in the current study were collected from February until July, whereas other studies had a longer collection period (unspecified months from 2004 to 2006, Chigerwe et al., 2008a; March 2008 to January 2009, Bielmann et al., 2010), or a warmer collection season (June to October 2010, Morrill et al., 2012b; June to August 2001, Quigley et al., 2013). The

highest levels of colostral IgG are reported to occur in the summer months (Gulliksen et al., 2008).

The findings from the sensitivity and specificity analysis suggest similar cut-points for the colostrometer and Brix refractometer compared to RID-measured IgG values to previously reported data. Some studies suggest that for the Brix 20 to 23% (Chigerwe et al., 2008a), 21% (Quigley et al., 2013) and 22% (Bielmannn et al., 2010) are appropriate cut-points for good quality colostrum. The present study suggests that 23% is appropriate. It is in the best interest of the calf to use a high cut-point in order to ensure high quality colostrum is correctly identified as such; therefore, 23% or higher should be used as the most appropriate cut-point.

For the colostrometer, the highest proportion of correctly identified good and poor quality samples occurred at the 80 mg/mL cut-point (Table 2-3), which is similar to previous data. Chigerwe et al. (2008a) suggests that a cut-point of 70 mg/mL offers high sensitivity and specificity (0.75 and 0.78, respectively) and recommends using a range of 60 to 90 mg/mL on the colostrometer to reduce misclassification of false negatives.

Positive predictive value indicates the probability that a sample is poor quality, given the prevalence of poor quality colostrum in the sample population. In this instance, if a sample tests lower than 80 mg/mL on the colostrometer there is a 60.6% chance that it is truly is poor quality (< 50 mg/mL IgG). The highest PPV occurred at the 50 mg/mL cut-point, indicating there is a 91.2% chance that a sample testing at 50 mg/mL or lower on the colostrometer truly has < 50 mg/mL IgG. Negative predictive values indicate the probability that a sample is good quality (≥ 50 mg/ml IgG) given the prevalence of good quality colostrum in the sample population. Using a cut-point of 80 mg/mL on the colostrometer, there is a 92.0% chance that it truly does contain ≥ 50 mg/ml IgG. Higher cut-points give a higher NPV. With regard to the Brix refractometer, the

PPV at the 23% cut-point indicates that 61.1% of samples contain < 50 mg/mL IgG. The highest PPV occurred at 19%. For the NPV, 23% indicates that there is a 85.8% chance the samples contains ≥ 50 mg/mL IgG. The NPV for the Brix refractometer increased with increasing cut-points as well. Using this information, producers are able to decide which cut-points to use depending on if they wish to reduce the number of either false positive or false negative misclassifications.

The colostrometer had a greater correlation than the Brix refractometer with RID-measured IgG concentrations. Other published studies also report higher correlation values for the colostrometer (Fleenor and Stott, 1980; Pritchett et al., 1994). This could be due to reasons described above, that non-IgG proteins affect the specific gravity of the colostrum and there may be differences in colostral composition between farms due to different nutritional and management practices of the cows.

The Pearson correlation coefficient between the RID data and Brix refractometer of 0.62 is very similar to the 0.64 reported by Chigerwe et al. (2008a). Other studies have reported higher correlation values between the Brix refractometer and IgG levels; 0.71 (Bielmannn et al., 2010), 0.73 (Morrill et al., 2012b), and 0.75 (Quigley et al., 2013). Kehoe et al. (2007) describes how several cow and farm factors (number of cows with high SCC, if vitamin injections are given, farm size) are associated with the level of nutrients in colostrum. As the Brix refractometer measures total dissolved solids, such as colostral nutrients (protein, fat, and vitamins) and not IgG specifically, the colostrum samples in this study may have different levels of dissolved solids other than IgG compared to the samples in the other studies, due to possible differences in cow management.

Several previous studies suggest between 60 and 80 mg/mL on the colostrometer should be used as the cut-point for good quality colostrum (Mechor et al., 1991; Pritchett et al., 1994; Chigerwe et al. 2008a). If a colostrometer is to be used on the farm the data from this study suggest that a cut-point of 80 mg/mL should be used.

The r^2 for Brix versus RID-measured IgG is lower compared to a previous report (0.81, Quigley et al., 2013) although Chigerwe et al. (2008a) reported an r^2 of 0.41 and Bielmann et al. (2010) reported 0.53 when comparing the same model of Brix refractometer with RID-measured IgG values. The r^2 in those studies did not account for the random effect of farm identification, which may explain why it is so much lower than the r^2 reported by Quigley et al. (2013) who did account for farm effects, and why it was similar to the r^2 in the current study.

Chigerwe et al. (2008a) reported an r^2 of 0.41 for colostrometer versus RID-measured IgG values, and Pritchett et al. (1994) reported an r^2 of 0.47. The difference in colostrometer versus IgG found in this and previous studies may be explained in several ways. There may have been variation in colostrum collection and handling procedures between the current and previous studies. For example, if colostrum samples were not refrigerated or frozen immediately there could be bacterial contamination or changes in colostrum pH that could influence protein levels and specific gravity. Previous studies show that specific gravity is better correlated with total colostral protein than IgG itself (Morin et al., 2001). There may be variable levels of non-IgG protein in the colostrum samples used in this study compared to other studies, though parameters other than IgG were not measured in the current study.

Though these results indicate the colostrometer has a higher correlation with actual IgG levels, the colostrometer is not used frequently on farms. Vasseur et al. (2010) stated that even though producers are aware of the benefits of using a colostrometer, utilization remains low,

possibly due to its extreme fragility and lack of ease of use. The digital Brix refractometer provides a user-friendly alternative to the colostrometer.

This study also confirmed others' findings that 3rd or higher parity cows had significantly higher IgG levels compared to 1st and 2nd parity cows, and that cows in the second parturition had significantly lower colostral IgG levels (Gulliksen et al., 2008).

2.5 Conclusion

As the amount of IgG that a calf consumes is one of the most important factors influencing passive transfer of immunity, it is essential to have a tool available for farmers to measure colostrum quality prior to feeding. The Brix refractometer is a suitable tool as it is user-friendly, its accuracy is independent of colostrum temperature, and it has a specificity of over 80%. The recommended cut-point for determining good quality colostrum is 23%. The colostrometer is better correlated with RID results but it overestimates colostrum quality; a cut-point of 80 mg/mL is recommended when using this tool.

Chapter Three: Associations Between Dairy Calf Management Practices and Calf Immune Status, Growth, and Risk of Morbidity and Mortality

3.1 Introduction

Newborn dairy calves must acquire their immunity passively through the consumption of colostrum. Calves are at high risk of FPT if consumption of colostral IgG within hours of birth is inadequate. This condition is associated with 39 to 50% of calf mortality in American dairy herds (Margerison and Downey, 2005), as well as an increased risk of morbidity, decreased growth rate, and decreased lifetime milk production (Chigerwe et al., 2009). Failure of passive transfer of immunity is a widespread problem in farms across the US and Canada; 19% of calves on US farms had FPT and at least one calf on 41% of the farms had FPT (Beam et al., 2009). In Ontario, the estimated prevalence across 112 farms was 37% (Trotz-Williams et al., 2008). Having 10% or fewer calves on a farm with FPT is a suggested achievable goal for dairy producers (Chigerwe et al., 2009). The prevalence of FPT for Alberta dairy calves is currently unknown.

The two methods by which FPT is commonly assessed are measurement of STP and measurement of serum IgG. Serum total protein levels < 5.2 g/dL (Beam et al., 2009), or serum IgG levels < 10 mg/mL (Godden et al., 2009) are commonly accepted cut-points for FPT.

Certain practices used to manage neonatal calves are known to increase the risk of FPT. Calves left with the dam for more than 3 hours have twice the risk of FPT compared to those removed within 3 hours (Trotz-Williams et al., 2008). The timing, volume, and quality of the first colostrum meal are considered the most important factors associated with risk of FPT (Godden, 2008). The objectives of this study were to: 1) determine the prevalence of FPT on Alberta dairies; 2) determine which management practices are used on the farms; and, 3)

determine which management practices are risk factors for FPT, morbidity, mortality, and low growth rate. This is the first study examining calf-level practices and the prevalence of FPT on Alberta dairy farms.

3.2 Materials and Methods

3.2.1 On-Farm Activities

Fourteen farms in central Alberta were recruited, with herd sizes ranging from 60 to 300 Holstein milking cows. The farms were representative of Alberta dairy farms in terms of herd size and breed. Each farm was visited weekly from February to September, 2012. At each weekly farm visit, bull and heifer calves between 0 and 7 days of age had blood collected via jugular venipuncture. Trotz-Williams et al. (2008) determined that calves younger than 1 day of age had a significantly lower STP content than calves over 1 day of age, so calves sampled within 24 hours of birth had a second blood sample taken 1 week later. Blood was collected into a sterile 10 mL Vacutainer tube without anticoagulant (Becton Dickinson and Co. NJ, USA), and stored on ice until returned to the University of Calgary laboratory.

Height and BW for each bull and heifer calf were measured at the initial visit, and heifers had these measurements taken weekly until 8 weeks of age. Weight was estimated by measuring heart girth using a measuring tape (Nasco Holstein Dairy Calf Weigh Tape, Nasco, Fort Atkinson, WI, USA), pulled snuggly around the thorax behind the forelimbs. Weight was calculated from the heart-girth measure according to a formula described by Heinrichs et al. (1992):

$$BW = 65.36 - (1.966 \times HG) + (0.01959 \times HG^2) + (0.00001691 \times HG^3)$$

Where BW = body weight in kg, and HG = heart girth measurement in cm.

For measuring height, the calf was made to stand on a level surface with head in a neutral position. The bottom of the measuring stick (Nasco, Fort Atkinson, WI, USA) was placed on a flat surface on the same level as the hooves. The crossbar was placed against the highest point on the withers and height measured to the nearest half centimeter.

Each calf was assessed by the researchers for respiratory illness and diarrhea. Respiratory disease was defined as discharge from the nose or eyes or repetitive coughing. Diarrhea was defined as the presence of watery feces. Any medications or treatments given to the calf were recorded. This was determined through notes made in the farm treatment logbook, if one was kept, or through discussion with the producer at the weekly visits. Calf deaths were also recorded.

At each visit, a sample of colostrum was collected that represented the MC or CR that was fed to the calf at the first feeding. Samples were collected by producers and kept frozen at -20°C on the farm until transported back to the University of Calgary laboratory. Of the 572 samples collected, 3 were CR. Samples were kept frozen in the laboratory at -80°C until processing. The farms that used CR submitted one sample that they thought was representative of the usual recipe used to reconstitute the CR powder.

A calf record sheet, similar to that developed by Trotz-Williams et al. (2008) was distributed to producers, and one was completed for each calf born. These were collected at each weekly farm visit (Appendix A).

3.2.2 Laboratory Analysis

Blood samples were kept on ice until centrifuged at 3,000 x *g* for 20 minutes at 4°C. The serum was harvested and divided into aliquots. One aliquot was used to determine STP with an optical refractometer (Atago Co. Ltd. WA, USA). The refractometer was used according to manufacturer's directions: 2 to 3 drops of serum were placed on the glass reading plate, the lid was closed to ensure proper spreading of the serum, and the STP value was recorded. Failure of passive transfer was defined as STP < 5.2 g/dL (Beam et al., 2009). A second aliquot was frozen at -80°C and shipped to a commercial laboratory for IgG analysis (Prairie Diagnostic Laboratories, University of Saskatchewan, Saskatoon, SK). Serum IgG levels were quantitatively determined using RID analysis as previously described (Chelack et al., 1993). Rabbit anti-bovine IgG (heavy and light chains) was used from rabbits immunized with whole and functional IgG of both isotypes. The IgG values reported refer to the sum of all IgG isotypes. The colostrum samples were also assessed using RID analysis at the same commercial laboratory.

3.2.3 Statistical Analysis

The statistical software used for analysis was SAS version 9.3 (SAS Institute Inc., Cary, NC). PROC UNIVARIATE was used to determine normality of the STP, serum IgG, colostral IgG, and calf BW and height. Assumptions of normality were tested using the Kolmogorov-Smirnoff, Anderson-Darling, Shapiro-Wilk and Cramervon Mises tests. Colostral IgG, serum IgG, and calf BW were not normally distributed, though transformations were performed but none improved the normality. To determine the optimal cut-point for FPT on the refractometer STP values between 4.6 to 5.6 g/dL in 0.2 g/dL intervals were compared to a serum IgG cut-point of 10 mg/mL using a chi-squared test. Sensitivity, specificity, PPV and NPV were

calculated. However, in order to compare results from this study with previous studies, the accepted cut-point of 5.2 g/dL was used to define FPT in the remainder of the analysis. Regression analysis comparing STP and RID-measured serum IgG was performed using PROC REG, and Spearmann correlation was performed using PROC CORR to determine correlation between STP and serum IgG.

Calf-level explanatory variables offered to the statistical model included: calving factors (location, time of day, assistance, difficulty, twins, abnormalities), calf sex, dam parity, colostrum management (timing, source, form, type, amount, method of delivery), calf management factors (time left with dam, medications given, type of housing), maternity pen management (type of pen, number of dams, cleaning protocol), and postnatal treatments (antibiotics or coccidostats given). All information offered to the model came from calf records filled out by farm personnel. Time of birth and time left with dam were recorded in intervals (6pm to 12am, 12am to 6am, 6am to 12pm, 12pm to 6pm). The amount of colostrum and the method by which it was fed were recorded at three separate intervals (0 to 6 hours of life, 6 to 12 hours of life, 12 to 24 hours of life).

Variables were screened for association with the continuous outcomes (serum IgG and STP, final height and weight) by univariable analysis (PROC MIXED in SAS). Variables with $p < 0.1$ were offered to the multivariable regression model. Variables with $p > 0.05$ were removed by backwards elimination. If the removal of a variable resulted in a > 30% change in the parameter estimate of the variables remaining in the model, the variable was considered a confounder and retained in the model. No predictors were found to be confounding. Farm was considered a random effect. For the growth outcomes, the same procedure was used as described above, with BW or height at 8 weeks as the response variable.

The same screening procedure was followed for the mortality and morbidity outcomes except the GLIMMIX procedure was used to accommodate the binary format of the outcome variables (death, respiratory event, diarrhea, treatment given). Only heifers were included in the growth, morbidity and mortality analyses as bulls left the farms within one week of birth.

Collinearity was assessed between all of the predictor variables that were significant in the univariable screening using PROC CORR. Colostral IgG (mg/mL), IgG consumed from 0 to 6 hours (mg), and IgG consumed from 6 to 12 hours (mg) had a high level of collinearity using a threshold of 0.7. Collinearity was also found between colostral IgG consumed from 0 to 6 hours and colostral IgG consumed from 12 to 24 hours, as well as between STP and serum IgG. Different models were created using each of the collinear variables. The models with the smallest AIC (Akaike Information Criterion) value were considered to be final.

3.3 Results

3.3.1 Descriptive Statistics

From the 14 farms enrolled, 199 bull calves and 556 heifer calves were sampled (755 calves total). One farm was inconsistent in providing reliable calf record data and did not submit any colostrum or CR product samples. This farm was only included in the descriptive analysis for serum IgG, STP, and growth descriptive analysis but not the management analysis.

The distribution of STP and serum IgG is shown in Figures 3-1 and 3-2, respectively. According to STP analysis, 44.2% of calves had FPT as 334 of 755 samples fell below the 5.2 g/dL cut-point. For the RID analysis, 15 samples were below the measurement detection limit of 0.7 mg/mL. Of the remainder, 195 were below the 10 mg/mL cut-point, so 210 of 755 or 27.8% had FPT. The age of the calf when sampled was associated with serum IgG; IgG of calves

sampled within 24 hours of birth was significantly lower (16.0 ± 1.94 mg/mL) than in calves sampled at greater than 1 day of age (21.1 ± 1.13 mg/mL, $p < 0.01$). Samples from calves taken on the day of birth were therefore removed from regression analysis but samples from day 8 were retained.

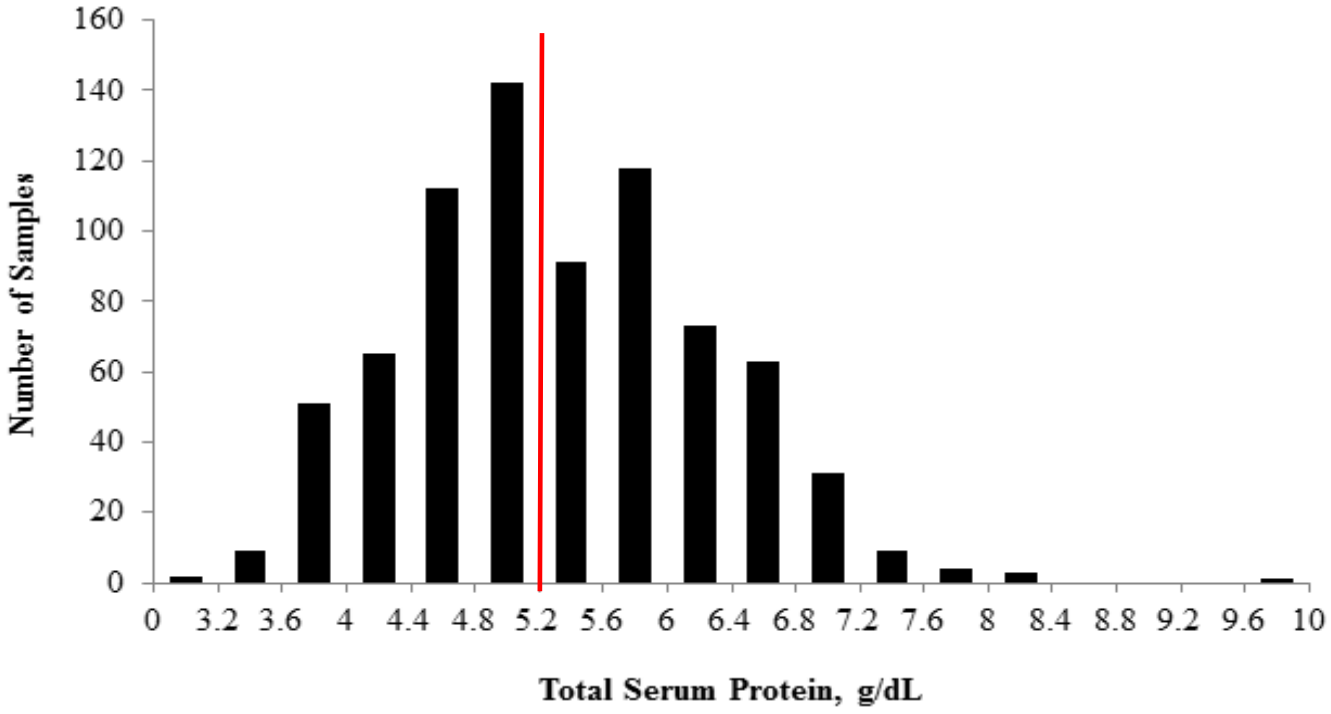

Figure 3-1. Histogram of the distribution of serum total protein analysed with an optical refractometer. Samples were collected at 1 to 8 days of age from 755 calves on 14 farms in central Alberta. The line indicates the cut-point for failure of passive transfer of immunity.

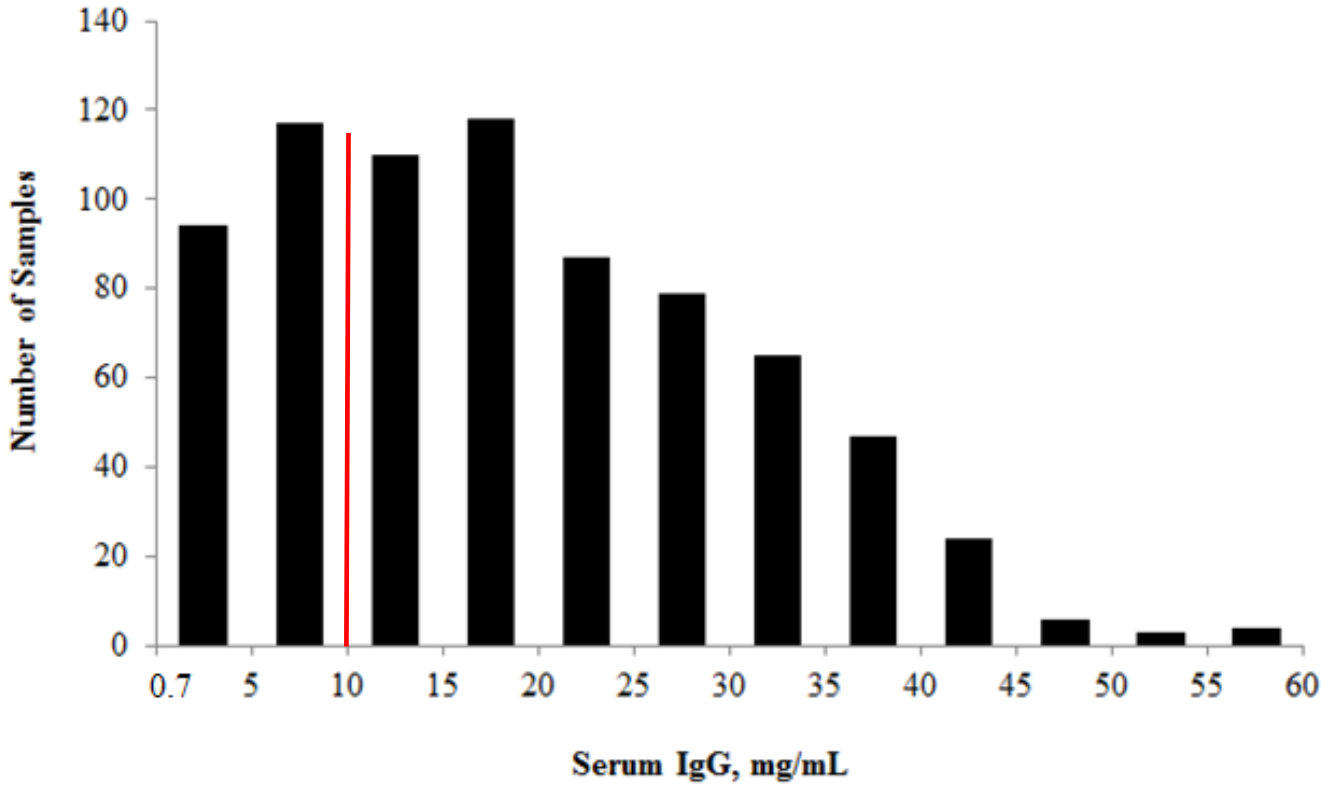

Figure 3-2. Histogram of the distribution of serum immunoglobulin G analysed by radial immunodiffusion. Samples were collected at 1 to 8 days of age from 755 calves on 14 farms in central Alberta. The line represents the cut-point for failure of passive transfer of immunity.

The relationship between STP and RID-measured IgG is shown in Figure 3-3. The Spearmann correlation coefficient was 0.89. Based on the regression equation, STP of 5.2 g/dL is equal to 16.6 mg/mL of IgG, and 10 mg/mL IgG is equal to 4.6 g/dL STP.

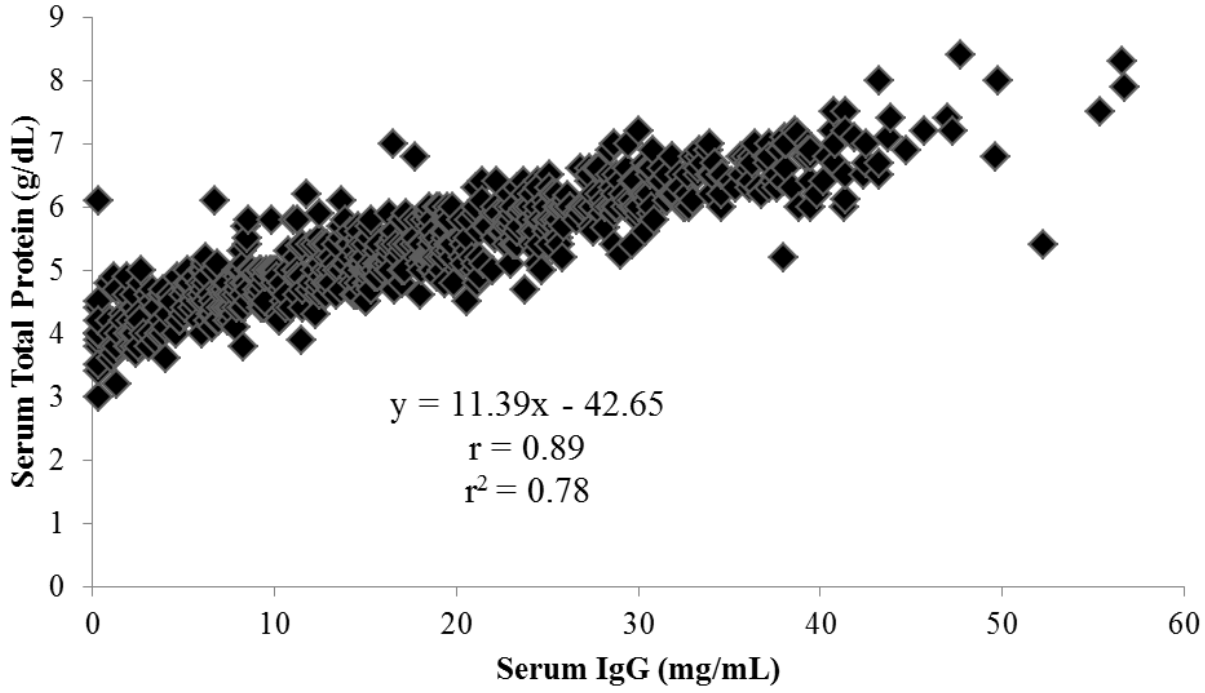

Figure 3-3. Scatterplot of serum total protein and immunoglobulin G values for 755 calves on 14 farms in central Alberta

Table 3-1 describes the diagnostic test characteristics of the optical refractometer compared to 10 mg/mL of RID-measured serum IgG. The highest combined sensitivity and specificity occurred at 5.1 g/dL. The summary of results of the calf records is shown in Appendix 2. Producers submitted calf records for 760 calves.

Table 3-1. Sensitivity, specificity, and positive and negative predictive values for different measurement cut-points of serum total protein on an optical refractometer compared to 10 mg/mL serum immunoglobulin G as measured by radial immunodiffusion. Data presented as means with 95% confidence intervals in the parenthesis.

Cut-point (g/dL)	Sensitivity (%)	Specificity (%)	Se + Sp[†]	PPV (%)	NPV (%)
4.9	76.4 (66.9-80.1)	90.3 (87.3-92.8)	1.67	73.9 (69.5-82.4)	91.4 (88.5-93.7)
5.0	81.5 (75.8-87.2)	87.7 (84.4-90.4)	1.69	70.4 (63.7-76.5)	92.0 (90.3-95.1)
5.1	92.7 (87.8-96.1)	80.8 (77.0-69.3)	1.73	63.5 (57.3-69.3)	96.8 (94.7-98.3)
5.2	94.4 (89.9-97.3)	76.7 (72.7-80.4)	1.71	59.4 (53.4-65.1)	97.4 (95.3-98.8)
5.3	94.9 (90.6-97.6)	71.1 (66.8-75.0)	1.66	54.2 (48.5-59.8)	97.5 (95.3-98.95)

PPV= positive predictive value; NPV= negative predictive value

[†]Sensitivity and specificity percentage was converted into a unit value

A large number of BW and height observations were unavailable between week 1 and 8 due to various reasons: 14 calves died, some calves were moved after weaning and could not be located, some weekly visits were missed due to weather conditions, and the type of calf hutches used on some farms did not allow researchers to safely and accurately measure large calves at 8 weeks of age. Initial and final BW are shown in Table 3-2 and heights are shown in Table 3-3. Table 3-4 describes the ADG for heifer calves from week 1 to week 8.

Table 3-2 Calf body weights from week 1 (n=556) to week 8 (n=381) for heifer calves and week 1 for bull calves (n=199) on 13 farms in central Alberta

		Mean (kg)[1]	Min (kg)	Max (kg)
Heifer	Week 1	40.5 (39.8 – 41.2)	25.8	74.4
	Week 2	42.8 (42.3 – 43.3)	27.6	57.5
	Week 3	45.5 (45.0 – 46.0)	28.5	59.4
	Week 4	50.2 (49.6 – 50.8)	31.7	72.1
	Week 5	55.1 (54.5 – 55.8)	36.5	81.6
	Week 6	60.7 (59.9 – 61.4)	34.0	84.0
	Week 7	67.0 (66.1 – 67.9)	42.1	89.2
	Week 8	72.5 (71.5 – 73.5)	42.1	94.5
Bull	Week 1	42.0 (41.3 – 42.7)	24.2	59.4

[1]95% confidence intervals shown in parenthesis

Table 3-3. Calf heights from week 1 (n=556) to week 8 (n=381) for heifer calves and week 1 for bull calves(n=199) on 13 farms in central Alberta

		Mean (cm)[1]	Min (cm)	Max (cm)
Heifer	Week 1	78.1 (77.8 – 78.3)	64.0	88.5
	Week 2	79.0 (78.7 – 79.3)	70.0	88.0
	Week 3	80.2 (79.9 80.5)	71.0	89.0
	Week 4	81.4 (81.1- 81.7)	71.0	90.0
	Week 5	83.1 (82.8 – 83.4)	73.0	92.0
	Week 6	84.4 (84.1 – 84.7)	75.0	93.0
	Week 7	86.2 (85.8 – 86.4)	76.0	96.0
	Week 8	87.7 (87.4 – 88.0)	76.0	99.0
Bull	Week 1	79.4 (78.9 – 79.8)	68.0	86.0

[1]95% confidence intervals shown in parenthesis

Table 3-4. Average daily gain and height increase in 556 heifer calves from 13 farms in central Alberta.

Week	Gain (kg/d)[1]	Height increase (cm/d)[1]
2	0.4 (0.3 – 0.5)	0.1 (0.1-0.2)
3	0.4 (0.3-0.5)	0.2 (0.1-0.2)
4	0.7 (0.6-0.8)	0.2 (0.1-0.2)
5	0.7 (0.6 – 0.8)	0.2 (0.2-0.2)
6	0.8 (0.8-0.9)	0.2 (0.2-0.3)
7	0.9 (0.8 – 1.0)	0.2 (0.2-0.3)
8	0.8 (0.7 - .9)	0.2 (0.2-0.3)

[1] 95% confidence intervals shown in parenthesis

Disease incidence and calf deaths over the first 8 weeks of life are shown in Table 3-5. Diarrhea was the primary disease observed. Although the total number of calves observed by the researchers to be sick was 83, the producers gave treatments for disease to 115 calves.

Table 3-5. Rates of morbidity and mortality of calves

Event	Calves (n)	Calves (%)
Death	12/465	2.6
Respiratory	33/466	7.1
Diarrhea	50/465	10.8

3.3.2 Regression Analysis

Variables significantly associated with STP in the univariable analysis included method of feeding from 6 to 12 hours of life, the type of colostrum fed from 0 to 6 and 6 to 12 hours of life, calf age when sampled, weight of colostral IgG consumed from 0 to 6, 6 to 12, and 12 to 24 hours of life, concentration of colostral IgG, if fresh or frozen colostrum was fed in the first 12 hours of life, type of milk fed after colostrum, and if medications were given in the first days of life. The final multivariable model is shown in Table 3-6. The type of colostrum fed from 6 to12 hours of life and type of milk fed after colostrum were important categorical predictors. As well, for every unit increase in IgG (mg) consumed in the first 6 hours of life, STP increased by 0.002 g/dL ($p < 0.01$).

Table 3-6. Linear regression model for variables associated with serum total protein in 704 dairy calves on 13 farms in central Alberta.

Variable	Estimate (CI[1])	Least Squares Mean of STP[2] g/dL (CI[1])	P-value
Intercept	6.05 (4.9 – 7.2)	-	-
Type of colostrum fed from 6-12 hours	-	-	< 0.002
Other cow colostrum	0.5 (-0.3-1.2)	6.1[a] (5.6-6.6)	
Dam colostrum	0.3 (-0.4-0.9)	5.9[a] (5.6-6.2)	
Not fed	0.9 (0.07-1.7)	5.9[a] (5.7-6.2)	
Colostrum replacer	0.4 (-0.5-1.2)	5.5[a] (4.9-6.1)	
Supplement	-0.6 (-1.44-0.1)	5.2[b] (4.5-5.9)	
Pooled colostrum	Referent	5.4[ab] (4.7-6.1)	
Type of milk fed after colostrum	-	-	< 0.04
Waste milk, sale milk, and milk replacer	Referent	6.7[a] (5.7-7.7)	
Sale milk	-1.2 (-2.1- -0.2)	5.4[b] (5.1-5.7)	
Waste milk	-1.2 (-2.2 - -0.02)	5.2[b] (4.8-5.6)	
Waste and sale milk	-1.3 (-2.3 - -0.4)	5.2[b] (4.9-5.5)	
Weight of IgG consumed from 0 to 6 hours	0.002 (0.0014 – 0.0041)	-	< 0.01

[1]95% confidence interval
[2]Serum total protein measured in calves
Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with serum IgG in the univariable analysis included method of feeding from 6 to 12 hours, if fresh or frozen colostrum was fed in the first 12 hours, calf age when sampled, type of milk fed after colostrum, if any medications were given, type of colostrum fed from 0 to 6 and 6 to 12 hours, weight of IgG consumed from 0 to 6, 6 to 12, and 12 to 24 hours, concentration of colostral IgG, and type of housing. The final multivariable analysis for serum IgG is shown in Table 3-7. Colostral IgG concentration (mg/mL) was significantly associated with serum IgG ($p < 0.01$); for each unit increase in colostral IgG, serum IgG increased by 0.2 mg/mL. For each mg of IgG consumed from 6 to 12 hours, serum IgG increased by 0.02 mg/mL ($p < 0.04$).

Table 3-7. Linear regression model for variables associated with serum IgG in 704 dairy calves on 13 farms in central Alberta

Variable	Estimate	Least Squares Means of Serum IgG (CI[1])	P-value
Intercept	23.2 (1.7-34.7)	-	-
Fresh or frozen colostrum fed from 0-12 hours	-	-	<0.01
Fresh and frozen	4.1 (-1.5-9.6)	29.3[a] (25.4-33.2)	
Frozen	2.9 (-3.7-9.6)	29.2[a] (24.2-34.3)	
Fresh	-0.7 (-5.6-4.2)	24.3[b] (21.6-27.0)	
Colostrum replacer	Referent	24.7[ab] (19.5-30.0)	
Type of milk fed after colostrum	-	-	< 0.02
Waste, sale, and milk replacer	Referent	37.1[a] (27.3-46.9)	
Sale milk	-15.0 (-25.1 - -4.9)	25.2[b] (21.5-28.8)	
Waste and sale milk	-15.1 (-25.4 - -4.7)	22.8[b] (20.1-25.5)	
Waste milk	-11.2 (-21.9 - -0.5)	22.5[b] (20.5-24.5)	
Colostral IgG (mg/mL)	0.24 (0.1 – 0.3)	-	< 0.01
Colostral IgG consumed from 6 to 12 hours	-0.03 (-0.04 - -0.008)	-	<0.04

[1]95% confidence interval

Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with BW at week 8 in the univariable analysis included calf health at birth, if fresh or frozen colostrum was given in the first 12 hours, STP concentration, if medications were given, volume of colostrum given from 6 to 12 hours, method of feeding from 0 to 6 and 6 to 12 hours, month of birth, dam parity, if milk was pasteurized, type of colostrum given from 6 to 12 hours, and weight in week 1. The final multivariable analysis of BW at week 8 is shown in Table 3-8. For every g/dL increase in STP, BW at 8 weeks increased by 1.65 kg. Also, for each kilogram increase in initial weight, BW at week 8 increased by 0.49 kg ($p < 0.01$).

Table 3-8. Linear regression model for variables associated with body weight at 8 weeks in 380 heifer dairy calves on 13 farms in central Alberta

Variable		Estimate (CI[1])	Least Squares Means of Weight at week 8 (kg)	P-value
Intercept		47.6 (36.7 – 58.5)	-	
Method of feeding from 0 to 6 hours		-	-	<0.02
	Bottle	Referent	74.5[a] (72.1 – 77.0)	
	Left with dam	-2.1 (-6.5 – 2.3)	72.2[a] (67.6-76.8)	
	Not fed	1.7 (-10.7 – 14.1)	71.8[ab] (59.1-84.6)	
	Bucket	-3.4 (-9.2 – 2.4)	71.0[ab] (65.3-76.6)	
	Tube	-7.2 (-12.0 - -2.4)	66.0[b] (61.3-70.7)	
Parity of dam		-	-	< 0.01
	3+	Referent	73.3[a] (69.52-77.08)	
	2	-2.8 (-5.4 - -0.2)	70.3[b] (66.63-74.05)	
	1	-3.5 (-5.9 - -1.2)	69.7[b] (66.02-73.27)	
Month of birth		-	-	0.02
	May	3.5 (-0.002 – 6.9)	75.4[a] (71.53-79.28)	
	June	1.3 (-2.1 – 4.8)	73.3[a] (69.37-77-22)	
	April	-0.5 (-4.0 – 3.0)	71.5[b] (67.58-75.41)	
	February	-1.2 (-5.4 – 2.9)	70.8[b] (66.86-74.81)	
	March	-1.8 (-5.5 – 1.9)	70.1[b] (66.02-74.24)	
	July	Referent	72.0[a] (67.61-76.29)	
Weight in week 1		(3.0 – 0.67)	-	< 0.01
Serum total protein		1.7 (0.01 – 0.5)	-	< 0.01

[1]95% confidence interval

Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with height at week 8 in the univariable analysis included type of milk fed after colostrum, method of feeding from 0 to 6, 6 to 12, and 12 to 24 hours, if fresh or frozen colostrum was fed in the first 12 hours, type of colostrum fed from 0 to 6, 6 to 12, and 12 to 24 hours, serum IgG level, volume of colostrum fed from 12 to 24 hours, and if medications were given. The final multivariable analysis for height at week 8 in shown in Table 3-9. Height in week 1 was significantly associated with height at week 8 ($p < 0.01$); for each 1 cm increase in height at week 1, height at week 8 increased by 0.48 cm.

Table 3-9. Linear regression model for categorical variables associated with height at 8 weeks in 380 heifers across 13 farms.

Variable	Estimate (CI[1])	Least squares means for height at week 8 in centimeters (CI[1])	P-value
Intercept	54.3 (46.2 – 62.4)	-	-
Method of feeding from 6 to 12 hours	-	-	<0.001
Not fed	2.0 (-0.1 – 4.0)	90.8[ab] (88.6-93.0)	
Bucket	1.7 (0.3 – 3.1)	90.4[a] (8.9-92.0)	
Left with dam	-0.6 (-2.0 – 0.7)	88.5[b] (86.9-90.0)	
Tube	-2.3 (-3.2 - -1.4)	86.7[c] (85.5 -87.8)	
Bottle	Referent	88.9[b] (88.2-89.8)	
Type of colostrum fed from 0 to 6 hours	-	-	<0.001
Not fed	1.9 (-2.0 – 5.7)	91.4[ab] (87.8-95.0)	
Pooled	Referent	89.5[ab] (87.8-91.2)	
Other cow	-0.4 (-2.3 – 1.5)	89.1[a] (88.0-90.2)	
Colostrum replacer	-1.6 (-3.4 -0.2)	87.9[bc] (87.1-88.7)	
Dam colostrum	-2.0 (-3.8 - -0.3)	87.4[c] (86.7-88.0)	
Height in week 1	0.5 (0.3 – 0.6)	-	< 0.01

[1]95% confidence interval

Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with death in the univariable analysis included serum IgG, weight in week 1, and method of feeding from 0 to 6 hours. The final multivariable analysis for risk of death is shown in Table 3-10. For each kilogram increase in BW in week 1, probability of death decreased by 13% ($p < 0.06$). The odds ratio (OR) for calves that were either not fed from 0 to 6 hours, or fed by tube, compared to bottle-fed is 59.8 (1.2 - 118.4) and 8.7 (1.2 – 65.1), respectively. The mean risk of mortality for calves that were not fed within 6 hours of birth was 32.7%

Table 3-10. Logistic regression model for categorical variables associated with death in 465 dairy calves on 13 farms in central Alberta

Variable	Estimate (CI[1])	Least Squares Means of % incidence risk of mortality (CI[1])	P-value
Intercept	0.2 (-5.1 – 5.5)	-	
Method of feeding from 0 to 6 hours	-	-	< 0.02
Not fed	4.1 (1.2 – 6.9)	32.7[a] (3.7 – 86.0)	
Tube fed	2.2 (0.7 – 3.6)	6.6[a] (3.0 – 14.4)	
Left with dam	1.5 (-0.8 – 3.8)	3.5[ab] (0.5 – 21.9)	
Bucket	1.3 (-1.0 -3.7)	3.0[ab] (0.4 – 19.3)	
Bottle	Referent	0.8[b] (0.2 – 2.7)	
Weight in week 1	-0.13	-	< 0.06

[1]95% confidence interval
Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with diarrhea in the univariable analysis included; if the calving pen was cleaned prior to birth, month of birth, if the calf received any treatments during the first 8 weeks of life, and if the calf experienced at least 1 incidence of respiratory disease. The final multivariable analysis is shown in Table 3-11. The OR of having diarrhea for

calves not born in an individual calving pen was 2.97 (0.8 – 10.6) compared to calves born in an individual calving pen that was cleaned before birth.

Table 3-11. Logistic regression for variables associated with diarrhea in the first 8 weeks of age in 465 calves on 13 farms in central Alberta

Variable	Estimate (CI[1])	Least Squares Means of % incidence risk of diarrhea (CI[1])	P-value
Intercept	-2.6 (-3.5 - -1.7)	-	-
Calving pen cleaned before birth	-	-	<0.01
Not born in individual pen	0.7 (-0.2 – 1.6)	12.9[a] (9.4-17.4)	
Born in a dirty individual calving pen	-0.4 (-1.7 – 0.9)	7.1[ab] (3.2 – 14.9)	
Born in a cleaned individual calving pen	Referent	4.8[b] (1.8 – 12.0)	

[1]95% confidence interval
Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with respiratory disease in the univariable analysis included volume of colostrum fed from 6 to 12 hours, colostral IgG concentration, if medications were given in the first days of life, type of colostrum fed from 6 to 12 hours, method of feeding from 6 to 12 hours, if the calf had at least 1 episode of diarrhea, and if milk fed after colostrum was pasteurized. The final logistic regression analysis for risk of respiratory disease is shown in Table 3-12. The OR of calves having at least on event of respiratory disease who received no colostrum from 6 to 12 hours was 98.2 (6.2 - 190.2) compared to calves fed a colostrum supplement, and 15.9 (2.3 – 29.5) compared to calves fed their own dam's colostrum.

Table 3-12. Logistic regression model for categorical variables associated with at least 1 event of respiratory disease in 466 heifer dairy calves on 13 farms in central Alberta

Variable	Estimate (CI[1])	Least Squares Means of % incidence risk of respiratory disease (CI[1])	P-value
Intercept	-16.6 (-4194.0-4161.0)	-	-
Type of colostrum fed from 6 to 12 hours	-	-	< 0.04
Not fed	16.9 (-3718.0-3751.9)	58.4[a] (17.5-90.3)	
Colostrum replacer	14.7 (-3720.2-3749.7)	13.8[ab] (3.3-43.2)	
Dam colostrum	14.1 (-3720.8-3749.1)	8.1[b] (4.9-13.2)	
Other cow colostrum	13.5 (-3721.5-3748.4)	4.3[ab] (0.6-25.9)	
Supplement	12.3 (-3722.6-3747.3)	1.4[b] (0.2-10.0)	
Pooled	Referent	6.36×10^{-6ab} (0.0-1.0)	

[1]95% confidence interval
Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

Variables significantly associated with receiving at least one treatment in the univariable analysis included colostral IgG consumed from 0 to 6 hours, location of birth, serum IgG, STP, if milk fed after colostrum was pasteurized, and if the calf experienced at least 1 episode of diarrhea. The final logistic regression model is shown in Table 3-13. For each g/dL increase in STP, the probability that a calf would receive at least one treatment decreased by 0.61. The OR of calves having at least one treatment was 4.67 (1.2 - 18.8) for calves given unpasteurized versus pasteurized milk, and 0.42 (0.2 – 1.0) for calves who experienced no observed diarrhea compared to those who had ≥ 1 incidence of diarrhea.

Table 3-13. Logistic regression model for categorical variables associated with at least 1 treatment series in 464 heifer dairy calves in 13 farms in central Alberta

Variable		Estimate (CI[1])	Least Squares Means of % incidence risk of respiratory disease (CI[1])	P-value
Intercept		1.6 (-1.1-4.3)	-	-
At least 1 diarrhea incident in the first 8 weeks		-	-	<0.05
	Yes	Referent	28.4[a] (11.7-55.1)	
	No	-0.9 (-1.7 - -0.02)	14.2[b] (6.4-28.5)	
Milk fed after colostrum was pasteurised		-	-	<0.04
	No	1.5 (0.1-2.9)	35.6[a] (13.3-66.5)	
	Yes	Referent	10.6[b] (4.1-24.6)	
Serum total protein		-0.61 (-1.0 - -0.2)	-	< 0.01

[1]95% confidence interval
Significance reported at $p < 0.05$. Superscripts with the same letters indicate no significant difference.

3.4 Discussion

3.4.1 Calf management practices

The results from this study can be compared to other Canadian data from similar calf management studies in Ontario (Trotz-Williams et al., 2008) and Quebec (Vasseur et al., 2010). A higher number of heifers than bulls were enrolled in the study, reflective of the fact that bulls are often sold within the first week of birth, sometimes prior to the weekly farm visit. This was also reported in the Ontario study. There was a generally even distribution of calves born within a 24 hour period, though fewer calves were reported born from 6pm to midnight. This could be indicative of when the calf was found rather than when it was born. Fewer calves in the current study were separated from the dam within one hour of birth compared to Ontario data (Trotz-Williams et al., 2008).

In AB, QC, and ON the most common volume of colostrum fed in the first 6 hours of life was 2 L, with a range from 0 to 6 or more litres. Most of this colostrum was from the calf's own dam or was a CR, which is comparable to Ontario data whereby 59.4% of calves received MC (Trotz-Williams et al., 2008). In Quebec, all farms reported using MC for the first feeding (Vasseur et al., 2010). In the current study most calves received fresh colostrum, which is similar to Ontario data: 77.4% of calves were given fresh colostrum and 5.5% were given frozen (Trotz-Williams et al., 2008). Most calves were housed in individual indoor pens, similar to housing reported in Quebec (Vasseur et al., 2010).

3.4.2 Serum Total Protein and Serum Immunoglobulin G

Calloway et al. (2002) suggested that for the refractometer the best measurement cut-points for the highest proportion of correctly identified FPT positive or negative samples occurs at 5.0 and 5.2 g/dL STP. Our study shows that 5.1 g/dL gives the highest proportion of correctly identified samples (Table 3-4) with a sensitivity and specificity of over 80%. Our results confirm that using cut-points between 5.0 and 5.2 are appropriate. According to the regression equation (Figure 3-3), a cut-point of 5.2 g/dL is equal to 16.6 mg/mL IgG, showing that the cut-point of 5.2 g/dL overestimates true IgG levels. The r^2 for STP and IgG was higher than the 0.54 reported by Calloway et al. (2002) and similar to the 0.76 reported by Tyler et al. (1996). Though the refractometer has good correlation with serum IgG, there was a large difference in prevalence of FPT depending on which of these measurements were used. It may be better to use serum IgG levels instead of STP to measure levels of passive transfer of immunity. Serum total protein measures total protein, not just IgG, and non-IgG protein will influence the STP results.

Positive and negative predictive values take into account the prevalence of FPT in the sample population. Positive predictive value indicates the probability that a calf with a test below a particular cut-point truly has FPT. In this instance, if a calf's STP is 5.1 g/dL, there is a 63.5% chance that the calf has serum IgG lower than 10 mg/mL. The highest PPV occurred at 4.9 g/dL, and PPV increased with decreasing cut-points. Negative predictive values indicate the probability that a calf with a negative test does not have FPT, or has serum IgG $\geq$ 10 mg/mL, given the prevalence of FPT in the sample population. The highest NPV occurred at 5.3 g/dL, indicating that if a calf tests at 5.3 g/dL, there is a 97.5% chance that it does not have FPT. With increasing cut-points, NPV also increases.

The herd-level portion of a study performed by Trotz-Williams et al. (2008) shows 37.1% of calves from 112 farms had FPT. In contrast, the calf-level portion of their study indicated that 8% of calves from 11 farms in Ontario had FPT. A recent study by Windeyer et al. (2012) reported that 11% of calves from 19 farms in Ontario and Minnesota had STP levels less than 5.2 g/dL, compared to our study where 44.2% of all calves had FPT. The difference in these results may be the result of the farms chosen; in studies with few farms, the influence of one farm with poor management practices and a high incidence of FPT would have a large influence on the overall mean. Using the conventional cut-point of 10 mg/mL of IgG, nearly 27.8% of calves in the current study had FPT. Data from the US indicated that 19.2% of calves have serum IgG levels < 10 mg/mL (Beam et al., 2009). There was no significant difference in serum IgG or STP levels between bull and heifer calves, similar to previous studies (Filteau et al., 2003; Trotz-Williams et al., 2008).

Regarding the multivariable analysis of STP, it is not surprising that calves fed CS exclusively had the lowest STP, since it has been suggested that supplements delivering less than

50 mg/mL of IgG per dose are an inadequate source of IgG and not designed to achieve > 10 mg/mL of serum IgG (Quigley et al., 2002; Godden 2008). In one study (Quigley et al., 2002), calves receiving supplement compared to CR had lower serum IgG though interestingly plasma protein at 24 hours was not significantly different. This might be attributable to a higher concentration of non-IgG proteins present in the supplement than CR.

In the present study, there was no significant difference in STP levels for calves not fed from 6 to 12 hours of life compared to calves fed any type of colostrum or CR product in this time period. This could be because calves had already received adequate amounts of IgG in the first 6 hours of life. Previous literature suggests that calves fed CR are at higher risk of FPT than those fed MC (Swan et al., 2007; Godden et al., 2009a). The current study demonstrates no significant difference in STP or serum IgG levels between calves fed MC or CR, although calves fed MC had a numerically higher average STP level than calves fed CR.

The amount of colostral IgG consumed was positively associated with STP in agreement with the results of Godden (2008). In regard to serum IgG, colostral IgG on its own, as well as amount of colostral IgG consumed were significantly associated. There have been numerous studies showing that there is a positive relationship between colostral IgG intake and serum IgG levels (Bush and Staley, 1980; Furman-Fratczak et al., 2011).

A significant predictor of both STP and serum IgG was type of milk fed after colostrum. Producers may not have fed colostrum for more than the initial feeing, so type of milk fed prior to 12 hours could have influenced STP levels if it was absorbed before gut closure was complete. Due to the nature of waste milk, it may contain an unknown amount and type of pathogen, or may be contaminated through improper handling and storage (Moore et al., 2009). A study by Moore et al. (2009) describes the quality of waste milk at a calf ranch, finding a range of somatic

cell count (SCC) from 1.5 x 10^6 to > 10 x 10^7 SCC/mL, with abundant coliform and streptococci growth on plates. Total colostral dissolved solids in that study were 11% as determined by Brix. The present study had an average of 24.3% Brix. High SCC results in low total milk solids, including IgG and non-IgG protein (Moore et al., 2009). If the waste milk on the farms in our study was similar to that reported by Moore et al. (2009), it would seem that waste milk may result in lower serum IgG and STP compared to calves ingesting cleaner milk types, such as sale milk or MR. The addition of MR powder to waste and sale milk, or feeding dissolved MR in addition to normal waste or sale milk feedings could account for higher serum IgG in several possible ways. Milk replacer would supply a consistent amount of protein and a pathogen-free source of milk. It may allow the uptake of more IgG as the MR would contain fewer pathogens that may bind IgG (Moore et al., 2009), or compete with IgG for pinocytotic uptake (Roy, 1980). Alternatively, the association between FPT and milk types fed after colostrum could be the result of a management practice not measured in the calf records; for example, if a farm gives MR in addition to regular milk feedings, or adds MR to supplement normal feedings, this could be considered a higher standard of care and would likely extend to other aspects of calf care not necessarily measured in this study.

Calves fed a combination of fresh and frozen colostrum or just frozen colostrum had significantly higher serum IgG compared to calves fed exclusively fresh colostrum in the first 12 hours of life. This could be due to producers saving and freezing good quality colostrum. Freezing colostrum has been shown to retain greater IgG content compared to other storage methods as it prevents the breakdown of the IgG (Foley and Otterby, 1978). Alternatively, it could also be due to the handling of fresh colostrum prior to feeding. Total solids in colostrum stored at room temperature decrease with increased storage time (Foley and Otterby, 1978). If

colostrum was left out at ambient temperatures for hours prior to feeding, colostral IgG levels could be diminished and bacterial counts exponentially increased. Clean handling of colostrum during collection may or may not have occurred, and improper handling could have allowed pathogen contamination, which also decreases colostral total solids. Serum IgG concentration increases with increased AEA (Johnson et al., 2007) and AEA is decreased by bacterial contamination of colostrum. The bacteria or other pathogens bind to colostral antibodies and prevent their uptake in the gut. There may also be competition between antibodies such as IgG and pathogens for enterocyte receptors (Elizondo-Salazar and Heinrichs, 2009).

Type of calving pen and whether or not the pen was cleaned prior to calving was not significantly associated with serum IgG, although type of calving pen was associated with risk of diarrhea. This is different than the study of Pithua et al. (2009) that found that there was no difference in risk of disease between calves born in single or multiple cow calving pens with varied levels of cleaning.

3.4.3 Weight and Height

Aust et al. (2012) found that in the first 14 days of life ADG for calves was negligible. However, between 15 and 28 days it was approximately 500 g/day, with the highest ADG occurring between 29 and 56 days. In the current study, ADG was also the highest between weeks 5 and 8, and lowest in the first 2 weeks. Reported average birth height is 74 cm (Kertz et al., 1998). Calves in this study had a higher average in week 1, with heifers at 78.1 cm, and bulls at 79.3 cm. Windeyer (2010) also reports a similar height for heifers, suggesting that Canadian Holsteins may be overall taller, or there may be effects of genetic selection on height since the older reported data.

In the current study, month of birth was significantly associated with both initial weight in week 1 and final weight at week 8. Calves born from May to July had significantly higher weights than calves born from February to April. This is in contrast to Godden et al. (2005), who reported that calves born in the winter months had significantly higher birth weight and weaning weight than calves born in the summer. The reason for the difference between this and the current study may be related to calf enrolment, as a greater number of calves were born in April to July than in February to March. In the current study, had there been more calves enrolled throughout the winter season (October through March) there may have been a different seasonal effect reported. The most significant factor affecting height and weight at 8 weeks was initial height and weight, respectively. The weight and height advantage at birth continued on during the preweaning period. Although not monitored in this study, it is possible that the farms used milk feeding programs that were specific to each calf, such that calves received milk allowances as a proportion of their BW.

Dam parity was associated with calf BW at weeks 1 and 8, with calves born from 1st or 2nd parity heifers having significantly lower weights than calves from 3rd parity or higher cows. Linden et al. (2009) also found that calves from 1st and 2nd parity dams had lighter calves. First and 2nd parity heifers are still growing themselves and may have smaller calves due to the division of energy resources between their own growth and fetal growth. The difference in calf weight may also be related to the fact that mature cows are larger than cows in their 1st and 2nd parities and larger cows tend to have larger calves (Linden et al., 2009). Additionally, calves from 1st parity cows have lower growth rates in the first 90 days compared to other calves (Lundborg et al., 2003).

Method of feeding in the first 6 hours after birth was associated with BW at 8 weeks: calves fed by bottle or left with the dam to suckle had significantly higher BW compared to calves fed by esophageal tube. The reasons for this may be 2-fold. First, feeding by bottle or suckling allows the closure of the esophageal groove, allowing the colostrum to bypass the reticulorumen and deposit directly into the omasum and abomasum. As a result, it takes less time for colostrum to reach the small intestine where absorption occurs. Secondly, although the current study did not attempt to culture bacteria from feeding equipment, the amount of bacteria cultured from esophageal tubes was greater than that cultured from milk taken directly from the udder (Stewart et al., 2005). It would seem bottles may be easier to sanitize compared to a tube. The early exposure to pathogens, or effects of not having esophageal closure in the first feedings could have an effect on growth rate at 8 weeks of age. Method of feeding was also significantly associated with height at week 8, with tube-fed calves having significantly lower height than calves that were bottle-fed or that suckled.

Bateman et al. (2012) suggested that there is no established link between STP and growth performance, however, in our study, STP was one of the most important predictors of weight at 8 weeks of age. Another Canadian study also found that STP was associated with BW (Windeyer, 2010).

Calf height may not be as useful a parameter for measuring growth and development as weight. In this study, height varied depending on the surface the calf was standing on, even when efforts were made to ensure the measuring stick was level with the bottom of the calf’s hooves. Girth measurements had more consistency compared to height. The challenge with measuring height is also described in another study (Linden et al., 2009).

3.4.4 Morbidity and Mortality

The odds of death for calves not fed colostrum or fed by esophageal tube in the first 6 hours of life was significantly higher than for calves that were bottle-fed. Wells et al. (1996) reported that calves not fed any colostrum or fed 1.9 to 3.8 L via esophageal tube in the first six hours of life had significantly higher odds of death compared to calves fed by bucket or that nursed. Those authors suggested that the reason for the higher odds of mortality in tube-fed calves was that these calves were weaker at birth, predisposing them to disease. This explanation could be relevant to the current study, with the exception of one farm that routinely tube-fed all calves. Although farm was included as a random effect this one farm may have influenced the results. This particular farm also had a high mortality, with 5 of the 12 calves that died. Another explanation for the higher odds or mortality with tube feeding could be unsanitary tube conditions; calves receiving colostrum via tube may be exposed to a greater number of pathogens compared to bucket fed or nursed calves, as described above. Wells et al. (1996) also hypothesized that poor sanitation of the tubes or improper feeding technique may have harmful effects on the calf, which could contribute to the high risk of mortality.

There may be other management factors or farm practices contributing to this relationship that were not measured in this study. For example, Swali and Wathes (2006) describe the factors of calf birth weight being related to cow body condition at time of conception, nutrition available to the fetus, and maternal food supply. This could be related to management practices not measured in this study. For example, perhaps cows that were offered a constant nutritious food supply and maintained good body condition during pregnancy would have larger calves, and these calves would likely also receive adequate nutrition after birth. High birth weight resulting in lowered risk of death could be a surrogate measure for other farm management practices.

The risk of having diarrhea at least once in the first 8 weeks of life was significantly associated with cleaning the calving pen prior to birth. Calves that were born in a clean individual calving pen had lower risk of diarrhea compared to those born in group maternity pens. This makes sense biologically as the calves will absorb any molecules they ingest in the first hours after birth, including bacteria. Cleaning out the calving pen prior to birth would remove dirt, feces, and birth material from previous calvings that could harbour pathogens. Frank and Kaneene (1993) showed that calves born in maternity pens that were cleaned, and calves born in individual maternity pens had lower incidence of diarrhea compared to those born in pens that were not cleaned and group maternity pens. They suggest this is due to the removal of fecal material that can contain bacterial pathogens that can cause diarrhea. In contrast, Pithua et al. (2007) examined the difference in calf mortality and morbidity between calves born in single or multiple calving pens. They found that there was no significant difference in morbidity between the groups; however, all dams were vaccinated with a multi-antigen vaccine three months prior to calving specifically to protect calves against diarrhea. The vaccination status of the dams in the current study is unknown.

Type of colostrum fed from 6 to 12 hours of life was significantly associated with respiratory disease in the first 8 weeks. Calves given no colostrum in this time period were 98.2 times more likely to have respiratory disease compared to those who received a colostrum supplement. Colostrum supplements are not designed to replace colostrum but clearly they provide some IgG that helps prevent respiratory illness. Calves fed no colostrum were 15.9 times more likely to have respiratory disease compared to calves fed their own dam's colostrum. This could indicate that maternal colostrum from their own dam has benefits outside of IgG levels. Colostrum from the dam may contain specific antibodies, cytokines, maternal leukocytes or other

proteins that could possibly benefit her own calf more than another calf. As well, fresh colostrum has immunologic benefits over frozen colostrum (Donovan et al., 2007). In one study in which dams were vaccinated with bovine viral diarrhea virus before parturition the calves that were fed fresh colostrum had a greater proliferative response to bovine viral diarrhea virus compared to calves that consumed frozen-thawed whole colostrum or cell-free colostrum, although each group of calves received equivalent amounts of IgG (Donovan et al., 2007). The authors suggest that live maternal cells other than IgG, such as maternal leukocytes, are responsible for this immune response, and that freezing colostrum destroys these cells. In the current study, calves fed colostrum from their own dams received it fresh after milking. Calves that were fed another cow's colostrum or pooled colostrum often received it in frozen-thawed form. This could contribute to the result that calves fed their own dam colostrum were less likely to receive any form of treatment, as live maternal cells they received through fresh colostrum could help reduce the chances they would become ill or show symptoms of disease when exposed to a pathogen.

Diarrhea and respiratory disease was determined as observed by researchers. Calves that experienced at least one diarrhea incident had a higher risk of receiving at least one treatment compared to calves with no diarrhea. Respiratory illness was not significantly associated with treatment, possibly indicating that producers may be more likely to treat calves with diarrhea than respiratory disease. The treatment information came from log books kept at the farm or discussions with farm personnel. Due to the nature of this type of data collection, there may be recall bias in this result. As well, because diarrhea and respiratory disease symptoms were recorded solely based on observations made at weekly farm visits, it is probable that the calves experienced disease events for which they were treated that researchers did not observe.

The odds of receiving treatment for calves fed unpasteurized milk was 4.7 more likely than calves fed pasteurized milk. This is likely because raw milk contains more bacteria than pasteurized milk (Godden et al., 2006).

For each unit increase in STP, the probability of receiving treatment decreased by 0.61. This is likely due to higher STP equating to better immunity, and these calves would be less likely to fall ill and exhibit symptoms requiring a treatment compared to calves with lower immunity (Calloway et al., 2012; Windeyer et al., 2012; Priestly et al., 2013).

3.5 Conclusion

The calf record results for management practices used in Alberta are comparable to other Canadian data. Prevalence of FPT is similar in this study compared to American and Canadian data, suggesting that FPT is a widespread problem throughout North America. In order to reduce the prevalence of FPT, producers should feed as much high quality colostrum as possible, either fresh or frozen, in the first 12 hours after birth. Tube-feeding should be avoided when possible and bottle-feeding used in order to reduce the chances of calfhood mortality and ensure calves reach their full weight and height potential. To reduce the likelihood of diarrhea, maternity pens should be cleaned prior to parturition.

Chapter Four: General Conclusions and Future Perspectives

4.1 Introduction

This study is the first to describe colostrum quality on Alberta dairies. It is also first to describe the types of calf management practices used in Alberta, and to evaluate how these management practices are associated with risk of FPT, morbidity and mortality, as well as height and weight growth.

4.2 Colostrum Quality and Measurement Tools

Nearly one third of colostrum samples measured by RID analysis were below 50 mg/mL. This is similar to American reports of colostrum quality (Morrill et al., 2012a; Quigley et al., 2013). The Brix refractometer had the highest combined sensitivity and specificity at a cut-point of 23%, which supports previous studies (Chigerwe et al., 2008a; Bielmannn et al., 2010; Quigley et al., 2013). For the colostrometer, the highest proportion of correctly identified good and poor quality samples occurred at the 80 mg/mL cut-point. This cut-point is similar to reported data (Chigerwe et al., 2008a). RID-measured IgG concentrations were better correlated with the colostrometer data than with the Brix refractometer data, however, the colostrometer is a better predictor of RID-measured IgG levels than is the Brix refractometer.

Farmers concerned with using the best colostrum available should consider using either the colostrometer or the Brix refractometer to estimate the quality of colostrum on their farm. The colostrometer may be more difficult to use on farm due to its fragility and temperature sensitivity, but it is better correlated with true IgG content compared to the Brix refractometer. Recommendations are to measure colostral IgG of all colostrum, (fresh and frozen) and

colostrum replacer, prior to feeding. Nearly 30% of colostrum samples contained insufficient IgG, highlighting the importance of checking quality prior to feeding to ensure calves are receiving an adequate mass of IgG.

4.3 Prevalence of Failure of Passive Transfer of Immunity

According to RID analysis, 27.8% of the calves had FPT, and according to STP, 44.2% had FPT. Serum total protein is often used on the farm, in clinic, or in the lab as an alternative to the labour and time intensive RID- analysis, however this study highlights the fact that there is a wide discrepancy between the values obtained with these 2 methods. Serum total protein tends to overestimate the prevalence of FPT. If using STP to determine prevalence of FPT, the results should be interpreted with caution. This study found that 5.1 g/dL on the refractometer offers the highest sensitivity and specificity, in line with reported values.

Although many of the factors associated with FPT have been identified, this study shows that the prevalence of FPT is still very high. A prevalence of 10% FPT on a farm is an achievable goal (Chigerwe et al., 2009), yet this is not being met. The first step in improving management is to benchmark existing practices. This study gives Alberta producers a starting point from which they can address management practices associated with FPT.

With regard to FPT, this study indicates that feeding a colostrum supplement from 6 to12 hours of life was associated with lower STP levels; feeding solely fresh colostrum, compared to feeding frozen-thawed, or a combination of fresh and frozen, in the first 12 hours was associated with low serum IgG, and feeding a combination of waste, sale, and milk replacer after colostrum, compared to a combination of waste and sale, or either on their own, was associated with higher STP and serum IgG levels. Based on these results, recommendations are to measure fresh

colostrum prior to feeding to ensure is will provide an adequate amount of IgG, and to not use a colostrum supplement in place of colostrum. It may be beneficial to add a milk replacer to the waste or sale milk that is fed after colostrum, although more research is needed to better understand the effect of combining waste, sale milk, and milk replacers.

Risk of death before 8 weeks was associated with not being fed within the first 6 hours of life, and calves that were bottle-fed had the lowest risk of death. The recommendation to feed calves as soon as possible after birth is obvious and tube-feeding is recommended only in cases whereby the calf is unable or unwilling to drink colostrum by any other method.

Calves born in the summer months and those born from 3rd or higher parity dams had the greatest BW at 8 weeks of age. Alternatively, calves that had lower 8-week weights were those born in the winter and those born to lower parity dams, suggesting that these calves may need different management practices to reach their growth potential. Although producers cannot control season of birth or dam parity, they can avoid tube-feeding calves as this practice was associated with lower height and weight at 8 weeks compared to bottle-feeding or suckling.

In order to reduce a calf's risk of diarrhea, individual calving pens should be used and cleaned after each calving. Calves born under these conditions are nearly 3 times less likely to have diarrhea compared to calves born in multiple cow calving pens. Calves also need to be fed additional colostrum from 6 to 12 hours of life in order to reduce the risk of developing respiratory disease. Calves fed pasteurized milk of any kind after colostrum were less likely to receive any kind of treatment compared to calves fed unpasteurized milk.

4.4 Implications

Overall, this study fills a gap in the literature by describing the prevalence of FPT in Alberta as well as describing practices commonly used and the quality of colostrum used on the farms. It also is the first study, to the author's knowledge, to run a 3-way comparison of the Brix refractometer, colostrometer, and RID analysis of colostrum using a large sample size and colostrum samples from individual cows that have not been pooled. As well, there is little published data available regarding pre-weaned changes in height; this study adds to the current body of literature by describing weekly gains in height and weight, and the practices associated with these changes.

This study is also valuable as it allows researchers to compare practices used in Western and Eastern Canada, as previously only data was available for the eastern provinces. The descriptive analysis is useful on its own to understand the different practices used around the country, and it can also be used to determine which types of practices may be more beneficial to calf performance.

4.5 Future Perspectives

Possible future studies may further evaluate the role of MR on serum IgG, and if there is any benefit to adding powdered or reconstituted MR to waste and sale milk. Further work could examine the time periods in which calves are introduced to milk after colostrum and if there are differences in calves fed colostrum for more than the initial two feedings compared to calves that are fed colostrum for a longer period of time. No farm in this study pasteurized colostrum – future studies may look at potential differences between calves fed pasteurized or raw colostrum in relation to other management practices. Other future studies may examine how any changes

made to farm practices on the sample farms as a result of this study may affect risk of morbidity, mortality, and preweaned growth.

The goal of raising replacement heifers is to ensure that the future herd will consist of healthy cows that can produce large volumes of milk. The results of this study will give producers knowledge about how to best to manage the newborn calf in the first 24 hours of life to ensure healthy and productive future herds. Hopefully, producers will be encouraged to utilize colostrum measuring tools that are already available, and that they will consider making improvements to their current management practices to better improve the health of neonatal calves.

References

Aust, V., K. Knappstein, H.J. Kunz, H. Kaspar, J. Wallmann, and M. Kaske. 2012. Feeding untreated and pasteurized waste milk and bulk milk to calves: effects on calf performance, health status and antibiotic resistance of faecal bacteria. *Journal of Animal Physiology and Animal Nutrition*. Available at http://www.ncbi.nlm.nih.gov/pubmed/23205592.

Bateman, H.G., T.M. Hill, J.M. Aldrich, R.L. Schlotterbeck and J.L. Firkins. 2012. Meta-analysis of the effect of initial serum protein concentration and empirical prediction model for growth of neonatal Holstein calves through 8 weeks of age. *Journal of Dairy Science*. 95, 363–369.

Beam, A. L., J.E. Lombard, C.A. Kopral, L.P. Garber, A.L. Winter, J.A. Hicks and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *Journal of Dairy Science*. 92, 3973–3980.

Besser, T. E., C.C. Gay, and L. Pritchett. 1991. Comparison of three methods of feeding colostrum to dairy calves. *Journal of the American Veterinary Medical Association*. 198, 419-422.

Besser T.E., and C.C. Gay. 1993. Colostral transfer of immunoglobulins to the calf. *Veterinary Annual*. 33, 53-61.

Bielmann, V., J.N.R. Gillan, A.L. Perkins, L. Skidmore, S. Godden, and K.E. Leslie. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *Journal of Dairy Science*. 93, 3713–3721.

Canadian Dairy Information Centre. 2011a. *Canadian Dairy Information Centre (CDIC) – Dairy Herd Improvement.* Government of Canada. April 4, 2011.

Canadian Dairy Information Centre. 2011b. *Canadian Dairy Information Centre (CDIC) – Number of Farms, Dairy Cows, and Heifers.* Government of Canada. September 16, 2011.

Calloway, C. D., J.W. Tyler, R.K. Tessman, D. Hostetler and J. Holle. 2002. Comparison of refractometers and test endpoints in the measurement of serum transfer status in calves. *Journal of the American Veterinary Medical Association*. 221, 1–4.

Cash, R.S.G. 1999. Colostral quality determined by refractometry. *Equine Veterinary Education*. 11, 36-38.

Chelack, B.J., P.S. Morley, and D.M. Haines. 1993. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves. *The Canadian Veterinary Journal*. 34, 407–412.

Chigerwe, M., J.W. Tyler, J.R. Middleton, J.N. Spain, J.S. Dill, and B.J. Steevens. 2008a. Comparison of four methods to assess colostral IgG concentration in dairy cows. *Journal of the American Veterinary Medical Association*. 233, 761–766.

Chigerwe, M., J.W. Tyler, L.G. Schultz, J.R. Middleton, B.J. Steevens, and J.N. Spain. 2008b. Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. *American Journal of Veterinary Research*. 69, 1158–1163.

Chigerwe, M., J.W. Tyler, M.K. Summers, J.R. Middleton, L.G. Schultz, and D.W. Nagy 2009. Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *Journal of the American Veterinary Medical Association*. 234, 785–789.

Davis C.L., and J.K. Drackley. 1998. *The Development, Nutrition, and Management of the Young Calf*. 1st Ed. Iowa State University Press: Ames, IA.

DeNise S.K., J.D. Robison, G.H. Stott, and D.V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. *Journal of Dairy Science.* 72, 552-554.

Donovan, G., L. Badinga, R.J. Collier, C.J. Wilcox, and R.K. Braun. 1986. Factors influencing passive transfer in dairy calves. *Journal of Dairy Science*. 69, 754–759.

Donovan G., I.R. Dohoo, D.M. Montgomery, and F.L. Benett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Preventive Veterinary Medicine.* 34, 31–46.

Donovan, D.C., A.J. Reber, J.D. Gabbard, M. Aceves-avila, K.L. Galland, K.A. Holbert, L.O. Ely, and D.J. Hurley. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. *American Journal of Veterinary research*. 68, 778-782.

Elizondo-Salazar, J.A., and A.J. Heinrichs. 2009. Feeding heat-treated colostrum to neonatal dairy heifers: effects on growth characteristics and blood parameters. *Journal of Dairy Science*. 92, 3265–3273.

Faber S.N., N.E. Faber, T.C. McCauley. 2005. Effects of colostrum ingestion on lactational performance. *The Professional Animal Scientist*. 21, 420–425.

Fleenor, W.A., and G.H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. *Journal of Dairy Science*. 63, 973–977.

Fleenor, W.A, and G.H. Stott. 1981. Single radial immunodiffusion analysis for quantitation of colostral immunoglobulin concentration. *Journal of Dairy Science*. 64, 740–747.

Frank, N. A, and J.B. Kaneene. 1993. Management risk factors associated with calf diarrhea in Michigan dairy herds. *Journal of Dairy Science*. 76, 1313–1323.

Furman-Fratczak, K., A. Rzasa, and T. Stefaniak. 2011. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. *Journal of Dairy Science*. 94, 5536–5543.

Gay C. C. 1983. The role of colostrum in managing calf health, p. 79–84. In Proceedings of the 16th Annual Convention of the American Association of Bovine Practitioners. American Association of Bovine Practitioners, Opelika, AL. (Ed)

Godden, S.M., S. Smith, J.M. Feirtag, L.R. Green, S.J. Wells, and J.P. Fetrow. 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. *Journal of Dairy Science*. 86, 1503–1512.

Godden, S.M., P. John, J.M. Feirtag, L.R. Green, and S.J. Wells. 2005. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. *Journal of the American Veterinary Medcal Association*. 226, 1547-1554.

Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat-treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G. *Journal of Dairy Science*. 89, 3476–3483.

Godden, S. 2008. Colostrum management for dairy calves. *The Veterinary Clinics of North America. Food Animal Practice*. 24, 19–39.

Godden, S.M., D.M. Haines, K. Konkol, and J. Peterson. 2009a. Improving passive transfer of immunoglobulins in calves. II: interaction between feeding method and volume of colostrum fed. *Journal of Dairy Science*. 92, 1758–1764.

Godden, S.M., D.M. Haines, and D. Hagman. 2009b. Improving passive transfer of immunoglobulins in calves. I: Dose effect of feeding a commercial colostrum replacer. *Journal of Dairy Science*. 92, 1750–1757.

Guy M.A., T.B. McFadden, D.C. Cockrell, and T.E. Besser. 1994. Effects of unilateral prepartum milking on concentrations of immunoglobulin G_1 and prolactin in colostrum. *Journal of Dairy Science.* 77, 3584–3591.

Hancock, D. D. 1985. Assessing efficiency of passive immune transfer in dairy herds. *Journal of Dairy Science*. 68, 163–183.

Heinrichs, A.J., G.W. Rogers, and J.B. Cooper. 1992. Predicting body weight and wither height in Holstein heifers using body measurements. *Journal of Dairy Science*. 75, 3576–3581.

Heinrichs, A.J. 2009. Feeding heat-treated colostrum to neonatal dairy heifers : Effects on growth characteristics and blood parameters 1. *Journal of Dairy Science*. 92, 3265–3273.

Heinrichs A.J., and C. Jones. 2011. *Colostrum Management Tools: Hydrometers and Refractometers.* Department of Dairy and Animal Science. PA, USA; The Pennsylvania State University

Holloway, N.M., J.W. Tyler, J. Lakritz, S.L. Carlson, and J. Holle. 2001. Serum immunoglobulin G concentrations in calves fed fresh and frozen colostrum. *Journal of the American Veterinary Medical Association.* 219, 1–3.

Johnson, J. L., S.M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. *Journal of Dairy Science*. 90, 5189–5198.

Kaske, M., A. Werner, H. Schuberth, J. Rehage, and W. Kehler. 2005. Colostrum management in calves : effects of drenching vs. bottle feeding. *Journal of Animal Physiology and Animal Nutrition.* 89, 151–157.

Kehoe, S. I., B.M. Jayarao, and A.J. Heinrichs. 2007. A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. *Journal of Dairy Science.* 90, 4108–4116.

Kertz A.F., L.F. Reutel, and J.H. Mahoney. 1984. Ad libitum water intake by neonatal calves and its relationship to calf starter intake, weight gain, fecal scores, and season. *Journal of Dairy Science.* 67, 2964-2969.

Klobasa, F., M.C. Goel, and E. Werhahn. 1998. Comparison of freezing and lyophilizing for preservation of colostrum as a source of immunoglobulins for calves. *Journal of Animal Science.* 76, 923–926.

Larson, B.L., H.L. Heary, and J.E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. *Journal of Dairy Science*. 63, 665–671.

Lee S.H., J. Jaekal, C.S. Bae, B.H. Chung, S.C.Yun, M.J. Gwak, G.J. Noh, and D.H. Lee. 2008. Enzyme-Linked immunoabsorbent assay, single radial immunodiffusion, and indirect methods for the detection of failure of transfer of passive immunity in dairy calves. *Journal of Veterinary Internal Medicine.* 22, 212-218.

Liebhaber, M., N.J. Lewiston, M.T. Asquith, L. Olds-Arroyo, and P. Sunshine. 1977. Alterations of lymphocytes and of antibody content of human milk after processing. *Journal of Pediatrics.* 91, 897-900.

Lombard, J. E., F.B. Garry, S.M. Tomlinson, and L.P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. *Journal of Dairy Science.* 90, 1751–1760.

Longenbach, J. and A.J. Heinrichs. 1998. A review of the importance and physiological role of curd formation in the abomasum of young calves. *Animal Feed Science and Technology*.*73*, 85–97.

Margerison J. and N. Downey. 2005. Guidelines for optimal dairy heifer rearing and herd performance. In PC Garnsworthy (Eds.), *Calf and heifer rearing – principles of rearing the modern dairy heifer (307-338).* London, UK: Nottingham University Press.

McGuirk, S.M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *The Veterinary Clinics of North America. Food Animal Practice*. 20, 593–603.

Mechor, G.D., Y.T. Gröhn, L.R. McDowell, and R.J. Van Saun. 1992. Specific gravity of bovine colostrum immunoglobulins as affected by temperature and colostrum components. *Journal of Dairy Science*. 75, 3131–3135.

Morin, D. E., G.C. McCoy, and W.L. Hurley. 1997. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in holstein bull calves. *Journal of Dairy Science.* 80, 747–753.

Morin, D.E., P.D. Constable, F.P. Maunsell, and G.C. McCoy. 2001. Factors associated with colostral specific gravity in dairy cows. *Journal of Dairy Science*. 84, 937–943.

Morin D.E., S.V. Nelson, E.D. Reid, D.W. Nagy, G.E. Dahl, and P.D. Constable. 2010. Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows. *Journal of the American Veterinary Medical Association.* 237, 420-428.

Morrill, K.M., E.M. Conrad, A. Lago, J. Campbell, J. Quigley, and H. Tyler. 2012a. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *Journal of Dairy Science*. 95, 3997–4005.

Morrill, K.M., J.D. Quigley, A. Lago, and H.D. Tyler. 2012b. Estimate of colostral IgG concentration using refractometry without or with caprylic acid fractionation. *Journal of Dairy Science.* 95, 3987–3996.

National Animal Health Monitoring System. Dairy 2007: Heifer Calf Health and Management Practices on U.S. Dairy Operations, 2007. United States Dept. of Agric., Animal Plant and Health Inspection Service, Veterinary Services, October 2007, Fort Collins, CO.

Pithua, P., S.J. Wells, S.M. Godden, and E. Raizman. 2009. Clinical trial on type of calving pen and the risk of disease in Holstein calves during the first 90 d of life. *Preventive Veterinary Medicine*. 89, 8–15.

Poulsen K.P., A.L. Foley, M.T. Collins, and S.M. McGuirk. 2010.Comparison of passive transfer of immunity in neonatal dairy calves fed colostrum or bovine serum-based colostrum

replacement and colostrum supplement products. *Journal of the American Veterinary Medical Association.* 237, 949–954.

Priestley, D., J.H. Bittar, L. Ibarbia, C. Risco, and K.N. Galvão. 2013. Effect of feeding maternal colostrum or plasma-derived or colostrum-derived colostrum replacer on passive transfer of immunity, health, and performance of preweaning heifer calves. *Journal of Dairy Science.* 96, 3247–3256.

Pritchett, L.C., C.C. Gay, T.E. Besser, and D.D. Hancock. 1991. Management and production factors influencing immunoglobulin G_1 concentration in colostrum from Holstein cows. *Journal of Dairy Science*. 74, 2336-2341.

Quigley J.D., C.S.T. III Nyabadza, G. Benedictus, and A. Brand. 1996. Monitoring replacement rearing: Objectives and materials and methods. In Brand A., Noordhuizen J.P.T.M., and Schukken Y.H. (Eds.), *Herd Health and Production Management in Dairy Practice* (75-102), Wageningen, the Netherlands: Wageningen Press.

Quigley, J.D., C.J. Kost, and T.M. Wolfe. 2002. Absorption of protein and IgG in calves fed a colostrum supplement or replacer. *Journal of Dairy Science*. 85, 1243–1248.

Quigley J.D., C.J. Hammer, L.E. Russell, and J. Polo. 2005. Passive immunity in newborn calves. In PC Garnsworthy (Eds.), *Calf and Heifer Rearing – Principles of Rearing the Modern Dairy Heifer* (135-156). London, UK: Nottingham University Press.

Quigley, J.D., A. Lago, C. Chapman, P. Erickson, and J. Polo. 2013. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. *Journal of Dairy Science*. 96, 1148–1155.

Rea, D.E., J.W. Tyler, D.D. Hancock, T.E. Besser, L. Wilson, D.S. Krytenberg, and S.G. Sanders. 1996. Prediction of calf mortality by use of tests for passive transfer of colostral immunoglobulins. *Journal of the American Veterinary Medical Association*. 208, 2047–2049.

Robison J.D., G.H. Stott, and S.K. DeNise. 1988. Effects of passive immunity on growth and survival of the dairy heifer. *Journal of Dairy Science*. 71, 1283-1287.

Roy, J.H.B. 1980. Factors affecting susceptibility of calves to disease. *Journal of Dairy Science*. 63, 650–664.

Stabel, J.R. 2008. Pasteurization of colostrum reduces the incidence of paratuberculosis in neonatal dairy calves. *Journal of Dairy Science*. 91, 3600–3606.

Steinbach G., B. Kreutzer, and H. Meyer. 1981. Effect of heating on the immunobiological value of bovine colostrum. *Monatshefte Fur Veterinarmedizin.* 36, 29-31.

Stelwagen K., E. Carpenter, B. High, A Hodgkinson, and T.T. Wheeler. 2009. Immune components of bovine colostrum and milk. *Journal of Animal Science.* 87, 3-9.

Swali, A., and D.C. Wathes. 2006. Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. *Theriogenology.*66, 1173–1184.

Swan, H., S. Godden, R. Bey, S. Wells, J. Fetrow, and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. *Journal of Dairy Science*. 90, 3857–3866.

Trotz-Williams, L.A., K.E. Leslie, and S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. *Journal of Dairy Science*. 91, 3840–3849.

Tyler J.W., B.J. Steevens, D.E. Hostetler, J. Holle, J. Denbigh. 1999. Colostral IgG concentrations in Holstein and Guernsey cows. *American Journal of Veterinary Research.* 60, 1136-1139.

Tyler, J.W., D.D. Hancock, S.M. Parish, D.E. Rea, T.E. Besser, S.G. Sanders, and L.K. Wilson. 1996. Evaluation of 3 assays for failure of passive transfer in calves. *Journal of Veterinary Internal Medicine*.10, 304–307.

Vasseur, E., F. Borderas, R.I. Cue, D. Lefebvre, D. Pellerin, J. Rushen, and A.M. de Passillé. 2010. A survey of dairy calf management practices in Canada that affect animal welfare. *Journal of Dairy Science*. 93, 1307–1315.

Vasseur, E., J. Rushen, A.M. de Passillé, D. Lefebvre, and D. Pellerin. 2010. An advisory tool to improve management practices affecting calf and heifer welfare on dairy farms. *Journal of Dairy Science*. 93, 4414–4426.

Virtala, A.M., G.D. Mechor, Y.T. Gröhn, and H.N. Erb. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. *Journal of Dairy Science*. 79, 1040–1049.

Virtala, A.M.K., Y.T. Gröhn,G.D. Mechor, and H.N. Erb. 1999. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. *Preventive Veterinary Medicine*. 39, 25–37.

Waltner-Toews, D., S.W. Martin, and A.H. Meek. 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. II. Age and seasonal patterns. *Preventive Veterinary Medicine.* 4, 125–135.

Weaver, D.M., J.W. Tyler, D.C. VanMetre, D.E. Hostetler, and G.M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*. 14, 569–77.

Windeyer, M.C. 2010. Vaccination and risk fctors for bovine respiratory disease in dairy heifer calves. Unpublished doctor of veterinary science thesis. University of Guelph, Guelph, Ontario

Windeyer, M.C., K.E. Leslie, S.M. Godden, D.C. Hodgins, K.D. Lissemore, and S.J. LeBlanc. 2012. The effects of viral vaccination of dairy heifer calves on the incidence of respiratory disease, mortality, and growth. *Journal of Dairy Science*. 95, 6731–6739.

Appendix 1: Individual Calf Record Form

Date: _____

Farm: _____ **Address**: _____

Name of person filling out questionnaire: _____

Calf information:

ID number: _____ **ID number of dam**: _____
Sex: M □ F □ FM □ (freemartin)

Date of birth: _____

Approximate time of birth: 6 pm to 12 midnight □ 12 midnight to 6 am □
6 am to 12 noon □ 12 noon to 6 pm □

1. How many calves were born at this calving? One □ Two (twins) □ Three (triplets) □

2. Is the calf's dam: a 1st-calf heifer □ a 2nd parity cow □ a mature cow □

3. How long was the calf left with the dam?
less than 1 hour □ 1 to 3 hours □ 3-12 hours □
12-24 hours □ more than 24 hours □

4. Was any colostrum fed to this calf in the first 24 hours after birth? Yes □ No □

If yes, how much was fed in the first **6** hours after birth? _____

If yes, how much was fed from **6** to **12** hours after birth? _____

If yes, how much was fed from **12** to **24** hours after birth? _____

5a. What type of colostrum was fed to this calf?

Commercial □ Colostrum from dam □
Colostrum from another cow □ Pooled colostrum from cows in herd □

5b. Was the colostrum: Fresh □ Frozen □

6. How was the colostrum fed to this calf? *(indicate more than one if applicable)*

Calf suckled the dam □ Tube □ Bucket □ Bottle □
Other □ If other, please describe method:
_____

Appendix 1 cont. Individual Calf Record Form

7. Did this calf have a difficult birth? Yes □ No □

If yes, what problems occurred at calving?

_____

8. Did this calf seem healthy at birth? Yes □ No □

If not, what seemed to be wrong with the calf?

---

9. Where was this calf born?
Individual calving pen/area □ General herd housing area □ Outdoors □

10. If this calf was born in an individual calving pen, was the pen cleaned before this birth?
Yes □ No □

If yes, how was it cleaned (ex: dirty bedding removed, hosed down, disinfected)

_____

If yes, how long after cleaning the pen was this calf born? _____

11. What was/will this calf be fed after colostrum?
Whole waste milk □ Whole sale milk □ Milk replacer □
If milk, is it pasteurized? Yes □ No □

12. Has this calf received any of the following additives in milk replacer or other feed?
Coccidiostat (eg: Deccox): Yes □ No □
Ionophores (eg. Rumensin or Bovatec): Yes □ No □
Antibiotics: Yes □ No □ If yes, which one?_____

13. Where is the calf housed?
Individual indoor pen □ Individual outdoor hutch □
Indoor group housing □ Outdoor group housing □

13. Any other comments about this calf?_____

Appendix 2: Summary of responses to individual calf record forms administered to 13 central Alberta dairy farms on calf management

Response Options	Calf (n)	Calf (%)
Sex		
Heifer	466	61.32
Bull	287	37.76
Free Martin	7	0.92
Month of birth		
January	3	0.39
February	119	15.66
March	138	18.16
April	121	15.92
May	144	18.95
June	140	18.42
July	95	12.5
At approximately what time was this calf born?		
18:00 to 0:00	120	15.98
0:00 to 6:00	226	30.09
6:00 to 12:00	204	27.16
12:00 to 18:00	201	26.76
Dam Parity		
1st Calf	293	39.07
2nd Calf	207	27.6
Mature Cow	250	33.33
Number of calves born		
Single	697	96.81
Twin	23	3.19
Did the calf seem healthy at birth?		
Yes	774	98.85
No	9	1.15
Where is the calf housed?		
Individual indoor	525	70.85
Individual outdoor	188	25.37
Indoor group	28	3.78

Appendix 2 cont. Summary of responses to individual calf record forms administered to 13 central Alberta dairy farms on calf management

Response Options	Calf (n)	Calf (%)
How long was the calf left with the dam?		
<1 h	88	11.76
1 to 3 h	321	42.91
>3 to 12 h	283	37.83
>12 to 24 h	54	7.22
>24 h	2	0.27
How many liters of colostrum were fed to this calf in the first 6 h after birth?		
Nothing	11	1.62
< 1 L	2	0.29
1 to 2 L	36	5.29
2 L	330	48.46
2 to 3 L	123	18.06
3 L	85	12.48
3 to 4 L	13	1.91
4L	72	10.57
> 4L	9	1.32
How many liters of colostrum were fed to this calf from 6 to 12 h after birth?		
Nothing	29	5.31
< 1 L	9	1.65
1 to 2 L	95	17.4
2 L	231	42.31
2 to 3 L	94	17.22
3 L	56	10.26
3 to 4 L	1	0.18
4L	20	3.66
> 4L	11	2.01
How many liters of colostrum were fed to this calf from 12 to 24 h after birth?		
Nothing	15	4.09
< 1 L	4	0.96
1 to 2 L	14	3.37
2 L	207	49.76
2 to 3 L	89	21.39
3 L	42	10.1
3 to 4 L	16	3.85
4L	25	6.01
> 4L	1	0.24

Appendix 2 cont. Summary of responses to individual calf record forms administered to 13 central Alberta dairy farms on calf management

Response Options	Calf (n)	Calf (%)
What type(s) of colostrum was fed to this calf in the first 6 hours?		
Nothing	7	0.96
Colostrum replacer	199	27.34
Supplement	1	0.14
Dam	410	56.32
Other Cow	77	10.28
Pooled	32	4.24
Replacer and maternal colostrum	2	0.27
What type(s) of colostrum was fed to this calf from 6 to 12 hours?		
Nothing	24	4.26
Colostrum replacer	20	3.55
Supplement	69	12.26
Dam	391	69.45
Other Cow	51	9.06
Pooled	7	1.24
CR and maternal colostrum	1	0.18
What type(s) of colostrum was fed to this calf from 12 to 24 hours?		
Nothing	15	3.6
Colostrum replacer	5	1.20
Dam	344	82.49
Other Cow	46	11.03
Pooled	7	1.68
Was this colostrum fresh or frozen?		
Fresh	480	63.58
Frozen	27	3.58
Fresh + Frozen	48	6.36
Product	200	26.49
What method was used to feed the calf from 0-6 hours of life?		
Nothing	7	0.96
Suckled	68	9.34
Tube	121	16.62
Bucket	45	6.18
Bottle	487	66.9

Appendix 2 cont. Summary of responses to individual calf record forms administered to 13 central Alberta dairy farms on calf management

Response Options	Calf (n)	Calf (%)
What method was used to feed the calf from 6 to 12 hours of life?		
Nothing	24	4.26
Suckled	34	6.04
Tube	101	17.94
Bucket	38	6.75
Bottle	366	65.01
What method was used to feed the calf from 12 to 24 hours of life?		
Nothing	13	3.15
Suckled	7	1.69
Tube	7	1.69
Bucket	42	10.17
Bottle	344	83.29
Did the calf have a difficult birth?		
No	730	93.23
Yes	53	6.77
Pull + backwards	3	0.38
Pull	20	2.55
Backwards	7	0.89
Twin	15	1.92
Other	8	1.02
Where was the calf born?		
Individual calving pen	256	35.02
Group Maternity Pen	442	60.48
Outdoors	33	4.51
If born in individual pen, was it cleaned before birth?		
Yes	143	19.67
No	128	17.61
N/A	456	62.72
What method was used to clean the maternity pen?		
N/A	457	76.42
Dirty bedding removed	31	5.18
New substrate added	56	9.36
More than one method	54	9.03

Appendix 2 cont. Summary of responses to individual calf record forms administered to 13 central Alberta dairy farms on calf management

Response Options	Calf (n)	Calf (%)
How long prior to calving was it cleaned?		
N/A	457	82.64
< 1 hour	3	0.54
1-3 hours	25	4.52
3-12 hours	48	8.68
12 or more hours	20	3.62
What will the calf be fed after colostrum?		
Waste milk	528	72.33
Sale milk	97	13.29
Waste and Sale	83	11.37
Waste milk, sale milk and milk replacer	21	4.02
Will it be pasteurized?		
Yes	284	40.51
No	417	59.49
Medication added to milk/colostrum		
None	585	86.67
Coccidiostat	21	3.11
Antibiotics	58	8.59
Coccidiostat + Antibiotics	11	1.63