EFFECT OF COLOSTRAL ADMINISTRATION PRACTICES ON SERUM IMMUNOGLOBULIN CONCENTRATION IN DAIRY CALVES

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by
MUNASHE CHIGERWE

Dr. Jeff W. Tyler, Dissertation Supervisor

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

EFFECT OF COLOSTRAL ADMINISTRATION PRACTICES ON SERUM IMMUNOGLOBULIN CONCENTRATION IN DAIRY CALVES

Presented by Munashe Chigerwe

A candidate for the degree of Doctor of Philosophy

And hereby certify that in their opinion it is worthy of acceptance

_____

Jeff W. Tyler, DVM, PhD

_____

John R. Middleton, DVM, PhD

_____

Loren G. Schultz, DVM, MS

_____

Jim Spain, PhD

_____

Barry J. Steevens, PhD

_____

Kristofer J. Hagglund, PhD

DEDICATION

First, all thanks goes to God who has kept me in good health till to this day. I will always praise him. To my Mom, who raised seven children with her strong hands and gave everything she had with love. Mom, you taught me to do the right thing always. I will always love you Mom. To my Dad, thank you for your hard work. To my twin sister Vimbai whom I shared a room in my mother's womb, you will always be special to me. My big brothers Andrew and Dominic, I followed your steps. To my little brothers Kudzanai and Takura, you are great brothers. My little sister Yeukai, your strength inspires me. And to my sister-in-laws, Melody and Grace and brother-in- law Simangaliso, you make our family great. To my nephews Tafadzwa, Tinashe and Tinotenda; you guys can become great people if you stay out of trouble. Nieces Nyasha, Tariro and Tinodaishe; you are beautiful.

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Munashe Chigerwe

Dr. Jeffrey W. Tyler, Dissertation Supervisor

ABSTRACT

Despite the accumulated understanding of the factors which affect passive transfer of colostral immunoglobulins and its recognized importance in dairy calves, approximately 35-40 % of US dairy calves have inadequate passive transfer of colostral immunoglobulins. The objectives of this research were 1) determine the frequency and role of precolostral serum immunoglobulin concentration in dairy calves, 2) Compare various methods in assessing colostral immunoglobulin concentration, 3) determine the amount of colostral IgG required for adequate passive transfer of colostral immunoglobulins in calves fed colostrum by oroesophageal tubing and evaluate other accepted factors on passive transfer of colostral immunoglobulins in dairy bull calves, and 4) determine factors affecting serum IgG concentrations in bottle fed heifer calves.

There was no apparent link between precolostral serum immunoglobulin against common infectious agents known to be transmitted transplacentally. The weight of first milking colostrum as a test method has low sensitivity, thus its use in identifying colostrum with low IgG concentration is not justified. At least 150 to 200 g of colostral IgG is required for adequate passive transfer of colostral immunoglobulins in tube fed calves. Probability of FPT in calves ingesting 3 L at first feeding and 3 L at 12 hours was < 0.05 even at low colostral IgG concentrations bottle fed calves.

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CHAPTER 1

INTRODUCTION

COLOSTRAL SYNTHESIS AND COLOSTRAL CONSTITUENTS IN DAIRY COWS

Colostrum is the secretions of the mammary gland prior to parturition (Kehoe and Heinrichs, 2007). Colostrum contains protein, fat, carbohydrates, water and fat soluble vitamins, growth factors, nucleotides (Kehoe and Heinrichs, 2007), cytokines (Hagiwara et al., 2000), complement (Osburn, 1980) and immunoreactive cells (Kmetz et al., 1970; Smith and Schultz, 1977; Concha et al., 1980).

The major proteins in colostrum include immunoglobulins, lactoferrin, transferrin, α-lactalbumin and β-lactoglobulin, and albumin (Kehoe and Heinrichs, 2007). The major class (85 %-90 %) of immunoglobulin in bovine colostrum is IgG (Larson et al., 1979). Bovine IgG is distributed between two subclasses, IgG_1 and IgG_2 (Larson et al., 1979). The predominant IgG subclass is IgG_1 which can fix complement while IgG_2 cannot fix complement (Beh, 1973). Immunoglobulin G_1 is transferred to colostrum from blood (Larson, 1958; Pierce and Feinstein, 1965; Butler, 1969; Sasaki et al., 1976; Barrington et al., 1997). Transport of IgG into colostrum begins 5 weeks before parturition and peaks at 1 to 3 days before parturition for IgG_1 and IgG_2 respectively (Brandon et al., 1971; Sasaki

et al., 1976). Transport of IgG_1 is facilitated through binding to receptors on IgG_1 binding mammary leukocytes and mammary epithelial cells (Barrington et al., 1997).

Other colostral immunoglobulins are IgM and IgA. Percentage of total colostral immunoglobulin of IgM and IgA are 7 % and 3 %, respectively (Larson et al., 1979). These are largely derived from local synthesis by plasmocytes in the mammary gland (Mach and Pahud, 1971). As a result of its large size, IgM is efficient in bacterial agglutination, complement fixation and opsonization (Pike, 1967). The concentration of IgM is higher in serum than colostrum (Butler et al., 1972; Duncan et al., 1972). However, due to its large size, IgM is more restricted to the intravascular compartment than other immunoglobulin classes (Waldman et al., 1970). Immunoglobulin A neither fixes complement nor opsonizes (Ziparsky et al., 1973), but prevents adherence of bacterial cells to epithelial surfaces (Williams and Gibbons, 1972).

Lactoferrin is glycoprotein that binds iron thereby reduces its availability to microbes (Lee et al., 1998; Elass-Rochard et al., 1998). Lactoferrin also inhibits lipolysaccharide binding to monocytes or macrophages (Lee et al., 1998; Elass-Rochard et al., 1998) and prevents gastrointestinal colonization by bacteria (Wada et al., 1999). Transferrin is a glycoprotein similar to lactoferrin capable of binding and transporting iron (Kehoe and Heinrichs, 2007). Apha-lactalbumin and β-lactoglobulin are synthesized by the mammary gland (Kehoe and Heinrichs, 2007). The functions of β-lactoglobulin are not well understood, but it has been suggested to bind retinol-binding protein and is involved in uptake of vitamin A from the calf's intestine (Papiz et al., 1986). Additionally, β-

lactoglobulin binds long-chain fatty acids *in vitro* and may be involved lipid transport in the mammary gland or the calf's intestine (Diaz de Villegas et al., 1987). Apha-lactalbumin is the functional subunit of lactase and is essential for lactose synthesis in the mammary gland (Perez et al., 1990). Albumin found in milk produced by the mammary tissue has the same amino acid sequence as plasma albumin and its presence has been used as marker for disruption of tight junctions in the mammary gland (Kehoe and Heinrichs, 2007). However, healthy mammary tissue secretes albumin in colostrum at an average concentration of 2.63 ± 0.86 ng/mg (Sanchez et al., 1988). Colostral albumin has similar functions with plasma albumin as a transport protein once absorbed from the calf's intestine.

Neonatal calves require carbohydrate, fat and protein for energy and muscle development (Roy, 1970). Bovine colostral lactose concentration is inversely proportional to other colostral constituents (Kehoe and Heinrichs, 2007). Most mammals' colostrum or milk contains lactose as the predominant carbohydrate and small amounts of oligosaccharides (Nakamura et al., 2003). Bovine colostrum contains less free lactose, but has higher oligosaccharide and sialyglycoconjugate concentrations than bovine milk (Rook and Campling, 1965). Sialylosaccharides and other sialylglycoconjugates are reported to have an antimicrobial effect against bacteria and viruses (Pacitti and Gentsch, 1987; Simon et al., 1997; Rolsma et al., 1998). Bovine sialylosaccharides have been recommended to the pharmaceutical industry as food additives (Nakamura et al., 2003). Colostral fat concentration ranges from 0.3 % to 18 % (Kehoe and Heinrichs, 2007), but the majority of cows normally secrete colostrum with higher fat levels than milk (Parish et al, 1950).

Iincreasing levels of fat in the diet during the dry period does not affect total colostral fat concentration (Weiss et al., 1990, 1992). However dietary manipulation may change fatty acid concentration (Kehoe and Heinrichs, 2007). In one study, calves born to beef cows fed safflower oil, which contains high levels of linoleic acids, had increased ability to maintain their body temperature (Dietz et al., 2003; Lammoglia et al., 1999). This indicates possible effect on calf ability to maintain body temperature by specific fatty acid concentrations in the diet.

Fat soluble vitamins are important in immunity and inadequate absorption of fat soluble vitamins may predispose calves to enteric infections (Kehoe and Heinrichs, 2007). The major colostral fat soluble vitamins include α-tocopherol, β-carotene and retinol (Kehoe and Heinrichs, 2007). Fat soluble vitamin concentrations in colostrum vary among cows and also are dependent on maternal reserves, diet and season (Kehoe and Heinrichs, 2007). Studies examining colostral concentrations of water soluble vitamins or the effect of their supplementation on bovine colostral composition are lacking. Water soluble vitamins supplementations have been reported to improve milk production in lactating dairy cows (Shaver and Bal, 1988; Girard and Matte, 1998).

Colostrum contains growth factors including insulin-like growth factor (IGF-1), transforming growth factor beta-2 (TGF β-2) and growth hormone (Buhler, et al., 1998). Ingestion of colostrum causes hyperplasia of intestinal epithelium and a decrease in crypt to villus ratios, indicating toward a shift to differentiated cells as a result of these growth factors in calf's diet (Buhler et al., 1998). Nucleotides constitute part of the non-protein

nitrogen fraction (Kehoe and Heinrichs, 2007). They consist of pyrimidine and purine nucleotides (Kehoe and Heinrichs, 2007). The role of these nucleotides remains unknown. Bovine colostrum contains cytokines such as interleukin 1, interleukin 6 and tumor necrosis factor alpha (Hagiwara et al., 2000). Exposure of neutrophils to these cytokines amplifies the oxidative burst and activates phagocytosis by bovine polymorphonuclear leukocytes (Sugisawa et al., 2001).

Total plasma complement activity in calves at birth for both classical and alternate pathways is 12 % to 60 % of that in adult bovines (Renshaw and Everson, 1979). Activity of complement increases after ingestion and absorption of colostrum and following *de novo* synthesis by the calf postnatally (Day et al., 1969). Complement reaches adult levels at 6 months of age in calves (Osburn, 1980). Cell-mediated immunity in the neonatal domestic mammals is poorly understood (Banks, 1982). The number of circulating B-lymphocytes at birth in calves is approximately 33 % of that in adult cattle (Banks, 1982) and reaches adult concentration after 20 days (Senogles et al., 1978). Lymphocyte responses reach adult activity by 14 days of age (Rossi et al., 1979). The role of colostral lymphocytes has been hypothesized to provide the neonate a transient local and systemic cell-mediated immunity that reflects antigenic exposure of the dam (Smith and Schultz, 1977). A recent study shows that transfer of live maternal cells from colostrum to the neonate enhances responses to antigens to which the dam had been previously exposed, but not to the antigens for which the dam was naïve (Douglas et al., 2007).

Colostrum has a lower water content compared with milk due to the lower concentration of lactose which results in colostrum being thicker than milk (Ontsouka et al., 2003).

ECONOMIC IMPORTANCE OF COLOSTRUM INGESTION AND ABSORPTION IN DAIRY CALVES

Although colostrum contains several components important to the calf, most studies have concentrated on the role of immunoglobulins. The structure of the placenta restricts the transfer of immunoglobulins from the cow to the fetus. Transport of large molecular weight nutrients depends on how many membranes separate the fetal and maternal blood supply (Senger, 1999). Ruminants have a synepitheliochorial placenta with eight membranes separating fetal blood from the maternal circulation, and thus very low concentration of immunglobulins cross the placenta (Fowden et al., 2006). Consequently calves are born hypogammaglobulinemic, making it essential to ingest and absorb colostral immunoglobulins to acquire passive immunity. The process of ingesting and absorbing colostral immunoglobulins is termed passive transfer of colostral immunoglobulins. The prevalence of failure of passive transfer (FPT) in dairy calves in the US is 35-40 % (USDA-APHIS, 1993; Tyler et al., 1998).

Failure of passive transfer (FPT) is responsible for approximately 50 % of calf death losses on US dairy farms (Tyler, 1999b). Calves with failure of passive transfer have increased mortality due to *Escherichia coli* septicemia (Sawyer et al., 1973). Inadequate ingestion of colostral immunoglobulins affects the calf's health and survival beyond the

neonatal period; calves with FPT have increased rate of mortality until at least 10 weeks of age (Tyler et., 1998). In another study, calves with FPT had 2-times higher odds of pneumonia than calves with adequate passive transfer during the first 3 months of life (Virtala., 1999). Higher serum IgG concentrations (> 1200 mg/dl) at 24 to 48 hours of age in heifer calves are associated with increased daily weight gain up to 180 days of life and higher weaning weights compared to calves with low serum IgG concentration (Robinson et al., 1988). Calves with FPT have lower milk production in their first lactation and are more likely to be culled for low production (DeNise et al., 1989).

FACTORS AFFECTING PASSIVE TRANSFER OF COLOSTRAL IMMUNOGLOBULINS

Several factors affect passive transfer of colostral immunoglobulins. The concentration of colostral immunoglobulins is one of the prime factors for successful transfer of colostral immunoglobulins. There is cow to cow to variation in first milking colostral immunoglobulin concentration (Gay, 1994). A total immunoglobulin mass of 100 g when calves are fed by bottle or oroesophageal tubing has been recommended for adequate passive transfer of colostral immunoglobulins (Besser et al., 1994; Gay 1994; McGuirk and Collins, 2004).

The volume of colostrum ingested by the calf also affects passive transfer of colostral immunoglobulins. A volume of 3 to 4 L is recommended (Besser et al., 1994). However if a fixed amount of colostrum is fed, the immunoglobulin concentration is the only

determinant of total immunoglobulin delivered and higher serum IgG concentrations are achieved by feeding higher IgG colostrum (Morin et al., 1997).

Efficiency of colostral immunoglobulin absorption decreases with increase in the age of the calf (Matte et al., 1982). In a previous study, 65.8 %, 49 %, 11.5 %, 6.7 % and 6.0 % of the ingested colostral immunoglobulin appeared in serum when calves were fed at 6, 12, 24, 36 and 48 hours of age respectively (Matte et al., 1982). Absorption of colostral components into the blood from the small intestine in the first 24-36 hours of life is non-selective and occurs by pinocytosis (Stott et al., 1979). Cessation of transfer of colostral components into the blood decreases rapidly after 12 hours of age with mean closure time at 24 hours of age (Bush and Staley, 1980). Absorption of colostral components may be extended to 36 hours of age if feeding is delayed (Stott et al., 1979). Secretion of immunoglobulins to the intestinal lumen and excretion through feces is major route for clearance of immunoglobulins (Matte et al., 1982; Besser et al., 1988). The transfer of immunoglobulins to the intestinal lumen results in antigen-binding antibody in the intestinal lumen (Besser et al., 1988). The transfer of immunoglobulins into the intestinal lumen has been hypothesized as an explanation for reduction in morbidity and mortality due to enteric disease in calves ingesting and absorbing higher colostral IgG concentration (Besser et al., 1988). Excretion through the kidney also contributes to loss absorbed immunoglobulins (Kickhofen et al., 1971).

Colostrum can be administered to the calf by oroesophageal tubing, nipple bottle or nursing. Colostral immunoglobulin absorption efficiency is improved when calves are fed through the nipple bottle due to the closure of the esophageal groove (Lateur-Rowet and

Breukink, 1983; Adams et al., 1985). However, sufficient absorption of colostral immunoglobulins has been reported in tube fed calves in the absence of closure of the esophageal groove because of rapid flow of colostrum from the forestomachs to the abomasum and small intestine (Lateur-Rowet and Breukink, 1983). Furthermore, there is no statistical difference between serum IgG concentrations of calves fed equal volume and colostral IgG mass using a nipple bottle or oroesophageal tubing (Adams et al., 1985). Reported prevalences of failure of passive transfer (FPT) of colostral immunoglobulins are 61.4, 19.3 and 10.8 % for dairy calves which nurse from their dams, suckled from a bottle and are tube fed, respectively (Besser et al., 1991).

Cows in their third lactation produce first milking colostrum with higher colostral IgG concentration compared to cows in their first or second lactation (Muller and Ellinger, 1981; Tyler et al., 1999a). Colostral immunoglobulin concentrations between 1st and 2nd lactation cows were not significantly different in some studies (Muller and Ellinger, 1981; Pritchett et al., 1992; Tyler et al., 1999a). However, other studies report no difference in colostral immunoglobulin concentration between 1^{st}, 2^{nd} and 4^{th} lactation cows (Muller and Ellinger, 1981) or between 1^{st} and 3^{rd} lactation cows (Pritchett et al., 1992). It is important to note that based on these studies, the practice of discarding colostrum based on parity of the cow is not optimal due to the inconsistency of the study results.

Different breeds of dairy cows produce first milking colostrum with varying immunoglobulin concentration (Muller and Ellinger, 1981; Quigley et al., 1994; Tyler et

al., 1999). Jersey and Aryshire breeds have significantly higher colostral IgG concentration compared with Holsteins (Muller and Ellinger, 1981). Colostral IgG concentration in Guernsey cows are higher compared with Holstein cows (Tyler et al., 1999).

Dairy calves remaining in the presence of the dams have higher serum IgG concentration compared to calves separated from their dams due to increased absorption of immunoglobulins (Selman et al., 1971). However the prevalence of FPT in dairy calves allowed to nurse their dams is very high compared to calves fed colostrum artificially (Besser et al, 1991), thus allowing dairy calves to suckle is not beneficial due to inadequate colostrum volume ingestion and IgG mass ingested (Weaver et al, 2000).

Decreased colostral immunoglobulin absorption has been demonstrated in calves with postnatal respiratory acidosis based on venous blood gas analysis (Besser at al., 1990). Postnatal respiratory acidosis occurs more commonly as a result of prolonged or difficult labor or dystocia (Garry, 1993). However arterial blood analysis by Drewry et al. (1999) demonstrated no differences in ability to absorb colostral immunoglobulins between calves with non-respiratory and respiratory acidosis. Although absorption of colostral immunoglobulin is delayed in hypoxic calves, period of absorption is extended to 40.5 hours from 20 hours in normoxic calves (Tyler and Ramsey, 1991). Thus hypoxia has no effect on absorptive capacity based on serum IgG concentration (Tyler and Ramsey, 1991).

Some management practices can affect transfer of colostral immunoglobulins. Freezing of colostrum for storage does not affect serum IgG concentration in calves (Holloway et al., 2002) but destroys colostral leukocytes (Donovan et al., 2007). Delay in milking the dam after parturition results in reduction in concentration of colostral immumoglobulins and colostrum collected after 2 hours has significantly lower IgG concentrations (Moore et al., 2005). Colostrum pooling will result in dilution of high immunoglobulin, low volume colostrum with low immunoglobulin, high volume colostrum and thus pooling of colostrum is not recommended (Weaver et al., 2000).

Pasteurization of colostrum is used to prevent transmission of infectious pathogens through contaminated colostrum. Pathogens which can be transmitted through milk and colostrum include *Mycobacterium avium* subsp. *paratuberculosis* (Streeter et al., 1995), *Salmonella* (Steele et al., 1997), *Mycoplasma* spp. (Farber et al., 1988; Steele et al., 1997; Walz et al., 1997), Escherichia *coli* (Clarke et al, 1989) and *Mycobacterium bovis* (Grant et al., 1996). Pasteurization of at 63^0 C did not eliminate *Mycobacterium avium* subsp. *paratuberculosis* in 2 out of 18 experimentally inoculated colostrum samples (Meylan et al., 1996). Studies investigating the effect of pasteurization on colostral components and passive transfer in calves report varying results. Ingestion of heat pasteurized colostrum (76^0 C for 15 minutes) resulted in decreased serum IgG and lactoferrin concentrations and neutrophil superoxide production indicating negative effects on passive transfer. (Lakritz et al., 2000). Pasteurization at 63^0 C for 30 minutes reduces colostral IgG concentration (Godden et al., 2003). However serum IgG concentration are not different between calves fed unpasteurized colostrum and calves fed pasteurized colostrum

(Godden et al., 2003). In another study the decrease in colostral immunoglobulin concentration was minimal when colostrum was pasteurized at 63^0 C while substantial decrease in colostral immunoglobulin concentration was reported when pasteurization was performed at 76^0 C (Tyler et al, 2000). However studies have not evaluated the viability of colostral leukocytes or biological activity of the immunoglobulins after pasteurization. Currently only 0.8 % of dairy producers pasteurize colostrum before feeding to the calves (USDA-APHIS, 2007)

Recently colostrum supplements and colostrum replacers have been formulated for use in dairy calves. Effects of colostrum replacers on serum IgG concentration indicate varying results depending on the type of product used due to the differences in IgG concentrations (Quigley et al., 2001; Wereme et al., 2001; Hammer et al., 2004; Foster et al., 2006). None of the studies on colostrum replacers have evaluated viability of leukocytes in the products.

Seasonal variation in passive transfer of colostral immunoglobulins has been reported (Gay et al., 1983). The lowest mean serum IgG concentration in calves was reported in winter and serum IgG concentrations in calves increased during spring and early summer on a farm which fed a fixed volume of colostrum by oroesophageal tubing (Gay et al., 1983). The reasons for this variation remain to be determined.

ASSESSMENT OF IMMUNOGLOBULIN CONCENTRATION IN DAIRY COW COLOSTRUM

Differentiating between low IgG concentration and high IgG concentration colostrum is useful to ensure adequate passive transfer. Several methods have been used to assess bovine colostral IgG concentration. Visual appearance of colostrum and hydrometers are the two most commonly used methods to evaluate colostrum quality in the United States (USDA-APHIS, 2007). Forty-one percent of dairy operations in the US use visual appearance to evaluate colostral quality (USDA-APHIS, 2007). However, visual colostral characteristics are of little value in predicting colostral immunoglobulin concentration (Maunsell et al., 1999). Hydrometer (colostrometer) estimation of colostral IgG concentrations are based on colostral specific gravity (Flennor and Stott, 1980). However colostral specific gravity is more correlated with colostral protein concentration than colostral IgG concentration (Flennor and Stott, 1980; Quigley et al., 1994; Morin et al., 2001). Additionally, colostral specific gravity is affected by several factors including month of calving, season, lactation number (Morin et al., 2001) and colostral temperature (Mechor et al., 1991, 1992). In the US, 43.7 % of dairy producers estimate colostral IgG concentrations using hydrometers. The sensitivity of the hydrometer in identifying low IgG colostrum samples (< 50 g/L) is only 32 % (Pritchett et al., 1994).

Weight of first milking colostrum is inversely related (r = -0.29) to colostral IgG1 concentration (Pritchett et al., 1991). First milking colostrum weighing less than 8.5 kg has significantly higher IgG concentration (Pritchett et al., 1991). In another study, colostral IgG1 concentration was weakly correlated with volume produced by the cow (Maunsell et al., 1999). Refractometry on colostral whey also is used to assess colostral IgG concentration (Molla, 1980). This method was not adaptable for use on farms. An

immunoassay to qualitatively assess colostral immunoglobulins is available (Chigerwe et al., 2005). The immunoassay is relatively expensive compared to use of hydrometers or weight of first milking colostrum. The radial immunodiffusion (RID) is the reference method for assessing colostral immunoglobulin (Flennor and Stott, 1981). The method is not farm adaptable and results from the test are interpreted after 48-72 hours.

ASSESSMENT OF PASSIVE TRANSFER OF COLOSTRAL IMMUNOGLOBULINS IN DAIRY CALVES

Several tests are available to assess passive transfer colostral immunoglobulin in dairy calves. The tests include RID, serum total protein by refractometry, sodium sulfite test, zinc sulfate turbidity test, whole-blood glutaraldehyde coagulation test, enzyme-linked immunosorbent assay (ELISA) and gamma glutamyl transferase (GGT) activity.

The RID is the reference method and measures serum IgG concentration directly (Fahey and McKelvey, 1965). Different serum IgG concentrations for the RID endpoints have been used to define adequacy of passive transfer (McGuire and Adams, 1982; Robinson et al., 1988; Besser et al., 1991; Selim et al., 1995; Tyler et al., 1996; Virtala et al., 1999). The disadvantage of the method is the prolonged time (48-72 hours) before results are available. Studies by McBeath et al (1971) and Tyler et al (1996) report a correlation of 0.71 and 0.84 respectively between serum immunoglobulin concentration and total serum protein. Serum total protein measurement by refractometry measures total globulins and other proteins. A total serum concentration of 5.2 g/L is equivalent to 1000 mg/dL serum

and this total serum IgG concentration or greater endpoint is used to indicate adequacy of passive transfer in clinically normal calves IgG (Tyler et al., 1996). In clinically ill animals a serum total protein endpoint of ≥5.5 g/L is used to indicate adequate passive transfer to account for dehydration (Tyler et al., 1999c). Serum total protein refractometry is the test recommended for herd monitoring of calves for adequate transfer of colostral immunoglobulins (Weaver et al., 2000).

Mixture of serum and a sodium sulfite solution causes precipitation of proteins of high molecular weight including immunoglobulins (Weaver et al., 2000). An 18 % sodium sulfite solution endpoint maximizes test sensitivity and specificity for identifying calves with inadequate passive transfer of colostral immunoglobulins. The zinc sulfate turbidity test is no longer widely used for assessing passive transfer in calves. The percentage with FPT correctly classified with the zinc sulfate test is less than observed with serum total protein refractometry or sodium sulfite test (Tyler et al., 1996). Other limitations of the zinc sulfate turbidity test include misclassification of samples in the presence of hemolysis and the instability of the solution when exposed to atmospheric caborn dioxide (Pfeiffer et al., 1977; Tyler et al., 1996; Hudgens et al., 1996). An ELISA which directly measures serum IgG concentration is available to assess passive transfer (Dawes et al., 2002). The sensitivity and specificity of the immunoassay in detecting calves with inadequate passive transfer of colostral immunoglobulins is 0.93 and 0.88 respectively. The ELISA can be used on farms and does not require additional instrumentation needed for refractometry and sodium sulfate tests.

Increase in serum GGT activity, an enzyme which is synthesized in the mammary gland, is observed after ingestion of colostrum in calves (Perino et al., 1993). Although increased activity of GGT confirms ingestion of colostrum, the association between the activity of the enzyme and serum IgG concentration is low (Perino et al., 1993). While a study by Parish et al (1997) made suggestions for predicting passive transfer status based on age and serum GGT levels, a study by Wilson et al (1999) found serum GGT activity of little value in predicting passive transfer in calves. Use of increase in serum GGT activity in calves is strongly discouraged as a method to assess passive transfer in calves (Weaver et al., 2000).

A whole-blood glutaraldehyde coagulation test used to estimate gamma-globulins in cattle has low sensitivity (0.00 – 0.41) and specificity ranging from 0.85 – 1.00 (Tyler et al., 1996b). The performance of the test in insufficient for routine diagnostic use (Tyler et al., 1996b).

MANAGEMENT OF FAILURE OF PASSIVE TRANSFER OF COLOSTRAL IMMUNOGLOBULINS IN DAIRY CALVES

Neonatal morbidity and mortality is a result of several factors. These include passive transfer status, housing, hygiene and nutrition (Radostits, 1983; Odde, 1988). Thus it is important to partition the mortality attributable to each of the risk factors when investigating calf morbidity and mortality on dairy farms (Tyler et al., 1999b). Consequently intervention strategies will be focused on areas with the greatest impact on

calf health. Calves with FPT will survive if housing, nutrition and hygiene are optimal (Weaver et al., 2000).

Decision to treat calves with FPT should be based on the calf age, value and presence of other risk factors for morbidity and mortality in calves (Weaver et al., 2000).

Management of FPT in clinically normal calves involves transfusion with plasma, serum or whole blood intravenously or intraperitoneally. However treatment is empirical and no controlled studies have evaluated the efficacy of plasma, serum or whole blood on morbidity and mortality in calves with FPT. Prophylactic antibiotics have been suggested as an adjunct treatment in calves with failure of passive transfer (Weaver et al., 2000).

Despite the accumulated understanding of the factors which affect passive transfer of colostral immunoglobulins and its recognized importance in dairy calves, approximately 35-40 % of US dairy calves have inadequate passive transfer of colostral immunoglobulins. The hypothesis for this dissertation is stated as follows:

Null hypothesis: Current recommendations on colostral administration practices and factors affecting passive transfer in dairy calves are sufficient.

Alternative hypothesis: Current recommendations on colostral administration practices and factors affecting passive transfer in dairy calves are insufficient and new recommendations are required.

Chapter 2 tested the hypothesis that presence of precolostral immunoglobulins in dairy calves are related to infectious agent transmitted in utero, calf birth weight, calf sex and season of calving. Chapter 3 tested the hypothesis that the diagnostic utility of an immunoassay kit was adequate in identifying colostral samples with low IgG concentrations (<50g/L). Chapter 4 tested the hypothesis that there was significant

differences in the sensitivity and specificity at different test endpoints of two hydrometers, an electronic refractometer and weight of first milking colostrum in identifying low IgG (<50g/L) colostrum samples. Chapter 5 tested the hypothesis that the recommended total colostral IgG of 100 g was insufficient for adequate colostral immunoglobulin transfer. Chapter 6 tested the hypothesis that calves fed immediately after birth were likely to ingest smaller volumes of colostrum when fed by bottle compared to calves allowed to acclimate for a period of hours.

The specific objectives of this research were 1) determine the frequency and role of precolostral serum immunoglobulin concentration in dairy calves, 2) Compare various methods in assessing colostral immunoglobulin concentration, 3) determine the amount of colostral IgG required for adequate passive transfer of colostral immunoglobulins in calves fed colostrum by oroesophageal tubing and evaluate other accepted factors on passive transfer of colostral immunoglobulins in dairy bull calves, and 4) determine factors affecting serum IgG concentrations in bottle fed heifer calves.

CHAPTER 2

FREQUENCY OF DETECTABLE SERUM IgG IN PRECOLOSTRAL CALVES

Munashe Chigerwe, BVSc, DACVIM; Jeff W. Tyler, DVM, PhD, DACVIM; Dusty W. Nagy, DVM, PhD, DACVIM; John R. Middleton, DVM, PhD, DACVIM

From the Departments of Veterinary Medicine and Surgery (Chigerwe, Tyler, Nagy, Middleton) and Veterinary Pathobiology (Tyler, Middleton), College of Veterinary Medicine, University of Missouri, Columbia, MO 65211.

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ABSTRACT

The objective of the study was to determine the prevalence of detectable serum IgG concentrations in calves prior to ingestion of colostrum and to assess whether a detectable IgG concentration was related to dam parity, calf birth weight, calf sex, season of calving, or infectious agents that can be transmitted transplacentally. One-hundred and seventy Holstein dairy calves were enrolled in the study. Serum samples were obtained from calves prior to ingestion of colostrum and serologic testing for bovine viral diarrhea virus (BVDV) and *Neospora caninum* performed. Relative risk, attributable risk, population attributable risk, and population attributable fraction for calves with a detectable serum IgG concentration attributable to positive results for *N caninum* and BVDV serologic testing were calculated. Logistic regression to determine whether dam parity, calf sex, season of calving, and calf weight were associated with precolostral IgG concentration was performed. Ninety (52.9%) calves had a detectable total serum IgG concentration (IgG ≥ 16 mg/dL). Relative risk, attributable risk, population attributable risk, and population attributable fraction for calves with a detectable serum IgG concentration attributable to positive results for *N caninum* serologic testing were 1.66, 0.34, 0.014, and 0.03, respectively. Calf sex, calf birth weight, and season of calving were not significant predictors for detection of serum IgG in precolostral samples. Prevalence of IgG concentrations in precolostral serum samples was more common than reported elsewhere. There was no apparent link between serum antibodies against common infectious agents that can be transmitted transplacentally and detection of measurable serum IgG concentrations

ABBREVIATIONS

BVDV	Bovine viral diarrhea virus
RR	Relative risk
AR	Attributable risk
PAR	Population attributable risk
PAF	Population attributable fraction
MAP	*Mycobacterium avium* subsp *paratuberculosis*
BLV	Bovine leukosis virus

INTRODUCTION

In primates, birds, carnivores, and rodents, there is transfer of maternal immunoglobulins to the fetus in utero across the placenta or yolk sac membrane (Larson et al, 1979). In ruminants, the maternal and fetal blood supply is separated by a syncytium formed by the syndesmochorial placenta, which prevents transplacental transfer of maternal immunoglobulins to the fetus (Arthur, 1999). Consequently, calves are hypogammaglobulinemic at birth (Besser and Gay, 1994; Drewry et al., 1999).

Precolostral immunoglobulin concentration for specific pathogens can be assessed as a component of a diagnostic investigation in cows that abort (Sawyer et al., 1973). In these circumstances, detection of an appreciable IgG concentration is deemed suggestive of in utero exposure to pathogens.

Detection of low serum concentrations of IgG, IgM, and IgA in calves at birth has been reported (Sawyer et al., 1973; Stott et al., 1979). In 1 of those studies (Stott et al., 1979), 37% of calves had detectable immunoglobulin concentrations in samples obtained before ingestion of colostrum; however, the lower limit for the detection of immunoglobulins was not indicated. The purpose of the study reported here was to determine the prevalence of a detectable serum IgG concentration in dairy calves prior to ingestion of colostrum and to assess whether the prevalence of a detectable precolostral IgG concentration was related to dam parity, calf birth weight, calf sex, season of calving, or

fetal serologic recognition of common infectious agents that can be transmitted transplacentally.

MATERIAS AND METHODS

Animals—One hundred seventy Holstein dairy calves (68 heifer calves and 102 bull calves) born sequentially from cows of various parities with confirmed breeding dates were selected from the University of Missouri Foremost Teaching and Research Dairy for use in the study. This herd used artificial insemination exclusively and recorded all estrus and breeding events. Pregnancy diagnosis was performed on all cows within 45 days after breeding. Consequently, accurate calving dates were available for all cows. Adult cows were vaccinated annually with a multivalent vaccine (infectious bovine rhinotracheitis virus, BVDV type 1 and 2, parainfluenza-3 virus, bovine respiratory syncytial virus, *Campylobacter fetus*, and *Leptospira* spp [serovars Canicola, Grippotyphosa, Hardjo, Pomona, and Icterohaemorrhagiae]) (Bovi-shield Gold FP VL5, Pfizer Animal Health, Exton, PA). Additionally, cows are vaccinated with *Escherichia Coli* bacterin (*Escherichia coli* J-5 strain, Boehringer Ingelheim, St Joseph, MO). at the end of lactation, 1 month before parturition, and at parturition. Calves are vaccinated with a multivalent vaccine (infectious bovine rhinotracheitis virus, BVDV type 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus) (Bovi-shield Gold 5, Pfizer Animal Health, Exton, PA) at 2, 4, 6 and 12 months of age. The research study was approved by the University of Missouri-Columbia Animal Care and Use Committee.

Sample collection—Only calves whose births were observed were enrolled in the study. After parturition, each calf was immediately separated from its dam, weighed, and assigned a unique identification number. Heifer and bull calves were enrolled in the study. Serum samples were obtained from all calves within 1 hour after birth but before calves were provided colostrum. Samples were stored at –20°C until processed for serum immunoglobulin determinations and serologic testing to detect antibodies against BVDV and *Neospora caninum*.

Serum IgG determination—Total serum IgG concentrations were determined by adaptation of a radial immunodiffussion technique described elsewhere (Tyler et al., 1999; Hostetler et al., 2003). Radial immunodiffusion plates for measuring IgG were prepared by dissolving 1% agarose (Agarose, Sigma-Aldrich Co, St Louis, MO) Louis, MO) in a sodium barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) containing 0.1% sodium azide (Sodium azide, Sigma-Aldrich Co, St Louis, MO). Rabbit–anti-bovine IgG (1%) (Anti-bovine IgG (whole molecule) developed in rabbit IgG fraction of anti-serum, Sigma-Aldrich Co, St Louis, MO) was added to thawed agarose solution. Eleven milliliters of the agarose solution was added to 10-cm Petri dishes. After the agarose solidified, 3-mm wells were cut in the agar. Serum samples were diluted 1:2 with barbital buffer, and 5 μL was inoculated in each well. Plates were incubated for 72 hours at 23°C, and diameter of the zone of precipitation was then recorded. Sample IgG concentrations were determined by comparing the diameter of zones of precipitation with a standard curve generated by use of serial dilutions of a bovine IgG standard (Bovine IgG (lyophilized), (Sigma-Aldrich Co, St Louis, MO).

The regression equation generated for this technique can accurately predict inoculum IgG concentration. Lower detection limit of the radial immunodiffusion assay was 16 mg/dL.

Serologic testing—A serum neutralization test for BVDV type 1 was performed**.** Serologic titers against BVDV were determined by adaptation of a technique reported elsewhere (Deregt et al, 1992). Briefly, heat-inactivated (56°C) serum samples were titered in duplicates, starting at a dilution of 1:4. The serum-virus mixture was incubated for 1 hour at 37°C. Trypsinized Madin-Darby bovine kidney cells (0.05 mL of cell suspension) in medium were added to microtiter plates containing growth media (2mM L-glutamine and Earle's buffered saline solution containing 1.5 g of sodium bicarbonate/L, 0.1mM nonessential amino acids, 1.0mM sodium pyruvate, and 10% horse serum). Microtiter plates were incubated for 48 to 72 hours at 37°C in a humid, 5% carbon dioxide environment. Test sample wells were assessed for cytopathic effect at 48 to 72 hours by use of an inverted ocular of a light microscope. Endpoint antibody titer was defined as the highest dilution of serum at which virus neutralization was detected.

A commercial competitive ELISA (*Neospora caninum* antibody test kit, cELISA, VMRD, Pullman, WA) for detecting antibody against *N caninum* also was performed on all serum samples from calves, as described elsewhere (Baszler et al., 2001). Diagnostic sensitivity and specificity of the competitive ELISA was 97.6% and 98.6%, respectively, by use of cutoff value of 30% inhibition (Baszler et al., 2001).

Data analysis—The proportion of calves with a detectable serum IgG concentration and the mean ± SEM serum IgG concentration in calves with a detectable IgG concentration were calculated. The RR, AR, PAR, and PAF for calves that had a detectable serum IgG concentration attributable to a positive result for serologic testing to detect *N caninum* or BVDV also were calculated (Smith, 1995; Dohoo et al., 2003). Forward stepwise logistic regression was used to determine whether dam parity, calf sex, calf weight, and season of calving were associated with detection of precolostral IgG concentrations (dependent variable) (PROC LOGISTIC, SAS for Windows, version 9.13, SAS Institute Inc, Cary, NC). Detection or nondetection of an IgG concentration was treated as a binomial variable (0, serum IgG was not detectable; 1, detectable serum IgG concentration). Dam parity was treated as a categoric independent variable (first lactation, second lactation, and ≥ 3 lactations). Calf sex was treated as an independent binomial variable (0, male; 1, female). Calf weight was treated as a continuous independent variable. Season of calving was treated as an independent categoric variable and was divided into 4 seasons (winter [December, January, and February], spring [March, April, and May], summer [June, July, and August], and autumn [September, October, and November]). Variables were included when the value to enter was $P < 0.05$ by use of the Wald-χ statistic (PROC LOGISTIC, SAS for Windows, version 9.13, SAS Institute Inc, Cary, NC). The variable with the smallest *P* value to enter was selected at each step. The goodness-of-fit of the final model was estimated through the Hosmer-Lameshow χ^2-test statistic (PROC LOGISTIC, SAS for Windows, version 9.13, SAS Institute Inc, Cary, NC)

RESULTS

The regression equation generated by comparing the diameter of zones of precipitation with a standard curve generated by use of serial dilutions of a bovine IgG standard accurately predicted IgG concentrations (R^2 = 0.98; **Figure 1)**. Ninety (52.9 %) calves had a detectable serum IgG concentration (IgG ≥ 16 mg/dL). Mean ± SEM serum IgG concentration for calves that had a detectable IgG concentration was 63.9 ± 4.9 mg/dL. The range of serum IgG concentrations in calves with a detectable serum IgG concentration prior to ingestion of colostrum was 16 to 234 mg/dL. The distribution of serum IgG concentrations in calves was determined **(Figure 2)**.

One of 170 (0.6 %) calves had positive results for a serum neutralization test (serum neutralization titer of 1:4) to detect BVDV type 1. Seven of 170 (4.1%) calves had positive results when serologically tested for *N caninum*. All serum samples that had positive results for BVDV type 1 or *N caninum*, except for 1 (positive result for *N caninum*), had a detectable serum IgG concentration. The RR, AR, PAR, and PAF for calves that had a detectable serum IgG concentration attributable to a positive result for *N caninum* were 1.66, 0.34, 0.014, and 0.03, respectively. The RR, AR, PAR, and PAF for calves that had a detectable serum IgG concentration attributable to a positive result for BVDV were not reported because only 1 calf had a positive result for BVDV for the serum neutralization test.

A logistic regression equation for the probability that a precolostral calf would have a detectable IgG concentration was generated: $P_{IgG} = 1/(1 + \exp[0.1457 - \{0.7996 \text{ X } 2\text{nd parity}\}])$, where P_{IgG} is the probability of a detectable IgG concentration, and exp is the exponential function. The probability that a precolostral calf from a second-lactation cow would have a detectable IgG concentration was 0.66, whereas the probability that a precolostral calf from a first-lactation cow or a cow in her third or greater lactation would have a detectable IgG concentration was 0.46. Thus, precolostral calves from second-lactation cows were 1.5 times as likely to have a detectable IgG concentration (RR = 0.66/0.46 = 1.4) than were precolostral calves from a first- lactation cow or a cow in her third or greater lactation. Calf sex, calf birth weight, and season of calving were not significant predictors of a detectable precolostral serum IgG concentration.

DISCUSSION

A detectable serum IgG concentration in calves before ingestion of colostrum indicates transplacental transfer, de novo IgG production by the fetus after transplacental exposure or transcervical exposure, or an invalid assay for measurement of serum IgG concentrations. The linear regression equation for predicting IgG concentration in serum samples was generated by use of a broad range of standard concentrations of bovine IgG. Results clearly indicated a linear dose-response relationship and lack of systematic error at IgG concentrations as low as 16 mg/dL **(Figure 1)**. Hence, transplacental transfer of IgG or production of IgG by the fetus appears to be more likely. Possible reasons for the wide range of IgG concentrations include the type of antigen, duration of exposure, and age of the fetus at the time of exposure to the antigen. An increase in IgG and IgM

concentrations has been detected in serum samples obtained from precolostral calves naturally or experimentally infected with BVDV, *Coxiella burnetii*, *Vibrio fetus*, *Chlamydia* organisms, and blue tongue virus, compared with immunoglobulin concentrations for control bovine fetuses and uninfected precolostral calves (Sawyer et al., 1973). It should be mentioned that only serum IgG was determined in this study, while serum IgM and IgA concentrations were determined in the previous study (Sawyer et al., 1973). Additionally, IgG subclassification was not performed. To further evaluate potential causes of precolostral serum IgG concentrations, infectious agents that can be transmitted transplacentally were considered. Common infectious agents that can be transmitted in utero in cattle include *N caninum*, (Hall et al., 2004), MAP (Nielsen et al., 2002), BLV (Everman et al., 1987) and BVDV (Dean et al., 2003). The study herd consisted of 200 lactating dairy cows. The seroprevalence of MAP for the study herd was 6%, as determined on the basis of results for an ELISA (CSL Veterinary/Biocur Animal Health, Omaha, NE). Reported rates of transplacental MAP transmission range from 21% to 37% in cows with clinical signs of paratuberculosis (Lawrence, 1956; Doyle, 1958; Seitz et al., 1989). However, a low prevalence (8.6 %) of fetal infections has been detected in calves born to cows without clinical signs of paratuberculosis (Sweeney et al., 1992). Consequently, MAP transmission and de novo serologic responses are unlikely causes of common detectable serum IgG concentrations in precolostral calves. Reported rates of transplacental BLV transmission range from 3% to 20% (Evermann et al., 1987), with lower estimates reported more often (Jacobbsen et al., 1982; Thurmond et al., 1983; Lassauzet et al., 1991). Accepting that there was an in utero transmission rate of 10%, as well as a reported BLV prevalence of 80% in this herd (Nagy et al 2003), it would appear

that BLV infection was an unlikely cause of serum IgG concentrations in precolostral calves because only 8% of calves would have been infected at birth. In addition, another study (Nagy et al., 2007) that involved this herd revealed a transplacental transmission rate of 0% in 32 cows. It is important to mention that serologic tests used in the study reported here were qualitative. Thus, it is possible that the serologic tests may have required a higher IgG concentration than was detected in our study to be considered serologically positive. Consequently, precolostral IgG concentrations against the agents tested may have existed but not been detected by the serologic assays used in the study; thus, the number of calves with IgG attributable to each of the agents may have been underestimated.

Cattle in the study herd were annually vaccinated against BVDV by use of a multivalent modified-live vaccine that contained both type 1 and 2 (Bovi-shield Gold FP VL5, Pfizer Animal Health, Exton, PA). All cattle, regardless of pregnancy status, were vaccinated at that time. Only 1 (0.6%) calf had positive results when tested to detect antibodies against BVDV type 1. If a positive serologic result was attributable to transfer of immunoglobulins from dam to calf in utero as a result of vaccination, all calves should have had positive results for BVDV type 1. Vaccination with BVDV type 1 protects a fetus from infection with heterologous virulent strains (Dean et al., 2003). Results for that single calf suggest that in utero BVDV infection and subsequent response to the infection in utero by fetuses is an uncommon event in this herd.

Seroprevalence for *N caninum* varies with country, region, and type of serologic test used (Dubey, 2005; Staubili et al., 2006). Historical prevalence of *N caninum* on the farm for the study reported here was 50%, as determined on the basis of whole-herd serologic testing performed before study samples were acquired. Hence, 85 precolostral calves typically would have been expected to be seropositive. However, detected rates of vertical transmission vary (Dubey et al., 2006) and infection can be transmitted intermittently (Guy et al., 2001). For any 100 calves in the herd, only 2 calves had detectable serum IgG concentrations attributable to positive results for *N caninum* serologic testing (PAR = 0.014). Consequently, *N caninum* was considered an unlikely cause of the high prevalence of detectable precolostral serum IgG concentrations.

The time at which a bovine fetus becomes immunocompetent ranges from 145 to 200 days of gestation (Dean et al., 2003; Osburn et al., 1982). Results of the study reported here suggested that a significant proportion of calves are gammaglobulinemic at birth prior to ingestion of colostrum. Calves from second-lactation cows were 1.4 times as likely to have a detectable precolostral IgG concentration than were calves from first-lactation cows or cows in their third or greater lactation. The cause for this result remains unknown. It should be mentioned that another study (Stott et al., 1979) as well as the study reported here were performed in single herds. Variation among herds with regard to prevalence of precolostral IgG concentrations may exist. On the basis of our results, it appeared that in utero production of detectable concentrations of IgG was more common than has been reported. Serologic testing was unable to identify links to any of agents that are believed to be commonly transmitted transplacentally in cattle. Consequently, fetal

production of IgG was associated with transplacental infection of a specific undetermined pathogen or, alternatively, should be considered a common event associated with in utero exposure and response to a broad range of antigens.

CHAPTER 3

EVALUATION OF A COW-SIDE IMMUNOASSAY KIT FOR ASSESSING IgG CONCENTRATION IN COLOSTRUM

Munashe Chigerwe, BVSc; Maisie E. Dawes, DVM; Jeff W. Tyler, DVM, PhD, DACVIM; John R. Middleton, DVM, PhD, DACVIM; Malantus P. Moore, BS; Dusty M. Nagy, DVM, MS, DACVIM

From the Departments of Veterinary Medicine and Surgery (Chigerwe, Dawes, Tyler, Middleton, Moore), Veterinary Pathobiology (Tyler, Middleton, Dawes), College of Veterinary Medicine, University of Missouri, Columbia, MO 65211; and the Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana IL 61802 (Nagy). Mr Moore's current address is School of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088.

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ABSTRACT

The objective of the study was to determine the sensitivity and specificity of a cow-side immunoassay kit fro assessing IgG concentration in colostrum. Colostrum samples from first, second, third milkings and colostrum from seventy-six dairy and 11 beef cows were collected. Colostral IgG was performed using radial immunodiffusion. The immunoassay was performed according to the manufacturer's instructions, and sensitivity and specificity were calculated by comparing results of the immunoassay (positive vs negative) with results of immunodiffusion (< 50 g/L vs ≥ 50 g/L). One-hundred and thirty five colostrum and milk samples were collected. Mean ± SD colostral IgG concentrations, determined by means of radial immunodiffusion for dairy and beef cows were 65.4 ± 51.4 g/L and 114.8 ± 42.7 g/L, respectively. Mean IgG concentrations for first, second and third-milking colostrum samples and milk samples were 92 ± 49.0 g/L, 74.6 ± 45.1 g/L, 47.5 ± 32 g/L, and 6.8 ± 3.8 g/L, respectively. Sensitivity of the immunoassay (ie, percentage with IgG concentration < 50 g/L with a positive immunoassay result) was 93 %, and specificity (ie, percentage with IgG concentration ≥ 50 g/L with a negative immunoassay result) was 76 %. The results suggest that the immunoassay kit is an acceptable cow-side test to identify colostrum samples with IgG concentrations < 50 g/L. The immunoassay is useful in screening colostrum for adequate IgG concentration before feeding to calves or storage.

INTRODUCTION

Ingestion and absorption of colostral immunoglobulins is an important determinant for calf health, survival and productivity (Besser and Gay, 1994; Virtala et al., 1999; Tyler et al., 1999b). Adequate passive transfer of colostral immunoglobulins hinges upon the formation of a high immunoglobulin concentration colostrum, ingestion of an adequate colostral volume by the calf and absorption of immunoglobulin by the calf's intestine (Besser and Gay 1994; Maunsell et al., 1999). To minimize inadequate passive transfer it has been recommended that calves receive a minimum of 100g of colostral IgG in the first 12 hours of life (Stott et al., 1979; Bush and Staley, 1980; Matte et al., 1982; Weaver et al., 2000; Besser et al., 1991). Optimum absorption of colostral immunoglobulin across the intestinal epithelium occurs within the first 4 hours of life and declines rapidly after 12 hours with minimal absorption occurring after 24 hours of life (Stott et al., 1979; Bush and Staley, 1980; Matte et al., 1982; Weaver et al., 2000).

Several methods have been recommended for assessing colostral IgG concentration. Refractometry on colostral whey has been described for estimating colostral immunoglobulin concentration (Molla, 1980). This procedure has limited utility because the preparation of the whey takes several hours and may delay the feeding of colostrum past the time of optimal IgG absorption (Molla, 1980). Furthermore, this technique is not readily adapted to on-farm conditions.

Hydrometers may be used to estimate colostral immunoglobulin concentration based on colostral specific gravity (Flennor and Stott, 1980: Pritchett et al., 1991) Although the hydrometer has been widely used in the field as a cow-side test, studies have shown that colostral specific gravity is more closely related to colostral protein concentration than colostral IgG concentration (Flennor and Stott, 1980; Morin et al., 2001). Colostral specific gravity also varies with temperature, (Mechor et al., 1991, 1992) month of calving, year of calving, milk protein yield in the previous lactation, cow breed (Quigley et al., 1994; Nardone et al., 1997; Morin et al., 2001) and lactation number (Oyeniyi and Hunter, 1978; Devery-Pocius and Larson, 1983; Morin et al., 2001). Defining the sensitivity of the hydrometer as the ability to correctly identify colostral samples with less than 50g/L of IgG, the sensitivity of the hydrometer is only 32% (Pritchett et al., 1994). Consequently 2 of every 3 colostral samples with less than 50g/L are classified as acceptable by this procedure (Pritchett et al., 1994). Test sensitivity can be improved by increasing the cut-off point specific gravity above the recommended 1.050. However, this adaptation results in excessive numbers of colostral samples with IgG concentrations ≥ 50g/L being falsely classified as having inadequate IgG concentration (Pritchett et al., 1994).

First milking colostrum weighing less than 8.5 kg in Holsteins have been reported to have higher IgG concentrations (Pritchett et al., 1991), hence, discarding first milking colostrum weighing more than 8.5 kg would likely increase the proportion of colostrum with acceptable IgG concentration.

The purpose of this study was to evaluate the sensitivity and specificity of a commercially available cow-side immunoassay in the detection of inadequate colostral IgG concentrations. For the purposes of this study inadequate IgG concentration was defined as colostral IgG concentration less than 50g/L.

MATERIALS AND METHODS

Sample collection─ Colostrum and milk samples were collected from dairy and beef cows hospitalized in the University of Missouri or University of Illinois Large Animal Clinics or housed at the university farms of the same institutions. A colostrum sample was collected from each quarter into one milking collecting tube. Immediately after colostrum collection a commercially available immunoassay (Colostrum Bovine IgG Quick test kit, Midlands Bio-Products, Boone, IA) was performed following the manufacturer's directions. The appearance of 2 lines in the test cassette was indicative of a colostral sample with <50g/L and the appearance of a single line in the test cassette was indicative of a colostral sample with ≥50g/L. Colostrum samples were then stored frozen at -20ºC for later IgG determination by radial immunodiffusion (RID).

Determination of colostral IgG─ Colostrum IgG concentration was determined by RID. The RID was performed as previously described (Tyler et al., 1999). Briefly, assay plates were prepared by dissolving 1% agarose (Agarose, Sigma-Aldrich Co, St Louis, MO) in sodium barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) containing 0.1% sodium azide (Sodium azide, Sigma-Aldrich Co, St Louis, MO). Rabbit anti-bovine

(1%) (Anti-bovine IgG (whole molecule) developed in rabbit IgG fraction of anti-serum, Sigma-Aldrich Co, St Louis, MO) was added to the agarose solution and the solution was transferred to 10-cm Petri dishes (11ml/dish). After the agarose solidified, 3-mm-diameter wells were cut in the agar. Colostrum samples were diluted 1:120 with barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) and 5µl aliquots of the diluted samples were added to the wells in the assay plates. The plates were incubated for 72 hours at 23ºC and the diameter of the observed zone of precipitation was recorded. Colostral IgG concentration of test samples was then determined by comparing the diameter of the zone of precipitation with a standard curve generated by assaying serial dilutions of a commercially available standard solution of bovine IgG (Bovine IgG (lyophilized), Sigma-Aldrich Co, St Louis, MO). The regression equation generated in this manner accurately predicted inoculum IgG concentration ($r^2 = 0.978$).

Data analysis─ Mean and standard deviation of colostral IgG concentration were calculated for beef and dairy cow colostrum, and for first ,second, third milking colostrum and milk samples. Colostrum samples were considered to have adequate immunoglobulin concentration if colostral IgG was ≥50g/L as determined by RID. Sensitivity and specificity of the immunoassay were determined by comparing the results of the immunoassay with the results of the RID using two-way frequency tables (Tyler and Cullor, 1989). A test result was considered positive (inadequate) for the detection of poor quality colostrum if the colostral IgG was <50g/L and a test result was considered negative (adequate) if the colostral IgG was ≥50g/L. Confidence intervals (95%

confidence interval, CI) for sensitivity and specificity we were also constructed (Daniel, 1983).

RESULTS

One hundred and thirty five colostrum samples were collected from 76 dairy and 11 beef cows of varying parities. In some instances first, second and third milking colostrums were obtained from a single cow. Samples included 70 first milking colostrum, 27 second milking colostrum, 16 third milking colostrum and 22 milk samples. Thirty nine cows were in their first lactation, 20 in the second lactation and 28 were in the third or greater lactation. Mean ± SD colostral IgG concentration as determined by RID for dairy and beef cows were 65.4 ± 51.4 and 114.8 ± 42.7 g/L respectively. Mean IgG concentration for first-, second-, and third milking colostrum samples were 92 ± 49 g/L, 74 ± 45.1 g/L, 47.5 ± 32 g/L and 6.8 ± 3.8 g/L, respectively. Of the 55 samples with IgG concentration < 50 g/L, 12 (22%) were first- milking colostrum samples, 10 (18%) were second-milking colostrum samples, 11 (20%) were third-milking colostrum samples and 22 (40%) were milk samples. Twelve of 70 (17%) first-milking colostrum samples, and 10 of 27 (37%) second-milking and 11 of 16 (69%) third milking colostrum samples had IgG concentrations < 50 g/L. All 22 milk samples had IgG concentrations < 50g/L. The immunoassay had a sensitivity of 93% (95% CI, 89% to 97%) and specificity of 76% (69% to 83%) **(Table 1)**.

DISCUSSION

Results of the present study suggested that the immunoassay kit had acceptable sensitivity to identify colostrum samples with IgG concentrations < 50 g/L. Even though the specificity of the immunoassay kit was less than optimal, our results suggests that the immunoassay kit was an acceptable cow-side test to identify colostrum samples with IgG concentration < 50 g/L. The immunoassay is sensitive for the detection of colostrum with IgG concentrations less than 50g/L. The specificity of the immunoassay is acceptable. Practically, the use of the immunoassay will result in low numbers of false negatives. A specificity of 76% means 24% samples with adequate IgG concentration (≥ 50g/L) could be classified as inadequate, which could limit the amount of colostrum available to feed calves on some farms. By contrast, the specificity of the hydrometry, when specific gravity of 1.050 is used as the cutoff, is 97% (Pritchett et al, 1991). On the other hand, the sensitivity of the immunoassay kit in the present study (93%) was substantially high the reported sensitivity of the hydrometer (32%) (Pritchett et al., 1994), suggesting that the immunoassay kit is better for identifying colostrum samples with inadequate IgG concentrations.

One limitation of the immunoassay kit is that it only yields positive (< 50g/L) and negative (≥ 50 g/L) and does not provide an estimate of the actual IgG concentration. It should be noted that the cutoff point defining adequate colostral IgG concentration is arbitrary. Lower colostral IgG concentration may still permit adequate passive transfer if sufficient colostral volumes are fed in a timely manner.

Predictive values of positive and negative tests are prevalence dependent (Tyler and Cullor, 1989). The samples in this study were collected from different farms and breeds with different prevalence of colostrum samples with less than 50g/L. Thus predictive values in this study are not useful in assessing performance of the immunoassay. Mean colostral IgG concentrations for cattle of various parities were not calculated because multiple samples were collected from some cows.

An interesting finding in the present study was the relative high proportions of second- and third-milking colostrum samples with apparently adequate IgG concentrations. Seventeen of 27 (63%) second-milking colostrum and 5 of 16 (31%) third milking colostrum samples had IgG concentrations ≥ 50g/L. This suggests the immunoassay could be used to screen second- and third-milking colostrum samples to determine whether they would be an adequate source of colostrum for calves.

The immunoassay is relatively cheap and offers on site results compared to the RID since a result can be read in 20 minutes using the test. Colostral specific gravity varies with colostral temperature (Mechor et al., 1991, 1992), hence one of the disadvantages of a hydrometer. At the manufacturer's recommendation, the result of immunoassay is not dependent on colostral temperature. Results of the study suggest that the immunoassay is useful in testing colostrum before feeding the calf or storage.

CHAPTER 4

COMPARISON OF FOUR METHODS TO ASSESS COLOSTRAL IgG CONCENTRATION IN DAIRY COWS

Munashe Chigerwe, BVSc, DACVIM; Jeff W. Tyler, DVM, PhD, DACVIM; John R. Middleton, DVM, PhD, DACVIM; James N. Spain, PhD; Jeffrey S. Dill; Barry J. Steevens, PhD

From the Departments of Veterinary Medicine and Surgery (Chigerwe, Tyler, Middleton, Dill), Veterinary Pathobiology (Tyler, Middleton) and Animal Sciences (Steevens, Spain) University of Missouri-Columbia, 900 East Campus Drive, Columbia, MO 65211, USA

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ABSTRACT

The objective of the study was to determine the sensitivity (Se) and specificity (Sp) of 4 methods to assess colostral IgG concentration in dairy cows and to determine the optimal endpoint for each method. Composite colostrum samples were collected from 171 Holstein dairy cows within 2 hours after parturition. Colostral IgG concentration was assessed using weight of first milking colostrum, two hydrometers and an electronic refractometer. Test results were compared with colostral IgG concentration as determined by radial immunodiffusion. Sensitivity and specificity of each method in detection of low colostral IgG concentration (<50g/L) was calculated across the range of outcomes for the four methods. Test endpoints were selected to maximize Se and Sp. The 2 hydrometers and the electronic refractometer performed similarly in the classification of colostrum with low IgG concentration. First milking colostrum weight had Se of 0.42 and Sp of 0.74 in the detection of low colostral IgG concentration using an endpoint of 8.5 kg. First milking colostrum weight was far less sensitive in the detection of poor quality colostrum, but had specificity similar to that of other methods examined. The two hydrometers and the electronic refractometer are acceptable test methods for screening colostrum for low IgG concentration; however, the instrument defined scale of both hydrometers overestimated colostral IgG concentrations. The weight of first milking colostrum as a test method has low sensitivity, thus its use in identifying colostrum with low IgG concentration is not justified.

INTRODUCTION

Importance of ingestion and absorption of colostral immunoglubins for calf survival and health has been documented (Besser and Gay, 1994; Tyler et al., 1999b; Virtala et al., 1999). Failure of passive transfer of colostral immunoglobulins is responsible for approximately one-half of calf death losses on US dairy farms (Tyler et al., 1999b). Previous reports and reviews suggest a minimum of 100g of immunoglobulin G (IgG) in colostrum is required for adequate passive transfer (Besser et al., 1994).

Differentiating between low IgG concentration and high IgG concentration colostrum is one useful tool to improve calf health. Several methods have been used to assess bovine colostral IgG concentration. Refractometry on colostral whey (Molla, 1980), hydrometers (Fleenor and Stott, 1980; Pritchett et al., 1994), weight of first milking colostrum (Pritchett et al., 1991) and an immunoassay (Chigerwe et al., 2005) may be used. A digital electronic refractometer has been marketed to assess equine colostral IgG concentration (Cash, 1999). This refractometer has not been used to evaluate bovine colostral IgG concentration. However, none of these procedures has gained widespread acceptance and application.

The purpose of this study was to determine the sensitivity and specificity of four methods adapted to on farm use to assess colostral IgG concentrations. The methods assessed included two commercially available hydrometers, weight of first milking colostrum and

a commercially available electronic refractometer. The optimal test endpoint for each method also was assessed.

MATERIALS AND METHODS

Animals– One hundred and seventy-one composite colostrum samples were collected from Holstein cows within 2 hours of observed parturition over a period of 3 years. The research study was approved by the University of Missouri-Columbia Animal Care and Use Committee. All cows were housed at the University of Missouri Foremost Dairy. All dry cows are housed in a single free stall barn and fed a total mixed ration. However the dry cows have access to grass pasture. All cows receive a commercially available intramammary dry cow therapy which incorporates a latex teat sealant. This practice minimizes the potential for colostrum to leak from the mammary gland. Cows which did not produce colostrum or had missing values for any of the test methods were excluded from the study. Subsequent colostral samples from lactations from the same cow were treated as independent events.

Experimental protocol– All cows received 20 IU of oxytocin before milking to ensure complete milking of the udder. Composite colostrum sample collection was performed using a portable bucket milking machine and the weight of the colostrum (kg) was determined. A 20 ml, mixed colostrum sample was obtained from the milking bucket. Temperature of the colostrum was determined using a digital thermometer (Traceable thermometer, Control Company, Friendswood, TX). Colostrum samples were evaluated

using hydrometer #1 (Colostrometer, Biogenics, Mapleton, OR), hydrometer #2 (Milking Tube Colostrum Scale, Waukee, IA) and an electronic refractometer (PAL-1 Refractometer, Atago USA Inc, Bellevue, WA). All methods were performed in accordance with the manufacturers' recommendations. The use of hydrometer #1 (H1) entailed floating the instrument in a column of colostrum. Colostrum was transferred into a graduated cylinder and colostral temperature was adjusted to 14ºC to 30ºC by cooling the colostrum in a refrigerator. The hydrometer was lowered into the cylinder until the hydrometer floated freely. The instrument scale provides an approximation of colostral IgG (g/L) concentration. To use hydrometer #2 (H2), the hydrometer was inserted into a glass tube with a crystal plastic tube tip on one end and a squeeze bulb on the other. The temperature of the colostrum was adjusted to fall between 20ºC to 25ºC by cooling in a refrigerator. The glass tube was inserted into the colostrum and pressure was released on the squeeze bulb so that the colostrum was drawn into the glass tube. The hydrometer was allowed to stabilize and a reading was obtained. The scale on this instrument provides an approximation of colostral IgG (g/L) concentration. Use of the electronic refractometer required the colostral sample to have a temperature of between 10^0C and 40^0C. An aliquot (0.3ml) was placed into the prism on the refractometer and a digital measurement was displayed on the instrument screen in Brix units. The colostrum samples were frozen at -20^0C for subsequent IgG determination.

Colostral IgG determination– Colostrum IgG determinations were performed using an adaptation of a previously reported radial immunodiffusion (RID) technique (Tyler et al., 1999). Radial immunodiffusion plates measuring colostral IgG were prepared by

dissolving 1% agarose (Agarose, Sigma-Aldrich Co, St Louis, MO) in a sodium barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) containing 0.1% sodium azide (Sodium azide, Sigma-Aldrich Co, St Louis, MO). Rabbit–antibovine IgG (1%) (Anti-bovine IgG, whole molecule, developed in rabbit IgG fraction of anti-serum, Sigma-Aldrich Co, St Louis, MO) was added to the agarose solution. Eleven milliliters of the agarose solution was added to 10-cm Petri dishes. After the agarose solidified, 3-mm wells were cut in the agar. Colostrum samples were diluted 1:120 using barbital buffer and 5 Ul inoculated in each well. The diameter of the zone of precipitation was recorded after 72 hours of incubation at 23^{o}C. Sample IgG concentrations were determined by comparing the diameter of zones of precipitation with a standard curve generated using serial dilutions of a bovine IgG (Bovine IgG (lyophilized), Sigma-Aldrich Co, St Louis, MO) standard. The regression equation generated in this manner accurately predicts inoculum IgG concentration ($r^2 = 0.98$).

Data analysis– Mean colostral IgG concentration, standard deviation and percentage of colostrum samples classified as having low IgG (<50g IgG /L) for all cows were calculated. Mean colostral IgG concentrations and standard deviation for cows in their first, second, third or greater lactation also were calculated. Sensitivity (Se) and specificity (Sp) of the methods were determined by comparing results of the different methods with results of the RID assay using a series of 2-way frequency tables (Tyler and Cullor, 1989). Sensitivity was defined as the probability that a colostrum sample with an IgG concentration of <50g IgG /L as determined by RID would be classified as low by each method. Specificity was defined as the probability that a colostrum sample with an

IgG concentration of ≥50g IgG /L as determined by RID would be classified as adequate by each method. The sensitivity and specificity of each method in detection of low colostral IgG concentration (<50g IgG /L) were calculated across the range of test outcomes for each of the four methods (Tessman et al., 2001). Ninety-five percent confidence intervals were calculated for sensitivity and specificity (Saxmose et al., 2004). In addition to sensitivity and specificity at each potential test endpoint, the proportion of colostrum samples accepted at each endpoint also was calculated. Sensitivity and specificity among the assays were compared using z-test for difference between population proportions (Daniel, 1983). Regression models predicting colostral IgG concentration (g/L) determined by RID as a function of H1, H2, electronic refractometer results or weight of first milking colostrum were developed using SAS (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC).

RESULTS

One hundred- seventy-one first milking composite colostral samples were collected. Seventy-seven (77) colostral samples were from first lactation cows, 50 from second lactation cows and 54 from third or greater lactation cows. Nineteen samples had missing results and 7 cows did not produce colostrum. Eleven cows had 2 colostral samples collected in subsequent lactations. Mean ± standard deviation of first milking colostral weight was 7.4 ± 3.9 kg. Mean temperature of fresh colostrum was 34.6 ± 2.6 ^{0}C. Mean colostral IgG concentration as determined by RID assay for all cows was 68.5 ± 32.4 g IgG /L. Mean colostral IgG concentration for 1st, 2nd and 3rd or greater lactation were 65.8

± 32.0, 66.3 ± 30.2 and 73.9 ± 34.6, respectively. Mean colostral IgG concentration in cows in their 3rd lactation was higher than cows in their 1st or 2nd lactation while there was no difference in mean colostral IgG concentration between 1st and 2nd lactation cows ($p < 0.05$). Thirty two percent of colostrum samples had colostral IgG concentration less than 50g IgG /L as determined by RID assay. Hydrometer #1 had a Se of 0.75 and Sp of 0.78 in the detection of low colostral IgG concentration as determined by the RID using an instrument endpoint of 70g IgG /L **(Table 2)**. H2 had a Se of 0.76 and Sp of 0.66 in the detection of low colostral IgG as determined by the RID concentration using an instrument endpoint of 87.5g IgG /L **(Table 3)**. The electronic refractometer had a Se of 0.75 and Sp of 0.78 in the detection of low colostral IgG concentration as determined by the RID using an instrument endpoint of 22 % Brix units **(Table 4)**. Weight of first milking colostrum had Se of 0.42 and Sp of 0.74 in the detection of low colostral IgG concentration as determined by the RID using an endpoint of 8.5 kg **(Table 5)**. Proportions of the total weight of colostrum accepted at each endpoint for the four different methods are shown **(Tables 2, 3, 4 and 5)**. Comparison of sensitivity and specificity among the tests at the chosen endpoints are shown in **Table 7**.

Hydrometer #1, H2, and refractometry results were directly related to colostral IgG concentration. Weight of first milking colostrum was inversely related to colostral IgG; however the relationship was weak ($r^2 = 0.03$) **(Table 6)**. Regression analysis of the relationship between weight of first milking colostrum and IgG concentration as determined by RID using square and logarithmic transformation to determine presence of non-linear relationship did not improve the fit.

DISCUSSION

The use of 50 g IgG /L endpoint to classify colostrum quality was based on studies by Kruse (1970) and Fleenor and Stott (1980). The proportion of colostral samples with IgG concentration less than 50g/L are variable in different studies reported (Tyler et al., 1999a; Chigerwe et al., 2005). Mean colostral IgG concentration reported here was substantially higher than reported in some studies (Pritchett et al., 1994), but lower than reported in others (Foster et al., 2006). Several explanations exist for differences observed among studies. First, timing of colostral collection was not reported in studies by Pritchett (1994). Colostrum collected after 2 hours had significantly lower IgG concentrations (Moore et al., 2005). Secondly a herd effect on colostral IgG concentration may exist. Suggested factors that might contribute to the herd effects include nutrition and environment (Tyler et al., 1999). While in this study cows were milked using a milking machine to obtain a representative composite sample, only small volumes within 4 hours were collected from each quarter in a previous study which reported higher colostral IgG concentrations (Tyler et al., 1999). Variation in IgG concentration exists throughout the first milking colostrum with lower IgG concentration in the last fractions of colostrum, hence collection method will have an effect on IgG concentration (Hostetler et al., 2003). Colostral IgG concentration between cows in their first lactation did not differ significantly from cows in their second, lactation consistent with previous studies (Tyler et al., 1999a).

Several factors were considered in choosing the optimal endpoint for each method. First endpoints chosen maximized both sensitivity and specificity. Additionally, the chosen endpoint ensured that sufficient colostrum was available to feed calves. A minimum 100 g of colostral IgG is recommended for adequate passive transfer (Besser et al., 1991). In the studies by Besser et al, only 36 % of colostrum samples evaluated had 100 g of IgG1 in 2 L while 66 % and 85 % of samples contained 100 g of IgG in 3 L and 4 L respectively hence feeding of 3 to 4 L liters of colostrum is recommended in dairy calves to minimize failure of passive transfer. Hence, we presumed that a minimum intake of 3 L of colostrum is a reasonable goal to assure adequate passive transfer in calves. Thus, the total amount of colostrum required to feed 171 calves produced by enrolled cows would range from 513 to 684 L if 3 to 4 L of colostrum were fed. Thus, the selection of endpoints in the present study also was based on the absolute requirement that a minimum of 513 L of colostrum were classified as acceptable. Based upon this requirement the maximum acceptable endpoint for H1 would be 90 g IgG/L. The highest potential endpoints for H2 and the electronic refractometer would be 100 g IgG/L and 23 Brix (%) respectively. Similarly, the highest acceptable cutpoint for weight of first milking colostrum would be 9 kg. Practical recommendations for the test methods would include a range of acceptable test endpoints. For H1 we recommend 60 to 90 g IgG /L test endpoints using the instrument scale. For H2 we recommend 75 to 100 g IgG /L test endpoints using the instrument scale. Endpoints from 20 to 23 Brix (%) were deemed appropriate when using the electronic refractometer. It should be noted that lower endpoints of the ranges will increase availability of colostrum, but compromise sensitivity in the detection of low IgG concentration colostrum, while higher endpoints

will decrease the amount of colostrum available, but increase sensitivity in the identification of low IgG concentration colostrum. Additionally, the limited availability of colostrum created by rigorous screening protocols will increase the necessity for colostrum storage facilities.

Weight of first milking was inversely related (r = -0.29) to colostral IgG1 concentration in previous studies (Pritchett et al., 1991). In the study presented here a negative correlation between weight of first milking colostrum and IgG concentration (r = -0.17) was reported, consistent with the previous studies; however the strength of the observed relationship was less. First milking colostrum weighing less than 8.5 kg had significantly higher IgG concentration (Pritchett et al., 1991). In the present study a test endpoint of 8.5 kg for the weight of first milking colostrum also appear to maximize both sensitivity and specificity consistent in this study. However, the sensitivity was low (0.42) in detecting colostral samples with low IgG concentration (<50 g IgG /L). Thus the use of weight of first milking colostrum as a screening test for colostrum with low IgG concentration cannot be justified. The two hydrometers and the electronic refractometer readings are colostral temperature sensitive; however, the recommended test conditions varied among instruments. The mean temperature of fresh colostrum in this study was $34.6 \pm 2.6\ ^{0}C$. Hence, using the electronic refractometer had the advantage of not requiring cooling of the colostrum. A higher sensitivity (0.93) in identifying colostral samples with low IgG concentration (<50g/L) has been reported (Chigerwe et al., 2005). The cost of performing an immunoassay is substantially higher per test compared to the methods evaluated in this study. Furthermore, the immunoassay is qualitative compared

to the hydrometers which indicate an approximation of the colostral IgG concentration. The immunoassay kit would be recommended on farms with a higher prevalence of low IgG colostrum samples.

Manufacturer's recommended endpoint for H1 and H2 for classifying low IgG concentration was < 50g IgG /L and 100 g IgG/L respectively. Substituting these endpoints in the respective regression models developed in this study yields colostral IgG concentrations of 46.8 g IgG/L and 73.4 g IgG/L for H1 and H2 respectively. This suggests that H1 and H2 systematically overestimate colostral IgG concentration. The sensitivity for both hydrometers reported in this study were higher than previously reported at the same endpoints of 50, 60 and 70 g IgG/L for H1 and 50 g IgG/ L for H2 (Pritchett et al., 1994). A number of possible explanations for this discrepancy are apparent. Firstly, colostral immunoglobulin concentration is related to colostral specific gravity (Fleenor and Stott, 1980). Furthermore, colostral specific gravity is more correlated with colostral protein concentration than colostral IgG concentration (Fleenor and Stott, 1980; Quigley et al., 1994; Morin et al., 2001). Additionally, colostral specific gravity is affected by several factors including month of calving, season, lactation number (Morin et al., 2001), and colostral temperature (Mechor 1991, 1992). All these factors affecting colostral specific gravity mentioned may vary from herd to herd resulting in the differences observed in these studies. Secondly, while colostrum collection was performed within 2 hours after calving in this study, collection was performed within 12 hours, (Morin et al., 2001) within 24 hours (Mechor 1991) or at an undisclosed time (Pritchett et al., 1994). Decreased IgG concentration in colostrum

collected after 2 hours has been reported (Moore et al., 2005). Thirdly, colostrum was equilibrated to room temperature (18 to 22 ^{0}C) while in a bucket before determination of IgG concentration using the hydrometer in another study (Pritchett et al., 1994). In contrast, colostrum was cooled to manufacturer recommended temperatures prior to quality assessments using the hydrometers in this study.

Prevalence of low IgG colostrum may vary from farm to farm. Since predictive values for positive and negative tests are prevalence dependent, their determination would only be applicable to the farm of study and are not reported here (Dohoo, 2003). A test method which maximizes sensitivity, yields low numbers of false negatives and identify most colostral samples with low IgG concentration but practical purposes, colostral samples identified as having low IgG concentration by a screening test are not routinely confirmed with a highly specific test. Thus, maximizing sensitivity for a test method to identify low IgG colostral samples is considered more critical.

In summary, sensitivity of test methods at different test endpoints should be considered when choosing optimum test endpoints. We recommend using H1 at endpoint range of 60 to 90 g IgG/L, H2 at 75 to 100 g IgG/L and the electronic refractometer at an endpoint range of 20 to 23 Brix (%). Weight of first milking colostrum is a poor test method for identifying low colostral IgG colostrum because of the low sensitivity.

CHAPTER 5

THE EFFECT OF COLOSTRUM ADMINISTRATION PRACTICES ON SERUM IgG CONCENTRATION IN TUBE FED HOLSTEIN BULL CALVES

Munashe Chigerwe, BVSc, DACVIM; Jeff W. Tyler, DVM, PhD, DACVIM; Loren Schultz, DVM, MS, DACVPM; John R. Middleton, DVM, PhD, DACVIM; Barry J. Steevens, PhD; James N. Spain, PhD

From the Departments of Veterinary Medicine and Surgery (Chigerwe, Tyler, Schultz, Middleton), Veterinary Pathobiology (Tyler, Middleton), Animal Sciences (Steevens, Spain) and the University of Missouri Public Health Program (Tyler, Schultz), University of Missouri-Columbia, 900 East Campus Drive, Columbia, MO 65211, USA

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ABSTRACT

The objective of this determine the amount of colostral IgG required for adequate passive transfer of colostral immunoglobulins in calves fed colostrum by oroesophageal tubing and to evaluate the impact of other accepted factors on passive transfer of colostral immunoglobulins in dairy calves. A randomized controlled study with120 Holstein bull calves was performed. Calves were randomly assigned without replacement to specific combinations of treatments (age of calf at feeding colostrum and volume of colostrum administered). A single feeding of colostrum was administered once by oroesophageal intubation. Equal numbers of calves received 1, 2 ,3 or 4 liters of their dam's colostrum and equal numbers of calves received colostrum at 2, 6, 10, 14, 18 or 22 hours of age. Serum samples were collected from calves at 48 hours of age for IgG determination by radial immunodiffusion. The effects of calf age at time of colostrum administration, volume of colostrum administered, colostrum IgG concentration, dam parity, colostrum weight produced by the dam, calf weight and interactions between these factors on calf serum IgG concentration at 48 hrs of age were determined using a stepwise multiple regression model and logistic regression models. A minimum requirement of 153 g of colostral IgG was required for optimum colostral transfer of immunoglobulins when calves were fed 3 L at 2 hours of age. Substantially larger IgG intakes are required by calves fed colostrum at ages greater than 2 hours. Feeding 100 g of colostral IgG by oroesophageal feeding is inadequate for adequate passive transfer of colostral immunoglobulins. At least 150 to 200 g of colostral IgG is required for adequate passive transfer of colostral immunoglobulins. Tube feeding calves 3 L of colostrum within 2 hours after birth is recommended.

INTRODUCTION

Several studies have described the importance of the ingestion and absorption of colostral immunoglobulins on the morbidity, mortality, growth and future productivity of dairy calves (Kruse V, 1980; Robison et al., 1988; Denise et al., 1989; Besser et al., 1991; Tyler et al., 1998; Virtala et al., 1999). Calves with inadequate passive transfer of colostral immunoglobulins have increased risk for mortality in the first 3 months of life (Tyler et al., 1998; Virtala et al., 1999). Additionally, decreased rate of gain (Robison et al., 1988), lower milk production (Denise et al., 1989) and decreased first lactation survival (Denise et al., 1989) have been reported in calves with failure of passive transfer of colostral immunoglobulins. Failure of passive transfer of colostral immunoglobulins (FPT) is responsible for approximately one-half of calf death losses on US dairy farms (Tyler et al., 1999b).

Colostrum administration practices which affect serum immunoglobulin concentration in calves include the timing of colostrum administration (Stott et al., 1979; Bush and Staley, 1979), the volume of colostrum fed, the method of administration (Besser et al., 1991), timing of colostral collection (Moore et al., 2005), colostral IgG concentration (Pritchett et al., 1991) and dam parity (Pritchett et al., 1991; Tyler et al., 1999). Previous reports have recommended that calves ingest at least 100 g of colostral IgG for adequate passive transfer of colostral immunoglobulins (Besser et al., 1991; Gay, 1994; McGuirk and Collins, 2004). However, this goal was based upon anecdotal or clinical observations rather than controlled studies.

Lower failure of passive transfer rates have been reported in calves fed colostrum by oroesophageal tube, relative to bottle fed calves or calves allowed to nurse from their dams (Besser et al., 1991). Administration of 3 to 4 L of colostrum by artificial methods has been recommended to ensure sufficient IgG mass is delivered (Besser et al., 1991). Tube feeding calves is an attractive alternative to bottle feeding on many large commercial dairies because calves can be fed colostrum in a rapid manner by experienced employees. Unfortunately, no prospective have critically examined the effects of volume of colostrum administered and the timing of colostrum administration on the serum IgG concentration in tube fed calves.

Despite the accumulated understanding of the factors which affect passive transfer and the recognized importance of passive transfer of colostral immunoglobulins in dairy calves, approximately 35-40 % of US dairy calves have inadequate passive transfer of colostral immunoglobulins (USDA-APHIS, 1993; Tyler et al., 1998). Based on this observed high prevalence of failure of passive transfer of colostral immunoglobulins in calves, we hypothesized that current recommendations regarding colostrum feeding practices are inadequate. Thus, either the total immunoglobulin mass being fed to the calves is inadequate or colostrum administration practices require more concerted effort than most farmers perform. The first objective of this study was to determine the amount of colostral IgG required for adequate passive transfer of colostral immunoglobulins in calves fed colostrum by oroesophageal tubing. The second objective was to evaluate the impact of calf age at time of colostrum administration, volume of colostrum

administered, colostrum IgG concentration, dam parity, colostrum weight produced by the dam, calf weight and their interactions on transfer of colostral immunoglobulins in dairy calves.

MATERIALS AND METHODS

Animlas– One-hundred-twenty (120) Holstein bull calves resulting from observed calvings were drawn from the University of Missouri Foremost Teaching and Research Dairy. The research study was approved by the University of Missouri-Columbia Animal Care and Use Committee. After parturition, calves were immediately separated from their dams, weighed and identified. Only calves whose births were observed were included in the study. All cows received 20 IU of oxytocin intramuscularly before milking to ensure complete milking. Cows were milked within 2 hours after parturition using a portable milking machine. A 20 ml mixed composite colostrum was collected and stored at -20^{o}C until processed for immunoglobulin determinations. The calves were randomly assigned without replacement to specific combinations of treatments (age of calf at feeding colostrum and volume of colostrum administered). Equal numbers of calves received 1, 2 ,3 or 4 liters of their dam's colostrum and equal numbers of calves received colostrum at 2, 6, 10, 14, 18 or 22 hours of age **(Table 8)**. A single feeding of fresh colostrum was administered once by oroesophageal intubation. Thereafter, all calves were fed 2 L of a commercial milk replacer every 12 hrs. Calves from cows which did not produce sufficient colostrum were fed colostrum from another, single cow. Calves with missing values during data collection or twin calvings were excluded from the study. Excluded

calves were replaced with additional single born calves assigned to the same treatment groups. Assisted calvings also were recorded. All calves enrolled survived until at least 48 hours of age. Serum samples were collected from calves at 48 hours of age. Serum samples were stored at -20^{o}C until processed for serum immunoglobulin determinations.

Serum and colostral IgG concentration determination– Colostral and total serum IgG concentrations were determined using adaptations of a previously reported radial immunodiffussion (RID) techniques (Tyler et al., 1999; Hostetler et al., 2003). Radial immunodiffussion plates for measuring IgG were prepared by dissolving 1% agarose (Agarose, Sigma-Aldrich Co, St Louis, MO) in a sodium barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) containing 0.1% sodium azide (Sodium azide, Sigma-Aldrich Co, St Louis, MO). Rabbit–antibovine IgG (1%) (Anti-bovine IgG (whole molecule) developed in rabbit IgG fraction of anti-serum, Sigma-Aldrich Co, St Louis, MO) was added to the agarose solution. Eleven milliliters of the agarose solution was added to 10-cm Petri dishes. After the agarose solidified, 3-mm wells were cut in the agar. Serum samples were diluted 1:20 and colostrum samples were diluted 1:120 using barbital buffer and 5 uL were inoculated in each well. The diameter of the zone of precipitation was recorded after 72 hours of incubation at 23^{o}C. Sample IgG concentrations were determined by comparing the diameter of zones of precipitation with a standard curve generated using serial dilutions of a bovine IgG standard (Bovine IgG (lyophilized), Sigma-Aldrich Co, St Louis, MO). The regression equation generated in this manner accurately predicted inoculum IgG concentration ($r^2 = 0.98$).

Data analysis– Mean colostral IgG concentrations (g/L) and standard error of mean for cows in their first, second, third or greater lactation were calculated. Mean ± standard error of mean for calf weight and colostral volume produced by the dam also was calculated. The cut point defining adequacy of passive transfer was 48 hour serum IgG concentrations ≥1340 mg/dl based on previous studies (Tyler et al., 1998; Virtal et al., 1999; Tyler et al., 1996). The effects of calf age at time of colostrum administration, volume of colostrum administered, colostrum IgG concentration, dam parity, colostrum weight produced by the dam, and calf weight on calf serum IgG concentration at 48 hrs of age were determined using a stepwise multiple regression model (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC). Calf weight (kg), colostral weight produced by the dam (kg), calf age at time of colostral feeding (hours) and colostrum IgG concentration (g/L) were treated as continuous independent variables. Colostrum volume fed to the calf (1, 2, 3 or 4 L) was treated as a categorical independent variable due to non-linear effects identified during preliminary regression model diagnostics (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC). Dam parity (1^{st}, 2^{nd} and $\geq 3^{rd}$) and calving assistance (assistance or no assistance) were treated as categorical variables. First parity cows and feeding of 1 L of colostrum were considered as baseline categories for parity and colostral volume fed respectively. Second order interactions between volume of colostrum administered and colostral IgG concentration were considered. Additionally, interactions between volume of colostrum fed and calf age at time of feeding colostrum were considered. The variable(s) or interaction with the lowest P-to-enter was added to the model until no variable had a P-to-enter < 0.1 (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC). At each step all variables

included in the model were checked to see if they remained significant ($p<0.1$) after a new variable had been added (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC). The average, total required colostral IgG intake for adequate passive transfer of colostral immunoglobulins was calculated by solving the final regression model predicting serum IgG for colostral IgG concentration and then multiplying this IgG concentration by the volume administered.

A logistic regression model predicting the probability of a calf having failure of passive transfer at 48 hours of age was developed as function of calf weight, calf age at time of colostrum administration, volume of colostrum fed, dam parity, calving assistance, colostral weight produced by the dam and colostral IgG concentration (PROC GENMOD, SAS for Windows, version 9.13, SAS Institute, Cary, NC). Second order interactions between volume of colostrum administered and colostral IgG concentration were considered. Interactions between volume of colostrum fed and calf age at time of feeding colostrum also were considered. The variable(s) or interaction with the lowest P-to-enter was added to the model until no variable had a P-to-enter < 0.1. The goodness-of-fit of the final model was estimated using the Pearson Chi-square statistic (PROC GENMOD, SAS for Windows, version 9.13, SAS Institute, Cary, NC).

RESULTS

Forty-four, 33 and 43 calves from 1st, 2nd and ≥3rd lactation cows respectively were enrolled into the study. The mean ± standard error of mean for colostral IgG

concentration for all cows, 1st, 2nd and ≥3rd lactation cows were 67.2 ± 2.8, 68.1 ± 2.7, 65.0 ± 3.0 and 68.0 ± 2.7 g/L respectively. Mean ± standard error of mean for colostral weight produced by the dam and calf weight were 8.8 ± 0.5 kg and 40.9 ± 0.6 kg, respectively. Mean ± standard error for serum IgG (mg/dl) concentration was 1136 ± 50.8.

Results of the multiple regression model predicting serum IgG concentration at 48 hours as a function of calf weight, time of colostrum administration, volume of colostrum fed to the calf and colostrum IgG concentration are represented in **Table 9**. Dam parity, calf birth weight, colostral weight produced by the dam and whether a calving was assisted were not significantly associated with serum IgG concentration ($p > 0.1$). Interactions between colostral IgG concentration and volume of colostrum fed, age of calf at time of feeding and volume of colostrum fed were not significant independent variables of 48-hour serum IgG concentration ($p>0.1$). Colostral IgG concentration and volume of colostrum fed to the calf were positively correlated to the 48-hour serum IgG concentration. Age of calf at time of feeding was negatively correlated to the 48-hour serum IgG concentration **(Table 9)**.

Using the regression model in Table 9, the average total colostral IgG concentration required to achieve a serum IgG concentration of 1340 mg/dl was calculated for all possible permutations of volume of colostrum fed, calf age at time of colostrum administration and various colostral IgG concentrations (25, 50, 75, 100, 125 g/L). In the

following example, the average, required total IgG mass was calculated for a calf receiving 3 L of colostrum at 2 hours of age:

$1340 \text{ mg/dl} = 509.048 + 5.795 \times \text{ColIgG} + 464.357 \times V_2 + 633.756 \times V_3 + 623.670 \times V_4 - 20.058 \times \text{Age}.$

where

V_2 = feeding 2 L of colostrum,

V_3 = feeding 3 L of colostrum,

V_4 = feeding 3 L of colostrum,

ColIgG = colostral IgG concentration,

Age = age of calf at feeding colostrum (hours).

$1340 = 509.048 + 5.795 \times \text{ColIgG} + 464.357 \times 0 + 633.756 \times 1 + 623.670 \times 0 - 20.058 \times 2.$

$237.311 = 5.795 \times \text{ColIgG}$

$40.951 \text{ g/L} = \text{ColIgG}$

Hence, feeding 3 L of colostrum at 2 hours of age will require on average $40.951 \times 3 = 122.85$ g of colostral IgG for adequate passive transfer of colostral immunoglobulins. Total average colostral IgG mass required for adequate passive transfer of colostral immunoglobulins when calves were fed 1, 2, 3 and 4 liter of colostrum at different ages is summarized in **Table 10**.

The logistic regression model to predict the probability of a calf having failure of passive transfer based on the 48-hour IgG concentration is represented in **Table 11**. Dam parity, calf weight, whether calving was assisted, colostral weight produced by the dam were not

significant predictors of 48-hour serum IgG concentration ($p>0.1$). Interactions between colostral IgG concentration and volume of colostrum fed, age of calf at time of feeding and volume of colostrum fed were not significant independent variables of 48-hour serum IgG concentration ($p>0.1$). The probability of calf having FPT was calculated using the following formula represented below:

$$p(FPT) = 1/[1 + \exp(0.3543 + 0.0283 \times ColIgG - 0.1052 \times Age - 3.8115 \times V_1)]$$

where

ColIgG = colostral IgG concentration,

Age = age of calf at feeding colostrum,

V_1 = feeding 1 L of colostrum.

Summary of probabilities of a calf having failure of passive transfer as a function of different predictors for 48-hour serum IgG concentration is represented in **Table 12**.

DISCUSSION

The threshold value for serum IgG concentrations defining failure of passive transfer in dairy calves varies among studies and reports. Serum IgG concentrations of <1000 mg/dl have been reported as an indication of failure of passive transfer colostral immunoglobulins (Besser et al., 1991; Tyler et al., 1996). Mortality was increased in calves with serum IgG concentrations of <1200 mg/dl (Robinson et al., 1988) and < 1500 mg/dl (Selim et al., 1995) in other studies. Calves with serum IgG concentrations of ≤1200 mg/dl have been reported to have a 2-times higher odds of pneumonia compared to calves with serum IgG concentration >1200 mg/dl (Virtala et al., 1999). Another report

has suggested a serum IgG concentration of >1600 mg/dl indicates adequate passive transfer of colostral immunoglobulins (McGuire and Adams, 1982). A field based study which examined mortality in 3, 479 dairy replacement heifers showed that calves with serum protein IgG concentration ≥ 5.0 and < 5.5 g/dl experienced a 1.3 relative risk of mortality compared to calves with serum protein ≥ 5.5 g/dl (equivalent to serum IgG concentration of 1340 mg/dl) (Tyler et al., 1998). On the basis of this study, a serum IgG concentration of 1340 mg/dl was chosen as the threshold which would define optimal passive transfer of colostral immunoglobulins. This cutpoint, although appropriate to guarantee optimal calf health is probably an excessive rigorous goal for most commercial dairies. On these farms FPT rates as high as 10 % probably are indicative of good colostrum administration practices and lower FPT rates are probably unreasonable and unattainable goals. Hence, less rigorous cutpoints, 5.2 g/dl serum total protein or 1000 mg/dl IgG are probably appropriate for routine monitoring.

The regression model predicting serum IgG concentration **(Table 9)** deserves careful consideration. The reported intercept of 509.049 mg/dl does not imply that a calf which receives no colostrum will have a serum IgG concentration of 509.049 mg/dl. The serum IgG concentration fed 0 L containing 0 g/L IgG at 22 hours of age can be estimated as 509.049 + (22 hours)(-20.058) = 67.773 mg/dl, confirming that calves will have very low detectable serum IgG concentration at birth. Hence, the presented model is both consistent with previous studies and intuitively logical (Sawyer et al., 1973; Stott et al., 1979).

It should be noted that the results of this study are applicable only to calves which are fed colostrum once by oroesophageal tubing between 2 and 22 hours of age. Smaller amounts of IgG may be adequate if calves receive a second colostral feeding early in life. Substantially larger IgG intakes are required by calves fed colostrum at ages greater than 2 hours. For calves fed at 6 hours of age, the required mean IgG intake varies from 164 to 226 g. For calves fed at 12 hours of age, the required mean IgG intake varies from 185 to 309 g. The results of this study suggests that on average, a minimum of 123 g of colostral IgG is required for adequate passive transfer when calves are fed 3 L of colostrum by tube feeding at 2 hours of age. This report contradicts previous reports which recommended that calves should receive ≥ 100 g of colostral IgG for adequate transfer of colostral immunoglobulins. It should be noted that multiple regression models in this study only predicted the mean colostral IgG required for adequate passive transfer of colostral immuniglobulins. Thus we anticipate that half of the calves fed 123 g of colostral IgG at 2 hours of age will have failure of passive transfer and half of the calves will have adequate passive transfer of colostral immunoglobulins. Previous studies have recommended feeding 3 to 4 L of colostrum by artificial methods (Besser et al., 1991). In the present study feeding of 4 L once was not beneficial compared to feeding of 3 L once based on the required colostral IgG concentration to achieve adequate passive transfer **(Table 9)**. Previous studies reported no significant increase in serum IgG concentrations at 24 or 48 hours of age when 4 L of low IgG concentration colostrum was fed within 3 hours compared to those calves fed 2 L (Morin et al., 1997).

Results of the logistic regression showed no differences in the probability of a calf having failure of passive of passive transfer of colostral immunoglobulins when fed 2, 3 or 4 L at a given age of the calf at feeding, and IgG concentration of colostral IgG fed **(Table 11)**. A possible explanation for the observed is the difference in the measured endpoints; serum IgG concentration in the multiple regression models (continuous dependent variable) compared to binomial (adequate or inadequate serum IgG concentrations) in the logistic regression. Calves tube fed 2, 3 or 4 liters of colostrum by 2 hours of age will have FPT of < 9 % when fed colostrum with IgG concentration of > 75 g/L concentration. An FPT rate of 17 % would be expected in calves fed 2, 3 or 4 L of colostrum containing > 50 g/L. The volume of colostrum administered and the colostral IgG concentration evident in **Table 12**. For instance feeding 1 L of colostrum with an colostral IgG of 100 g /L at 2 hours of age results in a FPT probability 0.68 while feeding 2 L of colostrum with a colostral IgG concentration of 50 g/L results in an FPT probability of 0.17.

Only bull calves were enrolled in the study since the study design was anticipated to create a substantial risk for FPT, and consequently, increased morbidity and mortality. In the present study calf birth weight did not influence serum IgG concentrations at 24 or 48 hours consistent with previous studies (Stott et a., 1979; Norman et al., 1971; Robinson et al., 1988). Assisted calving did not have an effect on serum IgG concentrations at 48 hours of age in this study. Previous studies showed no significant differences in colostral IgG concentration between cows in their first or second lactation (Pritchett et al., 1991; Tyler 1999a). However, cows in their third or greater lactation had significantly higher

colostral IgG concentration. In this study there was no significant difference in the mean colostral IgG concentration in the 1st, 2nd and ≥3rd lactation cows and parity was not a significant predictor of 48 hour serum IgG concentrations in calves. Thus the quality of colostrum with regards to the concentrations of IgG from first parity cows was equivalent to older cows' colostrum. Discarding colostrum from first lactation cows because of perceived lower IgG concentration is strongly discouraged. However it should be noted that conclusions on the differences in colostral IgG concentration due dam parity are based on studies performed on a single herd. Geographical and nutritional factors may potentially influence colostral IgG concentration.

The endpoints chosen for defining adequacy of passive transfer presented in this study are for optimal colostral transfer of colostral immunoglobulins. The reported average number of hours at which calves receive colostrum on all dairy operations in the US was 3.3 hours (USDA-APHIS, 2007). Thus, on most dairy farms in the US, a minimum of 137 g (106, 167; 95 % CI) of IgG, on average is required for adequate passive transfer when calves are fed 3 L. Since ingestion of 123 g will achieve adequate passive transfer in 50 % of the calves, the findings in this study suggest a minimum of 153 g (upper confidence interval limit for feeding 3 L at 2 hours of age) of colostral IgG is required for adequate passive transfer. In summary, 100 g of colostral IgG by oroesophageal feeding is inadequate for adequate passive transfer of colostral immunoglobulins. At least 150 to 200 g of colostral IgG is required for adequate passive transfer of colostral immunoglobulins. We recommend that tube fed calves should receive 3 L of colostrum within 2 hours after birth.

CHAPTER 6

FACTORS AFFECTING SERUM IgG CONCENTRATION IN BOTTLE FED HOLSTEIN HEIFER CALVES

Munashe Chigerwe, BVSc, DACVIM; Jeff W. Tyler, DVM, PhD, DACVIM; Marin K. Summers, BS; John R. Middleton DVM, PhD, DACVIM; Loren G. Schultz, DVM, MS, DACVPM; Dusty W. Nagy, DVM, PhD, DACVIM

From the Departments of Veterinary Medicine and Surgery (Chigerwe, Tyler, Summers, Middleton, Schultz, Nagy), and the MU Public Health Program (Tyler), University of Missouri-Columbia, 900 East Campus Drive, Columbia, MO 65211, USA

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ABSTRACT

The objectives of the study were to determine the effect of time interval from birth to first colostrum feeding on colostrum volume intake and serum IgG concentration and to determine the effect of varying colostral volume intakes and colostral IgG concentrations on the probability of failure of passive transfer (FPT) in bottle fed heifer calves. A prospective, randomized controlled study with 104 Holstein heifer calves was performed. Equal numbers of calves were randomly assigned to groups and fed up to 3 L of their dams' colostrum at 1, 2, 3 or 4 hours after birth using a nipple bottle. Calves were allowed to nurse for 15 minutes and actual intake was recorded. A second 3 L bottle feeding of the dam's colostrum was offered at 12 hours of age. Colostral intakes at 1, 2, 3 or 4 hours of age were compared using analysis of variance. A logistic regression model predicting the probability of a calf having FPT was developed. Calf age, up to 4 hours did not have a significant effect on the calf's ability to suckle colostrum or on 48-hour serum IgG concentrations. Colostral intake at 1, 2, 3 or 4 hours did not affect intake at the second feeding at 12 hours of age. Probability of FPT in calves ingesting 3 L at first feeding and 3 L at 12 hours was < 0.05 even at low colostral IgG concentrations. Allowing calves fed by nipple bottle to ingest as much colostrum as they can suckle within 4 hours and at 12 hours of age will substantially reduce the probability of FPT. Bottle fed calves which do not ingest 3 L within the first 4 hours of age should be targeted for more aggressive intervention.

INTRODUCTION

Increased risk for morbidity and mortality, reduced growth rate, and decreased milk production and survival in the first lactation has been described in dairy calves that fail to ingest and absorb adequate colostral immunoglobulins (Kruse, 1970; Robinson et al., 1988; DeNise et al., 1989; Besser and Gay, 1991; Tyler et al., 1998; Virtala et al., 1999). The method of colostrum administration can affect efficiency of transfer of colostral immunoglobulins (Besser et al., 1991; Virtala et al., 1999; Kaske and Schuberth, 2005). Reported prevalences of failure of passive transfer (FPT) of colostral immunoglobulins were 61.4, 19.3 and 10.8 % for dairy calves which nursed from their dams, suckled from a bottle and were tube fed, respectively (Besser et al., 1991). Timing of colostral feeding, colostral immunoglobulin concentration, dam parity, the volume of colostrum fed, and the method of administration affect colostral transfer of colostral immunoglobulins (Stott et al., 1979; Bush and Staley, 1980; Besser et al., 1991; Pritchett et al., 1991; Tyler et al., 1999; Moore et al., 2005) Increased efficiency of colostral immunoglobulin absorption has been reported in bottle fed calves because of closure of the esophageal groove (Lateur-Rowet and Breukink, 1983). However, sufficient absorption of colostral immunoglobulins was reported in tube fed calves in the absence of closure of the esophageal groove because of rapid flow of colostrum from the forestomachs to the abomasum and small intestine (Lateur-Rowet and Breukink, 1983). Although oroesophageal tube feeding has been recommended as a method to insure lower rates of FPT compared to bottle feeding, (Lateur-Rowet and Breukink, 1983; Gay, 1994; Kaske and Schuberth, 2005), 59.2 % of dairy operations in the US hand-fed colostrum from a

bucket or bottle compared to only 4.3 % which tube fed calves (USDA-APHIS, 2007). The remainder of farms allow calves to suckle from their dams (USDA-APHIS, 2007). This suggests that a substantial number of producers prefer bottle feeding of colostrum to tube feeding.

Calf vigor is anticipated to change within the first few hours after birth, consequently affecting voluntary intake of colostrum when calves are nipple bottle fed colostrum. Additionally, ingestion of colostrum using a bottle immediately after birth resulted in earlier cessation of absorption of colostral immunoglobulins from the intestine (Stott et al., 1979b). We hypothesized that calves fed immediately after birth would ingest smaller volumes of colostrum than calves which had been permitted to acclimate for a period of hours. The objectives of this study were to determine the effect of time interval from birth to first colostrum feeding on voluntary colostrum intake and subsequent serum IgG concentration in heifer calves, determine whether calf age and colostral volume ingested by the calf at first feeding had an effect on colostral volume intake at 12 hrs of age and to determine the effect of varying colostral intake and colostral IgG concentration on the probability of FPT in bottle fed Holstein heifer calves.

MATERIALS AND METHODS

Cows– One hundred–and–four (104) Holstein cows of varying parity with confirmed breeding dates were drawn from the University of Missouri Foremost Teaching and Research Dairy. The research study was approved by the University of Missouri Animal

Care and Use Committee. This herd uses artificial insemination exclusively and accurate breeding dates were available for all cows.

All calvings were observed and attended. After parturition, calves were immediately separated from the dam, weighed and permanently identified. Only heifer calves were enrolled in the study due to anticipated reduction in FPT rates with two colostral feedings. All cows received 20 IU of oxytocin intramuscularly before milking to ensure complete milking. All cows were milked completely within one hour after parturition using a portable milking machine and the volume of first milking colostrum was recorded. An aliquot (20 ml) of the composite mixed first milking colostrum from each cow was collected from the milking bucket for colostral IgG determination. Samples were stored at -20^{o}C for 7-12 months until processed for immunoglobulin determinations.

Calves– Initially 156 heifer calves were enrolled but 104 calves met the study criteria. Holstein heifer calves (n = 104) were assigned to groups using random assignment without replacement. Equal numbers of calves (n = 26/group) received fresh first milking colostrum collected from their dam at 1, 2, 3, or 4 hours of age. Calves were offered 3 liters of their dams' colostrum for 15 minutes total by nipple bottle. Undigested colostrum was measured with a graduated cylinder and actual intake was recorded. A second 15 minute-3 L bottle feeding of the dam's first milking colostrum was offered at 12 hours of age. Thereafter, calves were fed 2 liters of milk replacer every 12 hours. Blood was collected by jugular venipuncture at 48 hours of age to determine serum IgG concentration. Calves with missing values (52 calves) and twin calves were excluded

from the study. Calves which had failed to suckle colostrum at both the first and second feeding were tube fed 3 L of colostrum and excluded from the study.

Colostral and serum IgG determination– Colostrum and 48 hr serum IgG determinations were performed using adaptations of a previously reported radial immunodiffusion technique (RID) (Hostetler et al., 2003). RID plates measuring IgG were prepared by dissolving 1% agarose (Agarose, Sigma-Aldrich Co, St Louis, MO) in a sodium barbital buffer (Barbital buffer, Sigma-Aldrich Co, St Louis, MO) containing 0.1% sodium azide (Sodium azide, Sigma-Aldrich Co, St Louis, MO). Rabbit–antibovine IgG (1%) (Rabbit anti-bovine IgG (whole molecule), Sigma-Aldrich Co, St Louis, MO) was added to the agarose solution. Eleven milliliters of the agarose solution was added to 10-cm Petri dishes. After the agarose solidified, 3-mm wells were cut in the agar. Serum samples were diluted 1:20 and colostrum samples were diluted 1:120 using a barbital buffer and 5 μL were inoculated in each well. The diameter of the zone of precipitation was recorded after 72 hours of incubation at 23^{o}C. Sample IgG concentrations were determined by comparing the diameter of zones of precipitation with a standard curve generated using serial dilutions of a lyophilized bovine IgG standard (Bovine IgG (lyophilized), Sigma-Aldrich Co, St Louis, MO). The regression equation generated in this manner accurately predicts inoculum IgG concentration ($r^2 = 0.97$).

Statistical analysis–Mean colostral IgG concentrations (g/L) and standard error of mean for cows in their first, second, third or greater lactation were calculated. Mean ± standard error of mean for calf weight, first milking colostral volume produced by the dam and

48–hour serum IgG concentration for calves also was calculated. The cut point of serum IgG concentrations ≥ 1340 mg/dL was chosen for defining adequacy of passive transfer at 48 hours based on previous studies (Tyler et al., 1996, 1999). Colostral intake at first feeding across the four treatment groups (1, 2, 3 or 4 hours of age) was compared using one way analysis of variance ($P < 0.05$) (PROC GLM, SAS for Windows, version 9.13, SAS Institute, Cary, NC). A multiple regression model was developed to predict colostral intake (liters) at 12 hours of age as a function age of calf at first feeding (hours) and colostral intake volume at first feeding ($P < 0.1$) (PROC REG, SAS for Windows, version 9.13, SAS Institute, Cary, NC). A logistic regression model predicting the probability of a calf having FPT at 48 hours of age was developed as a function of calf weight, calf age at time of first colostrum feeding, volume of colostrum ingested at first feeding, volume of colostrum ingested at 12 hours, dam parity, colostral volume produced by the dam and colostral IgG concentration ($P < 0.1$) (PROC GENMOD, SAS for Windows, version 9.13, SAS Institute, Cary, NC).

RESULTS

Colostral IgG concentration did not differ significantly among cows in their first and second lactation. Cows in their third lactation had higher colostral IgG concentrations compared with cows in their first or second lactation. Two calves did not ingest colostrum at both the first colostrum feeding and 12 hours of age and were excluded from the study. Summary of the results are shown in **Table 13**. Proportion of colostrum samples with IgG concentration ≤ 25, ≤ 50, ≤ 75 and ≤ 100 g/L were 0.07, 0.40, 0.67 and

0.85, respectively. The mean ± standard error of mean for colostral volume produced by all cows was 9.5 ± 0.6 L. Twenty calves (19.2 %) had failure of passive transfer (serum IgG concentration < 1340 mg/dL). Eighteen calves (17.2 %) ingested 3 L of colostrum at first feeding and 3 L at 12 hours of age. First feeding voluntary colostral intake was not significantly different among calves fed at 1, 2, 3 or 4 hours of age ($p > 0.05$). Calf age at first feeding (1, 2, 3 or 4 hours) and colostral volume ingested at first feeding, nor their interactions were not significant predictors of colostral intake at 12 hours of age ($p > 0.1$). Results of the logistic regression model predicting the probability of a calf having failure of passive transfer (serum IgG concentration < 1340 mg/dl) are represented in **Table 14**. Colostral IgG concentration, first colostral volume intake, colostral volume intake at 12 hours of age, interaction between colostral IgG concentration and colostral volume intake at first feeding or at 12 hours were significant predictors of 48-hour serum IgG concentration ($p < 0.1$). Parity of the dam, calf age at first feeding, calf birth weight, colostral volume produced by the dam were not significant predictors of FPT based on the 48-hour serum IgG concentrations ($p > 0.1$). Interactions between calf age at first feeding and volume of colostrum ingested at first feeding were not significant predictors of failure of passive transfer ($p > 0.1$). The probability of a calf having failure of passive transfer (FPT) based on the 48-hour serum IgG was calculated as follows (Afifi and Clark, 1984):

$$p(FPT) = \frac{1}{1 + \exp(-12.894 + 0.230 \times ColIgG + 2.695 \times V_1 + 3.793 \times V_{12} - 0.029 \times ColIgG \times V_1 - 0.061 \times ColIgG \times V_{12})}$$

where,

ColIgG = colostral IgG concentration,

V_1 = colostrum volume intake (L) at initial feeding (1, 2, 3 or 4 hours of age),

V_{12} = colostrum volume intake (L) at 12 hours of age,

exp = exponential function.

Summary of the predicted probability of failure of passive transfer for selected combinations of independent variables is presented in **Table 15**.

DISCUSSION

Cows in their third lactation had higher colostral IgG concentrations compared with cows in their first or second lactation consistent with previous studies (Tyler et al., 1999a). However, some studies have reported no statistical difference in colostral IgG concentration among cows in their first, second or third lactation (Chigerwe et al., accepted manuscript)**.** Feeding calves for a time period of 15 minutes at each feeding was considered representative of the maximum effort a producer would spend trying to feed a newborn calf. The time may vary depending on available labor on a given farm and the perceived value of a calf. Twelve hours of age was considered optimal for the second feeding because closure of the intestine to colostral immunoglobulins occurs at an increased rate after 12 hours of age (Stott et al., 1979b). Several studies reported different serum IgG concentrations as cut points for defining adequacy of passive transfer (McGuire and Adams, 1985; Besser et al., 1991; Selim et al., 1995; Tyler et al., 1996; Virtala et al., 1999). A serum IgG concentration of ≥1340 mg/dL was chosen to indicate optimum passive transfer in this study based on previous, large, field studies (Tyler et al.,

1996, 1999). Although the 48 hour serum IgG concentration endpoint chosen in this study is for optimal colostrum administration practice, the prevalence of FPT in this study is clearly less than optimal. Nearly 20 % of the calves receiving colostrum in the described manner will be at increased risk for morbidity, mortality and decreased productivity (Robinson et al., 1998; DeNise et al., 1989; Tyler et al., 1998; Virtala et al., 1999). An FPT prevalence < 10 % is a rational and achievable goal.

Calf age, up to 4 hours did not have a significant effect on calf's ability to suckle colostrum from a nipple bottle or on serum IgG concentration at 48 hours of age. Thus within the first 4 hours, delaying feeding of calves to ensure adequate vigor is not warranted. The results of this study showed that calves can suckle up to 3 L of colostrum at first feeding through a nipple bottle, consistent with previous studies (Hopkins and Quigley 1997). Other studies reported calves suckling up to 4 L through a nipple bottle (Morin et al., 1997). Furthermore, the results of this study indicate that calves will often ingest up to 3 L of colostrum at 12 hours of age regardless of colostral volume of intake at first feeding. Potentially calves can ingest colostral volumes exceeding 3 L at each feeding, if offered. It is important to note that probability of FPT is low when calves ingest lower volumes of colostrum of high colostral IgG at 12 hours of age. However, the proportion of colostrum with high colostral IgG concentrations (> 50 g/L) is relatively low. The probability of FPT in calves which ingested higher volumes of colostrum at both first feedings and at 12 hours was substantially lower, even at low colostral IgG concentration. Consequently, allowing calves to ingest as much as they would suckle within 1 to 4 hours of age and then at 12 hours of age will substantially reduce the

probability of failure of FPT. It should be noted that the results of this study are based on a single farm and are only applicable to calves which are bottle fed colostrum twice within 12 hours.

A summarized flow chart illustrating recommended colostrum administration practices by nipple bottle is represented in **Figure 3**. The presented recommendations are premised upon the goal of maintaining FPT rates less than 10% and colostral IgG concentrations of 40 to 50 g/L. It should be noted that in the present study 40 % of cows studied had colostral IgG concentrations less than 50 g/L.

Calves should be offered the maximum volume of colostrum which they will voluntarily within 4 hrs of birth and calves which ingest ≥ 3 L at the first feeding will need to ingest 1 L of colostrum at 12 hours to have optimum colostral intake. Calves which fail to ingest at least 1 L at 12 hours should be tube fed with 2 L of colostrum. Calves which ingest greater than 2 L, but less than 3 L of colostrum at 1, 2, 3, or 4 hours of age will require a minimum intake of an additional 2 L of colostrum by 12 of age. Those calves which do not ingest at least 2 L at 12 hours should be tube fed with 2 L of colostrum at this time. Calves which ingest less than 2 L at their first feeding should be targeted for immediate oroesophageal tube feeding and a total volume of 3 L should be administered in the first feeding. Calves in this category will need to ingest ≥ 1 L of colostrum at the second feeding to have optimum colostral intake. If they ingest < 1 L of they should be tube fed 2 L of colostrum. Based on our previous study in tube fed Holstein bull calves, if we assume a colostral IgG concentration of 50 g/L, we anticipate that calves fed 3 L once

will have an FPT rate of only 17 %, even if no other colostrum is provided at 12 hours of age (Chigerwe et al., accepted manuscript). Based on the results of this study we do not expect feeding of colostrum volumes exceeding 3 L once by oroesophageal tubing to decrease the rate of FPT in calves (Chigerwe et al., accepted manuscript). It should be noted the FPT rates in calves which are fed colostrum by combinations of nipple bottle and oroesophageal tubing have not been critically investigated **(Figure 3)**.

Bottle feeding of colostrum is potentially labor intensive on large dairy farms. However, a considerable percentage (59.2 %) of dairy producers preferred to feed colostrum to calves through a bucket or nipple bottle compared to 4.3 % of producers who tube fed colostrum (USDA-APHIS, 2007). Possible reason for preference of bottle feeding colostrum over tube feeding includes the technical skills and experience required to feed calves in this manner. Although previous studies suggested tube feeding of colostrum reduced failure of passive transfer rates compared with bottle feeding or nursing (Besser et al., 1991), bottle feeding improved colostral immunoglobulin absorption due to closure of the esophageal groove (Lateur-Rowet and Breukink, 1983). Currently no studies have match-paired calves to receive equal amounts of colostrum by tube and bottle feeding for comparison. Such studies would critically compare the two methods to assess absorption efficiency of colostral immunoglobulins between the two methods. These studies will likely form the basis for future recommendations to dairy farmers regarding optimal colostrum administration practices.

CHAPTER 7

CONCLUSIONS

The results of the second chapter demonstrates that presence of serum IgG before feeding colostrum is not related to calf birth weight, season or calf sex. Although calves from cows in their second lactation were 1.5 more likely to have detectable precolostral IgG concentration the relevance of this finding is yet to be determined. It is not possible to determine from the results of this study whether the source of the IgG is the dam or fetal response to antigens in utero. The study reported in chapter 2 is a single farm study and replicating the same study design on a farm with a higher prevalence of MAP or other infectious agents which can be transmitted transplacentally, but not tested in this study, may generate different findings.

Methods to assess colostral immunoglobulins have variable drawbacks, and not surprisingly a large percentage (40 %) of producers use visual appearance characteristics of colostrum to assess quality. The results of the immunoassay in chapter 3 and the results of the study in chapter 4 cannot be compared. The immunoassay has high sensitivity identifying colostral samples with low IgG concentration (< 50 g/L). The current cost per test is $US 4.00 and the test kit expires after 2- 3 years. The two hydrometers and electronic refractometer have similar sensitivity in identifying low IgG colostrum samples while weight of first milking colostral has very low sensitivity. The current initial costs for the methods are similar ($US70-100). However the refractometer

durable and does not require cooling of colostrum. Based on the results and its advantages over other methods, the refractometer is recommended for routine use for assessing colostral IgG concentrations.

Results of chapter 5 demonstrate that a total mass of 150-200 g of colostral IgG is required for adequate passive transfer when calves are fed by oroesophageal tubing. This recommended amount of colostral IgG is higher than previously recommended. Chapter 5 demonstrated that when calves are fed once by oroesophageal tubing, feeding 3 L of colostrum is more beneficial than feeding 4 L. Results from chapter 5 and 6 of this dissertation can not be directly compared because of the differences in frequencies and volume of colostrum fed to the calves. Studies with pair-matched calves fed by oroesophageal tubing or nipple bottle will be required to critically compare the two methods. It has been hypothesized that because of the closure of the esophageal group, more colostrum reaches the abomasum and small intestine in a shorter period of time when calves are bottle fed compared to oroesophageal feeding, hence increased efficiency of absorption. However no critical studies have reported the comparison of the efficiency of absorption between the two methods using radiolabelling techniques.

Chapter 6 demonstrates that calf age up to 4 hours does not affect the ability of the calf to suckle colostrum when offered through a nipple bottle. Additionally the amount ingested at 1, 2, 3, or 4 hours of age does not affect intake at 12 hours. Calves can ingest 3 L of colostrum if offered by nipple bottle. A study investigating the volume of colostrum which a calf can ingest if offered *ad libitum* is warranted.

The studies presented in this dissertation with the exception of chapter 2 were completed on a single farm. While conclusions from the studies are valid, variable results may be reported if the studies are reproduced on a different farm. The endpoints for defining FPT based on serum IgG concentration are variable and morbidity and mortality in calves in these studies were evaluated up to 3 months of age. More studies are required to evaluated morbidity and mortality beyond 3 months of age. The studies presented in this dissertation focused on colostral and serum IgG only. Previous studies evaluated colostral and serum IgM and IgA. One major reason for focusing on IgG is a result of its high percentage (>90 %) in colostrum and in serum at 48 hours of age.

Most of the studies have focused on the effect of colostral IgG in calf health and other colostral components have been underestimated. One possible reason could be that immunoglobulin concentrations are easier to determine compared with other colostral components. With the introduction of colostrum supplements and replacers, no studies have investigated presence or activity of immureactive cells in the products. Studies on pasteurization have also shown detrimental effects on colostral cells. More studies are required to elucidate the role of colostral components other than immunoglobulins on calf health and survival.

The mechanism by which gut closure occurs in calves still not well known. Growth factors in colostrum have been hypothesized to cause hyperplasia and maturation of intestinal cells resulting in more mature intestinal epithelial cells that are not permeable to large molecules.

Existing controlled studies comparing the efficiency of absorption of colostral immunoglobulins between calves fed by oroesophageal tubing or bottle have some study design flaws. Studies by Besser (1991) compared three methods of administering colostrum on three different farms. In the study by Besser (1991), calves fed by bottle were offered colostrum several times for 48 hours, while tube fed were only fed once. Studies by Adams (1985) compared two methods of feeding colostrum in four different breeds of cattle.

Only one study determined the effect of colostral immunoglobulins on subsequent milk production (DeNise et al., 1988). However calves in this study were raised in Arizona for 6 months, moved to Idaho and then transferred back to Arizona at 7 to 8 months of gestation. Many confounding variables are present in this study and it is difficult to attribute the difference in milk production to serum IgG concentration at 24 to 48 hours of age. A repeat study involving calves raised on the same farm is more appropriate.

The results of data presented can be applied to dairy farms feeding colostrum by bottle and by oroesophageal tubing. The electronic refractometer is the recommended method to assess colostrum IgG concentrations. On farms feeding colostrum by oroesphageal tubing of 3 L of colostrum is recommended. On farms feeding colostrum by nipple bottle, offering colostrum *ad libitum* within 4 hours of age and at a second feeding at 12 hours is recommended. Calves that do not suckle < 2 through the bottle at the first feeding should be tube fed.

Table 1. Cross-classification of results of a cow-side immunoassay kit for assessing IgG concentration in 135 bovine colostrum and milk samples versus results of radial immunodiffusion.

Immunoassay result	Radial immunodiffusion	
	< 50 g/L	≥ 50 g/L
Positive	51	19
Negative	4	61

Sensitivity of the immunoassay = True positives/ (True positives + False negatives)

= 51/ (51 + 4)

= 93 %

Specificity of the immunoassay = True negatives/ (True negatives + False positives)

= 61/ (61 + 19)

= 76 %

Table 2. Sensitivity and specificity of hydrometer #1 for detection of low IgG concentration (< 50 g IgG/L) in colostrum collected from 171 Holstein cows of varying parity.

Hydrometer endpoint (g IgG/L)	Se (95 % CI)	Sp (95 % CI)	Total amount of colostrum accepted (L)	Proportion of samples accepted
≤ 10	0.05 (0, 0.11)	0.99 (0.81, 1)	1258.7	1.00
≤ 20	0.11 (0.03, 0.19)	0.98 (0.95, 1)	1220.1	0.97
≤ 30	0.21 (0.11, 0.33)	0.97 (0.94, 1)	1193.1	0.95
≤ 40	0.29 (0.17, 0.41)	0.95 (0.92, 0.99)	1126.1	0.91
≤ 50	0.47 (0.34, 0.60)	0.93 (0.88, 0.98)	1052.0	0.88
≤ 60	0.61 (0.49, 0.75)	0.83 (0.76, 0.90)	960.4	0.80
≤ 70	0.75 (0.63, 0.86)	0.78 (0.71, 0.86)	810.2	0.70
≤ 80	0.8 (0.69, 0.91)	0.66 (0.58, 0.75)	697.34	0.61
≤ 90	0.93 (0.86, 0.99)	0.52 (0.43, 0.62)	582.0	0.51
≤ 100	0.96 (0.91, 1.00)	0.40 (0.32, 0.49)	417.5	0.38
≤ 110	1.00 (*)	0.29 (0.21, 0.38)	303.0	0.29
≤ 120	1.00 (*)	0.25 (0.18, 0.34)	188.2	0.20
≤ 130	1.00 (*)	0.09 (0.04, 0.14)	117.7	0.12
≤ 140	1.00 (*)	0.00 (*)	55.5	0.06

* Confidence intervals could not be calculated due to Se =1 or Sp = 0.

Table 3. Sensitivity and specificity of hydrometer #2 for detection of low IgG concentration (< 50 g IgG/L) in colostrum collected from 171 Holstein cows of varying parity.

Hydrometer endpoint (g IgG/L)	Se (95 % CI)	Sp (95 % CI)	Total amount of colostrum accepted (L)	Proportion of samples accepted
≤ 25	0.11 (0.03, 0.19)	0.99 (0.97, 1.00)	1258.2	1.00
≤3 7.5	0.15 (0.05, 0.24)	0.97 (0.93, 1.00)	1198.7	0.96
≤ 50	0.35 (0.22, 0.47)	0.94 (0.90, 0.98)	1163.0	0.93
≤ 62.5	0.36 (0.24, 0.49)	0.88 (0.85, 0.91)	1076.8	0.85
≤ 75	0.67 (0.54, 0.80)	0.74 (0.66, 0.82)	991.9	0.79
≤ 87.5	0.76 (0.65, 0.88)	0.66 (0.58,0.75)	692.9	0.61
≤ 100	0.89 (0.81, 0.97)	0.53 (0.44, 0.63)	607.2	0.53
≤ 112.5	0.92 (0.86, 1.00)	0.40 (0.31, 0.49)	452.1	0.40
≤ 125	1.00 (*)	0.00 (*)	318.7	0.29

* Confidence intervals could not be calculated due to Se =1 or Sp = 0.

Table 4. Sensitivity and specificity of the refractometer for detection of low IgG concentration (< 50 g IgG/L) in colostrum collected from 171 Holstein cows of varying parity.

Refractometer endpoint (Brix %)	Se (95 % CI)	Sp (95 % CI)	Total amount of colostrum accepted (L)	Proportion of samples accepted
≤ 14	0.07 (0.04, 0.11)	1.00 (*)	1214.9	0.97
≤ 15	0.13 (0.04, 0.21)	0.99 (0.97, 1.00)	1176.6	0.95
≤ 16	0.16 (0.07, 0.26)	0.97 (0.93,1.00)	1158.4	0.94
≤ 17	0.22 (0.11, 0.33)	0.97 (0.93, 1.00)	1107.1	0.91
≤ 18	0.31 (0.19, 0.43)	0.97 (0.92, 0.99)	1062.4	0.88
≤ 19	0.40 (0.27, 0.53)	0.93 (0.88, 0.98)	1003.7	0.82
≤ 20	0.52 (0.40, 0.66)	0.92 (0.87, 0.97)	929.3	0.79
≤ 21	0.64 (0.51, 0.76)	0.90 (0.84, 0.95)	854.5	0.73
≤ 22	0.75 (0.63, 0.86)	0.78 (0.70, 0.85)	730.1	0.63
≤ 23	0.80 (0.69, 0.91)	0.65 (0.56, 0.73)	591.9	0.51
≤ 24	0.84 (0.74, 0.93)	0.58 (0.49, 0.67)	500.6	0.44
≤ 25	0.87 (0.78, 0.96)	0.47 (0.38, 0.57)	393.9	0.36
≤ 26	0.91 (0.83, 0.99)	0.42 (0.33, 0.51)	353.4	0.32
≤ 27	0.93 (0.86, 1.00)	0.33 (0.24, 0.41)	293.8	0.26
≤ 28	0.93 (0.86, 1.00)	0.27 (0.19, 0.35)	226.3	0.20
≤ 29	0.96 (0.91, 1.00)	0.20 (0.13, 0.270	161.8	0.15
≤ 30	0.96 (0.91, 1.00)	0.16 (0.10, 0.23)	134.0	0.13
≤ 31	0.98 (0.95, 1.00)	0.10 (0.05, 0.16)	81.9	0.08
≤ 32	1.00 (*)	0.07 (0.02, 0.13)	48.9	0.05

* Confidence intervals could not be calculated due to Se or Sp = 1.

Table 5. Sensitivity and specificity of weight of first milking colostrum for detection of low IgG concentration (< 50 g IgG/L) in colostrum collected from 171 Holstein cows of varying parity.

Colostral weight (kg)	Se (95 % CI)	Sp (95 % CI)	Total amount of colostrum accepted (L)	Proportion of samples accepted
>3	0.80 (0.69, 0.91)	0.12 (0.06, 0.18)	1225.4	0.90
>4	0.72 (0.61, 0.84)	0.27 (0.19, 0,35)	1133.0	0.74
>5	0.61 (0.49, 0.75)	0.34 (0.26, 0.43)	1071.0	0.68
>6	0.54 (0.41, 0.68)	0.46 (0.37, 0.55)	974.4	0.56
>8.0	0.42 (0.35, 0.48)	0.69 (0.61, 0.77)	741.4	0.36
>8.5	0.42 (0.28, 0.55)	0.69 (0.61, 0.77)	692.1	0.33
>9	0.36 (0.23, 0.49)	0.81 (0.74, 0.88)	606.4	0.27
>10	0.31 (0.19, 0.43)	0.86 (0.80, 0.92)	494.4	0.20
>15	0.11 (0.03, 0.19)	0.97 (0.94, 1)	214.5	0.06
>20	0.07 (0.04, 0.11)	1 (*)	77.3	0.02

* Confidence intervals could not be calculated due to Sp = 1.

Table 6. Comparison of sensitivity and specificity of four methods at the chosen endpoints.

Method	Se	Sp
Hydrometer #1	0.75^{a}	0.78^{c}
Hydrometer #2	0.76^{a}	0.66^{d}
Refractometer	0.75^{a}	0.78^{c}
Weight of first milking colostrum	0.42^{b}	$0.74^{c,d}$

$^{a-d}$ = Se and Sp with different superscripts differ ($P < 0.05$).

Table 7. Linear regression equations for the relationship between H1, H2, the electronic refractometer and weight of first milking colostrum and IgG concentration as determined by RID ($p<0.05$).

Method	Regression equation	R^2
H1	Colostral IgG = 15.3 + 0.63 x H1	0.41
H2	Colostral IgG = 14.4 + 0.59 x H2	0.30
Refractometer	Colostral IgG = -24.7 + 3.96 x Brix	0.41
Weight of first milking colostrum	Colostral IgG = 77.6 – 1.2 x Weight	0.03

Table 8. Assignment to time and volume defined groups using random assignment without replacement of the 120 calves.

Age of calf (hours)	Volume of colostrum fed (L)				
	1	**2**	**3**	**4**	**N**
2	n = 5	n = 5	n = 5	n = 5	20
6	n = 5	n = 5	n = 5	n = 5	20
10	n = 5	n = 5	n = 5	n = 5	20
14	n = 5	n = 5	n = 5	n = 5	20
18	n = 5	n = 5	n = 5	n = 5	20
22	n = 5	n = 5	n = 5	n = 5	20
N	30	30	30	30	120

Table 9. Results of a multiple regression analysis predicting serum IgG concentration (mg/dl) at 48 hours in 120 bull calves fed different colostral volumes at different times generated from the multiple regression analysis ($r^2 = 0.39$).

Predictor	Parameter estimate	Standard error	F-value	P-value
Intercept	509.049	146.833	12.02	0.0007
Colostral IgG concentration (g/L)	5.795	1.359	18.18	< 0.0001
Feeding 2 L of colostrum	464.357	115.712	16.10	0.0001
Feeding 3 L of colostrum	633.756	115.834	29.93	< 0.0001
Feeding 4 L of colostrum	623.670	116.051	28.88	< 0.0001
Age of calf at colostrum feeding	- 20.058	5.983	11.24	0.0011

Table 10. Average of total mass of colostral IgG (g) required for adequate passive transfer when fed varying colostrum volumes at various ages after calving in 120 bull calves.

Age of calf at feeding (hours)	Feeding 1 L colostrum	Feeding 2 L colostrum	Feeding 3 L colostrum	Feeding 4 L colostrum
2	150	140	123	171
3	154	147	133	185
4	157	154	144	198
5	161	161	154	212
6	164	168	164	226
7	168	175	175	240
8	171	182	185	254
9	175	189	196	268
10	178	196	206	282
11	181	203	216	295
12	185	210	227	309
13	188	217	237	323
14	192	223	248	337
15	195	230	258	351
16	199	237	268	365
17	202	244	279	379
18	206	251	289	392
19	209	258	299	406
20	213	265	310	420
21	216	272	320	434
22	220	279	331	448

Table 11. Results of a logistic regression model predicting the probability of a calf having failure of passive transfer (serum IgG concentration <1340 mg/dl) based on 48-hour serum IgG concentration in 120 dairy bull calves ($p<0.1$).

Variables	Coefficients (95 % CI)	P-value
Intercept	-0.3543 (-4.0188 , 3.3101)	0.8497
Colostral IgG concentration	- 0.0283 (-0.0459, -0.0106)	0.0017
Age of calf at feeding colostrum (hours)	0.1052 (0.0296, 0.1809)	0.0187
Feeding 1 L of colostrum	3.8115 (1.5561, 6.0669)	<0.0001

Table 12. Probabilities of a calf fed colostrum by oroesophageal tubing having failure of passive transfer of colostral IgG (serum IgG concentration <1340 mg/dl) as a function of colostral IgG concentration, volume of colostrum fed and time of colostrum administration in 120 dairy bull calves.

Volume of colostrum fed (L)		Colostral IgG concentration (g/L)				
		25	50	75	100	125
1 L	2 h	0.95	0.90	0.81	0.68	0.51
	6 h	0.97	0.94	0.88	0.78	0.63
	10 h	0.99	0.96	0.92	0.84	0.73
	14 h	0.99	0.97	0.94	0.89	0.80
	18 h	0.99	0.98	0.96	0.93	0.86
	22 h	0.99	0.99	0.97	0.95	0.90
2, 3 or 4 L	2 h	0.29	0.17	0.09	0.05	0.02
	6 h	0.39	0.24	0.14	0.07	0.03
	10 h	0.50	0.33	0.19	0.11	0.06
	14 h	0.60	0.43	0.27	0.15	0.08
	18 h	0.70	0.53	0.36	0.22	0.12
	22 h	0.78	0.63	0.46	0.30	0.17

Table 13. Summary of means ± standard error of means for colostral IgG concentrations of 104 cows and voluntary colostral intake, calf birth weight and 48-hour serum IgG concentrations of 104 heifer calves.

Cows			Calves	
	Number of cows	Colostral IgG concentration (g/L)	Initial colostral volume intake (L)	2.3 ± 0.1
1st Lactation cows	40	63.5 ± 5.2[a]	Colostral volume intake at 12 hours of age (L)	2.2 ± 0.1
2nd Lactation cows	28	60.7 ± 6.5[a]	Calf weight (kg)	38.9 ± 0.6
≥3rd Lactation cows	46	72 ± 5.6[b]	Serum IgG (mg/dL) concentration at 48 hours of age	1777.3 ± 57.8
All cows	104	66 ± 3.4	Total number of calves	104

Means with the same superscript are not significantly different ($p > 0.05$).

Table 14. Results of the logistic regression analysis predicting the probability of a calf having failure of passive transfer (serum IgG concentration <1340 mg/dl) based on 48-hour serum IgG concentration in 104 Holstein heifer calves ($p < 0.1$)

Predictor	Coefficient (95 % CI)	P-value
Intercept	12.894 (2.224, 23.563)	0.018
Colostral IgG concentration	-0.230 (-0.388, -0.072)	0.004
First colostral volume intake by calf	-2.695 (-5.048, -0.343)	0.025
Colostral volume intake at 12 hours of age	-3.793 (-6.507, -1.080)	0.0061
Interaction between initial colostral volume intake by calf and colostral IgG concentration	0.029 (-0.002, 0.059)	0.064
Interaction between colostral IgG concentration and colostral intake at 12 hours of age	0.061 (0.013, 0.109)	0.013

Table 15. Probabilities of failure of passive transfer (FPT) in Holstein heifers fed varying amounts of colostrum with varying IgG concentrations calculated using a logistic model derived from observations in 104 heifer calves bottle fed initially at 1, 2, 3 or 4 hours of age followed by a second feeding at 12 hours of age. Calculated FPT rates exceeding 20% are reported in a bold font.

Volume of colostrum ingested (L)		Colostral IgG concentration (g/L)				
Initial colostral volume (L) intake at 1, 2, 3 or 4 hours of age	Volume intake (L) at 12 hours of age	25	50	75	100	125
0	1	**0.99**	**0.65**	0.03	<0.001	<0.001
0	2	**0.93**	**0.46**	0.05	<0.001	<0.001
0	3	**0.57**	**0.27**	0.10	0.03	0.009
1	0	**0.99**	**0.56**	0.008	<0.001	<0.001
1	1	**0.95**	**0.36**	0.02	<0.001	<0.001
1	2	**0.65**	**0.20**	0.03	0.005	<0.001
1	3	0.16	0.10	0.06	0.04	0.02
2	0	**0.96**	**0.27**	0.005	<0.001	<0.001
2	1	**0.72**	0.14	0.01	<0.001	<0.001
2	2	**0.21**	0.07	0.02	0.006	0.002
2	3	0.03	0.03	0.04	0.05	0.07
3	0	**0.79**	0.10	0.003	<0.001	<0.001
3	1	**0.27**	0.05	0.007	<0.001	<0.001
3	2	0.04	0.02	0.01	0.008	0.005
3	3	0.04	0.02	0.01	0.008	0.005

Figure 1. Regression line generated from serial dilutions of a standard bovine IgG. Regression equation: IgG concentration = 0.0209 + 0.0039 x (zone diameter)^2. $R^2 = 0.98$.

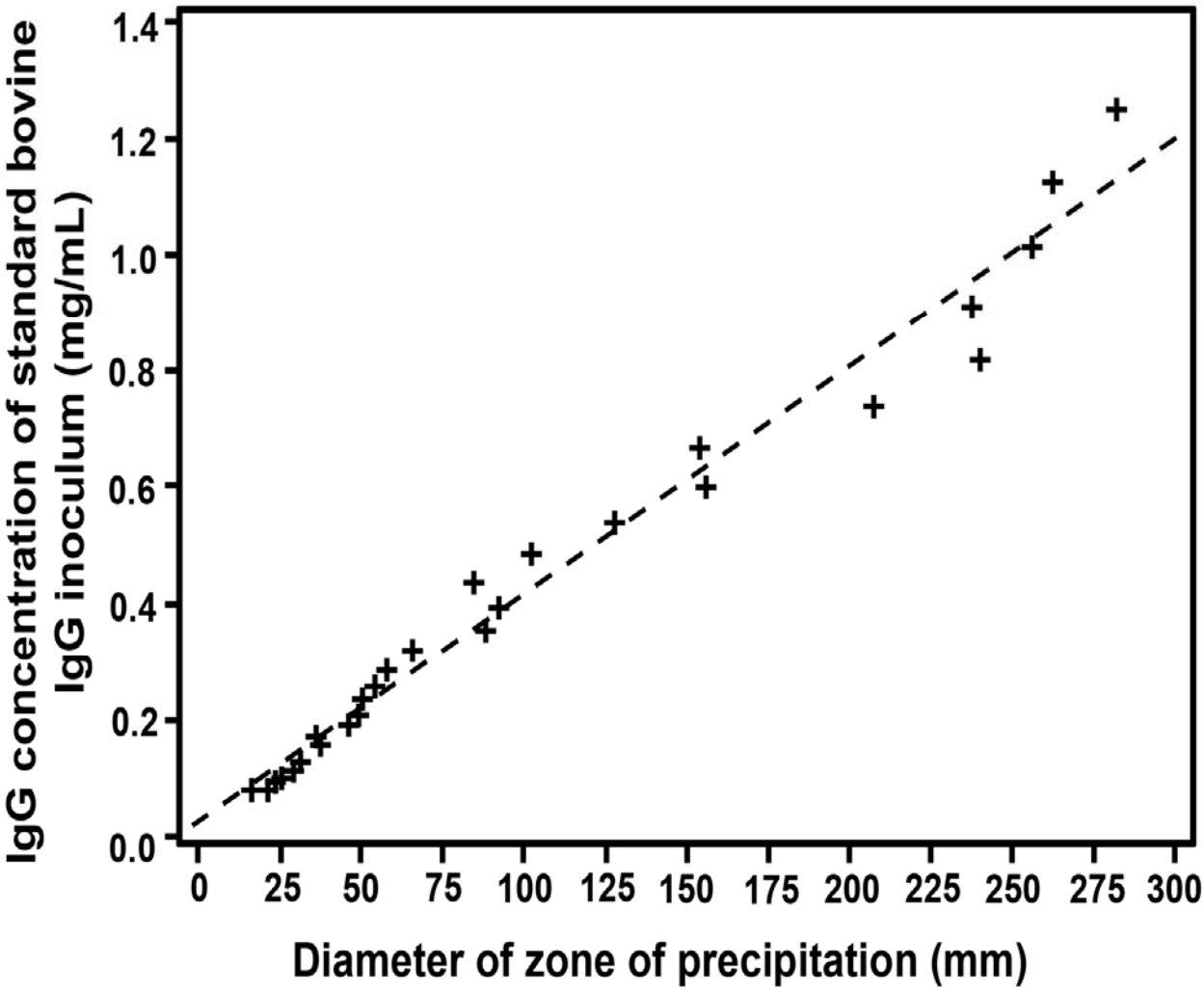


Figure 2. Histogram illustrating the relative frequency of various immunoglobulin concentrations in 170 dairy calves distribution of immunoglobulin concentration prior to feeding colostrum.

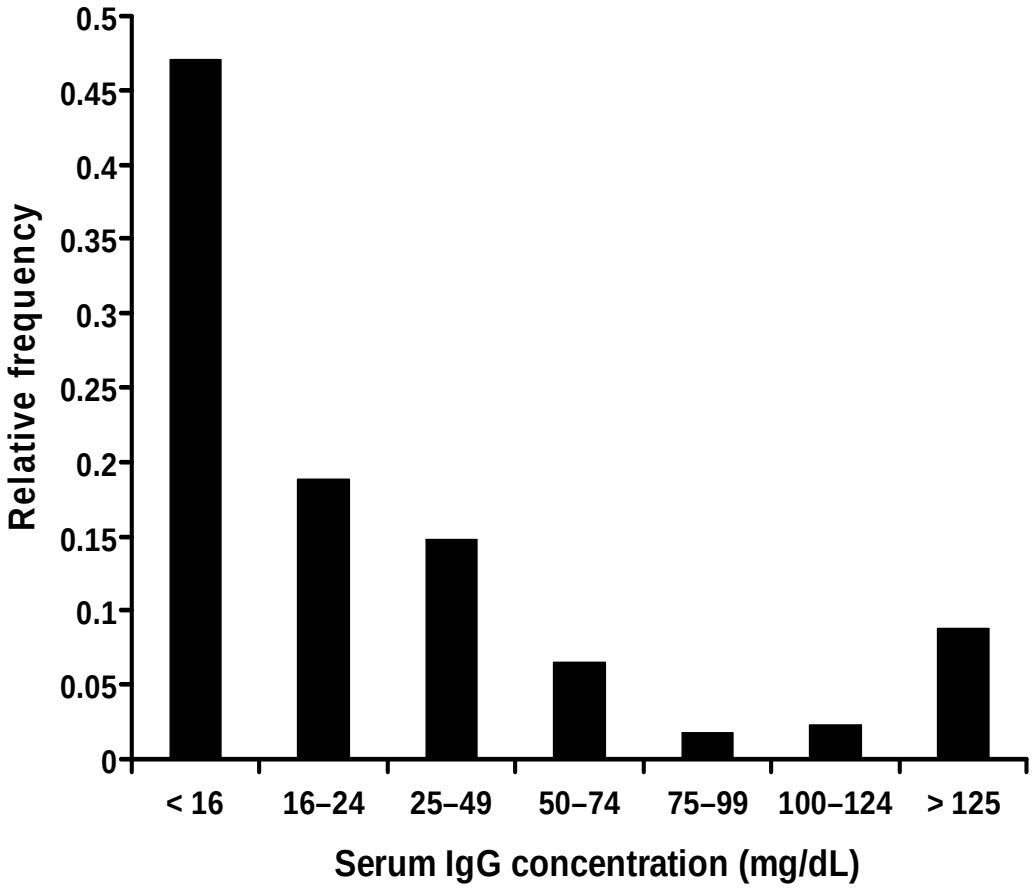
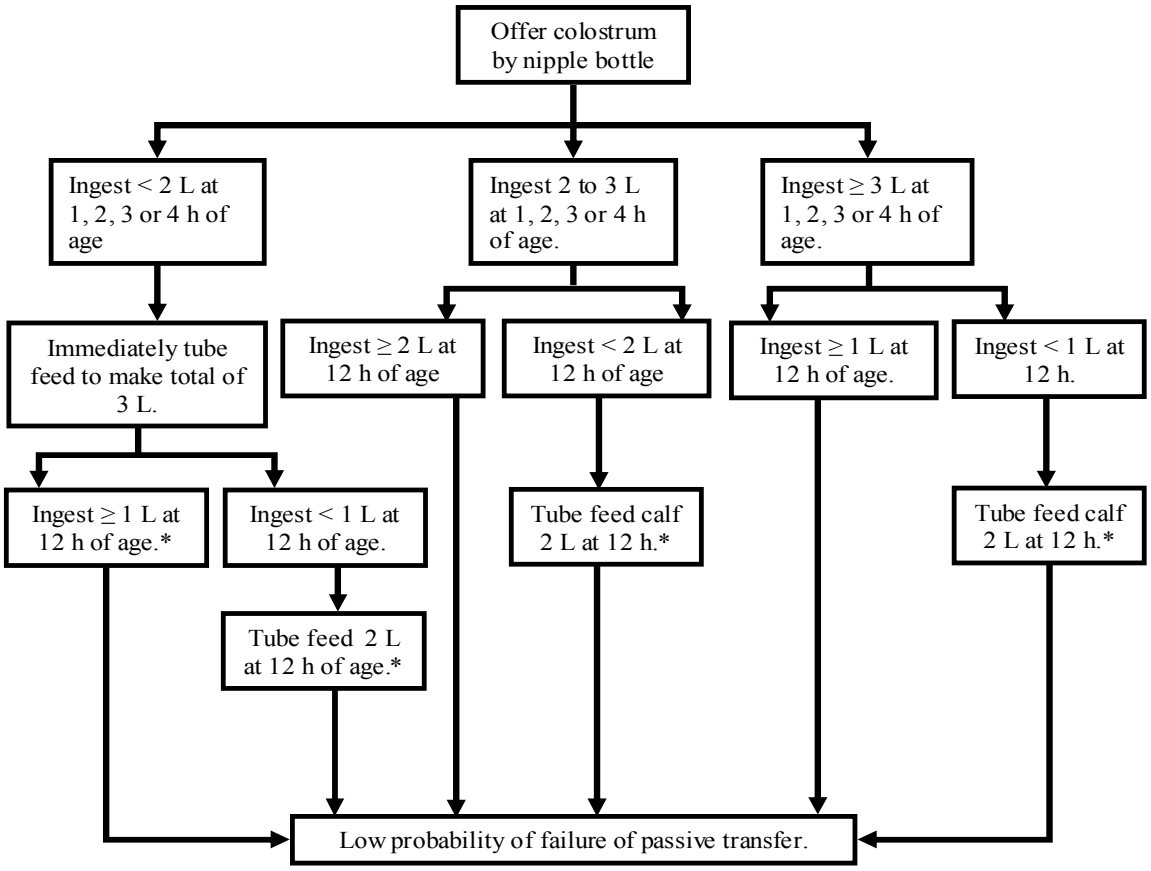


Figure 3. Flow chart summarizing recommended standard operating procedures for the feeding of colostrum by nipple bottle based on colostral intake at first feeding (1, 2, 3 or 4 hours of age) and intake at 12 hours of age.



*The FPT rates in calves which are fed by nipple bottle and oroesophageal tubing can not be calculated.

BIBLIOGRAPHY

1. Adams GD, Bush LD, Horner JL, et al. Two methods for administering colostrum to newborn calves. J Dairy Sci 1985;68:773-775.

2. Afifi AA, Clark V. Logistic regression: another method of classification. In: Beal C, ed. Computer-aided multivariate analysis. Belmont California: Lifetime Learning Publications, 1984;287-307.

3. Arthur GH. The development of the conceptus. In: Arthur GH, Noakes DE, Pearson H, et al, eds. Pregnancy and parturition in veterinary reproduction and obstetrics. 2nd ed. Philadelphia: WB Saunders, 1999;51–109.

4. Banks KL. Host defense in the newborn animal. J Am Med Assoc 1982;1053-1056.

5. Baszler TV, Adams S, Vander-Schalie J, et al. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. J Clin Microbiol 2001;39:3851–3857.

6. Barrington GM, Besser TE, Davis WC, et al. Expression of immunoglobulin G_1 receptors in bovine mammary epithelial cells and mammary leukocytes. J Dairy Sci 1997;80:86-93.

7. Ber KJ. Distribution of Brucella antibody among immunoglobulin classes and a low molecular weight antibody fraction in serum and whey of cattle. Res Vet Sci 1973;14:381-384.

8. Besser T, McGuire TC, Gay CC, et al. Transfer of functional immunoglobulin G antibody into the gastrointestinal tract accounts for IgG clearance in calves. J Virol 1988;62:2234-2237.

9. Besser T, Szenci O, Gay CC. Decreased colostral immunoglobulin absorption in calves with postnatal respiratory acidosis. J Am Vet Med Assoc 1990;196:1239-1243.

10. Besser TE, Gay CC, Pritchett L. Comparison of three methods of feeding colostrum to dairy calves. J Am Vet Med Assoc 1991;198:419-422.

11. Besser TE, Gay CC. The importance of colostrum to the health of the neonatal calf. Vet Clin North Am Food Anim Pract 1994;10:107–115.

12. Brandon MR, Watson DL, et al. The mechanism of transfer immunoglobulin into mammary secretion of cows. Aust J Exp Biol Med 1971;49;613-623.

13. Buhler C, Hammon H, Rossi GL, et al. Small intestinal morphology in eight-day-old calves fed colostrum for different durations on only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. J Anim Sci 1998;76:758-765.

14. Bush LJ, Staley TE. Absorption of colostral immunoglobulins in newborn calves. J Dairy Sci 1980;63:672-680.

15. Butler JE. Bovine immunoglobulins: a review. J Dairy Sci 1969;52:1895.

16. Butler JE, Kiddy CH, Pierce CS, et al. Quantitative charges associated with calving in the levels of bovine immunoglobulins in selected body fluids.1. Changes in the levels of IgA, IgG, and total protein. Can J Comp Med 1972;36:243-242.

17. Cash RSG. Colostral quality determined by refractometry. Equine Vet Ed 1999; 11:36-38.

18. Chigerwe M, Dawes ME, Tyler JW, et al. Evaluation of a cow-side immunoassay kit for assessing IgG concentration in colostrum. J Am Vet Med Assoc 2005; 227:129-131.

19. Chigerwe M, Tyler JW, Schultz LG, et al. The effect of colostrum administration practices on serum IgG concentrations in tube fed Holstein bull calves. Am J Vet Res. Accepted for publication

20. Clark RC, McEwen SA, Gannon VP, et al. Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in South-Western Ontario. Epidemiol Infect 1989;102:253-260.

21. Concha C, Holmberg D, Morein B. Characterization and response to mitogens of mammary lymphocytes from bovine dry-period secretion. J Dairy Res 1980;47:305-311.

22. Daniel WW. Biostatistics: a foundation for analysis in the health sciences.3rd ed. New York: John Wiley and Sons, 1983; 125-135.

23. Day NKB, Pickering RJ, Gewurz H, et al. Ontogenic development of the complement system. Immunology 1969;16:319-326.

24. Dean HJ, Hunsaker BD, Bailey OD, et al. Prevention of persistent infection in calves by vaccination of dams with noncytopathic type-1 modified-live bovine viral diarrhea virus prior to breeding. *Am J Vet Res* 2003;64:530–537.

25. Deregt D, Smithson S, Kozub GC. A short incubation serum neutralization test forbovine viral diarrhea virus. Can J Vet Res 1992;56:161–164.

26. DeNise SK, Robison JD, Stott GH, et al. Effects of passive immunity on subsequent production in dairy heifers. J Dairy Sci 1989;72:552-554.

27. Devery-Pocius JE, Larson BL. Age and previous lactations as factors in the amount of bovine colostral immunoglobulins. J Dairy Sci 1983;66:221-226.

28. Diaz de Villegas MC, Oria R, Sara FJ, et al. Lipid binding by beta-lactoglobulin of cow milk. Milchwissenschaft 1987;42:357-358.

29. Dietz RE, Hall JB, Whittier WD, et al. Effects of feeding supplemental fat to beef cows on cold tolerance in newborn calves. J Anim Sci 2003;885-894.

30. Dohoo I, Martin W, Stryhn H. Measures of association. In: Veterinary epidemiologic research. Charlottetown, PEI: AVC Inc, 2003;85–149.

31. Douglas DC, Reber AJ, Gabbard JD, et al. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. Am J Vet Res 2007;68:778-782.

32. Doyle TM. Foetal infection in Johne's disease. Vet Rec 1958;70:238.

33. Drewry JJ, Quigley JD, Geiser DR, et al. Effect of high arterial carbondioxide tension on effciciency of immunoglobulin G absorption in calves. Am J Vet Res 1999;60:609-613.

34. Dubey JP. Neosporosis in cattle. Vet Clin North Am Food Anim Pract 2005;21:473–483.

35. Dubey JP, Buxton D, Wouda W. Pathogenesis of bovine neosporosis. J Comp Pathol 2006;134:267–289.

36. Duncan JR, Wilkie BN, Hiestand F, et al. The serum and secretory immunoglobulins of cattle: Characterization and quantitation. J Immun 1972;108:965-976.

37. Elass-Rochard E, Legrand D, Salmon V, et al. Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. Infect Immun 1998;66:486-491.

38. Evermann JF, DiGiacomo RF, Hopkins SG. Bovine leukosis: understanding viral transmission and the methods of control. Vet Med 1987;10:1051–1058.

39. Fahey JL, McKelvey EM. Quantitative determination of serum immunoglobulin in anti-body agar plates. J Immunol 1965;94;84.

40. Farber JM, Sanders GW, Malcolm SA, et al. The presence of *Listeria* spp. In raw milk in Ontario. Can J Microbio 1988;34:95-100.

41. Fleenor WA, Stott GH. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. J Dairy Sci 1980;63:973-977.

42. Fleenor WA, Stott GH. Single radial immunodiffusion analysis for quantitation of colostral immunoglobulin concentration. J Dairy Sci 1981;64:740-747.

43. Foster DM, Smith GW, Truman SR, et al. Serum IgG concentrations in dairy calves fed two colostrum replacement products. J Am Med Assoc 2006;229:1282-1285.

44. Fowden AL, Ward JW, Woodling FPB, et al. Programming placental nutrient transport capacity. J Physiol 2006;572:5-15.

45. Garry FB. Clinical aspects of acidosis in newborn calves, in Proceedings. Int Conf Vet Perinatol 1993;60-63.

46. Gay CC. Colostrum research says feed 4 quarts for healthier calves. Hoard's Dairyman 1994;139:256.

47. Girard CL, Matte JJ. Dietary supplements of folic acid during lactation: Effects on the performance of dairy cows. J Dairy Sci 1998;81:1412-1419.

48. Godden SM, Smith, S, Feirtag JM, et al. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum IgG concentration in dairy calves. J Dairy Sci 2003;86:1503-1512.

49. Grant IR, Ball HJ, Rowe MT. Thermal inactivation of several Mycobacterium spp. in milk by pasteurization. Appl Microbiol 1996;22:253-256.

50. Guy CS, Williams DJL, Kelly DF, et al. *Neospora caninum* in persistently infected, pregnant cows: spontaneous transplacental infection is associated with an acute increase in maternal antibody. Vet Rec 2001;149:443–449.

51. Hagiwara K, Kataoka S, Yamanaka H, et al. Detection of cytokines in bovine colostrum in bovine colostrum. Vet Immuno and Immmunopath 2000;76:183-190.

52. Hall CA, Reichel MP, Ellis JT. *Neospora* abortions in dairy cattle: diagnosis, mode of transmission and control. Vet Parasitol 2004;128:231–241.

53. Hammer CJ, Quigley JD, Rebeiro et al. Characterization of a colostrum replacer and a colostrum supplement containing IgG concentrate and growth factors. J Dairy Sci 2004;87:106-111.

54. Holloway NM, Tyler JW, Lakritz J, et al. Serum immunoglobulin G concentrations in calves fed fresh and frozen colostrum. J Am Vet Med Assoc 2001;219:357-358.

55. Hostetler D, Douglas VL, Tyler JW, et al. Immunoglobulin G concentrations in temporal fractions of first milking colostrums. J Appl Res Vet Med 2003;1:168–171.

56. Hudgens KA, Tyler JW, Besser TE, et al. Optimizing performance of a qualitative zinc sulfate turbidity test for passive transfer of immunoglobulin G in calves. Am J Vet Res 1996;57;1171-1173.

57. Jacobsen KL, Bull RW, Miller JM, et al. Transmission of bovine leukemia virus: prevalence of antibodies in precolostral calves. Prev Vet Med 1982;1:265–272.

58. Lakritz J, Tyler JW, Hostetler DE. Effects of pasteurization of colostrum on subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. A J Vet Res 2000;61:1021-1025.

59. Lammoglia MA, Bellow RA, Grings EE, et al. Effects of feeding beef females supplemental fat during gestation on cold tolerance in newborn calves. J Anim Sci 1999;77:824-834.

60. Lateur-Rowet HJM, Breukink HJ. The failure of the oesophageal groove reflex when fluids are given with an oesophageal feeder to newborn and young calves. Vet Quarterly 1983;5:68-74.

61. Lawrence WE. Congenital infection with *Mycobacterium johnei* in cattle. Vet Rec 1956;68:312–314.

62. Kaske M, Werner A, Schuberth HJ, et al. Colostral management in calves: effects of drenching vs. bottle feeding. J Anim Physiology and Nutrition 2005;89:151-157.

63. Kehoe SI, Heinrichs AJ. Bovine colostrum nutrient composition. CAB Reviews:Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2007;2:1-9.

64. Kickhofen B, Hammer DK, Westphal M. Occurrence of IgG fragments in the urine of the newborn calves. Eur J Immunol 1971;1:49.

65. Kmetz M, Dunne H, Schultz RD. Leukocytes as carriers in transmission of bovine leukemia: invasion of the digestive tract of the newborn by ingested, cultured, leukocytes. Am J Vet Res 1970;100:445-449.

66. Kruse V. Yield of colostrum and immunoglobulin in cattle at first milking after parturition. Anim Prod 1970;12:619-626.

67. Larson BL. Transfer of specific blood serum protein to lacteal secretions near parturition. J Dairy Sci 1958;41:1033.

68. Larson BL, Heary HL, Devery JE. Immunoglobulin production and transport by the mammary gland. J Dairy Sci 1979;63:665-671.

69. Lassauzet MLG, Thurmond MC, Johnson WO, et al. Factors associated with in utero or periparturient transmission of bovine leukemia virus in calves on a California dairy. Can J Vet Res 1991;55:264–268.

70. Lee WJ, Farmer JL, Hilty M, et al. The protective effects of lactoferrin feeding against endotoxin lethal shock in germfree piglets. Infect Immun 1998;66:1421-1426.

71. McGuire TC, Adams DS. Failure of colostral immunoglobulin transfer to calves: prevalence and diagnosis. *Comp Cont Educ* 1982;4:S35-S39.

72. Mach JP, Pahud JJ. Secretory IgG: A major immunoglobulin in most bovine external secretions. J Immunol 1971;106:552.

73. Matte JJ, Girard, Seoane JR. Absorption of colostral immunoglobulin G in the newborn dairy calf. J Dairy Sci 1982;65:1765-1770.

74. Maunsell FP, Morin DE, Constable PD, et al. Use of mammary gland and colostral characteristics for prediction of colostral IgG1 concentration and intramammary infection in Holstein cows. J Am Vet Med Assoc 1999;214:1817-1823.

75. McGuirk SM, Collins M. Managing the production, storage and delivery of colostrum Vet Clin of North Am Food Anim 2004;20:593-603.

76. Meylan M, Rings DM, Shulaw WP, et al. Survival of *Mycobaterum paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. A J Vet Res 1996;57:1580-1585.

77. Mechor GD, Grohn YT, Van Saun RJ. Effect of temperature on colostrometer readings for estimation of immunoglobulin concentration in bovine colostrum. J Dairy Sci 1991;74:3940-3943.

78. Mechor GD, Grohn YT, McDowell LR, et al. Specific gravity of bovine colostrum immunoglobulins as affected by temperature and colostrum components. J Dairy Sci 1992;75:3131-3135.

79. Molla A. Estimation of bovine colostral immunoglobulins by refractometry. Vet Rec 1980;107:35-36.

80. Moore M, Tyler JW, Chigerwe M, et al. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. J Am Vet Med Assoc 2005;226:1375-1377.

81. Morin DE, McCoy GC, Hurley WL. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G_1 absorption in Holstein bull calves. J Dairy Sci 1997;80:747-753.

82. Morin DE, Constable PD, Maunsell FP, et al. Factors associated with colostral specific gravity in dairy cows. J Dairy Sci 2001;84:937-943.

83. Muller LD, Ellinger DK. Colostral immunoglobulin concentration among breeds of dairy cattle. J Dairy Sci 1981;64:1727-1730.

84. Nagy DW, Tyler JW, Kleiboeker SB, et al. Use of a polymerase chain reaction assay to detect bovine leukosis virus in dairy cattle. J Am Vet Med Assoc 2003;222:983–985.

85. Nagy DW, Tyler JW, Kleiboeker SB. Decreased periparturient transmission of bovine leukosis virus in colostrum. J Vet Intern Med 2007;21:1104-1107.

86. Nakamura T, Kawase H, Kimura K, et al. Concentration of sialyoligosaccharides in bovine colostrum and milk during prepartum and early lactation. J Dairy Sci 2003;86:1315-1320.

87. Nardone A, Lacetera N, Bernabucci U, et al. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and early postpartum period. J Dairy Sci 1997;80:838-844.

88. Nielsen SS, Grohn YT, Quass RL, et al. Paratuberculosis in dairy cattle: variation of the antibody response in offspring attributable to the dam. J Dairy Sci 2002; 85:406–412.

89. Norman JE, Hohenboken WD, Kelley KW. Genetic differences in concentration of immunoglobulins G1 and M in serum and colostrum of cows and in serum of neonatal calves. J Anim Sci 1981;53:1465-1472.

90. Odde KG. Survival of the neonatal calf. Vet Clin North Am Food Anim Pract 1988:4:501-508.

91. Osburn BI: The ontogeny of the ruminant immune system and its significance in the understanding of maternal-fetal-neonatal relationships: In Butler JE (ed): The ruminant Immune System. New York, Plenum Press, 1980;91-103.

92. Osburn BI, MacLachlan NJ, Terrell TG. Ontogeny of the immune system. J Am Vet Med Assoc 1982;1049-1052.

93. Ontsouka CE, Bruckmaier RM, Blum JW. Fractioned milk composition during removal of colostrum and mature milk. J Dairy Sci 2003;86:2005-2011.

94. Oyeniyi OO, Hunter AG. Colostral constituents including immunoglobulins in the first three milkings postpartum. J Dairy Sci 1978;61:44-48.

95. Pacitti AF, Gentsch JR. Inhibition of reovirus type 3 binding to host cells by sialylated glycoproteins is mediated through the viral attachment protein. J Virol 1987;61:1407-1415.

96. Papiz MZ, Sawyer L,Eliopoulos EE, et al. The structure of beta-lactoglobulin and its similarity to plasma retinol-binding protein. Nature 1986;324:383-385.

97. Parish DB, Wise GH, Hughes JS, et al. Properties of the colostrum of the dairy. V. Yield, specific gravity and concentration of total solids and its various components of colostrum and early milk. J Dairy Sci 1950;33:457-65.

98. Parish SM, Tyler JW, Besser TE, et al. Prediction of serum IgG_1 concentration in Holstein calves using serum gamma glutamyltransferase activity. J Vet Intern Med 1997;11;344-347.

99. Perez MD, Sanchez L, Aranda P, et al. Synthesis and evolution of concentration of beta-globulin and alpha-lactalbumin from cow and sheep colostrum and milk throughout early lactation. Cell Molec Bio 1990;36:205-212.

100. Perino LJ, Sutherland RL, Woollen NE. Serum γ-glutamyltransferase activity and protein concentration at birth and after suckling in calves with adequate and inadequate passive transfer of immunoglobulin G. A J Vet Res 1993;54:56-59.

101. Pfeiffer NE, McGuire TC, Bendel RB, et al. Quantitation of bovine immunoglobulins:Comparison of single radial immunodiffusion, zinc sulfate turbidity, serum electrophoresis, and refractometer methods. Am J Vet Res 1977;38:693-698.

102. Pierce AE, Feinstein A. Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. Immunol 1965;8:106-123.

103. Pike RM. Antibody heterogeneity and serological reactions. Bact Rev 1967;31:157-174.

104. Pritchett LC, Gay CC, Besser TE, et al. Management and production factors influencing immunoglobulin G1 concentration in colostrum from Holstein cows. J Dairy Sci 1991;74:2336-2341.

105. Pritchett LC, Gay CC, Hancock DD et al. Evaluation of the hydrometer for testing immunoglobulin G_1 in Holstein colostrum. J Dairy Sci 1994;77:1761-1767.

106. Quigley JD, Martin KR, Dowlen HH, et al. Immunoglobulin concentration, specific gravity, and nitrogen fractions of colostrum from Jersey cattle. J Dairy Sci 1994;77;264-269.

107. Quigley JD, Strohbehn RE, Kost CJ, et al. Formulation of colostrum supplements, colostrum replacers and acquisition of passive immunity in neonatal calves. J Dairy Sci 2001;84:2059-2065.

108. Radostits OM. Control of acute undifferentiated diarrhea of newborn beef calves. Vet Clin North Am Food Anim Pract 1983;5:143-145.

109. Reinshaw HW, Everson DO. Classical and alternate complement pathway activities in paired dairy cow/newborn calf sera. Comp Immunol Microbiol Infect Dis 1979;1:259-267.

110. Robison JD, Stott GH, DeNise SK. Effects of passive immunity on growth and survival in the dairy heifer. J Dairy Sci 1988;71:1283-1287.

111. Rolsma MD, Kuhlenschmidt HB, Gelberg HB, et al. Structure and function of a ganglioside receptor for porcine virus. J Virol 1998;72:9079-9091.

112. Rook JAF, Campling RC. Effect of stage and number of lactation on the yield and composition of cow's milk. J Dairy Res 1965;32:45-55.

113. Rossi CR, Kiesel, Hudson RS. Kinetics of detection of blastogenic responses of neonatal calves inoculated in utero with tetanus toxoid, killed *Mycobacterium bovis*, and killed *Brucella abortus*. Am J Vet Res 1979;40;576-579.

114. Roy JHB. The calf: Mangement and feeding. 5th ed. London: UK Butterworths, 1970.

115. Sanchez L, Aranda P, Perez MD, et al. Concentration of lactoferrin and transferring throughout lactation in cow's colostrum and milk. Biol Chem Hoppe-Seyler 1988;369:1005-1008.

116. Sasaki M, Davis CL, Larson BL. Production and turnover of IgG1 and IgG2 immunoglobulins in the bovine around parturition. J Dairy Sci 1976;59:2046-2055.

117. Sawyer M, Osburn BI, Knight HD, et al. A quantitative serologic assay for diagnosing congenital infections. Am J Vet Res 1973;34:1281-1284.

118. Saxmose SH, Houe H, Ersboll AK, et al 2004. Evaluating diagnostic tests. In: Houe H, Ersboll AK, Toft N. (eds.) Introduction to veterinary epidemiology. Frederiksberg: Biofolia, 2004;133-151.

119. Seitz SE, Heider LE, Heuston WD, et al. Bovine fetal infection with *Mycobacterium paratuberculosis*. J Am Vet Med Assoc 1989;194:1423–1426.

120. Selim SA, Smith BP, Cullor JS, et al. Serum immunoglobulins in calves: their effects and two easy reliable means of measurement. Vet Med 1995;387-404.

121. Selman IE, McEwan AD, Fisher EW. Absorption of immune lactoglobulin by newborn dairy calves. Attempts to produce consistent immune lactoglobulin absorptions in newborn dairy calves using standardized methods of colostrum feeding and management. Res Vet Sci 1971;12:205-210.

122. Senogles DR, Muscoplat CC, Paul PS, et al. Ontogeny of circulating lymphocytes in neonatal calves. Res Vet Sci 1978;25:34-36.

123. Senger PL. Pathways to pregnancy and parturition. 1st ed. Current Conceptions Inc., Pullman, WA; 1999

124. Shaver RD, Bal MA. Effect of dietary thiamin supplementation on milk production by dairy cows. J Dairy Sci 1988;83:2335-2340.

125. Simon PM, Goode A, Mobasseri A, et al. Inhibition of Helicobacter pylori binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. Infect Immun 1997;65:750-757.

126. Smith JW, Schultz RD. Mitogen and antigen-responsive milk lymphocytes. Cell Immunol 1977;29;165-173.

127. Smith RD. Risk assessment and prevention. In: Smith RD, ed. Veterinary clinical epidemiology. 2nd ed. New York: CRC Press, 1995;91–109.

128. Staubili D, Sager H, Haerdi C, et al. Precolostral serology in calves born from *Neospora*-seropositive mothers. Parasitol Res 2006;99:398–404.

129. Stott GH, Marx DB, Menefee BE, et al. Colostral immunoglobulin transfer in calves. II. Rate of absorption. J Dairy Sci 1979;62:1766-1773.

130. Stott GH, Marx DB, Menefee BE, et al. Colostral immunoglobulin transfer in calves I. Period of absorption. J Dairy Sci 1979b;62:1632-1638.

131. Steele ML, McNab WB, Poppe C, et al. Survey of Ontario bulk tank raw milk for food-borne pathogens. J Food Protection 1997;60:1341-1346.

132. Streeter RN, Hoffsis S, Bech-Nielsen S, et al. Isolation of *Mycobaterum avium paratuberculosis* from colostrum and milk of subclinically infected cows. Am J Vet Res 1995;56:1222-1324.

133. Sugisawa H, Itou T, Saito M, et al. A low-molecular-weight fraction of bovine colostrum and milk enhances the oxidative burst activity of polymorphonuclear leukocytes. Vet Res Comm 2003;27;453-461.

134. Tessman RK, Lakritz J, Tyler JW, et al. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. J Am Vet Med Assoc 2001;218:756-760.

135. Thurmond MC, Carter DM, Puhr MJ, et al. An epidemiological study of natural in utero infection with bovine leukemia virus. Can J Comp Med 1983;47: 316–319.

136. Tyler JW, Cullor JS. Titers, tests, and truisms: rational interpretation of diagnostic serologic testing. J Am Vet Med Assoc 1989;194:1550-1558.

137. Tyler JW, Hancock DD, Parish SM, et al. Evaluation of 3 assays for failure of passive transfer in calves. J Vet Intern Med 1996;10:304-307.

138. Tyler JW, Besser TE, Wilson L, et al. Evaluation of a whole blood glutaraldehyde coagulation test for detection of failure of passive transfer in calves. J Vet Intern 1996b;10:82-84.

139. Tyler JW, Hancock DD, Wiksie SE, et al. Use of serum protein concentration to predict mortality in mixed-source dairy replacement heifers. J Vet Intern Med 1998;12:79-83.

140. Tyler JW, Steevens BJ, Hostetler DE, et al. Colostral IgG concentrations in Holstein and Guernsey cows. Am J Vet Res 1999a;60:1136-1139.

141. Tyler JW, DD Hancock, J Thorne, et al. A model partitioning the risk of mortality associated with inadequate passive transfer in dairy calves. J Vet Intern Med 1999b;13:335-337.

142. Tyler JW, Parish SM, Besser TE, et al. Detection of low serum immunoglobulin concentration in clinically ill calves. J Vet Intern Med 1999c;13;40-43.

143. Tyler JW, Lakritz J, Hostetler D, et al. Effect of pasteurization at 76 and 63 °C on the absorption of colostral IgG in calves. J Dairy Res 2000;67:619-623.

144. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. Transfer of maternal immunity to calves. Highlights of the National Dairy Heifer Evaluation 1993; Bull. No N118.0293.

145. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. Part 1: Reference of dairy cattle health and management practices in the United States 2007; Bull. No N480.1007:38-46.

146. Virtala AM, Grohn YT, Mechor GD, et al. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. Prev Vet Med 1999;39:25-37.

147. Wada T, Aiba Y, Shimizu K, et al. The therapeutic effect of bovine lactoferrin in the host infected with *helicobacter pylori*. Scand J Gasteroenterol 1999;34:238-243.

148. Waldman TA. Disorders of immunoglobulin metabolism. New Engl J Med 1969;281:1170-1177.

149. Walz PH, Mullaney TP, Render JA, et al. Otitis media in preweaned Holstein dairy calves in Michigan due to *Mycoplasma bovis*. J Vet Diagn Invest 1997;9:250-254.

150. Weaver DM, Tyler JW, VanMetre DC, et al. Passive transfer of colostral immunoglobulins in calves. J Vet Intern Med 2000;14:569-577.

151. Weiss WP, Todhunter DA, Hogan JS, et al. Effect of duration of supplementation of selenium and vitamin E in periparturient dairy cows. J Dairy Sci 1990;73:3187-3194.

152. Weiss WP, Hogan JS, Todhunter DA, et al. Effect supplementing periparturient cows with vitamin E on distribution of alpha-tocopherol in blood. J Dairy Sci 1992;73:3479-3485.

153. Wereme AN, Strabel M, Grongnet JF, et al. Immunoglobulin G absorption from pooled maternal colostrum, commercial powder and freeze dried colostrum by newborn. Anim Res 2001;50:315-323.

154. Williams RC, Gibbons RJ. Inhibition of bacterial adherence by secretory immunoglobulin A. A mechanism of antigen disposal. Science 1972;177:697-699.

155. Wilson LK, Tyler JW, Besser TE, et al. Prediction of serum IgG_1 concentration in beef calves based on age and serum gamma glutamyl transferase activity. J Vet Intern Med 1999;13:123-125.

156. Ziparsky AE, Brown EJ, Bienenstock J. Lack of opsonization potential of 11s human secretory α A. Proc Soc Exp Biol Med 1973;142:181-184.

VITA

Munashe Chigerwe was born in Chiredzi, Zimbabwe on 15 September 1977 to Adonia and Pelagia Chigerwe. Munashe attended primary school at Gandami Primary School in Chivhu. He completed his secondary and high schools at Gokomere High School in Masvingo in 1995. He attended University of Zimbabwe, Faculty of Veterinary Science for veterinary school in 1996 and graduated with Bachelor of Veterinary Science in 2001. He worked at the University of Zimbabwe as a resident veterinarian from 2001 to 2003.

Munashe moved to the United States, University of Missouri-Columbia in 2003 to complete a one year internship in Food Animal Medicine and Surgery and Production Medicine. He then completed a 3-year residency in Food Animal Medicine and Surgery, a Master of Public Health and a doctoral program. Munashe was awarded board certification in Large Animal Internal Medicine in March 2007. Munashe is single and resides in Columbia and currently employed by the University of Missouri-Columbia.