



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Sede Amministrativa: Università degli Studi di Padova

Dipartimento di Medicina Animale, Produzioni e Salute

CORSO DI DOTTORATO DI RICERCA IN: Scienze Animali e Agroalimentari

CURRICOLO: Scienze Animali

CICLO XXIX

**MANAGEMENT OF NEWBORN CALVES
IN ITALIAN DAIRY FARMS**

Coordinatore: Ch.mo Prof. Stefano Schiavon

Supervisore: Ch.ma Prof. Flavia Gottardo

Dottorando: Isabella Lora

ABSTRACT

Despite of their great impact on animal welfare and on farm incomes, morbidity and mortality rates of dairy replacement animals are often underestimated by dairy farmers. The highest risk of morbidity and mortality is usually recorded within the first months of the calf life. Because calves are born nearly deprived of antibodies, they strictly depend on colostrum ingestion to acquire the immune protection against infectious pathogens. Improper colostrum provision could lead to failure of transfer of passive immunity (FTPI), which is recognized to be the first relevant factor that affect calf health and survival. There is a lack of knowledge on mortality rates of young stock in Italian dairy farms and on the prevalence of FTPI in Italian dairy calves. Aims of this thesis were to preliminary investigate mortality rates of calves and heifers in Italian dairy farms, and then to evaluate different colostrum management practices in relationship to FTPI. Finally, it was investigated the association between passive immunity and health in calves. The median value of mortality rate in dairy farms was 5% in young stock of less than <12 mo of age and 0% in heifers from 12 to 24 mo of age. However, the wide variability among farms (n = 95) pointed out a serious situation in about one third of them, which showed excessively high mortality rates in at least one of the two age categories. The overall percentage of calves with FTPI was 41%, with a generally high within-farm prevalence (>20% in 71% of the 21 enrolled farms). Colostrum management practices, in terms of delivery time, amount, and quality of the first colostrum meal, were strictly related to calf serum Ig concentration: the level of passive immunity in calves increased linearly as the management of colostrum improved. To allow the calf to nurse colostrum from the dam, supported by the farmer assistance, was the best practice of colostrum provision in terms of transfer of passive immunity. However, it implied considerable calf-dam separation distress. The use of commercial colostrum supplements (CS) could be valuable in improving the transfer of passive immunity in calves. However, the CS tested in this thesis failed to prevent FTPI and to reduce the occurrence of calf neonatal diseases. Finally, outcomes of this thesis confirmed the importance of passive immunity to calf health, and particularly to calf neonatal diarrhea. In conclusion, great improvements are needed in newborn calf management in Italian dairy farms, and proper colostrum provision is the first step to improve calf health and welfare.

RIASSUNTO

I tassi di morbilità e mortalità negli animali da rimonta sono spesso sottovalutati dagli allevatori di vacche da latte, nonostante il loro considerevole impatto sul benessere animale e sui profitti dell'azienda. Il periodo di maggior rischio per la salute dei vitelli si concentra nei primi mesi di vita. Poiché i vitelli nascono praticamente privi di anticorpi, essi devono assumere il colostro per poter acquisire la protezione immunitaria contro gli agenti patogeni. La non corretta somministrazione del colostro può comportare il fallimento del trasferimento dell'immunità passiva (FTPI), che è considerato uno dei principali fattori di rischio per la salute e la sopravvivenza dei vitelli. Nelle aziende di vacche da latte italiane, i tassi di mortalità del giovane bestiame e la prevalenza di FTPI non sono ben noti. Gli obiettivi di questa tesi sono stati la definizione di tali tassi e la valutazione dell'associazione tra diverse pratiche di colostratura e FTPI, e tra immunità passiva e salute dei vitelli. La mediana del tasso di mortalità negli allevamenti di vacche da latte è stata di 5% per gli animali di età <12 mesi, e di 0% per le manze tra i 12 e i 24 mesi di vita. Tuttavia, l'ampia variabilità tra le aziende (n = 95) ha rivelato, per entrambe le categorie di età, tassi di mortalità elevati in circa un terzo degli allevamenti. La percentuale complessiva dei vitelli con FTPI è stata del 41%, con una prevalenza aziendale generalmente elevata (>20% nel 71% dei 21 allevamenti indagati). Le pratiche di colostratura, in termini di tempo di somministrazione, quantità e qualità del primo pasto dopo la nascita, erano strettamente correlate alla concentrazione sierica di Ig dei vitelli, che aumentava in modo lineare al migliorare della gestione del colostro. La colostratura dei vitelli sotto la madre, con l'assistenza dell'allevatore, è risultata essere il metodo migliore di somministrazione del colostro in termini di trasferimento dell'immunità passiva. Tuttavia, questo metodo comporta un notevole stress alla separazione degli animali. L'uso di prodotti commerciali che integrano il colostro materno potrebbe essere utile per migliorare il trasferimento dell'immunità passiva nei vitelli. Tuttavia, il prodotto testato in questa tesi non è risultato efficace nella prevenzione di FTPI e patologie neonatali. Infine, è stata confermata l'importanza dell'immunità passiva per la salute del vitello, in particolare verso le diarree neonatali. In conclusione, la gestione del vitello neonato nelle aziende di vacche da latte italiane necessita di importanti miglioramenti, a partire dalla corretta somministrazione del colostro.

CONTENT

Chapter 1	1
General introduction	
Chapter 2	29
Survey on mortality rate of young stock on dairy farms of the Province of Padova	
Chapter 3	39
Factors associated with failure of transfer of passive immunity in dairy calves: combined effect of delivery time, amount and quality of the first colostrum meal	
Chapter 4	61
Dairy calves allowed to nurse colostrum from dams: effects on transfer of passive immunity, behavior and health	
Chapter 5	85
Passive immunity and health in dairy calves fed maternal colostrum or a commercial colostrum supplement	
Chapter 6	99
Association between passive immunity and occurrence of disease and mortality in calves of less than 30 days of age	
Chapter 7	115
General conclusions	
Supplementary material	119

CHAPTER 1

General introduction



CHAPTER 1

General introduction

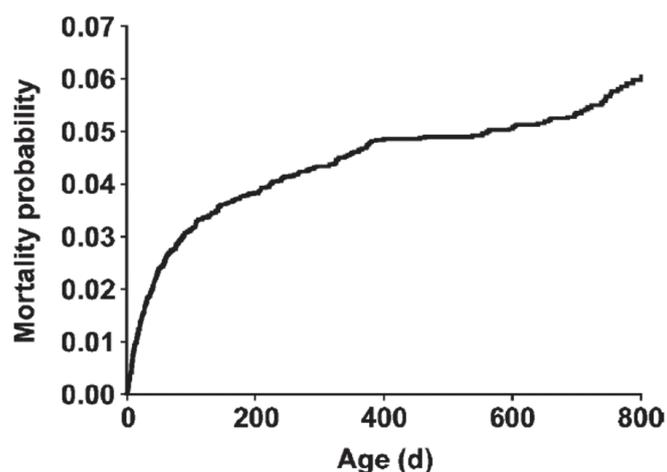
Despite the good level of management that generally characterizes Italian intensive dairy farms, poor attention is paid to welfare and health conditions of calves and replacement heifers. However, considering that the culling rate for Italian Holstein cows is around 30%, nearly a half of the number of animals reared on each Italian dairy farm is young stock, with replacement costs that can reach 15% to 25% of the total costs for milk production (Campiotti, 2012). Therefore, even if rarely quantified, the economic losses related to poor management of calves and heifers until they enter in production could have great impact on farm incomes. Beside the culling rate of the cows, which directly determine the number of replacement heifers needed to be reared, main factors that can affect replacement costs are mortality rate and age at death of young stock, and age at first calving of heifers. Particularly, the economic losses due to calf and heifer mortality can be relevant, and they are directly proportional to the age at death of the animals. For example, the rearing and veterinary costs incurred until the moment of death could range from 250 € for a calf dead at 1 mo of age, to about 1,800 € for a heifer dead soon before the first service (Campiotti, 2012). To those costs, further expenses must be added for purchasing new replacement animals, which could range from 1,800 to 2,500 € (Campiotti, 2012). Additional risk of introducing new infectious diseases in the herd should be considered too. Beside the economic impact, calf mortality is considered an important indicator of animal welfare (Mee, 2013; Uetake, 2013). However, Mee (2013) reported that not only morbidity and mortality rates of young stock were often underestimated by dairy farmers, but also the majority of them (94%) did not consider calf mortality as a problem, even if the average loss rate at birth was considerably high (9%). Therefore, there is a need of greater awareness by the farmers about young stock rearing and welfare.

Calf Mortality

Mortality rates in young stock tend to vary according to the age of the animals. In general, the highest mortality rate is observed in calves between 0 and 3 mo of age, with a peak within the first month of life, and then it tends to decrease progressively towards

the first year of age (Sivula et al., 1996; Wells et al., 1996b; Svensson et al., 2006) (Figure 1). This trend can be explained by the higher susceptibility to diseases (especially enteric and respiratory) of calves in the first months of life, due both to a poorly competent immune system and to stressful conditions (e.g. changing in housing and feeding) that might act as predisposing factors to disease outbreak and consequently to mortality (Sivula et al., 1996; Stull and Reynolds, 2008; Zucali et al., 2013). Therefore, according to the Gold Standards by the Dairy Calf and Heifer Association (2009, 2010), mortality rates should not exceed 5.0% for calves from 1 d to 2 mo of age, 2.0% for calves from 2 to 4 mo of age, 1.0% for calves from 4 to 6 mo of age and for heifers from 6 to 12 mo of age, and 0.5% for heifers from 12 mo of age to freshening. However, similarly to other countries (Mee, 2013; Uetake, 2013), Zucali et al. (2013) found that mortality rates in Italian dairy farms are characterized by wide variability, with losses in unweaned calf above 10% in nearly one third of the surveyed herds. Many factors can affect calf mortality rate, such as calving ease, navel treatment, colostrum feeding management, time at separation of calf from the dam, herd size, person caring for calves, calf housing management, and season (Wells et al., 1996a; Gulliksen et al., 2009; Uetake, 2013). Above all, proper colostrum management is of primary importance to minimize calf susceptibility to diseases and mortality (Tyler et al., 1999; Berge et al., 2005; Furman-Fratczak et al., 2011).

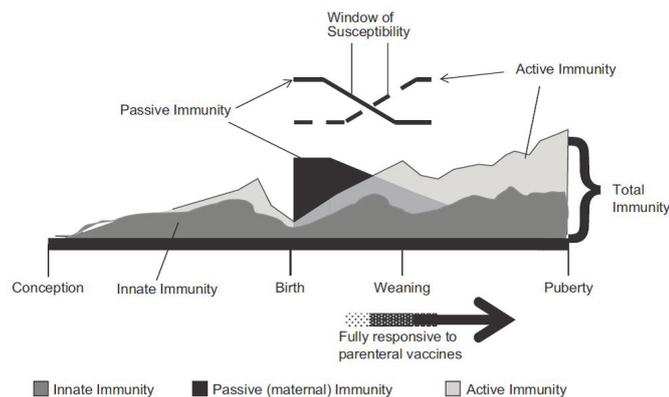
Figure 1. Cumulative probability of dying at 1 to 810 d of age in heifer calves and replacement heifers (From Svensson et al., 2006)



Calf Immune Development

Because of the syndesmochorial structure of bovine placenta, that prevents in utero transfer of large molecules to the fetus, calves are born nearly devoid of antibodies: serum immunoglobulin (**Ig**) concentration at birth ranges indicatively from 1.2 to 2.9 g/L (Klaus et al., 1969; Bush et al., 1971; Weaver et al., 2000). Therefore, newborn calf strictly depends on colostrum ingestion to acquire the maternal immune protection (“passive immunity”) against infectious diseases (Weaver et al., 2000; Godden, 2008). At birth, calf immune system is naïve to environmental pathogens and it develops progressively until about 2 mo of age, to be completely competent at 6 mo. Maternal immunity protects the calf against diseases until approximately the second month of life. After this period, the maternal Ig concentration in calf blood starts to decline significantly, as the capacity of the calf itself to respond to a variety of antigenic stimuli (“active immunity”) increases (Mallard et al., 1998; Chase et al., 2008). That particular period represents a “window of susceptibility” (Figure 2), when the calf is exposed to a higher risk for infectious diseases (particularly respiratory) that is often enhanced by the concomitance with the stressful period of weaning (Chase et al., 2008). The length of the “window of susceptibility” varies among calves and depends on the initial level of maternal antibodies in calf blood: maternal Ig can be found in calves until 6 mo of age (Chase et al., 2008).

Figure 2. *Development of the immune response in calves from conception to weaning (From Chase et al., 2008)*



Failure of Transfer of Passive Immunity (FTPI)

Improper colostrum administration to calves could lead to failure of transfer of passive immunity (**FTPI**), which is an important predisposing factor for calf disease and mortality occurrence. The FTPI condition is defined when calf serum Ig concentration is

lower than 10.0 g/L between 24 and 48 h of life (Jaster, 2005; Godden, 2008; Furman-Fratczak et al., 2011). However, calves with serum Ig concentration of 10.1 g/L are not much more protected than calves with 10.0 g/L, so a minimum serum Ig concentration of 16.0 g/L seems to be required to ensure a good immune protection (Waldner and Rosengren, 2009; Furman-Fratczak et al., 2011). Even if the within-farm prevalence of FTPI should not exceed 20% (McGuirk and Collins, 2004), it is estimated that about 35 to 40% of dairy calves suffer from FTPI (Weaver et al., 2000; Godden, 2008). From many years, the FTPI condition has been recognized to be a major risk factor for the incidence and the severity of calf disease and for calf mortality (Maunsell and Donovan, 2008). For example, Furman-Fratczak et al. (2011) reported that calves with serum Ig concentration >10.0 g/L at 30 to 60 h of life did not develop diseases before 14 d of life and showed a lower morbidity and severity of disease course, whereas calves with serum Ig concentration >15.0 g/L avoided respiratory infections at all (Figure 3). Moreover, mortality risk for calves with FTPI is at least two times higher than for calves with an adequate level of passive immunity (Figure 4) (Donovan et al., 1998a; Robison et al., 1988; Quigley and Drewry, 1998), and 31 to 39% of calf mortality occurring within the first month of life is attributable to FTPI (Wells et al., 1996a; Tyler et al., 1999). The effects of FTPI on calf health could have also long-term effects: for example, calves that suffered from diarrhea before 14 d of life were reported to be afterward more susceptible to respiratory tract infections (Curtis et al., 1988), and heifers treated for pneumonia during the first 3 mo of life were 2.5 times more likely to die after 90 d of age (Waltner-Toews et al., 1986). Moreover, negative effects of calf disease occurrence were reported on growth rate during the first 6 mo of life, on age at first calving, and on production in first-lactation (Waltner-Toews et al., 1986; Donovan et al., 1998b; Heinrichs and Heinrichs, 2011). Finally, Berge et al. (2005) pointed out that the use of antibiotic treatments on dairy calves could be minimized or even avoided if an adequate transfer of passive immunity is ensured. Therefore, the within-farm prevalence of FTPI should be monitored, and actions should be taken to reduce it below the threshold of acceptability. Rapid and cheap tools are available for on-farm screening of Ig concentration in calf serum at few hours of life, such as the Brix refractometer. That optical instrument requires only a drop of calf serum obtained without centrifuge needing, and has an optimal

sensitivity and specificity for FTPI detection at Brix percentage $\leq 8.5\%$ (Deelen et al., 2014; Hernandez et al., 2016).

Figure 3. Rate and intensity of enteric and respiratory diseases of calves grouped by serum Ig concentration. (Group 1: <5 g/L; Group 2: 5–10 g/L; Group 3: 10–15 g/L; Group 4: >15 g/L) (From Furman-Fratczak et al., 2011)

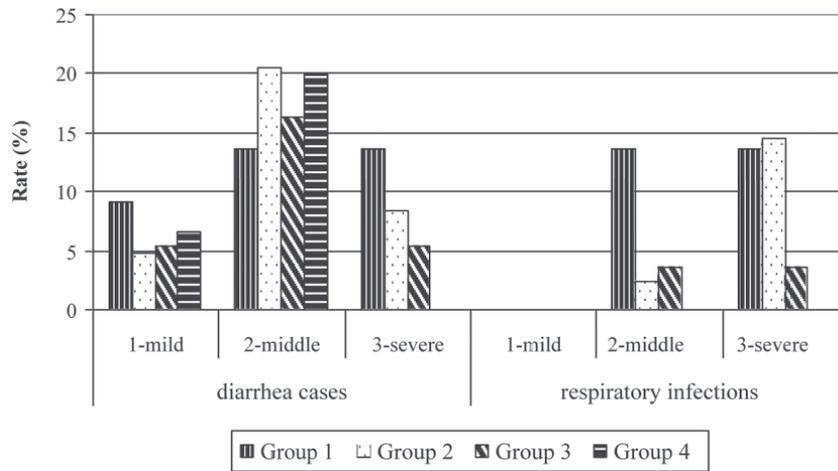
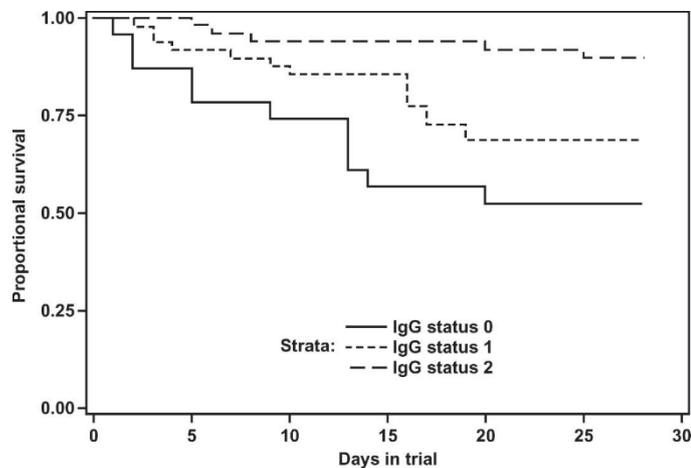


Figure 4. Rate of survival of calves according to the level of passive immunity (IgG status 0 = 0.86 g/L of serum IgG; IgG status 1 = 0.87 to 9.99 g/L of serum IgG; IgG status 2 = ≥ 10.00 g/L of serum IgG) (From Berge et al., 2005)



Colostrum and Colostrum Composition

Colostrum is defined as the secretion of the mammary gland during the first 24 h after calving (Jaster, 2005). Colostrum composition differs from that of the whole milk for the higher concentration of nutrients and for the presence of specific constituents such as immune components (particularly Ig), hormones, and enzymes (Figure 5). Colostrum (i.e., the pre-partum transfer of Ig from maternal circulation into

mammary gland secretions) is a process under hormonal control that begins about 5 wk prior to calving and stops abruptly at parturition (Barrington et al., 2001; Godden, 2008). Particularly, during this period, the IgG1 accumulates selectively from the blood circulation into the colostrum by an active receptor mediated transfer across the secretory epithelium of the mammary gland (Korhonen et al., 2000). It is reported that up to 500 g per week of IgG1 accumulates in the mammary gland, with a final concentration in colostrum 5- to 10-fold higher than in maternal serum (Korhonen et al., 2000; Barrington et al., 2001). As prolactin concentration increases at the onset of lactation, the alveolar epithelial cells of the mammary gland cease expressing the receptors (Barrington and Parish, 2001). Therefore, Ig concentration in colostrum declines rapidly in the hours following parturition (Figure 5). Concentrations of the other colostrum components, as well, decrease significantly from the second to the sixth milking (transition milk), and the typical composition of the whole milk is observed at the fourth day after parturition (Godden, 2008).

Differently from the whole milk, in which Ig account for only 2% of the total proteins, Ig in colostrum constitute about 85% of the total protein content (Korhonen et al., 2000). Moreover, due to the mechanism of selective transport discussed above, up to 85% of the total colostrum Ig is constituted by IgG, with 80 to 90% make up of IgG1, whereas IgM and IgA account for 7%, and 5%, respectively (Larson et al., 1980). There is evidence that colostral transfer of IgE occurs as well (McGuirk and Collins, 2004; Godden, 2008). The importance of colostrum Ig to calf health has been previously discussed. However, other immune components such as leucocytes (which colostral concentration is higher than 1×10^6 cells/mL), cytokines and nonspecific antimicrobial factors (e.g., lactoferrin, lysozyme and lactoperoxidase) accumulate in colostral secretion and are immunologically active (Reiter, 1978; Barrington and Parish, 2001). Besides immune components, colostrum contains hormones (particularly insulin and IGF-I) which are responsible of the development and maturation of newborn calf gastrointestinal tract (Blum and Hammon, 2000). Furthermore, trypsin inhibitor, that is found in colostrum in concentrations almost 100 times higher than in the whole milk, plays a key role in preservation of Ig and other proteins from the degradation in the calf gastrointestinal tract (Quigley et al., 1995). Colostrum energy content is also important for the neonate in the first hours of life, because newborn calf generally has poor

capabilities of thermic isolation and heat production. It has been reported that newborn calf summit metabolism could be supported by stored endogenous lipid for about 15 h, whereas glycogen reserves would be depleted in less than 3 h after birth (Quigley and Drewry, 1998). Therefore, even if relatively poor in lactose, the high content in fat and proteins of colostrum is crucial for the calf to support gluconeogenesis, protein synthesis and thermoregulation (Quigley and Drewry, 1998). Particularly, Vermorel et al., (1983) reported that in the first and the second hour after colostrum consumption, calf heat production at 10°C environment was increased by 18% and 9%, respectively. Finally, a variety of vitamins and minerals are highly concerted in colostrum (Figure 5).

Figure 5. *Composition of colostrum, transition milk and whole milk of Holstein cows (From Godden, 2008)*

Parameter	Colostrum	Transition milk (milking postpartum)		Milk
	1	2	3	6
Specific gravity	1.056	1.040	1.035	1.032
Total solids (%)	23.9	17.9	14.1	12.9
Fat (%)	6.7	5.4	3.9	4.0
Total protein (%)	14.0	8.4	5.1	3.1
Casein (%)	4.8	4.3	3.8	2.5
Albumin (%)	6.0	4.2	2.4	0.5
Immunoglobulins (%)	6.0	4.2	2.4	0.09
IgG (g/100 mL)	3.2	2.5	1.5	0.06
Lactose (%)	2.7	3.9	4.4	5.0
IGF-I (µg/L)	341	242	144	15
Insulin (µg/L)	65.9	34.8	15.8	1.1
Ash (%)	1.11	0.95	0.87	0.74
Calcium (%)	0.26	0.15	0.15	0.13
Magnesium (%)	0.04	0.01	0.01	0.01
Zinc (mg/100 mL)	1.22	—	0.62	0.3
Manganese (mg/100 mL)	0.02	—	0.01	0.004
Iron (mg/100 g)	0.20	—	—	0.05
Cobalt (µg/100 g)	0.5	—	—	0.10
Vitamin A (µg/100 mL)	295	190	113	34
Vitamin E (µg/g fat)	84	76	56	15
Riboflavin (µg/mL)	4.83	2.71	1.85	1.47
Vitamin B ₁₂ (µg/100 mL)	4.9	—	2.5	0.6
Folic acid (µg/100 mL)	0.8	—	0.2	0.2
Choline (mg/mL)	0.7	0.34	0.23	0.13

Factors Affecting Colostrum Quality

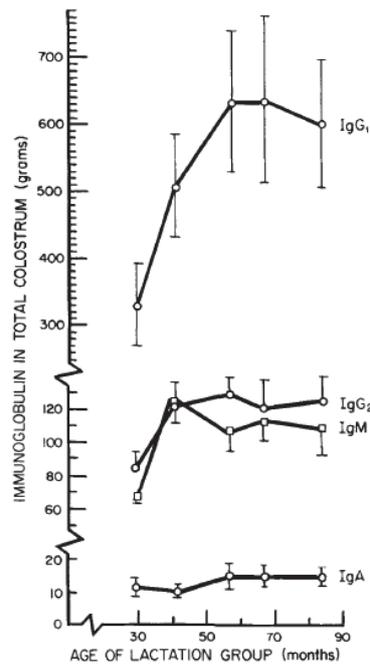
The quality of colostrum is commonly evaluated based on the Ig concentration, and particularly the IgG concentration, because of its importance to calf health and because that is the major protein fraction. Therefore, colostrum is considered as of good quality if IgG concentration is higher than 50 g/L (Godden, 2008). On field, dairy producers can easily screen the quality of colostrum by a colostrometer or a Brix refractometer, which are rapid and inexpensive tools. The colostrometer is a hydrometer that estimates the IgG content of colostrum by measuring the colostrum specific gravity (specific gravity >1.050 corresponds to IgG concentrations >50g/L) (Fleenor and Stott, 1980). It is the most used tool for quick colostrum evaluation on field, even if the sensibility is not particularly high (32%) (Pritchett et al., 1994) and the measure could be influenced by colostrum temperature (Mechor et al., 1991). Differently from the colostrometer, the application of the Brix refractometer to colostrum quality evaluation is relatively new, but that instrument seems to be characterized by a high specificity (85% at the cut-of value for colostrum quality of 22% Brix) and not to be influenced by colostrum temperature (Bielmann et al., 2010).

Many factors can affect colostrum quality, starting from a high individual variability. For example, a recent study reported colostrum IgG1 concentrations for Holstein cows ranging from 9 to 121 g/L, with a mean value of 41 g/L (Morin et al., 2010). Other factors that influence the quality of colostrum are summarized below.

Breed. Quality of colostrum tend to vary among breeds. Particularly, beef cows produce colostrum with higher Ig concentrations than dairy cows (Guy et al., 1994), probably due to the lower milk yield, and, among dairy cows, Jersey is reported to produce colostrum of a higher quality (Muller and Ellinger, 1981; Quigley et al., 1994).

Parity. The parity of the cow is recognized to be a major factor affecting colostrum quality. It is generally observed that the maximum Ig content of colostrum is reached at the third lactation (Figure 6), whereas colostrum of primiparous cows is often of poor quality. The reason is on one hand that the third lactation of the cow usually coincides with the complete development of the mammary gland and therefore with the maximum efficiency of IgG concentration in colostrum secretion, and, on the other hand, that older cows have been longer exposed to environmental pathogens and thus have a higher immunological memory (Devery-Pocius and Larson, 1983; Quigley, 2002).

Figure 6. Mean and SD of the total amount of Ig in colostrum of the first four milkings of 87 dairy cows as a function of age. The five points for each Ig class are plotted at the mean age of the cows in each lactation group, which represent, with increasing age, first, second, third, fourth, and fifth and later lactations, respectively (From Devery-Pocius and Larson, 1983)



Immune Status of the Cow. Not only the amount but also the specificity of colostrum antibodies is important to calf protection against diseases. Generally, older cows produce more specific antibodies against the pathogens that are typical of the farm where they are bred. Moreover, specific antibodies produced by vaccination can accumulate in colostrum. For that reason, vaccination of the cows during the dry period against enteric pathogens could be an effective tool for the prevention of calf neonatal diarrhea (Kohara et al., 1997; Jayappa et al., 2008).

Management of the Dry Period. Because colostrogenesis begins during the dry period, the management of that delicate phase can influence colostrum production. Particularly, dry period length inferior to 28 d or no dry period could lead to a reduction in colostrum IgG concentration up to 35% (Rastani et al., 2005; Santschi and Lefebvre, 2014), whereas dry period shortening from 60 to 40 d could lead to a lowered colostrum yield at first milking of about 2 L (Santschi and Lefebvre, 2014). Moreover, there is concern that protein content of the pre-partum diet may affect colostrum quality or intestinal absorption of Ig by the calf (Quigley and Drewry, 1998; Mann et al., 2016). However, further investigations are needed to clarify the relationship between dry-period diet and colostrum quality.

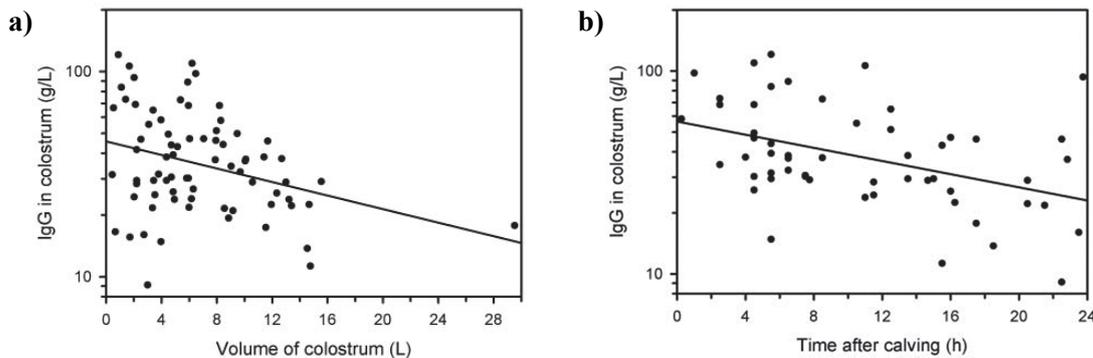
Season of Calving. In the countries that are characterized by marked climate variations among the year, colostrum quality may be affected by the season of calving,

due to the changing in feeding regimen, indoor climate, and disease occurrence (Gulliksen et al., 2008). In addition, Ig and nutrients content of colostrum seems to be negatively affected by the exposure of late pregnant cows to high environmental temperatures (heat-stress) (Nardone et al., 1997).

Volume of Colostrum. Even if the relationship is not constant, the volume of colostrum produced is generally negatively associated with the IgG concentration (Pritchett et al., 1991), probably due to a dilution effect. Particularly, Morin et al. (2010) found that IgG concentration decrease by 3.7% for each additional liter of colostrum produced (Figure 7.a).

Time at the First Milking. In order to maximize colostrum quality, the cow should be milked as soon as possible after birth. In fact, the IgG concentration in colostrum starts to decline from 2 h after calving, probably due to a passive diffusion of IgG into the cow's blood circulation (Moore et al., 2005). Particularly, a decrease by 3.7% in colostrum IgG concentration is reported for each hour of delay from calving of the first milking (Figure 7.b) (Morin et al., 2010).

Figure 7. Relationship between colostrum IgG concentration and colostrum volume at first milking ($r^2 = 0.11$; $P = 0.004$) (a) and relationship between colostrum IgG concentration and the interval between calving and first milking ($r^2 = 0.18$; $P = 0.001$) (b) for Holstein cows (From Morin et al., 2010)



Bacterial Contamination. Despite the Ig content, bacterial contamination of colostrum is a main factor to be considered. Proper hygienic practices during milking are needed in order to avoid bacterial contamination of colostrum, which could represent the first source of pathogen exposure for the newborn calf. For the same reason, colostrum from cows suffering from clinical mastitis should not be fed to the calves, despite colostrum IgG concentration may not be altered by the mastitis (Maunsell et al., 1998).

Moreover, free Ig in colostrum could bind bacteria, not being more available for absorption in calf intestine. Total bacteria count in colostrum should be lower than 100,000 cfu/mL, with less than 10,000 cfu/mL of fecal coliforms (McGuirk and Collins, 2004). If not fed within 2 h of collection, colostrum should be refrigerated at 4°C or frozen in order to minimize the bacterial growth. Colostrum can be refrigerated for up to one week and frozen for maximum one year, and it should be thawed gradually at a temperature not exceeding 60°C in order to avoid the denaturation of proteins and Ig (McGuirk and Collins, 2004; Godden, 2008). If no other sources of good quality colostrum are available, a long-time and low-temperature pasteurization (60°C for 60 min) could be useful to minimize or eliminate pathogens in colostrum without altering colostrum viscosity and Ig activity (Godden et al., 2006).

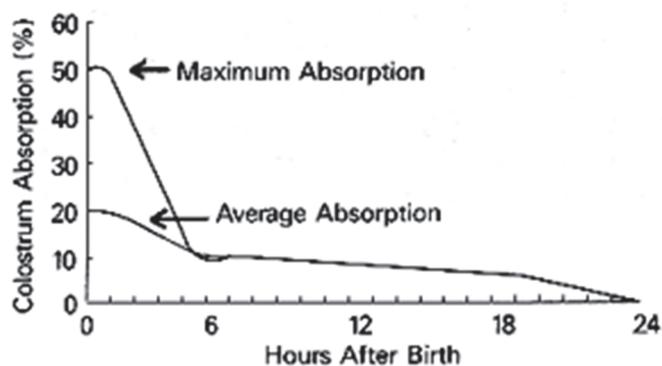
Pooling. Pooling colostrum without checking its quality (Ig content and bacterial contamination) is largely discouraged, both for the risk of Ig dilution (due to the presence of high-volume and low-quality colostrum) and for the risk of exposing a large number of calves to potential pathogens carried by colostrum (Weaver et al., 2000; McGuirk and Collins, 2004; Godden, 2008).

Intestinal Absorption of Ig

The small intestine of the neonate calf is temporarily able to absorb intact macromolecules such as Ig (“open gut”) (Godden, 2008). The enterocytes absorb Ig from the gut lumen by receptor-mediated pinocytosis, and release them in the lymphatics by exocytosis. Thus, through the thoracic duct, Ig gain the blood circulation of the calf (Weaver et al., 2000; Chase et al., 2008). Theoretically, the maximum efficiency of Ig absorption in calf is of 50% (Quigley, 2002). That could be estimated by the apparent efficiency of adsorption (**AEA**), which is calculated as following: $AEA (\%) = \text{serum IgG (g)} / \text{IgG intake (g)} \times 100$ (Quigley and Drewry, 1998). The mass of serum IgG is obtained by estimating the serum volume of the calf, which is usually assumed to be 7% or 9% of the calf’s body weight at 24 h of life [serum IgG (g) = serum IgG concentration (g/L) \times serum volume (L)], whereas the IgG intake depends on colostrum IgG concentration and on the amount of colostrum consumed (Quigley and Drewry, 1998; Quigley, 2002). Actually, the average AEA reported in literature for colostrum ranges from 25 to 35% (Quigley, 2002). Moreover, the efficiency of absorption of intact Ig from colostrum starts

to decrease from 6 to 12 h after birth, to be over at 24 h (Chase et al., 2008) (Figure 8). The loss of intestine absorptive capacity (“closure”) is related to the rapid development of the gastrointestinal tract, due to the cell turnover and to the main effect of the colostrum IGF-I (Quigley, 2002; Godden, 2008). For that reason, feeding colostrum soon after birth may result in earlier closure, whereas delaying the first feeding could postpone it up to 36 h. However, not feeding calves within 12 h after birth expose them to the risk that closure occurs before any absorption could take place (Stott et al., 1979a). Therefore, proper colostrum provision is of fundamental importance to ensure an adequate transfer of passive immunity to newborn calves. However, after the closure, feeding colostrum to calves until the third day of age could still provide a “local effect” that may reduce the risk of scours in the first week of life (Quigley, 2002). In fact, colostrum IgG, that are not inactivated by the gastric acid and are relatively resistant to proteolytic digestive enzymes (Korhonen et al., 2000), still reach the gut lumen and could prevent the bacterial attach to the intestinal wall by competing for the same binding sites on the erythrocytes’ membranes. (Quigley, 2002).

Figure 8. Efficiency of Ig absorption in calves by time after birth (From Moss et al., 1991)



Factors Affecting the Transfer of Passive Immunity in Calf

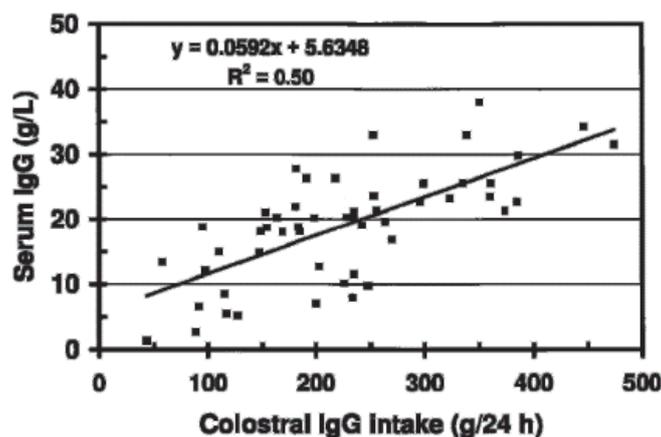
Many factors can affect the successful transfer of passive immunity in calves, and often combinations of two or more of them are responsible for FTPI occurrence. Proper management of newborn calves requires attention to several aspects, which are reviewed below.

Age at First Colostrum Feeding. As previously discussed, the time after birth of the first colostrum feeding is one of the major factors affecting the successful transfer of passive immunity in calves. Due to the progressive gut closure, colostrum should be fed

within 6 h after birth, considering that the maximum rate of Ig absorption occurs within the first 4 h of life (Stott et al., 1979b). Particularly, Rajala and Castrén (1995) found that a delay of 30 min in first colostrum meal lead to a decrease by 2 g/L in calf serum Ig concentration. Therefore, if the first colostrum feeding is delayed, larger amounts of Ig will be needed to achieve an adequate transfer of passive immunity in the calf. The delivery time of the first colostrum meal is such important to calf health that even the Council Directive 2008/119/EC on calf protection (European Commission, 2008) requires that each calf receives bovine colostrum as soon as possible after birth and in any case within the first 6 h of life.

Colostrum Quality. The Ig concentration of colostrum is the other major factor affecting the successful transfer of passive immunity in calves. The relationship between colostrum Ig intake and calf serum Ig concentration is linear (Stott and Fellah, 1983) (Figure 9), and a minimum mass of 150 g of colostral Ig seems to be required to ensure an adequate transfer of immunity in calves (Chigerwe et al., 2008). However, beside colostrum Ig concentration, colostrum Ig intake is affected also by the amount of colostrum fed.

Figure 9. Relationship between colostral IgG intake and calves' serum IgG concentration (From Quigley and Drewry, 1998)



Amount of Colostrum Fed. Without knowing colostrum Ig concentration, the common recommendation is to provide calves with 10% to 12% of their body weight of colostrum within 6 h of birth, corresponding to approximately 3 or 4 L of colostrum for an Holstein calf (McGuirk and Collins, 2004; Godden, 2008). Anyway, equal or less than 2 L of colostrum are inadequate to avoid FTPI in calves (Quigley, 2002). Generally, 60%

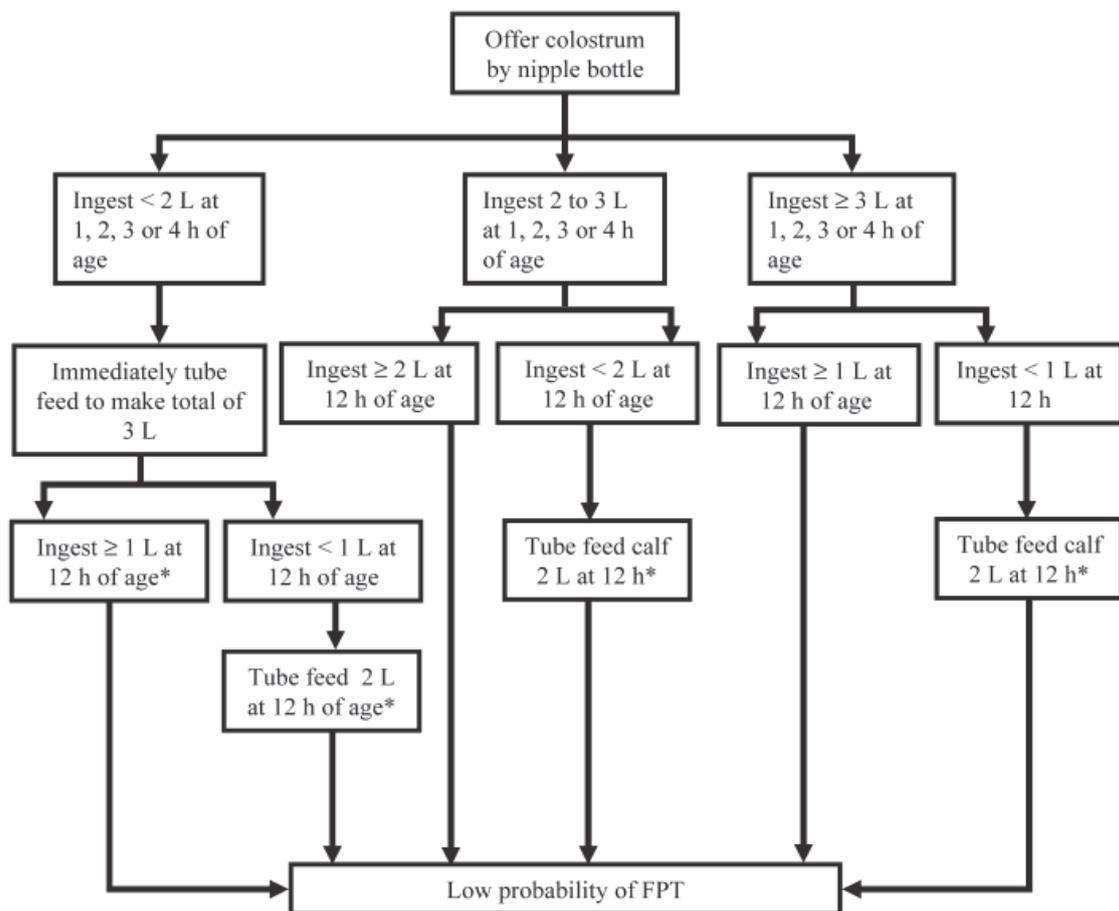
of the calves spontaneously consume at least 3 L of colostrum at the first feeding (Godden et al., 2009; Vasseur et al., 2009), but it could be difficult in dairy practice to provide all calves with 4 L of colostrum within few hours after birth in a unique feeding. Therefore, the method of colostrum provision is important to address the objectives of an effective colostrum management.

Method of Colostrum Provision. To maximize the AEA, ingested colostrum should reach the small intestine as soon as possible. That is guaranteed in the calf by the esophageal groove reflex, which drives colostrum from esophagus directly into the abomasum, avoiding the forestomachs, and allowing the rapid passage into the small intestine (Godden et al., 2009). Suckling is needed to trigger that reflex. Providing colostrum by nipple -bottle or -bucket is therefore an effective method for colostrum provision, if delivery time, quality and amount of the colostrum meal are adequate.

In order to provide all calves with 4 L of colostrum in a single feeding soon after birth, the use of the esophageal tube for colostrum administration is becoming increasingly common among dairy producers. That is an effective tool for prevention of FTPI occurrence in calves (Besser et al., 1991; Godden et al., 2009; Chigerwe et al., 2012); however, there is concern that this method could lead to a reduced AEA (Quigley, 2002). That is because the use of the esophageal tube does not trigger the esophageal groove reflex, leading to deposition of colostrum in the rumen before entering the abomasum and the intestine. In that way, colostrum could reach the intestine with 2 to 4 h of delay, a period long enough to allow the intestine maturation in the meanwhile (Quigley, 2002; Godden et al., 2009). Moreover, due to the receptor-mediated mechanism of Ig absorption in calf intestine, it has been hypothesized that a maximum amount of colostral Ig that can be absorbed exist (Quigley and Drewry, 1998). Even if the limit of Ig absorption is supposed to be over the range of typical Ig intake (Quigley and Drewry, 1998), Jaster (2005) suggested that large amounts of colostrum (e.g. 4 L), potentially of low quality, would not be as adequately absorbed as limited amounts of high quality colostrum. Therefore, the most effective practice for colostrum administration to maximize the Ig absorption could be to provide the calf with 4 L of high quality colostrum within 6 h after birth in more than one feeding. Even if no studies have been conducted in calves based on those parameters, Jaster (2005) reported a positive effect of feeding small amounts of high quality colostrum (2 L) in two separate feedings at 0 and 12 h of

life, whereas Chigerwe et al. (2009) suggested a protocol for colostrum feeding based on colostrum intake at the first meal (Figure 10). Anyway, when small amounts of colostrum are fed, the nipple-bottle ensures higher AEA than the esophageal tube, whereas when large amounts of colostrum are fed in a single administration (3 or 4 L) no differences are found in AEA between the two methods of colostrum provision (Godden et al., 2009; Chigerwe et al., 2012; Sakai et al., 2012).

Figure 10. Flow chart summarizing recommended standard operating procedures for the feeding of colostrum to calves via nipple bottle based on colostrum intake at first feeding (1, 2, 3, or 4 h of age) and intake at 12 h of age (From Chigerwe et al., 2009)



Allowing the calf to nurse colostrum from the dam ensure the highest AEA: calf could eat until satiety and suckle more than one time within few hours after birth (Stott et al., 1979c; Kälber and Barth, 2014). However, a prevalence of FTPI higher than 50% has been reported in calves that nursed from the dam without human assistance, because a considerable percentage of them did not stand or reach the teats or manage to consume enough colostrum within the first hours of life (Brignole and Stott, 1980; Besser

et al., 1991; Kälber and Barth, 2014). Moreover, leaving the calf with the dam in the calving pen has been associated with an increased risk of scours, due to the higher pathogen exposure (McGuirk and Collins, 2004; Maunsell and Donovan, 2008). Finally, even if the European Food Safety Authority (2006) identified the early separation from the dams as a welfare issue for the calves, due to the privation of the maternal cares, separation distress is considerably high in calves that are left with the dams for the first hours of life (Lidfors, 1996; Weary and Chua, 2000; Stěhulová et al., 2008). Therefore, allowing calves to nurse colostrum from the dams could have several positive effects, but it requires managerial efforts linked to the need of assistance to the neonate, hygiene of the calving pen, and gradual weaning for the reduction of the separation distress.

Environment. Extreme cold environment, outside the thermoneutral range for the calf, could negatively affect the Ig absorption, due either to a direct effect on the intestinal uptake and transport of Ig and to an indirect effect on calf ability to stand and nurse (Olson et al., 1981). Newborn calves are particularly exposed to cold stress, due to their poor insulation (i.e., hair coat, skin and subcutaneous fat), to their high body surface to body mass ratio (which facilitates heat loss), and to the lack of heat production by ruminal fermentation. Thermogenesis is even more impaired in dystocial calves (up to 36% lower than in eutocial calves), due to muscle shivering, inhibited vasoconstriction and reduced activity (Roland et al., 2016). Therefore, adequate housing (dry bedding and protection from drafts) and nutritional energy provision are needed to allow the calf successfully cope with the cold environment. Infrared lamps are useful to provide warm environment especially for diseased or weak calves (Roland et al., 2016).

Metabolic Status. Respiratory or metabolic acidosis, which usually occurs in calves that experienced prolonged parturition, is generally associated to a reduced Ig absorption (Weaver et al., 2000; Quigley, 2002; Godden, 2008). Therefore, dystocia and twin-birth could be risk factors for FTPI. The acidosis is not considered to directly affect the intestinal absorptive capacity of the calf, but it affects the calf vitality and consequently its capacity to stand and nurse. Particularly, Furman-Fratczak et al. (2011) demonstrated the importance of calf vitality for the successful transfer of passive immunity, founding a higher rate of FTPI in calves with a poor vitality score.

Breed and Sex. The effects of calf breed and sex on transfer of passive immunity are not clear. Different results about FTPI occurrence among breeds and sexes are

reported in literature. Probably, the main discriminating factor is the calf body weight, as linked to the serum volume (higher weight corresponds to a larger blood volume and therefore to a higher IgG dilution), but beside it, there are many other individual factors to be considered, such as the metabolic status, the quality, the amount and the delivery time of the first colostrum meal, and the method of colostrum provision. (Quigley and Drewry, 1998; Quigley, 2002; Vogels et al., 2013)

Colostrum Supplements and Colostrum Replacers

Good quality maternal colostrum is always the best source of Ig for the calf. However, under particular conditions, farmers could decide to substitute or integrate maternal colostrum with a commercial product. That would be useful, for example, when maternal colostrum is not available, is of poor quality, is positive to pathogens (e.g. *Mycobacterium avium* subsp *paratuberculosis*, *Staphylococcus aureus* or *Mycoplasma bovis*), or whenever correct colostrum management for newborn calf can not be ensured. There are two main types of commercial products: colostrum supplements (**CS**) and colostrum replacers (**CR**). Both types contain bovine Ig that are usually colostrum- or plasma-derived; both are pathogen free and may contain specific antibodies against calf diseases. Colostrum supplements are studied to provide only an integration of Ig to calves and, therefore, they have to be administered in addition to maternal colostrum (e.g. when it is of a poor quality or in a scarce amount). Usually, CS provide less than 100 g of IgG/dose and they are added with particular nutrients (e.g. Vitamin E) (Quigley, 2002). Colostrum replacers, instead, are designed to completely replace maternal colostrum. They provide at least 100 g of IgG/dose and contain a nutrient pack (proteins, carbohydrates, lipids, vitamins, and minerals) similar to the levels found in maternal colostrum (Quigley, 2002; Swan et al., 2007).

Studies on the efficacy of both CS and CR reported different results, but in many cases the IgG contents of the commercial products were not sufficient to ensure an adequate level of immunity in calves when administered according to the label instructions (Santoro et al., 2004; Smith and Foster, 2007; Fidler et al., 2011). Due to the variability on the characteristics and on the efficacy of different types of CS and CR available in trade, it is important for dairy producers to be adequately informed on the characteristics of the product before purchasing and using it.

REFERENCES

- Barrington, G. M., and S. M. Parish. 2001. Bovine neonatal immunology. *Vet. Clin. North Am. Food Anim. Pract.* 17:463–476. doi:10.1016/S0749-0720(15)30001-3.
- Barrington, G. M., T. B. McFadden, M. T. Huyler, and T. E. Besser. 2001. Regulation of colostrogenesis in cattle. *Livest. Prod. Sci.* 70:95–104. doi:10.1016/S0301-6226(01)00201-9.
- Berge, A. C. B., P. Lindeque, D. A. Moore, and W. M. Sisco. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. *J. Dairy Sci.* 88:2166–2177. doi:10.3168/jds.S0022-0302(05)72892-7.
- Besser, T. E., C. C. Gay, and L. Pritchett. 1991. Comparison of three methods of feeding colostrum to dairy calves. *J. Am. Vet. Med. Assoc.* 198:419–22.
- Bielmann, V., J. Gillan, N. R. Perkins, A. L. Skidmore, S. Godden, and K. E. Leslie. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *J. Dairy Sci.* 93:3713–3721. doi:10.3168/jds.2009-2943.
- Blum, J. W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livest. Prod. Sci.* 66:151–159. doi:10.1016/S0301-6226(00)00222-0.
- Brignole, T. J., and G. H. Stott. 1980. Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. *J. Dairy Sci.* 63:451–456. doi:10.3168/jds.S0022-0302(80)82952-3.
- Bush, L. J., M. A. Aguilera, G. D. Adams, and E. W. Jones. 1971. Absorption of colostral immunoglobulins by newborn dairy calves. *J. Dairy Sci.* 54:1547–1549. doi:10.3168/jds.S0022-0302(71)86063-0.
- Campiotti, M. 2012. Calcola il costo di sostituzione della tua stalla. *Inf. Agrar.* 14:12–16.
- Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. *Vet. Clin. North Am. Food Anim. Pract.* 24:87–104. doi:10.1016/j.cvfa.2007.11.001.
- Chigerwe, M., D. M. Coons, and J. V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. *J. Am. Vet. Med. Assoc.* 241:104–109. doi:10.2460/javma.241.1.104.

- Chigerwe, M., J. W. Tyler, L. G. Schultz, J. R. Middleton, B. J. Steevens, and J. N. Spain. 2008. Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. *Am. J. Vet. Res.* 69:1158–1163. doi:10.2460/ajvr.69.9.1158.
- Chigerwe, M., J. W. Tyler, M. K. Summers, J. R. Middleton, L. G. Schultz, and D. W. Nagy. 2009. Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *J. Am. Vet. Med. Assoc.* 234:785–789. doi:10.2460/javma.234.6.785.
- Curtis, C. R., J. M. Scarlett, H. N. Erb, and M. E. White. 1988. Path model of individual-calf risk factors for calfhooed morbidity and mortality in New York Holstein herds. *Prev. Vet. Med.* 6:43–62. doi:10.1016/0167-5877(88)90025-6.
- Dairy Calf and Heifer Association (DCHA). 2009. DCHA Gold Standards: Production and performance standards established for Holstein calves, from birth to 6 months of age, across the United States. Accessed May 11, 2013. www.calfandheifer.org.
- Dairy Calf and Heifer Association (DCHA). 2010. DCHA Gold Standards II: Production and performance standards established for Holstein heifers, from 6 months of age to freshening, across the United States. Accessed May 11, 2013. www.calfandheifer.org.
- Deelen, S. M., T. L. Ollivett, D. M. Haines, and K. E. Leslie. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. *J. Dairy Sci.* 97:3838–3844. doi:10.3168/jds.2014-7939.
- Devery-Pocius, J. E., and B. L. Larson. 1983. Age and previous lactations as factors in the amount of bovine colostrum immunoglobulins. *J. Dairy Sci.* 66:221–226. doi:10.3168/jds.S0022-0302(83)81780-9.
- Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998a. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev. Vet. Med.* 34:31–46. doi:10.1016/S0167-5877(97)00060-3.
- Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998b. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. *Prev. Vet. Med.* 33:1–10. doi:10.1016/S0167-5877(97)00059-7.
- European Commission. 2008. Council directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves. *Official Journal of the*

European Union. L 10, 15.1.2009, pages 7–13.
<http://data.europa.eu/eli/dir/2008/119/oj>.

- European Food Safety Authority (EFSA). 2006. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on the risks of poor welfare in intensive calf farming systems. An update of the Scientific Veterinary Committee Report on the Welfare of Calves. EFSA-Q-2005-014. The EFSA Journal. 366:1–36. doi:10.2903/j.efsa.2006.366.
- Fidler, A. P., M. L. Alley, and G. W. Smith. 2011. Short communication: Serum immunoglobulin G and total protein concentrations in dairy calves fed a colostrum-replacement product. *J. Dairy Sci.* 94:3609–3612. doi:10.3168/jds.2011-4358.
- Fleenor, W. A., and G. H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. *J. Dairy Sci.* 63:973–977. doi:10.3168/jds.S0022-0302(80)83034-7.
- Furman-Fratczak, K., A. Rzasa, and T. Stefaniak. 2011. The influence of colostrum immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* 94:5536–5543. doi:10.3168/jds.2010-3253.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* 24:19–39. doi: 10.1016/j.cvfa.2007.10.005.
- Godden, S. M., D. M. Haines, K. Konkol, and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. *J. Dairy Sci.* 92:1758–1764. doi:10.3168/jds.2008-1847.
- Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat-treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G. *J. Dairy Sci.* 89:3476–3483. doi:10.3168/jds.S0022-0302(06)72386-4.
- Gulliksen, S. M., K. I. Lie, L. Sølvørød, and O. Østerås. 2008. Risk factors associated with colostrum quality in Norwegian dairy cows. *J. Dairy Sci.* 91:704–712. doi:10.3168/jds.2007-0450.
- Gulliksen, S. M., K. I. Lie, T. Løken, and O. Østerås. 2009. Calf mortality in Norwegian dairy herds. *J. Dairy Sci.* 92:2782–2795. doi:10.3168/jds.2008-1807.

- Guy, M. A., T. B. McFadden, D. C. Cockrell, and T. E. Besser. 1994. Regulation of colostrum formation in beef and dairy cows. *J. Dairy Sci.* 77:3002–3007. doi:10.3168/jds.S0022-0302(94)77241-6.
- Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336–341. doi:10.3168/jds.2010-3170.
- Hernandez, D., D. V. Nydam, S. M. Godden, L. S. Bristol, A. Kryzer, J. Ranum, and D. Schaefer. 2016. Brix refractometry in serum as a measure of failure of passive transfer compared to measured immunoglobulin G and total protein by refractometry in serum from dairy calves. *Vet. J.* 211:2–87. doi:10.1016/j.tvjl.2015.11.004.
- Jaster, E. H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. *J. Dairy Sci.* 88:296–302. doi:10.3168/jds.S0022-0302(05)72687-4.
- Jayappa, H., R. Davis, L. Dierks, D. Sweeney, and T. Wasmoen. 2008. Demonstration of passive protection in neonatal calves against colibacillosis following immunization of pregnant heifers at 3 months of gestation. *Vet. Ther.* 9:283–289.
- Kälber, T., and K. Barth. 2014. Practical implications of suckling systems for dairy calves in organic production systems - a review. *Appl. Agric. Forestry Res.* 64:45–58. doi:10.3220/LBF_2014_45-58.
- Klaus, G. G. B., A. Bennett, and E. W. Jones. 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. *Immunology.* 1969, 16:293–299.
- Kohara, J., T. Hirai, K. Mori, H. Ishizaki, and H. Tsunemitsu. 1997. Enhancement of passive immunity with maternal vaccine against newborn calf diarrhea. *J. Vet. Med. Sci.* 59:1023–1025. doi:10.1292/jvms.59.1023.
- Korhonen, H., P. Marnila, and H. S. Gill. 2000. Milk immunoglobulins and complement factors. *Br. J. Nutr.* 84(Suppl. 1):75–80.
- Larson, B. L., H. L. Heary, and J. E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. *J. Dairy Sci.* 63:665–671. doi:10.3168/jds.S0022-0302(80)82988-2.

- Lidfors, L. M. 1996. Behavioural effects of separating the dairy calf immediately or 4 days post-partum. *Appl. Anim. Behav. Sci.* 49:269–283. doi:10.1016/0168-1591(96)01053-2.
- Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J. Dairy Sci.* 81:585–595. doi:10.3168/jds.S0022-0302(98)75612-7.
- Mann, S., F. A. Leal Yepes, T. R. Overton, A. L. Lock, S. V. Lamb, J. J. Wakshlag, and D. V. Nysdam. 2016. Effect of dry period dietary energy level in dairy cattle on volume, concentrations of immunoglobulin G, insulin, and fatty acid composition of colostrum. *J. Dairy Sci.* 99:1515–1526. doi:10.3168/jds.2015-9926.
- Maunsell F. P., D. E. Morin, P. D. Constable, W. L. Hurley, G. C. McCoy, I. Kakoma, and R. E. Isaacson. 1998. Effects of mastitis on the volume and composition of colostrum produced by Holstein cows. *J. Dairy Sci.* 81:1291–1299. doi:10.3168/jds.S0022-0302(98)75691-7.
- Maunsell, F., and G. A. Donovan. 2008. Biosecurity and risk management for dairy replacements. *Vet. Clin. North Am. Food Anim. Pract.* 24:155–190. doi:10.1016/j.cvfa.2007.10.007.
- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North Am. Food Anim. Pract.* 20:593–603. doi:10.1016/j.cvfa.2004.06.005.
- Mechor, G. D., Y. T. Gröhn, and R. J. Van Saun. 1991. Effect of temperature on colostrometer readings for estimation of immunoglobulin concentration in bovine colostrum. *J. Dairy Sci.* 74:3940–3943. doi:10.3168/jds.S0022-0302(91)78587-1.
- Mee, J. F. 2013. Why do so many calves die on modern dairy farms and what can we do about calf welfare in the future? *Animals.* 3:1036–1057. doi:10.3390/ani3041036.
- Moore, M., J. W. Tyler, M. Chigerwe, M. E. Dawes, and J. R. Middleton. 2005. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. *J. Am. Vet. Med. Assoc.* 226:1375–1377. doi:10.2460/javma.2005.226.1375.
- Morin, D. E., S. V. Nelson, E. D. Reid, D. W. Nagy, G. E. Dahl, and P. D. Constable. 2010. Effect of colostral volume, interval between calving and first milking, and

photoperiod on colostrum IgG concentrations in dairy cows. *J. Am. Vet. Med. Assoc.* 237:420–428. doi:10.2460/javma.237.4.420.

- Moss, B. R., D. A. Coleman, and J. Floyd. 1991. Feeding and management of the dairy calf: birth to 6 months. Alabama Cooperative Extension System, Alabama A&M University and Auburn University. Accessed May 05, 2016. <http://www.aces.edu/pubs/docs/A/ANR-0609/index2.tmpl>.
- Muller, L. D., and D. K. Ellinger. Colostrum immunoglobulin concentrations among breeds of dairy cattle. 1981. *J. Dairy Sci.* 64:1727–1730. doi:10.3168/jds.S0022-0302(81)82754-3.
- Nardone, A., N. Lacetera, U. Bernabucci, and B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *J. Dairy Sci.* 80:838–844. doi:10.3168/jds.S0022-0302(97)76005-3.
- Olson, D. P., R. C. Bull, L. F. Woodward, and K.W. Kelley. 1981. Effects of maternal nutritional restriction and cold stress on young calves: absorption of colostrum immunoglobulins. *Am. J. Vet. Res.* 42:876–880.
- Pritchett, L. C., C. C. Gay, D. D. Hancock, and T. E. Besser. 1994. Evaluation of the hydrometer for testing immunoglobulin G1 concentrations in Holstein colostrum. *J. Dairy Sci.* 77:1761–1767. doi:10.3168/jds.S0022-0302(94)77117-4.
- Pritchett, L. C., C. C. Gay, T. E. Besser, and D. D. Hancock. 1991. Management and production factors influencing immunoglobulin G1 concentration in colostrum from Holstein cows. *J. Dairy Sci.* 74:2336–2341. doi:10.3168/jds.S0022-0302(91)78406-3.
- Quigley, J. 2002. Passive immunity in newborn calves. *Advances in Dairy Technology: proceedings of the Western Canadian Dairy Seminar.* 14:273–292.
- Quigley, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. *J. Dairy Sci.* 81:2779–2790. doi:10.3168/jds.S0022-0302(98)75836-9.
- Quigley, J. D., K. R. Martin, and H. H. Dowlen. 1995. Concentrations of trypsin inhibitor and immunoglobulins in colostrum of Jersey cows. *J. Dairy Sci.* 78:1573–1577. doi:10.3168/jds.S0022-0302(95)76780-7.

- Quigley, J. D., K. R. Martin, H. H. Dowlen, L. B. Wallis, and K. Lamar. 1994. Immunoglobulin concentration, specific gravity, and nitrogen fractions of colostrum from Jersey cattle. *J. Dairy Sci.* 77:264–269. doi:10.3168/jds.S0022-0302(94)76950-2.
- Rajala, P., and H. Castrén. 1995. Serum immunoglobulin concentrations and health of dairy calves in two management systems from birth to 12 weeks of age. *J. Dairy Sci.* 78:2737–2744. doi:10.3168/jds.S0022-0302(95)76904-1.
- Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004–1014. doi:10.3168/jds.S0022-0302(05)72768-5.
- Reiter, B. 1978. Review of nonspecific antimicrobial factors in colostrum. *Ann. Rech. Vet.* 9:205–224.
- Robison, J. D., G. H. Stott, and S. K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. *J. Dairy Sci.* 71:1283–1287. doi:10.3168/jds.S0022-0302(88)79684-8.
- Roland, L., M. Drillich, D. Klein-Jöbstl, and M. Iwersen. 2016. Invited review: Influence of climatic conditions on the development, performance, and health of calves. *J. Dairy Sci.* 99:2438–2452. doi:10.3168/jds.2015-9901.
- Sakai, R. R., D. M. Coons, and M. Chigerwe. 2012. Effect of single oroesophageal feeding of 3 L versus 4 L of colostrum on absorption of colostral IgG in Holstein bull calves. *Livest. Sci.* 148:296–299. doi:10.1016/j.livsci.2012.06.011.
- Santoro, H. M., P. S. Erickson, N. L. Whitehouse, A. M. McLaughlin, C. G. Schwab, and J. D. Quigley. 2004. Evaluation of a colostrum supplement, with or without trypsin inhibitor, and an egg protein milk replacer for dairy calves. *J. Dairy Sci.* 87:1739–1746. doi:10.3168/jds.S0022-0302(04)73328-7.
- Santschi, D. E., and D. M. Lefebvre. 2014. Review: practical concepts on short dry period management. *Can. J. Anim. Sci.* 94: 381–390. doi:10.4141/CJAS2013-194.
- Sivula, N. J., T. R. Ames, W. E. Marsh, and R. E. Werdin. 1996. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. *Prev. Vet. Med.* 27:155–171. doi:10.1016/0167-5877(95)01000-9.

- Smith, G. W., and D. M. Foster. 2007. Short communication: absorption of protein and immunoglobulin G in calves fed a colostrum replacer. *J. Dairy Sci.* 90:2905–2908. doi:10.3168/jds.2006-682.
- Stěhulová, I., L. Lidfors, and M. Špinka. 2008. Response of dairy cows and calves to early separation: effect of calf age and visual and auditory contact after separation. *Appl. Anim. Behav. Sci.* 110:144–165. doi:10.1016/j.applanim.2007.03.028.
- Stott, G. H., and A. Fellah. 1983. Colostral immunoglobulin absorption linearly related to concentration for calves. *J. Dairy Sci.* 66:1319–1328. doi:10.3168/jds.S0022-0302(83)81941-9.
- Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979a. Colostral immunoglobulin transfer in calves. I. Period of absorption. *J. Dairy Sci.* 62:1632–1638. doi:10.3168/jds.S0022-0302(79)83472-4.
- Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979b. Colostral immunoglobulin transfer in calves. II. The rate of absorption. *J. Dairy Sci.* 62:1766–1773. doi:10.3168/jds.S0022-0302(79)83495-5.
- Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979c. Colostral immunoglobulin transfer in calves. IV. Effect of suckling. *J. Dairy Sci.* 62:1908–1913. doi:10.3168/jds.S0022-0302(79)83522-5.
- Stull, C., and J. Reynolds. 2008. Calf welfare. 2008. *Vet. Clin. North Am. Food Anim. Pract.* 24:191–203. doi:10.1016/j.cvfa.2007.12.001.
- Svensson, C., A. Linder, and S.-O. Olsson. 2006. Mortality in Swedish dairy calves and replacement heifers. *J. Dairy Sci.* 89:4769–4777. doi:10.3168/jds.S0022-0302(06)72526-7.
- Swan, H., S. Godden, R. Bey, S. Wells, J. Fetrow, and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. *J. Dairy Sci.* 90:3857–3866. doi:10.3168/jds.2007-0152.
- Tyler, J. W., D. D. Hancock, J. G. Thorne, C. C. Gay, and J. M. Gay. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. *J. Vet. Intern. Med.* 13:335–337.
- Uetake, K. 2013. Newborn calf welfare: a review focusing on mortality rates. *Anim. Sci. J.* 84:101–105. doi:10.1111/asj.12019.

- Vasseur, E., J. Rushen, and A. M. de Passillé. 2009. Does a calf's motivation to ingest colostrum depend on time since birth, calf vigor, or provision of heat? *J. Dairy Sci.* 92:3915–3921. doi:10.3168/jds.2008-1823.
- Vermorel, M., C. Dardillat, J. Vernet, Saido, and C. Demigne. 1983. Energy metabolism and thermoregulation in the newborn calf. *Ann. Rech. Vet.* 14:382–389. doi:10.4141/cjas89-014.
- Vogels, Z., G. M. Chuck, and J. M. Morton. 2013. Failure of transfer of passive immunity and agammaglobulinaemia in calves in south-west Victorian dairy herds: prevalence and risk factors. *Aust. Vet. J.* 91:150–158. doi:10.1111/avj.12025.
- Waldner, C. L., and L. B. Rosengren. 2009. Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. *Can. Vet. J.* 50:275–281.
- Waltner-Toews, D., S. W. Martin, and A. H. Meek. 1986. The effect of early calthood health status on survivorship and age at first calving. *Can. J. Vet. Res.* 50:314–317.
- Weary, D. M., and B. Chua. 2000. Effects of early separation on the dairy cow and calf 1. Separation at 6 h, 1 day and 4 days after birth. *Appl. Anim. Behav. Sci.* 69:177–188. doi:10.1016/S0168-1591(00)00128-3.
- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:596–577. doi:10.1111/j.1939-1676.2000.tb02278.x.
- Wells, S. J., D. A. Dargatz, and S. L. Ott. 1996a. Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Prev. Vet. Med.* 29:9–19. doi:10.1016/S0167-5877(96)01061-6.
- Wells, S. J., L. P. Garber, and G. W. Hill. 1996b. Health status of preweaned dairy heifers in the United States. *Prev. Vet. Med.* 29:185–199. doi:10.1016/S0167-5877(96)01078-1.
- Zucali, M., L. Bava, A. Tamburini, M. Guerci, and A. Sandrucci. 2013. Management risk factors for calf mortality in intensive Italian dairy farms. *Ital. J. Anim. Sci.* 12:162–166.

CHAPTER 2

Survey on mortality rate of young stock on dairy farms of the Province of Padova



CHAPTER 2

Survey on mortality rate of young stock on dairy farms of the Province of Padova

I. Lora^{1,2}, P. Paparella³, M. Brscic², and F. Gottardo²

¹*PhD Course in Animal and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

²*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

³*Breeders' Association of the Padova Province (APA), Corso Australia 67, 35136 Padova, Italy*

Acta Agraria Kaposváriensis, 2014. 18:69–74 (Further revised)

ABSTRACT

The present study aimed to preliminarily investigate the mortality rate of replacement calves and heifers in 95 dairy farms of the Province of Padova (Italy). Data regarding total number of cows and replacement stock reared in 2012 were gathered from the Provincial Breeders' Association records, whereas data on number of replacement animals dead in the same year were collected from insurance records. Results showed that the median value of the overall mortality rate of replacement cattle under 24 mo of age was 3.3% (0.0 - 6.7%, first - third quartile), with a maximum of 28.6%. Considering age categories, mortality of 0 to 12 mo old replacement cattle was higher than that of 13 to 24 mo old ones. The median value of mortality for cattle of 0 to 12 mo of age was 4.9% (0.0 - 11.8%, first - third quartile), with a maximum of 72.1% in one farm. The median value of mortality for the older age category (13 to 24 mo) was of 0% (0.0 - 1.6%, first - third quartile), with a maximum of 25.0%. However, the high variability among farms arisen from this survey pointed out serious problems in some farms. Moreover, despite results showed a higher risk of mortality at the early stage of cattle life, a considerable number of farms showed mortality rates exceeding 0.5% for the heifers between 13 and 24 mo of age. High mortality rates at a late stage indicated a serious situation in those farms, which could lead also to relevant economic losses. In conclusion, it would be useful to

investigate on risk factors for mortality at different ages in order to develop specific recommendations to overcome such problems in dairy farms.

INTRODUCTION

Italian intensive dairy farms are generally characterized by a good management for lactating cows, but little attention is paid to calves and heifers, with negative effects on their welfare. Since dairy farms' profit depends on the incomes from milk sale, the improvement in cows' welfare conditions have a direct and tangible economic return. Replacement heifers start to generate profit only after the first calving, so the economic losses related to poor management of those animals until that moment are rarely quantified. Due to a culling rate of about 30% for Italian Holstein cows, at least a half of the animals raised on each Italian dairy farm is made by young stock, and replacement costs can therefore reach 15 to 25% of the total costs for milk production (Mourits et al., 1999). Factors that can affect the replacement rearing costs are culling rate of the cows, age at first calving, and mortality and age at death of young stock. Among these factors, the mortality of young stock is very often underestimated by farmers. A recent study by Mee (2013) reported that farmers can underestimate the incidence of calf diseases by up to 40% and loss rates till 50%, and found a very low correlation ($r = 0.01$) between actual and perceived mortality rates. Farmers often do not apply the recommendations that they receive from veterinarians and technicians, mostly because they do not perceive calf mortality as a problem, still considering the calves as by-product of milk production and not as "the cow of the future". The result is that, despite the modernization of intensive dairy farms, young stock losses are still rising in many European countries (Mee, 2013). The present study investigated the mortality rate of replacement calves and heifers in 95 dairy farms of the Province of Padova, with the aim to lay the groundwork for future investigations on the main critical points of replacement rearing (from birth to first calving) that can cause losses of young stock.

MATERIALS AND METHODS

Ninety-five Italian Holstein dairy farms belonging to the Provincial Breeders Association (APA) and located in the Province of Padova (northeastern Italy) were considered in the study. All selected farms subscribed an insurance that refunded the

farmers for the loss of animals and their carcass disposal costs. For each farm, data referred to year 2012 were gathered by the APA and the insurance records. Particularly, data about total number of cows reared and number of young stock from 0 to 12 mo of age (0–12 mo) and from 13 to 24 mo of age (13–24 mo) were obtained from the APA records. Number of animals dead in each of the four age categories 0 to 2 mo (0–2 mo), 3 to 6 mo (3–6 mo), 7 to 12 mo (7–12 mo), and 13 to 24 mo (13–24 mo) were collected from the insurance records.

Data Processing and Statistical Analysis

Overall replacement mortality of cattle under 24 mo of age was calculated as a ratio between the number of dead animals and the total number of young cattle reared (0 to 24 mo). The same approach was used to calculate the mortality rates of the two age categories 0–12 mo and 13–24 mo. The percentage of animals dead in each age category (0–2, 3–6, 7–12, 13–24 mo) was then calculated over the total number of young animals dead, in order to highlight potential risks due to the age. All data obtained in the previous steps were firstly submitted to descriptive statistics to assess location parameters. The Kruskal-Wallis test was then used to investigate the effect of the herd size, classified into three categories (≤ 50 , 51 to 100, and > 100 dairy cows reared), on mortality rates.

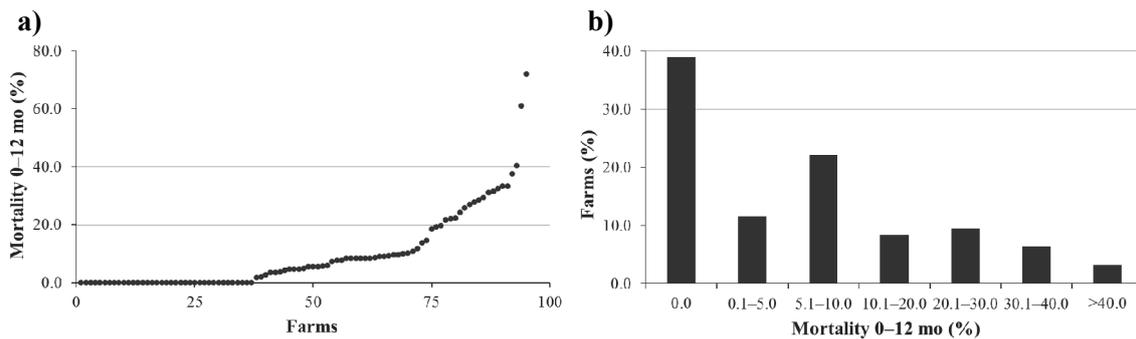
RESULTS AND DISCUSSION

The farms included in the study showed a wide range of herd size, from a minimum of 15 to a maximum of 523 cows. The average number of cows reared in 2012 was 92.6 ± 78.7 (\pm SD). Based on the classes of herd size, 26 farms were small (≤ 50 cows), 39 farms were medium (51–100), and 30 farms were large (> 100 cows).

Overall mortality rate of replacement cattle under 24 mo was not normally distributed and the median value was 3.3% (0.0 - 6.7%, first - third quartile). That seemed a positive outcome considering that the Gold Standards by the Dairy Calf and Heifer Association (DCHA Gold Standards, 2013) suggest that the cumulative mortality rate should not exceed 10% for calves and heifers from one day of life to the first calving. However, the high variability arisen from this survey pointed out serious problems in some farms. The loss rates are even more alarming if considering the single age categories (Figures 1 and 2). Distribution of mortality of the younger age category (0–12 mo) in the

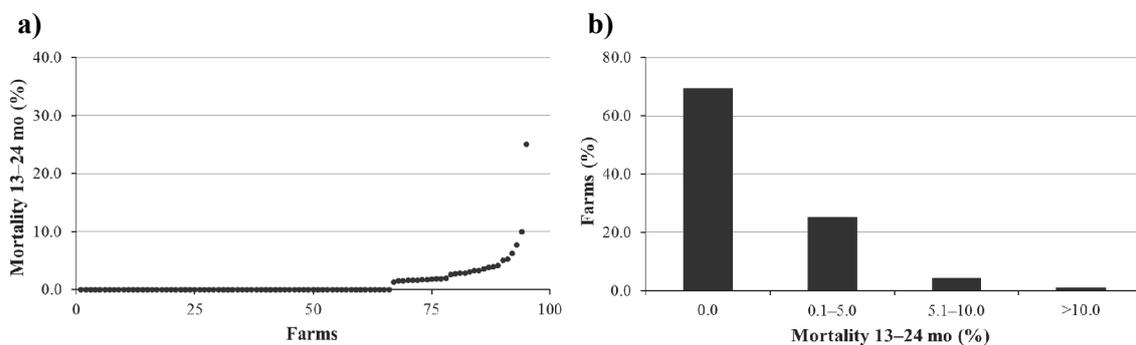
95 farms showed a median value of 4.9% (0.0 - 11.8%, first - third quartile), with a maximum value of 72.1% in one farm (Figure 1.a). Twenty-seven percent of the farms (Figure 1.b) had a mortality rate higher than the threshold value of acceptability (DCHA Gold Standards, 2013). Such farms, in particular, need an investigation of the risk factors for young stock mortality in order to take specific actions to reduce animal losses.

Figure 1. Mortality of replacement cattle between 0 and 12 mo of age: distribution of mortality rates of the 95 farms (a) and distribution of farms according to classes of mortality (b)



As expected from a previous study (Svensson et al., 2006), the mortality rate was lower for the older age category (13–24 mo), with a median value of 0.0% (0.0 - 1.6%, first - third quartile), and a maximum value of 25.0% (Figure 2.a). However, 31% of the farms had mortality rates above the 0.5% threshold value that is acceptable for replacement cattle older than 12 mo of age and until freshening (DCHA Gold Standards, 2013).

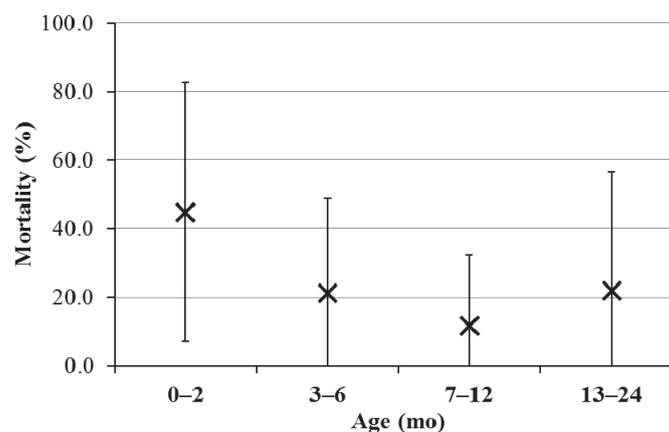
Figure 2. Mortality of replacement cattle between 13 and 24 mo of age: distribution of mortality rates of the 95 farms (a) and distribution of farms according to classes of mortality (b)



Analyzing into more detail the percentage of animals dead in each of the four age categories 0–2, 3–6, 7–12 and 13–24 mo over the overall dead replacement cattle, it must

be pointed out that the highest mortality rate was observed for calves between 0 and 2 mo of life (Figure 3). That finding was in accordance with the results of previous studies carried out on replacement cattle either in the USA (Sivula et al., 1996; Wells et al., 1996), in Sweden (Svensson et al., 2006) and in Italy (Colnago et al., 2007). A plausible explanation to the higher mortality rate of calves observed in the first months of life could be their susceptibility to diseases, especially enteric and respiratory, due to a poorly competent immune system (Sivula et al., 1996; Wells et al., 1996). Moreover, stressful conditions, such as calf separation from the dam and changes in housing (individual vs group) and feeding (weaning), might act as important predisposing factors to disease outbreak and consequently to mortality (Wells et al., 1996; Stull and Reynolds, 2008; Zucali et al., 2013). In accordance with results by Svensson et al. (2006), mortality rate in the current study tended to decrease progressively from the third month of life to one year of age, and to increase again after that age interval (Figure 3). In the current study, indeed, the average mortality rate reached 22% for the 13–24 mo age category. Causes of mortality for this age category could not be the same of those acting at an earlier stage, but they could be identified among housing facilities or management. Trauma as consequence of overcrowding, hierarchy establishment and inappropriate flooring, and peripartum disorders are reported to be the main predisposing factors for mortality at this age (Bøe and Færevik, 2003; Svensson et al., 2006; Dorigo et al., 2009).

Figure 3. Distribution of mortality (average percentage \pm SD) at different ages over the total number of replacement cattle dead in 95 dairy farms



Regardless of farm size, that did not affect mortality rates ($P > 0.05$), and age category in which mortality occurred, the high variability among farms made it necessary

to differentiate between good and bad performing farms. In order to identify the best and the worst situations, farms were distributed according to the mortality rates of the two age categories 0–12 and 13–24 mo within the mortality thresholds defined by the DCHA Gold Standards (2013). Twenty-six farms (27.4%) could be considered the best performing ones, having mortality rates lower than 1.0% for both the age categories (Table 1). Forty-seven farms (49.5%) could be considered as good performing because they fell in the acceptable range of mortality below 10.0% for the calves (0–12 mo) and below 1.0% for the heifers (13–24 mo). None of the farms had mortality rates over 10.0% for both the age categories, which would have been the worst possible situation. However, a considerable percentage of farms (22.1%) showed low or acceptable rates of mortality for the age category 0–12 mo, but mortality rates exceeding 1.0% for the heifers between 13 and 24 mo of age. Such mortality rates at a late stage indicated a serious situation in those farms, which could lead to relevant economic losses. The latter are proportional to the age at death of the animals, due to the incurred rearing costs and the purchasing of new replacement heifers (Campiotti, 2012). Regardless of the age category in which mortality occurs, the loss of replacement cattle is not only an economic problem but it also constitutes a health issue, because the introduction of new heifers from external herds increase the risk of introducing new diseases.

Table 1. *Distribution of 95 dairy farms (%) according to replacement cattle mortality rates between 0 and 12 mo of age and between 13 and 24 mo of age, and based on to the mortality thresholds of acceptability defined by the DCHA Gold Standards (2013). Darker color of filling indicates worse situations*

		Mortality 13–24 mo			
		≤1.0%	1.1–5.0%	5.1–10.0%	>10.0%
Mortality 0–12 mo	≤1.0%	27.4	7.4	1.1	1.1
	1.1–5.0%	6.3	6.3		
	5.1–10.0%	15.8	4.2	2.1	
	>10.0%	18.9	7.4	2.1	

CONCLUSIONS

The results of this preliminary investigation indicated that management of young cattle is still a critical point in a large number of dairy farms considering the high variability of the mortality rates. Although results pointed out a higher risk of mortality at the early stage of cattle life, a considerable number of farms showed mortality rates exceeding 0.5% for the heifers between 13 and 24 mo of age. High mortality rates at a

late stage indicated a particularly serious situation in those farms, which could lead to relevant economic losses. In conclusion, further investigations are needed on risk factors for young stock mortality at different ages, in order to develop specific recommendations to reduce calf and heifer mortality in dairy farms.

ACKNOWLEDGEMENTS

The Authors wish to thank Dr. Barbara Contiero for her statistical support.

REFERENCES

- Bøe, K. E., and G. Færevik. 2003. Grouping and social preferences in calves, heifers and cows. *Applied Animal Behaviour Science*. 80:175–190.
- Campiotti, M. 2012. Calcola il costo di sostituzione della tua stalla. *L'Informatore Agrario*. 14:12-16.
- Colnago, S., N. Colombo, and A. Tamburini. 2007. Meno perdite tra i vitelli con la giusta gestione alimentare. *L'Informatore Agrario*. 29:5–12.
- Dairy Calf and Heifer Association Gold Standards. 2013. Retrieved from www.calfandheifer.org.
- Dorigo, M., P. Dalvit, and I. Andrighetto. 2009. Una buona gestione della manza è costosa, ma è un investimento. *Terra e Vita*. 46:68–72.
- Mee, J. F. 2013. Why do so many calves die on modern dairy farms and what can we do about calf welfare in the future? *Animals*. 3:1036–1057.
- Mourits, M. C. M., R. B. M. Huirne, A. A. Dijkhuizen, A.R. Kristensen, and D.T. Galligan. 1999. Economic optimization of dairy heifer management decisions. *Agricultural Systems*. 61:17–31.
- Sivula, N. J., T. R. Ames, W. E. Marsh, and R. E. Werdin. 1996. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. *Preventive Veterinary Medicine*. 27:155–171.
- Stull, C., and J. Reynolds. 2008. Calf welfare. *Veterinary Clinics of North America: Food Animal Practice*. 24:191–203.
- Svensson, C., A. Linder, and S. O. Olsson. 2006. Mortality in Swedish dairy calves and replacement heifers. *Journal of Dairy Science*. 89:4769–4777.

- Wells, S. J., D. A. Dargatz, and S. L. Ott. (1996). Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Preventive Veterinary Medicine*. 29:9–19.
- Zucali, M., L. Bava, A. Tamburini, M. Guerci, and A. Sandrucci. 2013. Management risk factors for calf mortality in intensive Italian dairy farms. *Italian Journal of Animal Science*. 12:162–166.

CHAPTER 3

Factors associated with failure of transfer of passive immunity in dairy calves: combined effect of delivery time, amount and quality of the first colostrum meal



CHAPTER 3

Factors associated with failure of transfer of passive immunity in dairy calves: combined effect of delivery time, amount and quality of the first colostrum meal

I. Lora^{1,2}, A. Barberio³, B. Contiero², P. Paparella⁴, L. Bonfanti³, M. Brscic², A. L. Stefani³, and F. Gottardo²

¹*PhD Course in Animal and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

²*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

³*Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD), Italy*

⁴*Associazione Veneta Allevatori (AVA), Corso Australia 67, 35136 Padova, Italy*

Submitted to Animal

ABSTRACT

The adequate transfer of colostral immunoglobulins (**Ig**) is the first event after birth that affects calf health and survival. Aims of this study were to investigate the management factors associated with failure of transfer of passive immunity (**FTPI**) in newborn dairy calves, and particularly to evaluate the combined effect of delivery time, amount, and quality of the first colostrum meal. From March to August 2014, blood samples were collected from 244 calves (1 to 5 d old) born from Holstein cows on 21 Italian dairy farms (herd size ranging from 50 to 150 cows). A sample of the first colostrum meal of each calf included in the study was collected too. A questionnaire on calf management at the farm-level was administered to the farmers, whereas individual information on calves and the respective colostrum management were collected for each calf sampled. Immunoglobulin concentration of both serum and colostrum samples was assessed by electrophoresis. A mixed effects multivariable logistic regression model was used to investigate the association with FTPI (calf serum Ig concentration <10.0 g/L) of the variables obtained both from the management questionnaire and from the individual calf data. A cumulative score of colostrum management (**SCM**) that combined delivery

time, amount and quality of the first colostrum meal was generated for 236 calves. Higher values of the SCM corresponded to an overall better management of colostrum. The overall percentage of calves with FTPI was of 41.0%, and the within-farm percentage of calves with FTPI was >20.0% in 71.4% of the farms. Factors associated with FTPI were calf breed and sex, with higher risk for Holstein compared to crossbred calves and for female compared to male calves, time after birth of the first colostrum meal (regression coefficient = 0.13), and amount and quality of colostrum provided at the first meal (regression coefficients = -0.59 and -0.03, respectively). The level of passive immunity in calves improved as the SCM increased (regression coefficient = 1.67). To avoid FTPI at all, calves should receive at least 2.5 L of high quality colostrum (Ig concentration >87.6 g/L) within 1.0 h of birth. Considerable improvements can be done on dairy farms about colostrum management for newborn calves, and results of this study will help in the development of specific recommendations for dairy producers in order to reduce the prevalence of FTPI.

INTRODUCTION

Morbidity and mortality rates of dairy calves are often underestimated by farmers (Vasseur et al., 2012; Mee, 2013), despite their great impact on animal welfare and on farm profits. In general, mean values of calf mortality hide the right skewed distribution of mortality data, which identify a very large inter-farm variability ranging from minimal losses to over 20% of calf mortality (Mee, 2013; Uetake, 2013). Many factors can be involved in the occurrence of calf disease and mortality, but the first step to prevent calf losses is the appropriate administration of colostrum. In fact, calves are born almost agammaglobulinemic and they depend on colostrum ingestion to acquire the maternal immune protection against infectious diseases (Weaver et al., 2000; Lorenz et al., 2011). The efficiency of the calf intestine in the absorption of intact immunoglobulins (**Ig**) from colostrum (“open gut”) is the greatest within 6 h after birth, and it decreases steadily from 6 to 12 h of life, essentially ending by 24 h (“closure”) (Godden, 2008; Lorenz et al., 2011). Therefore, the common recommendations for proper colostrum management in order to obtain an adequate transfer of passive immunity in newborn calves are: feeding colostrum within 6 h of birth, in an adequate amount (i.e., 10.0 to 12.0% of the calf body weight, corresponding to about 3.0 or 4.0 L of colostrum for a Holstein calf), and of a

good quality (i.e., with a Ig concentration ≥ 50.0 g/L and a bacterial count $< 100,000$ cfu/mL) (Weaver et al., 2000; McGuirk and Collins, 2004; Godden, 2008). Inadequate colostrum administration leads to failure of transfer of passive immunity (**FTPI**), a condition which is defined when calf serum Ig concentration is lower than 10.0 g/L at 24 to 48 h of life (Jaster, 2005; Godden, 2008; Furman-Fratczak et al., 2011). However, a minimum serum Ig concentration of 16.0 g/L seems to be required for a good immune protection (Waldner and Rosengren, 2009; Furman-Fratczak et al., 2011). Many studies indicate FTPI as a major risk factor for the incidence and severity of calf diseases, both enteric and respiratory (Donovan et al., 1998; Maunsell and Donovan, 2008; Furman-Fratczak et al., 2011), and it is reported that 31.0% to 39.0% of calf mortality could be attributable to FTPI (Wells et al., 1996; Tyler et al., 1999; Godden, 2008). Moreover, FTPI could have long term consequences, such as negative effects on age at first calving and on performances in first lactation of heifers calves (Heinrichs and Heinrichs, 2011), or negative effects on health and performances of male calves that will enter the veal or the beef industry. Recent studies carried out on dairy calves in different countries reported a prevalence of FTPI still ranging from 35.0 to 40.0% (Weaver et al., 2000; Trotz-Williams et al., 2008; Vogels et al., 2013). Aims of this study were to investigate the management factors associated with FTPI in newborn calves at the farm level, and particularly to evaluate the combined effect of delivery time, amount, and quality of the first colostrum meal. Results of this study can be used to improve the current indications on proper colostrum management practices for dairy calves and to develop farm-specific programs for the reduction of the prevalence of FTPI.

MATERIALS AND METHODS

The study was carried out from March to August 2014 on a convenience sample of 21 dairy farms located in the northeast of Italy (Veneto Region). Farms were selected according to the following criteria: herd size (ranging from 50 to 150 cows), breed reared (Italian Holstein Friesian), housing system (loose), and farmer's willingness to be part of the study. In order to minimize calves' suffering and handling, farm selection included also the adoption of a voluntary plan for the control of bovine viral diarrhea (**BVD**), which already required blood sampling in newborn calves. Therefore, calf blood samples were analyzed for both BVD virus and Ig concentration.

Data Collection and Sampling

At first, a questionnaire about newborn calf management practices at the farm level was administered as an interview to the farmers. Each farm was then visited two times per week by the person in charge to carry out the study (data collection and samples delivery to the laboratory) and by the farm veterinarian responsible for the plan of BVD control (blood sampling). The veterinarian performed blood sampling on calves between 1 and 5 d of age, and, for the purpose of this study, a number of calves equal to 10% of the overall expected calving per year was considered in each farm. Blood samples were collected from the jugular vein using a 10 mL Vacutainer® tube without anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA), and they were stored at 4°C until the delivery to the laboratory. Both male and female, Holstein purebred and Holstein crossbred calves were included in the study. For each of them, data about sex and breed, parity of the nursing cow, occurrence of dystocia and management of colostrum (delivery time from birth and amount of the first colostrum meal) were recorded at the moment of the farm visit. Moreover, the farmers were in charge to collect, into a 100 mL tube, a sample of the first colostrum meal provided to each calf included in the study, and to store it at -20°C until the next farm visit, when the person in charge of samples collection gathered it. Colostrum samples were maintained at -20°C until the delivery to the laboratory.

Laboratory Analysis

At the laboratory, within 2 h of collection, blood samples were centrifuged at $3076 \times g$ for 10 min at 20°C. Serum was then transferred into 2 mL tubes and it was stored at -20°C until the day of the analysis. Serum and colostrum Ig concentrations were quantified by the method described in Tóthová et al. (2013). To perform the analysis, blood serum and colostrum samples were thawed in a water-bath at 20°C and at 37°C, respectively. Colostrum samples were then processed according to the procedure reported by Ceniti et al. (2016): in order to separate albumin and globulin fractions from casein fraction, 40.0 µL of a commercial rennet solution (Naturen, CHR Hansen, Hoersholm, Denmark) were added to 4.5 mL of each colostrum sample and incubated at 37°C for 5 min. Thereafter, the clot was disaggregated with a plastic stick and each sample was centrifuged at $3076 \times g$ for 15 min at 15°C. The supernatant (colostrum whey) was

collected and it was added with distilled water to restore the initial volume of extraction (4.5 mL). Total protein concentration (g/L) of both blood serum and colostrum whey samples were firstly assessed by the biuret method using an automatic analyzer (Cobas C501, Roche Diagnostics, Mannheim, Germany). Protein fractions (%) of the same samples were then analyzed by a semi-automated agarose gel system (Hydrasys LC Sebia, Bagno a Ripoli, FI, Italy) associated with Phoresis software, as described in Tóthová et al. (2013). For each serum and colostrum whey sample, the percentage of the Ig fraction resulted from the electrophoretic analysis was converted into the absolute concentration (g/L) based on the total protein concentration (g/L) obtained by the biuret method.

Data and Statistical Analysis

Of the total 247 calves sampled, three calves of different farms were excluded from the study (one due to a congenital intestinal atresia and two due to blood sample hemolysis), so the final dataset consisted of 244 calves (mean \pm SD: 12 ± 3 calves sampled per farm). Colostrum samples collected throughout the study were 223 (mean \pm SD: 11 ± 2 colostrum samples per farm), but, because 15 calves were fed colostrum from another cow included in the study, analyses of the first colostrum meal were available for 238 calves. None of the calves considered in the study received commercial colostrum supplements or replacers. Data about time and amount of the first colostrum meal were missing for two calves that were born during the night.

For the descriptive statistics, based on literature (Godden, 2008; Furman-Fratczak et al., 2011), three levels of calf serum Ig concentrations were defined: <10.0 g/L (FTPI), from 10.0 to 15.9 g/L (adequate transfer of passive immunity), and ≥ 16.0 g/L (optimal transfer of passive immunity - **OTPI**). Similarly, colostrum samples were divided into two main classes according to the Ig concentration: <50.0 g/L (poor quality colostrum) and ≥ 50.0 g/L (good quality colostrum) (Godden, 2008).

Because parity is one of the main factors that could affect the quality of colostrum, a Chi-square test was used to preliminarily investigate if there were differences in the percentage of poor quality colostrum samples between primiparous and pluriparous cows. Then, it was evaluated the association between FTPI (calf serum Ig <10.0 g/L) and the variables obtained from the management questionnaire and the individual calf data. At first, the relationship between single variable and FTPI was screened by univariable

analysis (PROC GLIMMIX, SAS Institute Inc., Cary, NC), including the farm as random effect and considering the calf as the statistical unit. Variables with $P < 0.05$ at the univariable analysis were subsequently included into a mixed effects multivariable logistic regression model with farm as random effect (PROC GLIMMIX, SAS Institute Inc., Cary, NC). Odds ratio for FTPI occurrence and 95% confidence interval were calculated for the binary variables that entered the multivariable model, whereas regression coefficients and standard errors were calculated for the continuous ones.

After that, a cumulative score of colostrum management (**SCM**) was generated for 236 calves, where higher values corresponded to an overall better management of colostrum. The SCM was calculated for each calf according to the ensuing three-step procedure. As first step, based on the quartiles distribution, specific scores from “0” to “3” were assigned, for each calf, to delivery time, amount, and quality of the first colostrum meal, as reported in Table 1. In the second step, the effects of the three main factors (delivery time, amount, and quality of the first colostrum meal) on FTPI occurrence were tested by a Classification and Regression Tree analysis (**C&RT**) (Dell™ Statistica™), in order to obtain a weighted coefficient for each of them. As last step, for each calf, the three specific scores were multiplied by the respective weighted coefficient obtained from the C&RT analysis, and the SCM was finally calculated as the sum of the three weighted specific scores. The effect of the SCM on calf serum Ig concentration was finally evaluated by a multilevel linear regression model (PROC MIXED, SAS Institute Inc., Cary, NC), including also the effects of calf sex and breed, and considering the farm as random effect.

Table 1. *Specific scores assigned to delivery time, amount, and quality (immunoglobulin concentration) of the first colostrum meal provided to the calves*

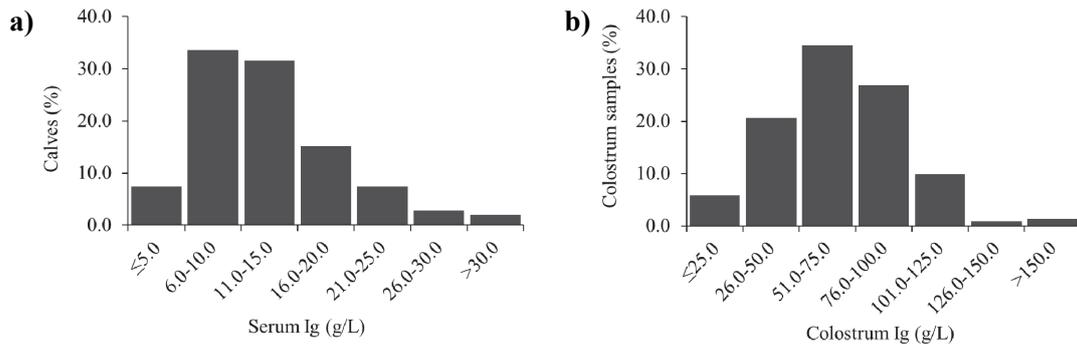
	Score 0	Score 1	Score 2	Score 3
Characteristics of the first colostrum meal:				
time after birth (h)	>5.5	5.5–2.6	2.5–1.1	≤1.0
amount (L)	≤1.5	1.6–2.0	2.1–2.5	>2.5
immunoglobulin concentration (g/L)	≤49.4	49.5–69.0	69.1–87.6	>87.6

RESULTS

Serum Ig concentrations of the 244 calves ranged from 1.4 g/L to 38.5 g/L, with a mean value of 12.4 g/L (Figure 1.a). Forty-one percent of calves had FTPI, 34.8% had adequate transfer of passive immunity, and 24.2% had OTPI (Figure 2.a). The distribution

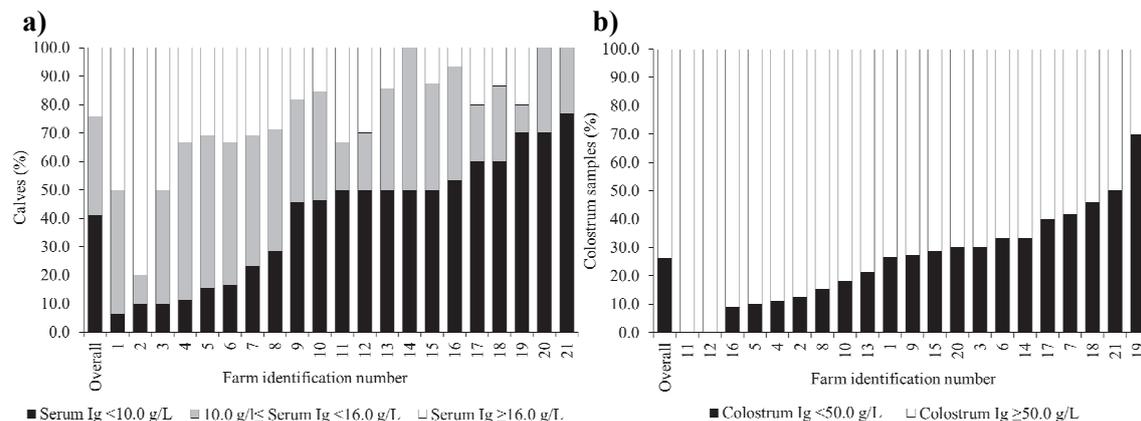
of the calves according to the serum Ig concentration among farms showed a wide variability, especially when considering the OTPI group (Figure 2.a). Only six farms had less than 20.0% of the calves with FTPI, whereas in two farms none of the calves reached the OTPI.

Figure 1. Distribution of immunoglobulin (Ig) concentrations in serum of 244 calves from 1 to 5 d of age (a) and in colostrum of 223 Holstein cows (b)



Of the 223 colostrum samples collected, 78 (35.0%) were from primiparous cows and 145 (65.0%) were from pluriparous cows. Colostrum Ig concentrations ranged from 10.9 g/L to 169.7 g/L, with a mean value of 68.1 g/L (Figure 1.b). The overall percentage of poor quality colostrum samples was 26.5%. No differences in the percentage of poor quality colostrum samples were detected between primiparous (34.6%) and pluriparous (22.1%) cows ($\chi^2 = 3.48$; $P = 0.062$). As observed for serum Ig concentration, the distribution of colostrum samples according to their quality was characterized by a considerable variability among farms (Figure 2.b). Only two farms did not have any samples of poor quality colostrum, whereas in nine farms 30.0% or more of the colostrum samples had an Ig concentration <50.0 g/L.

Figure 2. Overall and within-farm distribution of calves ($n = 244$) with different serum immunoglobulin (Ig) concentrations (a) and of colostrum samples ($n = 223$) according to Ig concentration (b) in 21 dairy farms



Calf Management Practices and Association with FTPI

At the univariable analysis, none of the calf management practices of the 21 surveyed farms was associated with FTPI occurrence in calves (Table 2).

Individual information that were collected at the calf-level are reported in Table 3. The final sample of calves consisted of 136 female and 108 male calves, 205 Holstein purebred and 39 Holstein crossbred. Of the tested calves, nearly one-third (33.6%) were fed colostrum from primiparous cows, and 7.0% was born with difficulty (Table 3). The most of the calves (80.2%) were fed the first colostrum meal within 6 h of life (overall mean \pm SD: 4.0 \pm 4.1 h of birth), but 65.3% of them consumed equal or less than 2.0 L of colostrum at the first meal (overall mean \pm SD: 2.1 \pm 0.7 L). However, colostrum provided to the calves was of a good quality in 74.4% of the cases. Parity of the nursing cow and occurrence of dystocia were not associated with FTPI at the univariable analysis (Table 3). Both at the univariable and at the multivariable analysis, the occurrence of FTPI resulted to be affected by calf breed and sex, and by time after birth, amount and quality of the first colostrum meal. Particularly, Holstein purebred calves were 3.2 times more likely to have FTPI than Holstein crossbred ones ($P = 0.034$), and female calves had an odds of FTPI 1.96 times higher than male calves ($P = 0.041$). Moreover, the risk of FTPI increased of 0.13 times ($P = 0.002$) for every hour of delay from birth of the provision of the first colostrum meal; it decreased of 0.59 times for every liter of colostrum given more to the calves ($P = 0.028$); and it decrease of 0.03 times for every gram per liter of Ig contained more in the colostrum fed ($P < 0.001$).

Table 2. Descriptive statistics of the responses to the questionnaire about calf management practices administered to 21 dairy farmers in the study. Percentage of calves with failure of transfer of passive immunity (FTPI - serum Ig concentration <10.0 g/L) by response options, and effects of the variables on FTPI occurrence are reported too

Variables (questions)	Response options	Farms	Calves exposed	Calves with FTPI	P-value
		n	n	%	
Overall		21	244	41.0	
Milking system	Milking parlour	18	211	39.8	ns
	Automatic milking system	3	33	48.5	
Calving pen	Present	14	177	40.7	ns
	Absent	7	67	41.8	
The caregiver for the calves is	Farmer or a family member	19	216	43.1	ns
	Employee	2	28	25.0	
Sex of the calf caregiver is	Female	2	26	50.0	ns
	Male	19	218	39.9	
Calves are left with their dams for at least 1 h	Yes	5	61	34.4	ns
	No	16	183	43.2	
Method of provision of the first colostrum meal	Nipple-bottle	11	129	46.5	ns
	Nipple-bucket	10	115	34.8	
	Esophageal tube	0	0	0.0	
More than one colostrum meal within 6 h of birth	Yes	0	0	0.0	ns
	No	21	244	41.0	
Type of colostrum fed	From the dam	18	203	39.9	ns
	Commercial supplement/replacer	3	41	46.3	
	From another cow	0	0	0.0	
	Pooled colostrum	0	0	0.0	
	Frozen colostrum	0	0	0.0	
Check of colostrum temperature at the first meal	Yes	8	93	33.3	ns
	No	13	151	45.7	
Main cause of calf mortality	None	5	55	30.9	ns
	Enteric disease	7	73	46.6	
	Respiratory disease	9	116	42.2	
Disinfection of the navel	Yes	18	211	40.8	ns
	No	3	33	42.4	
Vaccination of dry cows for calf diarrhea prevention	Yes	12	155	44.5	ns
	No	9	89	34.8	
Calf vaccination against respiratory diseases	Yes	11	137	38.7	ns
	No	10	107	43.9	
Use of prophylactic antibiotic treatments on young calves	Yes	10	119	41.2	ns
	No	11	125	40.8	

Table 3. Descriptive statistics of individual data about 244 calves of 21 dairy farms. Odds ratio and 95% CI, or regression coefficient and $S_{y,x}$, are reported for the variables associated with failure of transfer of passive immunity (FTPI - serum Ig concentration <10.0 g/L)

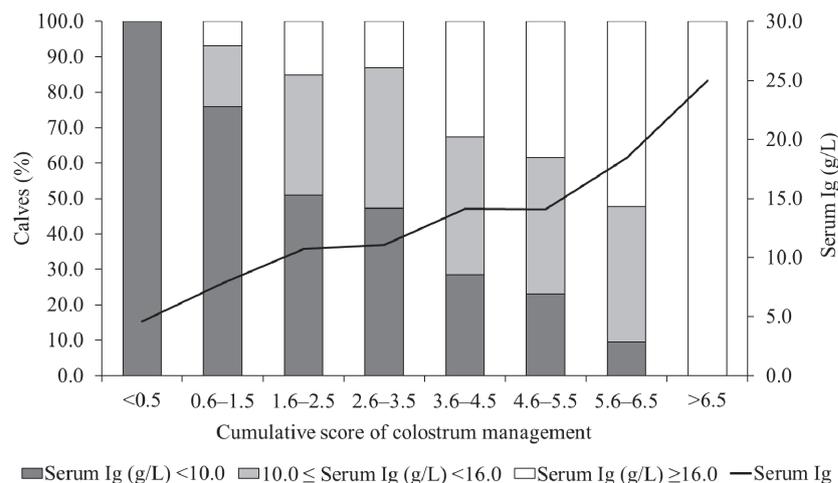
Variables	Levels	Calves	Calves with FTPI	Odds ratio	95% CI	Regression coefficient	$S_{y,x}$	P-value
		n	%					
Overall		244	41.0					
Calf breed	Crossbred	39	17.9	1.00	–	–	–	0.034
	Holstein	205	45.4	3.15	1.09–9.11	–	–	
Calf sex	Male	108	33.3	1.00	–	–	–	0.041
	Female	136	47.1	1.96	1.03–3.74	–	–	
Parity of the nursing cow	1	82	43.9	–	–	–	–	ns
	>1	162	39.5	–	–	–	–	
Dystocia	Yes	17	41.2	–	–	–	–	ns
	No	227	41.0	–	–	–	–	
Time of birth of the first colostrum meal (h)	Continuous variable	242	NA ¹	–	–	0.13	0.04	0.002
Amount of colostrum fed at the first meal (L)	Continuous variable	242	NA	–	–	–0.59	0.27	0.028
Colostrum Ig concentration (g/L)	Continuous variable	238	NA	–	–	–0.03	0.01	<0.001

¹NA = not applicable.

Cumulative Score of Colostrum Management

Results from the C&RT analysis showed that the most important factor associated with the occurrence of FTPI was the quality of colostrum provided at the first meal (weighted coefficient = 1.00), the second one was the time after birth of the first colostrum meal (weighted coefficient = 0.68), and the third one was the amount of colostrum fed at the first meal (weighted coefficient = 0.60) (Supplementary Figure S1). Therefore, the final formula used to calculate the SCM for each calf was: [(score of time at the first colostrum meal) × 0.68] + [(score of amount of colostrum fed) × 0.60] + [(score of quality of colostrum fed) × 1.00]. The SCM resulted to influence calf serum Ig concentration (regression coefficient = 1.67; $S_{y.x} = 0.24$; $P < 0.001$). Particularly, in case of completely defective management of colostrum in terms of delivery time, amount and quality of the first colostrum meal (SCM <0.5), 100% of the calves suffered from FTPI (Figure 3). As colostrum management improved, with different combinations of time after birth, amount, and quality of the first colostrum meal, the percentage of calves with FTPI decreased substantially, whereas the fraction of calves with OTPI increased. With the best SCM (>6.5) none of the calves had FTPI and all of them reached the OTPI (Figure 3).

Figure 3. *Distribution of dairy calves (n = 236) with different serum immunoglobulin (Ig) concentrations according to the cumulative score of colostrum management calculated for each of them based on delivery time, amount, and Ig concentration of their first colostrum meal. Distribution of serum Ig concentration of the calves according to the cumulative score of colostrum management is reported too (regression coefficient = 1.67; P < 0.001).*



DISCUSSION

The overall percentage of calves with FTPI found in the current study was higher than the one reported by Cavirani et al. (2005) for Italian dairy calves (35.0%). Slightly lower percentages of calves with FTPI were reported even by Vogels et al. (2013) for Australian calves (38.0%) and by Trotz-Williams et al. (2008) for Canadian calves (37.1%), whereas Beam et al. (2009) reported a value of 19.2% for U.S. heifer calves. Moreover, the low fraction of calves with OTPI found in this study clearly indicated the presence of generalized deficiencies in colostrum management for Italian dairy calves. The problem was even more evident when considering the within-farm percentage of calves with FTPI, which was over the 20% threshold of acceptability recommended by McGuirk and Collins (2004) in more than two-thirds of the farms. That result was in line with the findings by Cavirani et al. (2005), who reported a within-farm prevalence of FTPI $\geq 30.0\%$ in 70.6% of the Italian dairy farms investigated (n = 85). It appeared, therefore, that Italian farmers did not make any progress about colostrum management for newborn calves in the last 10 years.

The mean colostrum Ig concentration of Holstein cows found in this study was in line with the data reported by Swan et al. (2007) on colostrum quality of U.S. Holstein cows (Ig concentration of 76.7 ± 30.0 g/L, mean \pm SD), whereas the overall percentage of poor quality colostrum samples was higher than the one found by Cavirani et al. (2005) for Italian Holstein cows (17.1%). Godden (2008) reported that colostrum quality is characterized by a high individual variability, and it is mainly affected by breed (dairy vs beef-breeds), parity (older cows produce colostrum of a higher quality) and dry period management (length and nutrition). Although investigation of the factors that influence colostrum quality was not an aim of this study, the possibility that parity affected colostrum Ig concentration was considered. However, no differences in the percentage of poor quality colostrum samples were detected between primiparous and pluriparous cows.

Distributions among farms of calves by classes of serum Ig concentration and of colostrum samples by their quality were characterized by a considerable variability. However, it should be noticed that several farms with a high percentage of poor quality colostrum samples had a low percentage of calves with FTPI, and vice versa (i.e., farms

number 3, 6, 11 and 12; Figures 2.a and 2.b), indicating that other factors beyond the quality of colostrum could be responsible for the occurrence of FTPI in calves.

Calf Management Practices and Association with FTPI

None of the calf management practices considered in the farm questionnaire resulted to be associated with FTPI. Anyway, it was interesting to notice that none of the farmers used the esophageal tube for routine colostrum administration, nor offered the calves more than one colostrum meal within the first 6 h of life. Particularly, many farmers stated that they intentionally fed calves with a scarce amount of colostrum and did not offer them more than one colostrum meal within 6 h of life, because they were convinced that it was not suitable for newborn calves to eat more than 2.0 L of colostrum soon after birth. However, in a study by Vasseur et al. (2009), 42.0% of calves spontaneously consumed more than 4.0 L of colostrum at the first feeding, and 25.0% consumed 3.0 to 4.0 L. It was also interesting to point out that most of the farmers declared to have problems of calf mortality due to enteric or respiratory diseases. Even if no statistical association was found, the latter result was not surprising considering the high overall prevalence of FTPI found in this study, because FTPI is recognized to be the most important predisposing factor for calf diseases and mortality (Maunsell and Donovan, 2008; Furman-Fratczak et al., 2011). For the same reason, it was expected that in many cases calves were vaccinated against respiratory diseases and they were fed milk with added antibiotics to prevent neonatal diarrhea. However, Berge et al. (2005) pointed out that prophylactic antibiotic treatments in dairy calves could be minimized or even avoided if there is an adequate transfer of passive immunity. Vaccination of dry cows to prevent calf neonatal diarrhea was also widely adopted by the farmers, but it should be emphasized that the benefits of this practice on calf health (Kohara et al., 1997; Jayappa et al., 2008) could not be effective without proper colostrum administration.

About individual calf data, the lack of association of parity of the dam with FTPI was not surprising, because, in the current study, parity of the cow did not affect colostrum quality. Dystocia also did not affect the occurrence of FTPI, even if it is reported in literature that lower levels of passive immunity can be observed in calves born with difficulties, due to the poor vitality of the calves and to the postnatal acidosis that frequently occurs in such cases (Godden, 2008; Murray and Leslie, 2013). Among factors

that resulted to be associated with FTPI, the effects of calf breed and sex were unclear. The higher risk of having FTPI for Holstein and for female calves compared to crossbred and to male calves, respectively, was not expected, because it was reported that male and crossbred calves should be more at risk of having FTPI due to their larger size and, therefore, to their higher Ig requirement (Quigley and Drewry, 1998; Vogels et al., 2013). Other factors, probably linked to farmers' habits and to other aspects of farm management, could be responsible for that result.

On the other hand, it was expected that the delay from birth of the first colostrum meal had negative effects on transfer of passive immunity in calves, because of the progressive closure of intestinal guts and the consequent reduction in intestinal Ig absorption (Weaver et al., 2000; Godden, 2008). In this study, the risk of FTPI increased also when the amount of colostrum fed at the first meal decreased. Because farmers frequently do not measure colostrum Ig concentration, it was probable that when calves were fed a scarce amount of colostrum it might not be of a sufficient quality to provide the minimum Ig mass required for a successful transfer of passive immunity. Therefore, for example, Quigley and Drewry (1998) and Chigerwe et al. (2009) suggested that at least 3.0 L of colostrum should be provided at the first meal to minimize the percentage of calves suffering from FTPI. It was widely demonstrated that colostrum Ig concentration is of great importance for a successful transfer of passive immunity in calves, especially when the amount of colostrum fed within a few hours after birth is lower than 3.0 L (Stott and Fellah, 1983; McGuirk and Collins, 2004). Hence, it was not surprising that in this study the risk of FTPI was reduced when colostrum of a higher quality was provided to the calves.

Cumulative Score of Colostrum Management

Outcomes of this study revealed that, among the latter three factors discussed above (time after birth, amount and quality of the first colostrum meal), the quality of colostrum fed at the first meal was the most important one affecting the occurrence of FTPI in calves. However, in this study the good quality of colostrum did not ensure low percentages of calves with FTPI at the farm level (e.g. farms number 11, 12, and 16; Figures 2.a and 2.b), indicating that other factors, such as delivery time and amount of the first colostrum meal, were relevant for the successful immunization of the calves. Therefore, due to their

synergic action (Quigley and Drewry, 1998; Weaver et al., 2000; Godden, 2008), time of provision, amount, and quality of colostrum fed to the calves at the first meal should be considered not as single factors, but in combination. The SCM calculated in the present study was an expression of that combination, and it showed a wide range of values, each one representing a different solution for colostrum provision. The relationship between SCM and calf serum Ig concentration found in this study clearly demonstrated the importance of the synergic action of the three main factors (delivery time, amount and quality of the first colostrum meal) on transfer of passive immunity in calves. Particularly, the extreme values of the SCM emphasized that, combining a high quality colostrum with the best delivery practices, FTPI was completely avoided and most of calves reached OTPI, whereas the opposite happened when bad delivery practices were associated to a poor quality colostrum provision. The highest value of SCM obtained in this study suggested that optimal colostrum management practices, in order to maximize the transfer of passive immunity in calves, should include the administration of at least 2.5 L of high quality colostrum (Ig concentration >87.6 g/L) within 1.0 h after birth. Those indications were in line with the findings reported by Morin et al. (1997) and by Jaster (2005) in two studies addressed to evaluate, under experimental condition, the effects of quality, quantity and timing of colostrum feeding on Holstein and on Jersey calves, respectively. However, even the intermediate values of the SCM were of practical interest. In fact, considering that the same value of SCM could result from the sum of different single scores of the three main factors, the values of SCM that were near to the highest one represented different solutions of colostrum provision in which a high calf serum Ig concentration was obtained. The latter finding demonstrated that, in the dairy practice, a deficiency in one of the three main factors (delivery time, amount or quality of the first colostrum meal) should be compensated by adjusting the other two. Therefore, a further development of the SCM could be its on field application for the production of farm-specific recommendations to reduce the prevalence of FTPI.

CONCLUSIONS

Despite the widespread knowledge on importance of successful transfer of passive immunity in calves, the present study underlined the poor awareness of the farmers toward an effective management of newborn calves and of their colostrum provision. The

most important management factors that resulted to be associated with the occurrence of FTPI in calves were delivery time, amount, and quality of the first colostrum meal. The combined effect of those three factors resulted to be strictly related to calf serum Ig concentration. To prevent FTPI at all, calves should receive at least 2.5 L of high quality colostrum (Ig concentration >87.4 g/L) within 1.0 h after birth. A great effort is needed to increase the consciousness of dairy farmers about prevention of FTPI through the correct practices of colostrum management, in order to improve the health of both female and male calves and possibly to reduce the antimicrobial use in young stock rearing. The SCM created in this study could be developed in dairy practice as a tool to generate farm-specific indications for FTPI prevention.

ACKNOWLEDGEMENTS

This study was supported by the grants obtained from the Italian Ministry of Health for the research project (RC IZSVE 10/2011). The authors want to thank the “Associazione Veneta Allevatori - AVA” and the “Associazione Veterinari Buiatri ORUS” for their support to the data and samples collection.

REFERENCES

- Beam, A. L., J. E. Lombard, C. A. Koprak, L. P. Garber, A. L. Winter, J. A. Hicks, and J. L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *J. Dairy Sci.* 92:3973–3980. doi:10.3168/jds.2009-2225.
- Berge, A. C. B., P. Lindeque, D. A. Moore, and W. M. Sischo. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. *J. Dairy Sci.* 88:2166–2177. doi:10.3168/jds.S0022-0302(05)72892-7.
- Cavirani, S., S. Taddei, C. S. Cabassi, G. Donofrio, F. Toni, F. Ghidini, C. Piancastelli, E. Schiano, and C.F. Flammini. 2005. Deficit di IgG colostrali e di trasferimento di immunità passiva colostrale in allevamenti bovini da latte ad alta produzione. *Large Animal Review.* 11:17–21.
- Ceniti, C., F. Trimboli, G. Massimini, V. M. Morittu, T. Gigliotti, C. Cicino, and D. Britti. 2016. Valutazione della concentrazione delle immunoglobuline nel colostro ovino

mediante strumento di routine per l'elettroforesi capillare. *Large Animal Review*. 22:33–36.

- Chigerwe, M., J. W. Tyler, M. K. Summers, J. R. Middleton, L. G. Schultz, and D. W. Nagy. 2009. Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *J. Am. Vet. Med. Assoc.* 234:785–789. doi:10.2460/javma.234.6.785.
- Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev. Vet. Med.* 34:31–46. doi:10.1016/S0167-5877(97)00060-3.
- Furman-Fratczak, K., A. Rzasas, and T. Stefaniak. 2011. The influence of colostrum immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* 94:5536–5543. doi:10.3168/jds.2010-3253.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* 24:19–39. doi:10.1016/j.cvfa.2007.10.005.
- Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336–341. doi:10.3168/jds.2010-3170.
- Jaster, E. H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. *J. Dairy Sci.* 88:296–302. doi:10.3168/jds.S0022-0302(05)72687-4.
- Jayappa, H., R. Davis, L. Dierks, D. Sweeney, and T. Wasmoen. 2008. Demonstration of passive protection in neonatal calves against colibacillosis following immunization of pregnant heifers at 3 months of gestation. *Vet. Ther.* 9:283–289.
- Kohara, J., T. Hirai, K. Mori, H. Ishizaki, and H. Tsunemitsu. 1997. Enhancement of passive immunity with maternal vaccine against newborn calf diarrhea. *J. Vet. Med. Sci.* 59:1023–1025. doi:10.1292/jvms.59.1023.
- Lorenz, I., J. Fagan, and S. J. More. 2011. Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. *Ir. Vet. J.* 64:9. doi:10.1186/2046-0481-64-9.
- Maunsell, F., and G. A. Donovan. 2008. Biosecurity and risk management for dairy replacements. *Vet. Clin. North Am. Food Anim. Pract.* 24:155–190. doi:10.1016/j.cvfa.2007.10.007.

- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North Am. Food Anim. Pract.* 20:593–603. doi:10.1016/j.cvfa.2004.06.005.
- Mee, J. F. 2013. Why do so many calves die on modern dairy farms and what can we do about calf welfare in the future? *Animals.* 3:1036–1057. doi:10.3390/ani3041036.
- Morin, D. E., G. C. McCoy, and W. L. Hurley. 1997. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in Holstein bull calves. *J Dairy Sci.* 80:747–753. doi:10.3168/jds.S0022-0302(97)75994-0.
- Murray, C. F., and K. E. Leslie. 2013. Newborn calf vitality: risk factors, characteristics, assessment, resulting outcomes and strategies for improvement. *The Vet. J.* 198:322–328. doi:10.1016/j.tvjl.2013.06.007.
- Quigley, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. *J Dairy Sci.* 81:2779–2790. doi:10.3168/jds.S0022-0302(98)75836-9.
- Stott, G. H., and A. Fellah. 1983. Colostral immunoglobulin absorption linearly related to concentration for calves. *J Dairy Sci.* 66:1319–1328. doi:10.3168/jds.S0022-0302(83)81941-9.
- Swan, H., S. Godden, R. Bey, S. Wells, J. Fetrow, and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. *J. Dairy Sci.* 90:3857–3866. doi:10.3168/jds.2007-0152.
- Tóthová, C., O. Nagy, H. Seidel, and G. Kováč. 2013. Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. *Comp. Clin. Path.* 22:15–20. doi:10.1007/s00580-011-1363-8.
- Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. *J. Dairy Sci.* 91:3840–3849. doi:10.3168/jds.2007-0898.
- Tyler, J. W., D. D. Hancock, J. G. Thorne, C. C. Gay, and J. M. Gay. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. *J. Vet. Intern. Med.* 13:335–337.

- Uetake, K. 2013. Newborn calf welfare: a review focusing on mortality rates. *Anim. Sci. J.* 84:101–105. doi:10.1111/asj.12019.
- Vasseur, E., D. Pellerin, A. M. de Passillé, C. Winckler, B. J. Lensink, U. Knierim, and J. Rushen. 2012. Assessing the welfare of dairy calves: outcome-based measures of calf health versus input-based measures of the use of risky management practices. *Anim. Welf.* 21:77–86.
- Vasseur, E., J. Rushen, and A. M. de Passillé. 2009. Does a calf's motivation to ingest colostrum depend on time since birth, calf vigor, or provision of heat? *J. Dairy Sci.* 92:3915–3921. doi:10.3168/jds.2008-1823.
- Vogels, Z., G. M. Chuck, and J. M. Morton. 2013. Failure of transfer of passive immunity and agammaglobulinaemia in calves in south-west Victorian dairy herds: prevalence and risk factors. *Aust. Vet. J.* 91:150–158. doi:10.1111/avj.12025.
- Waldner, C. L., and L. B. Rosengren. 2009. Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. *Can. Vet. J.* 50:275–281.
- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:596–577. doi:10.1111/j.1939-1676.2000.tb02278.x.
- Wells, S. J., D. A. Dargatz, and S. L. Ott. 1996. Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Prev. Vet. Med.* 29:9–19. doi:10.1016/S0167-5877(96)01061-6.

CHAPTER 4

Dairy calves allowed to nurse colostrum from dams: effects on transfer of passive immunity, behavior and health



CHAPTER 4

Dairy calves allowed to nurse colostrum from dams: effects on transfer of passive immunity, behavior and health

I. Lora^{1,2}, F. Gottardo², L. Bonfanti³, A. L. Stefani³, E. Soranzo^{2,3}, B. Dall'Ava³, K. Capello³, M. Martini², and A. Barberio³

¹*PhD Course in Animal and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

²*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

³*Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD), Italy*

Submitted to Journal of Animal Science

ABSTRACT

In intensive dairy farming, the calf is usually separated from the dam immediately after birth and colostrum is hand-fed by the caregiver. In this study, three methods of colostrum provision were adopted and their effects on failure of transfer of passive immunity (**FTPI**) in calves, on calf-dam separation distress, and on health of both calves and dams were considered. In two dairy farms in Northeastern Italy, 107 calf-dam pairs were randomly assigned to one of the following methods of colostrum provision: “hand-fed method” (**HFM** - calf was separated from the dam right after birth and colostrum was provided by nipple-bottle, n = 50), “nursing method” (**NM** - calf nursed from the dam for the first 12 h of life without farmer assistance, n = 30), or “mixed method” (**MM** - nursing calf received a supplementary colostrum meal by nipple-bottle, n = 27). Serum of calves (from 1 to 5 d of age) and samples of their first colostrum meal were analyzed by electrophoresis to determine the immunoglobulin (**Ig**) concentration. Behavioral indicators of separation distress in the following 24 h (vocalizations of calves and dams, calf refusal of the first meal administered by nipple-bottle, and abnormal dam behavior at milking) were collected as binomial variables by farmer interviews. Health status of calves (disease and mortality occurrence) and dams (postpartum disorders and mastitis occurrence) were monitored for the first 3 mo of life and for 7 d after parturition,

respectively. The greatest percentage of calves with FTPI (serum Ig concentration <10.0 g/L) was found for the NM (60.0%) compared to both the MM (11.1%) and the HFM (22.0%) ($P < 0.05$). The main factors associated with FTPI within all the colostrum provision methods were parity of the dam and Ig concentration of colostrum ($P < 0.05$). The overall lowest separation distress was observed for the HFM, compared to both other colostrum provision methods ($P < 0.05$). The HFM had the greatest percentage of calf disease (64.0%), whereas the NM had the lowest one (33.3%) ($P < 0.05$). No effect of the colostrum provision method was observed on dam health or calf mortality ($P > 0.05$). Results from this study indicated that allowing calves to nurse colostrum from dams could be an effective practice of colostrum provision only when supported by farmer assistance. However, separation systems should be studied to minimize separation distress.

INTRODUCTION

The common practice of separating the calf from the dam as soon as possible after birth is adopted in intensive dairy farms essentially to facilitate the first care to the calf, to minimize calf-dam separation distress, and for biosecurity reasons (McGuirk and Collins, 2004; Maunsell and Donovan, 2008; Stěhulová et al., 2008). In this context, calves should be fed at least 4.0 L of good quality colostrum (i.e., with immunoglobulin - **Ig** - concentration ≥ 50.0 g/L) within 6 h of life to achieve a sufficient level of passive immunity for protection against infectious diseases (Weaver et al., 2000; Godden, 2008). Inadequate colostrum provision leads to failure of transfer of passive immunity (**FTPI**), a condition that is defined when Ig concentration in calf serum is <10.0 g/L at 48 h of life, and which has detrimental effects on calf health and survival (Tyler et al., 1999; Furman-Fratczak et al., 2011). The prevalence of FTPI in dairy calves is estimated to be high, ranging from 35% to 40% (Weaver et al., 2000; Cavarani et al., 2005; Vogels et al., 2013). Moreover, the European Food Safety Authority (2006) identified early separation of calves from dams as a main calf welfare issue, due to the deprivation of maternal care. Because calves allowed to nurse colostrum from their dams have a more efficient intestinal absorption of Ig (Kälber and Barth, 2014) and can suckle many times during the day (Lidfors, 1996), the practice of leaving the calf with the dam for the first hours of life could be helpful in improving the transfer of passive immunity in dairy calves while also complying with the demands of public opinion. Therefore, the aim of this study was

to investigate whether the practice of allowing calves to nurse colostrum from dams (with or without farmer assistance) could be effective in terms of successful transfer of passive immunity, considering also the potential effects on separation distress and on health of both calves and dams.

MATERIALS AND METHODS

Farm Description

All procedures adopted in the study were checked by the *Organismo preposto al benessere degli animali* (O.P.B.A.) of Padova University.

The study was carried out from October 2014 to April 2015 in two dairy farms located in Northeastern Italy (Veneto Region). One farm reared 77 Holstein cows; the other reared 105 Holstein cows. In both farms, the housing system was loose with cubicles, the feeding technique was total mixed ration distributed twice daily, and cows were milked twice daily at a milking parlour. Management of the cows at calving and the newborn calves was similar in both farms. Two days before the expected calving, cows were moved into a calving pen (total area of 14 m²) placed next to the milking parlour and bedded with straw added daily. Newborn calves were usually left in the calving pen with the dams for the first day of life and allowed to nurse colostrum freely. When possible, the farmers assisted nursing calves by offering them a supplementary colostrum meal by nipple-bottle within a few hours of birth.

Study Design

To conduct the study, newborn calves of each farm were alternately separated from the dams right after birth or left in the calving pen with their mothers for at least the first 12 h of life. In the first case, the farmers fed the calves their dams' colostrum by nipple-bottle; in the second case, the calves nursed colostrum freely from the dams. Additionally, according to their habits, farmers were allowed to administer alternately a supplementary meal of the own dams' colostrum to the calves left with their mothers. Therefore, three methods of colostrum provision were defined: the "hand-fed method" (**HFM**), when the calf was separated from the dam right after birth and fed its dam colostrum by nipple-bottle; the "nursing method" (**NM**), when the calf was left in the calving pen with the

dam for at least 12 h after birth and was not assisted by the farmer at all; and the “mixed method” (**MM**), when the calf was left in the calving pen with the dam for at least 12 h after birth and the farmer administered a supplementary colostrum meal by nipple-bottle within 6 h of life. Calves were included in the study irrespective of sex and genotype (Holstein purebred or Holstein-beef crossbred). During the study, the calving pen housed only one cow at a time, to guarantee a clean and quiet environment at parturition. Moreover, regardless of the colostrum provision method, the cows were routinely milked two times per day at the milking parlour starting from the day of parturition, whereas, after separation from the dams, the calves were ear tagged for individual identification and housed in single crates until 8 wk of age. After this period, calves were moved in straw bedded group pens that housed five pen mates. Milk was fed individually until weaning (9 to 11 wk of age), whereas water and solid feed (grass hay and commercial calf starter) were available starting from 7 d of age. All calves included in the study remained in the farm for at least the first 3 mo of life.

Sampling and Data Collection

During the study, the farms were visited twice per week to take blood and colostrum samples, to evaluate animal health, and to interview the farmers. To avoid further suffering and handling to the calves, the farms included in the study already adopted a voluntary plan for the control of bovine viral diarrhoea (**BVD**) that required blood sampling from newborn calves. The farm veterinarian responsible for the BVD control plan agreed to visit both farms twice a week together with the person in charge of conducting the study to take blood samples from calves between 1 and 5 d of age. Calf blood samples were therefore analyzed for both BVD virus and serum Ig concentration to assess the level of transfer of passive immunity. Blood was sampled from the jugular vein by a 10 mL Vacutainer® tube without anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA) and the samples were stored at 4°C until delivery to the laboratory. To evaluate the quality of colostrum (Ig concentration) provided to the calves, farmers were asked to collect a sample of the colostrum consumed at the first meal by each calf included in the study in a 100 mL tube and store it at –20°C. To obtain the colostrum samples for the calves left with their dams, farmers manually milked the cows within 3 h of parturition. Frozen colostrum samples were collected at every farm visit by the person in charge of

conducting the study and stored at -20°C until delivery to the laboratory. For each calf included in the study, farmers recorded, in a report form, data on calf sex and breed, dam parity, occurrence of dystocia at birth, type of calf management (separated vs left with the dam), and time and amount of the colostrum meals provided by nipple-bottle within 12 h of birth. Farmers were interviewed at every farm visit to gather information on behavioral indicators of separation distress in the 24 h after calf-dam separation. Two indicators were considered for the calves (vocalizations and refusal of the nipple-bottle) and two indicators were considered for the cows (vocalizations and behavior at milking). All these indicators were recorded as binomial variables (presence/absence) based on the following criteria: calf and cow vocalizations were considered present when the farmer reported abnormally frequent and loud vocalizations of the animals throughout the day after separation that clearly indicated that the dam was looking for the calf or vice-versa; refusal of the nipple-bottle was considered present when the calf refused to take at least the first meal after separation administered by nipple-bottle; abnormal behavior at milking was considered present when the cow was evidently nervous, when it kicked or detached the milking unit, or when it had poor or absent milk ejection during routine milking at the milking parlour after separation. Health data for each calf-dam pair included in the study were also collected. The cows were monitored for 7 d after calving for the occurrence of mastitis and postpartum disorders such as puerperal collapse and placenta retention, whereas the calves were monitored throughout the first 3 mo of life for the occurrence of disease (i.e., presence or absence, and type) and mortality (i.e., presence or absence, and cause). At each farm visit, in case of calf diarrhea (i.e., clinical manifestation of feces softer than normal) and if the calf was not treated yet, a fecal sample was collected by the veterinarian from calf rectal ampulla into a 100 mL tube and stored at 4°C until delivery to the laboratory for analysis of positivity to *Escherichia coli* K99, rotavirus, coronavirus, and *Cryptosporidium* spp.

Laboratory Analysis

At the laboratory, blood samples were centrifuged (within 2 h of collection) at $3076 \times g$ for 10 min at 20°C and serum was transferred into 2 mL tubes. Samples of serum, colostrum and feces were stored at -20°C until the day of analysis. Serum and colostrum Ig concentrations were determined using the method described by Tóthová et

al. (2013). Prior to performing analysis, blood serum samples were thawed in water-bath at 20°C, whereas colostrum samples were processed according to the method by Ceniti et al. (2016). After thawing in water-bath at 37°C, 4.5 mL of each colostrum sample was added with 40 µL of a commercial rennet solution (Naturen, CHR Hansen, Hoersholm, Denmark) and incubated at 37°C for 5 min to separate the albumin and globulin fractions from the casein fraction. A plastic stick was then used to disaggregate the clot, and each sample was centrifuged at $3076 \times g$ for 15 min at 15°C. The supernatant (colostrum whey) was collected and the initial total volume of extraction (4.5 mL) was restored by adding distilled water. Both blood serum and colostrum whey samples were first examined to determine the total protein concentration (g/L) using the biuret method on an automated analyzer (Cobas C501, Roche Diagnostics, Mannheim, Germany). A semi-automated agarose gel system (Hydrasys LC Sebia, Bagno a Ripoli, FI, Italy) associated with Phoresis software was then used to assess the protein fractions (%) of each sample as described by Tóthová et al. (2013). Finally, for each blood serum and colostrum whey sample, the total protein concentration (g/L) obtained by the biuret method was used to convert the percentage of Ig measured by electrophoretic analysis into absolute concentration (g/L).

Fecal samples were thawed at room temperature for analysis by ELISA test for antigens of *E. coli* K99, rotavirus, coronavirus, and *Cryptosporidium* spp. The “Rota-Corona-K99” kit and the “Cryptosporidium Ag test” kit (Idexx, Montpellier, France) were applied as specified by the manufacturer. Plates were then read by spectrophotometer at a wavelength of 450 nm, and the sample to positive (S/P) percentage was calculated for each well: the sample was considered positive to *E. coli* K99 if S/P $\geq 7\%$, to rotavirus if S/P $\geq 14\%$, to coronavirus if S/P $\geq 14\%$, and to *Cryptosporidium* spp. if S/P $\geq 20\%$.

Data and Statistical Analysis

A total of 108 calf-dam pairs were included in the study. Only one pair was discarded from the dataset due to the haemolysis of the calf blood sample. Of the remaining 107 calf-dam pairs, 50 belonged to the HFM, 30 to the NM, and 27 to the MM. Colostrum samples were missing for nine calves, five in the HFM, three in the MM, and one in the NM group. Three levels of transfer of passive immunity were defined based on

calf serum Ig concentration (Godden, 2008; Furman-Fratczak et al., 2011): <10.0 g/L (FTPI), 10.0 to 15.9 g/L (adequate transfer of passive immunity), and \geq 16.0 g/L (optimal transfer of passive immunity - **OTPI**). Colostrum was classified as being of poor quality when Ig concentration was <50.0 g/L (Godden, 2008).

Statistical analysis aimed at first excluding background differences (e.g., in calf sex and breed, parity of the dam, etc.) from among the three methods of colostrum provision, and was performed by Chi-square test and multiple comparisons. The effect of the method of colostrum provision on calf serum Ig concentration was tested by univariate analysis (PROC MIXED, SAS Institute Inc., Cary, NC) including the farm as random effect, and post hoc multiple comparisons between least squares means were performed by Bonferroni adjustment option. Chi-square test and multiple comparisons were then used to evaluate the differences among the three colostrum provision methods in the levels of transfer of passive immunity in calves, in behavioral indicators of separation distress, and in health of calves and dams. Additional effects of dam parity on the overall percentage of poor quality colostrum samples and of FTPI on the occurrence of calf disease and mortality were submitted to Chi-square test. The interaction between FTPI and the colostrum provision method was also evaluated for calf disease occurrence. Lastly, a study of the factors (e.g. sex, breed, dam parity, etc.) affecting FTPI within each colostrum provision method was made by Chi-square test, and the relative risk (**RR**) and 95% confidence interval were calculated for each factor with $P < 0.05$. Moreover, multiple comparisons of the percentage of calves with FTPI were made among methods of colostrum provision within each level of the factors considered. The threshold for significance was set at $P < 0.05$ for all the statistical analyses performed.

RESULTS

The characteristics of the calf-dam pairs assigned to each colostrum provision method are reported in Table 1. Calf sex and breed, dam parity, and colostrum quality (Ig concentration) were distributed equally among the three methods ($P > 0.05$). A difference was found only in the occurrence of dystocia: the greatest percentage of difficult calving was observed in the HFM, whereas no cases occurred in the MM ($P < 0.05$). As regards colostrum quality, Ig concentration ranged from 24.4 to 146.3 g/L, with a mean value of

68.0 g/L. Overall percentage of poor quality colostrum samples was 24.5%, and was greater for primiparous (41.4%) than for pluriparous (17.4%) cows ($P < 0.05$).

Table 1. Characteristics of the calf-dam pairs assigned to each method of colostrum provision

Characteristic	Levels	Overall calf-dam pairs		Calf-dam pairs by method of colostrum provision (%)		
		n	%	Hand-fed	Mixed	Nursing
Overall calf-dam pairs (n)		107	–	50	27	30
Overall calf-dam pairs (%)		–	100.0	46.7	25.2	28.0
Calf sex	Female	62	57.9	58.0	59.3	56.7
	Male	45	42.1	42.0	40.7	43.3
Calf breed	Holstein	56	52.3	52.0	44.4	60.0
	Crossbred	51	47.7	48.0	55.6	40.0
Dam parity	1	31	29.0	36.0	25.9	20.0
	>1	76	71.0	64.0	74.1	80.0
Dystocia	No	96	89.7	82.0	100.0	93.3
	Yes	11	10.3	18.0 ^a	0.0 ^b	6.7 ^{ab}
Colostrum Ig concentration ¹	≥50.0 g/L	74	75.5	77.8	83.3	65.5
	<50.0 g/L	24	24.5	22.2	16.7	34.5

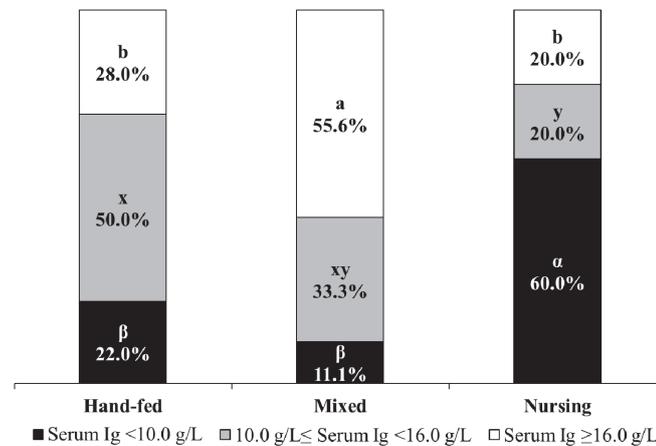
¹Colostrum analysis were available for 98 calves: 45 belonging to the hand-fed method (45.9%), 24 to the mixed method (24.5%), and 29 to the nursing method (29.6%).

^{a-b}Values within a row with different superscripts are different ($P < 0.05$).

Transfer of Passive Immunity

The method of colostrum provision affected considerably the transfer of passive immunity in calves ($P < 0.001$), with greater mean serum Ig concentration for calves of the HFM (13.9 g/L, ranging from 3.8 to 30.9 g/L) and of the MM (17.1 g/L, ranging from 4.6 to 42.2 g/L) than for calves of the NM (10.0 g/L, ranging from 1.6 to 25.2 g/L). The greatest percentage of calves with FTPI was found in the NM compared to both the MM and the HFM ($P < 0.05$), whereas the greatest fraction of calves with OTPI was observed in the MM (Figure 1). The HFM had the greatest percentage of calves with adequate transfer of passive immunity, and the NM had the lowest ($P < 0.05$) (Figure 1). Calves of the HFM received 1.9 ± 0.8 L (mean \pm SD) of colostrum within 6 h of life and the first meal was fed at 2.2 ± 0.1 h (mean \pm SD) of birth. Colostrum supplementation provided to the MM calves was 2.0 ± 0.8 L (mean \pm SD), with single administration at 1.4 ± 0.1 h (mean \pm SD) after birth.

Figure 1. Distribution of calves according to serum immunoglobulin (Ig) concentration and to the method of colostrum provision. Different letters for the same class of serum Ig concentration identify differences ($P < 0.05$) among methods of colostrum provision



Factors that affected FTPI occurrence in calves within each colostrum provision method are reported in Table 2. In the HFM, the amount of colostrum fed to calves within 6 h of life affected FTPI occurrence: calves fed less than 2.0 L of colostrum were more at risk of having FTPI than those that received at least 2.0 L ($P = 0.044$). Moreover, HFM and MM calves that were given poor quality colostrum (Ig concentration <50.0 g/L) were more at risk of having FTPI than those fed good quality colostrum ($P < 0.001$ and $P = 0.013$, respectively). Regardless of the method of colostrum provision, calves born from primiparous cows were more at risk of having FTPI than those born from pluriparous cows ($P < 0.001$, $P = 0.015$, and $P = 0.025$ for HFM, MM, and NM, respectively). Lastly, the percentage of FTPI for calves fed good quality colostrum (Ig concentration ≥ 50.0 g/L), female calves, Holstein purebred calves, calves born from both primiparous and pluriparous cows, and calves that did not experience dystocia at birth was greater in NM than in both HFM and MM ($P < 0.05$). Considering male calves, the percentage of FTPI was greater in both the NM and the HFM than in the MM, whereas the greatest percentage of FTPI in crossbred calves was found in the NM, with an intermediate value in the HFM ($P < 0.05$) (Table 2).

Table 2. Relative risk (RR) and 95% CI of factors affecting failure of transfer of passive immunity (FTPI) in calves (calf serum immunoglobulin - Ig - concentration <10.0 g/L) within method of colostrum provision. Differences in FTPI occurrence for the same factor and among methods of colostrum provision are reported too.

Factor	Levels	Method of colostrum provision								
		Hand-fed (n = 50 calves)			Mixed (n = 27 calves)			Nursing (n = 30 calves)		
		FTPI (%)	RR	95% CI	FTPI (%)	RR	95% CI	FTPI (%)	RR	95% CI
Overall (%)		22.0 ^b			11.1 ^b			60.0 ^a		
Time of the first colostrum meal	≤6 h from birth	21.3			11.1	— ²		—		
	>6 h from birth	33.3			—			—		
Amount of colostrum fed within 6 h of life	≥2.0 L	11.1	*	1.00	—	11.8		—		
	<2.0 L	34.8		3.13	0.94–10.44	10.0		—		
Colostrum Ig concentration ¹	≥50.0 g/L	11.4 ^b	***	1.00	—	5.0 ^b	*	1.00	—	47.4 ^a
	<50.0 g/L	70.0		6.13	2.24–16.78	50.0		10.00	1.17–85.60	80.0
Calf sex	Female	20.7 ^b				18.8 ^b				64.7 ^a
	Male	23.8 ^a				0.0 ^{b3}				53.8 ^a
Calf breed	Holstein	26.9 ^b				25.0 ^b				66.7 ^a
	Crossbred	16.7 ^{ab}				0.0 ^{b3}				50.0 ^a
Dam parity	>1	3.1 ^b	***	1.00	—	0.0 ^{b3}	*	1.00	—	50.0 ^a
	1	55.6 ^b		17.78	2.47–127.85	42.9 ^b		8.57	1.06–69.52	100.0 ^a
Dystocia at birth	No	17.1 ^b				11.1 ^b	— ²			57.1 ^a
	Yes	44.4				—				100.0

^{a-b}Values within a row with different superscripts are different ($P < 0.05$).

¹Colostrum analysis were missing for five calves belonging to the hand-fed method (overall calves with FTPI = 24.4%), for three calves belonging to the mixed method (overall calves with FTPI = 12.5%), and for one calf of the nursing method (overall calves with FTPI = 58.6%).

²The test was not applicable.

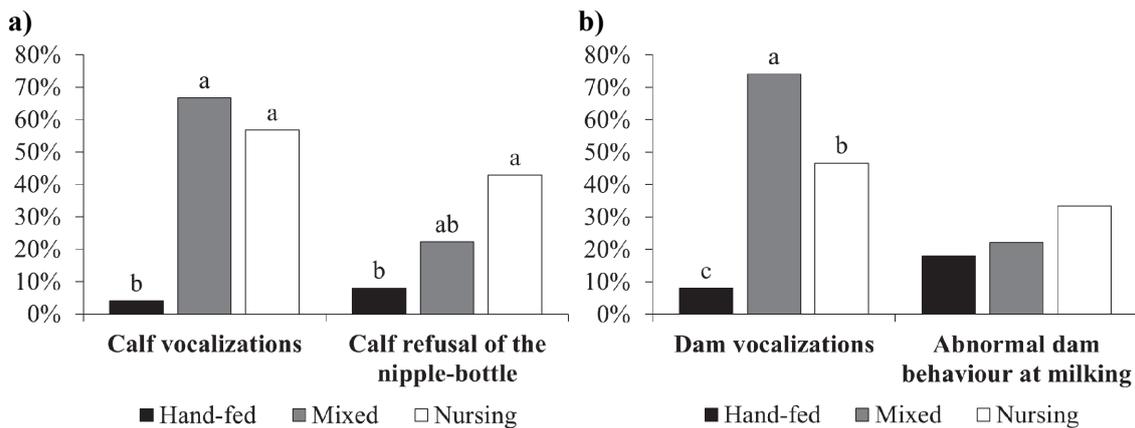
³one entry was moved in the “case and not-exposed” category in order to perform the test.

* $P < 0.05$, *** $P < 0.001$.

Calf-Dam Separation Distress

The influence of the colostrum provision method on behavioral indicators of separation distress is reported in Figures 2.a and 2.b. Both for calves and dams, the lowest percentages of vocalization events were observed in the HFM (4.0% for calves and 8.0% for dams) compared to the MM (66.7% for calves and 74.1% for dams) and the NM (56.7% for calves and 46.7% for dams) ($P < 0.001$). Refusal of the nipple-bottle also differed among the methods of colostrum provision ($P = 0.001$), with the maximum percentage of events observed in the NM (42.9%), an intermediate value recorded in the MM (22.2%), and the minimum in the HFM (8.0%). Dam behavior at milking was not affected by the method of colostrum provision ($P = 0.288$).

Figure 2. Effect of the method of colostrum provision on calf (a) and dam (b) behavior after separation. Different letters for the same behavioral indicator identify differences ($P < 0.05$) among methods of colostrum provision



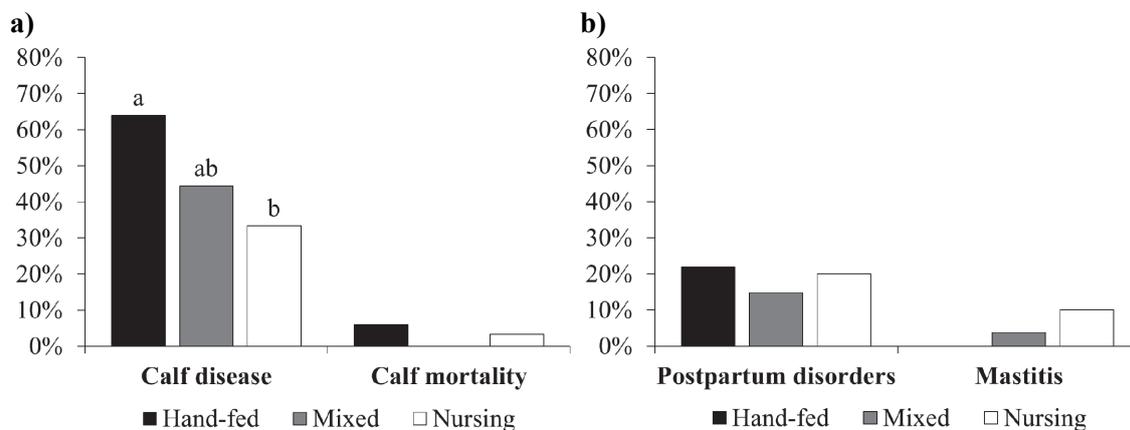
Calf and Dam Health

During the study, 50.5% of the calves suffered from disease, 94.4% from enteric and 5.6% from respiratory disease. All except one case of disease were recorded within 30 d of age, with the greatest percentage of disease occurrence observed within 8 d of life (46.3%). Fecal samples were collected from 78.4% of the calves with diarrhea: 47.5% were positive to *Cryptosporidium* spp., 15.0% to rotavirus, 7.5% to both *Cryptosporidium* spp. and rotavirus, and 30.0% were negative to all the pathogens. None of the fecal samples showed a positivity to *E. coli* K99 or to coronavirus. Four calves (3.7%) died during the study, all within 15 d of life and due to enteric disease. The occurrence of calf disease was affected by colostrum provision method ($P < 0.05$) (Figure 3.a), with the greatest percentage of ill calves observed for the HFM (64.0%), an intermediate value for

the MM (44.4%), and the lowest percentage for the NM (33.3%). The overall occurrence of calf disease was not affected by the FTPI condition ($P = 0.719$); however, a greater risk of disease occurrence for calves with FTPI was observed within the HFM (RR = 1.08; 95% CI = 0.72–1.60; $P = 0.035$). On the contrary, although calf mortality was unaffected by the method of colostrum provision ($P = 0.412$) (Figure 3.a), it was affected by the FTPI condition, which was believed to be linked to a greater risk of mortality (RR = 7.03; 95% CI = 0.76–65.07; $P = 0.045$).

Regarding dams, 19.6% showed postpartum disorders within 7 d after parturition (of which 19.0% were puerperal collapse and 81.0% were placenta retention), whereas 3.7% showed signs of mastitis. The colostrum provision method did not influence the occurrence of postpartum disorders ($P = 0.749$) or the occurrence of mastitis within 7 d after parturition ($P = 0.074$) (Figure 3.b). However, no cows in the HFM had mastitis.

Figure 3. Effect of the method of colostrum provision on calf health within 3 mo of life (a) and on dam health within 7 d after parturition (b). Different letters for the same health parameter identify differences ($P < 0.05$) among methods of colostrum provision



DISCUSSION

Together with rearing environment, colostrum management is one of the factors that most affects the occurrence of calf disease and mortality (Svensson et al., 2003; Maunsell and Donovan, 2008; Furman-Fratczak et al., 2011). The prevalence of FTPI in calves remains an issue in dairy farming, and in addition to colostrum quality, the main risk factors are known to be the time at feeding and the amount of colostrum fed (Weaver et al., 2000; Godden, 2008). Enabling dairy calves to nurse colostrum from dams in the first hours of life could be a useful practice in improving the transfer of passive immunity,

even if potential effects on health and welfare of both calves and dams should be considered as well. In this study, the practice of colostrum provision most commonly adopted by dairy farmers (HFM) was compared to a nearly natural condition (NM) and to a combined situation between the previous two (MM).

Except for the number of dystocia cases, the lack of differences among the characteristics of the calf-dam pairs assigned to each colostrum provision method allowed the results of this study to be considered unaffected by variability in sample features.

Transfer of Passive Immunity

The method of colostrum provision affected substantially the transfer of passive immunity and, although the percentage of calves with OTPI was not as great as in the MM, the HFM was shown to be effective in ensuring a successful transfer of passive immunity to calves. According to literature (Morin et al., 1997; Jaster, 2005; Godden, 2008), it is important that hand-fed calves receive more than 2.0 L of good quality colostrum within 6 h of life for optimized intestinal absorption of Ig and the avoidance of FTPI. The effect of dam parity on the transfer of passive immunity in hand-fed calves was probably due to the quality of colostrum: as expected from literature (Weaver et al., 2000; Morin et al., 2001; Gulliksen et al., 2008), we found overall colostrum of primiparous cows to be of poorer quality than that of pluriparous cows. The fairly high percentage of FTPI observed among male and crossbred calves in the HFM than in the other colostrum provision methods may be attributed to the relatively higher requirements of Ig typical of these kinds of calf because they are larger in size than female and Holstein calves (Quigley and Drewry, 1998; Vogels et al., 2013), a requirement that was probably not satisfied by the limited amount of colostrum provided by nipple-bottle. However, the hypothesis that farmers paid less attention to accurate colostrum administration with these categories of calf, which are not replacement animals and therefore are destined for sale within a few weeks of life, cannot be excluded. Although the greatest percentage of cases of dystocia was found in the HFM, this factor did not appear to be responsible for FTPI in hand-fed calves. Dystocia has been associated with an increased risk of FTPI in calves, due to their poor vitality and to the postnatal acidosis that frequently occurs in such cases (Godden, 2008; Murray and Leslie, 2013). In this study, it was likely that the immediate

calf assistance after birth given by the farmer (typical of the HFM) and correct colostrum administration might have compensated for potentially poor calf vitality.

The NM showed the greatest percentage of calves with FTPI, regardless of colostrum quality, calf sex and breed, dam parity and dystocia occurrence, and the lowest percentage of calves that reached the OTPI. Similar findings about suckling calves were reported by Besser et al. (1991), Rajala and Castrén (1995) and Filteau et al. (2003), likely due to the fact that many calves were unable to spontaneously consume enough colostrum within 6 h of life. Under natural conditions, a calf attempts to stand within 30 min of birth and reaches the udder within 90 min, managing to suckle by 2 h (McGuirk and Collins, 2004). Calf vitality is therefore crucial in finding the teat, and affects both the time after birth of first suckling and the amount of colostrum ingested (Lidfors, 1996; Furman-Fratczak et al., 2011). Particularly, Rajala and Castrén (1995) found that a delay of 30 min in first suckling lead to a 2.0 g/L decrease in serum Ig concentration. Furthermore, the scarce mothering instinct and poor udder conformation for the nursing purposes of high-producing cows could pose additional obstacles to calves in successfully reaching the teats (Brignole and Stott, 1980; Kälber and Barth, 2014). The mothering instinct and the udder conformation tend to vary also with dam parity (Lidfors, 1996; Flower and Weary, 2001; Kälber and Barth, 2014). According to that, the greater risk for calves born from primiparous cows of having FTPI than those born from pluriparous dams in the NM was probably more attributable to the less developed mothering instinct and inexperience of younger cows, which may have prevented calves to suckle successfully, than to udder conformation, which instead should be more favorable in younger cows. Unlike as in other colostrum provision methods, colostrum quality was not associated with FTPI occurrence in the NM, and it is likely therefore that the factor affecting FTPI most in the NM was the amount of colostrum consumed by the calf within 6 h of birth, even if this was not measured in the present study.

A strategy to overcome the limits of the NM could be farmer assistance to suckling calves, as has been done with the MM. The feeding of a supplementary colostrum meal to suckling calves within few hours of birth, in fact, was shown to be an effective practice in maximizing the transfer of passive immunity. The MM was not only the method with the lowest percentage of calves with FTPI (even disregarding the different factors considered in this study), but it was also the one with the greatest percentage of calves

that reached the OTPI (more than 50%). Similar findings were reported by Petrie (1984) in calves given early assistance in suckling colostrum to satiation. It was evident that providing a supplementary colostrum meal was vitally important in avoiding FTPI in calves that would not have successfully suckled within a few hours after birth. Moreover, it could be hypothesized that, having more energy to spend for the purpose, calves that suckled from the nipple-bottle were more motivated to search for the udder afterward. The MM therefore combined the advantages of the HFM and of the NM: calves were aided by the farmer immediately after birth and the provision of the first colostrum meal was ensured; additionally, calves could suckle more often during the day (even nine times per day) (Lidfors, 1996; Jensen, 2011), managing to consume a larger amount of colostrum. They may also have greater intestinal Ig absorption efficiency, which is typical of the nursing calves (Quigley et al., 1995; Kälber and Barth, 2014). Quality of colostrum consumed by the calves and parity of the dam were the factors that most affected the occurrence of FTPI in the MM. Consistent with findings by Petrie (1984), thanks to farmer assistance, nearly all the calves would have consumed enough colostrum within 6 h of birth, signifying that the remaining discriminating factor for FTPI occurrence was the quality of colostrum ingested. In that case, dam parity probably had a dual effect, influencing both colostrum quality and mothering behavior, as seen before for the HFM and the NM, respectively.

Calf-Dam Separation Distress

Regarding separation distress, the HFM showed the lowest negative effects on the behavior of both calves and dams, whereas the MM and the NM both increased such distress. These results were not surprising, given that the cow-calf bond is made soon after birth, and 5 min of contact seems to be enough for its establishment (Hudson and Mullord, 1977). Moreover, previous studies have reported that the longer the calf stayed with the dam, the greater separation distress was seen to be (Lidfors, 1996; Weary and Chua, 2000; Stěhulová et al., 2008). Therefore, calf-dam pairs assigned to the HFM showed less signs of separation distress likely due to the lower strength of their cow-calf bond. Furthermore, nearly half the calves in the NM refused at least the first meal provided by nipple-bottle after the separation from the dam. That kind of behavior could be an issue in terms of both animal welfare and dairy practice. However, results from the

current study suggested that offering nursing calves a supplementary colostrum meal by nipple-bottle, as in the MM, could reduce the occurrence of such behavior to the level observed for HFM calves. None of the three colostrum provision methods affected dam behavior at milking. The latter finding suggests that, regardless of the method adopted, routinely milking the dams from the day of parturition might be an effective practice in avoiding undesirable behavior at milking following calf-dam separation.

Calf and Dam Health

As expected from results reported in literature (Wells et al., 1996; Svensson et al., 2003; Windeyer et al., 2014), the occurrence of disease in sampled calves was concentrated within the first month of life. However, the overall percentage of calves that fell ill in the current study was greater than the 23% prevalence reported by Svensson et al. (2003) and by Windeyer et al. (2014). On the other hand, only few calves died, and unlike disease occurrence, calf mortality was not associated with the method of colostrum provision. The percentage of calves that fell ill, mainly of enteric disease, was particularly high in the HFM. Although it is true that FTPI was a predisposing factor for disease occurrence in HFM, a result that was in line with findings by Maunsell and Donovan (2008) and Furman-Fratczak et al. (2011), such a great percentage of disease was somehow unexpected considering the overall good level of passive immunity reached by the calves that were hand-fed colostrum. Moreover, calf disease prevention is actually one of the main reasons why calves are separated from dams soon after birth, because the practice of leaving them in the calving pen was commonly associated with increased risk of diarrhea (McGuirk and Collins, 2004; Maunsell and Donovan, 2008). Considering that FTPI affected overall calf mortality but not overall disease occurrence, it was likely that other factors besides the level of passive immunity acted as predisposing for infections in calves, such as cleanliness of crates and hygiene of equipment for colostrum and milk provision (Svensson et al., 2003; Maunsell and Donovan, 2008). Furthermore, most of cases of diarrhea in this study were caused by *Cryptosporidium* spp., against which an adequate level of passive immunity in calves has not yet been demonstrated to provide effective protection (Trotz-Williams et al., 2007), whereas other studies found no association between FTPI and disease occurrence in calves (Rajala and Castrén, 1995; Filteau et al., 2003; Trotz-Williams et al., 2007). Further investigations are therefore

required to identify the management factors associated with the great percentage of disease occurrence found in this study.

Regarding dam health, the lack of differences among methods of colostrum provision suggests that the presence of the calf and the frequent suckling typical of both the MM and the NM did not have positive effects on it, such as for example lower percentage of placenta retention due to the frequent stimulation of oxytocin release, but neither negative effects were observed, such as mastitis due to the frequent opening of the teat channels. In terms of dam health, farmers should feel free to choose any of the three methods of colostrum provision.

CONCLUSIONS

Results of this study indicated that, to maximize the transfer of passive immunity in newborn calves and to minimize calf-dam separation distress, the best method of colostrum provision is to separate the calf from the dam immediately after birth (before the cow-calf bond is made) and to scrupulously follow the rules for effective colostrum provision (4.0 L of good quality colostrum administered within 6 h of life). To avoid health problems in calves however, the method must be associated with proper calf rearing environment management. Allowing the calf to nurse colostrum from the dam for at least the first 12 h of life is the most effective practice in maximizing the transfer of passive immunity only when supported by farmer assistance: a supplementary colostrum meal should be offered to the calves by nipple-bottle within 6 h of birth. However, this method of colostrum provision seemed to augment calf-dam separation distress, and therefore a separation system should be developed. Lastly, it seemed that the NM should be avoided, due to its negative effects on both the transfer of passive immunity and the calf-dam separation distress.

ACKNOWLEDGMENTS

This study was supported by the grants obtained from the Italian Ministry of Health for the research project (RC IZSVE 10/2011).

REFERENCES

- Besser, T. E., C. C. Gay, and L. Pritchett. 1991. Comparison of three methods of feeding colostrum to dairy calves. *J. Am. Vet. Med. Assoc.* 198:419–22.
- Brignole, T. J., and G. H. Stott. 1980. Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. *J. Dairy Sci.* 63:451–456. doi:10.3168/jds.S0022-0302(80)82952-3.
- Cavirani, S., S. Taddei, C. S. Cabassi, G. Donofrio, F. Toni, F. Ghidini, C. Piancastelli, E. Schiano, and C.F. Flammini. 2005. Deficit di IgG colostrali e di trasferimento di immunità passiva colostrale in allevamenti bovini da latte ad alta produzione. *Large Animals Review.* 11:17–21.
- Ceniti, C., F. Trimboli, G. Massimini, V. M. Morittu, T. Gigliotti, C. Cicino, and D. Britti. 2016. Valutazione della concentrazione delle immunoglobuline nel colostro ovino mediante strumento di routine per l'elettroforesi capillare. *Large Animal Review.* 22:33–36.
- European Food Safety Authority (EFSA). 2006. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on the risks of poor welfare in intensive calf farming systems. An update of the Scientific Veterinary Committee Report on the Welfare of Calves. EFSA-Q-2005-014. *The EFSA Journal.* 366:1–36. doi:10.2903/j.efsa.2006.366.
- Filteau, V., É. Bouchard, G. Fecteau, L. Dutil, and D. DuTremblay. 2003. Health status and risk factors associated with failure of passive transfer of immunity in newborn beef calves in Québec. *Can. Vet. J.* 44:907–913.
- Flower, F. C., and D. M. Weary. 2001. Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. *Appl. Anim. Behav. Sci.* 70:275–284. doi:10.1016/S0168-1591(00)00164-7.
- Furman-Fratczak, K., A. Rzasa, and T. Stefaniak. 2011. The influence of colostrum immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* 94:5536–5543. doi:10.3168/jds.2010-3253.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North. Am. Food. Anim. Pract.* 24:19–39. doi:10.1016/j.cvfa.2007.10.005.

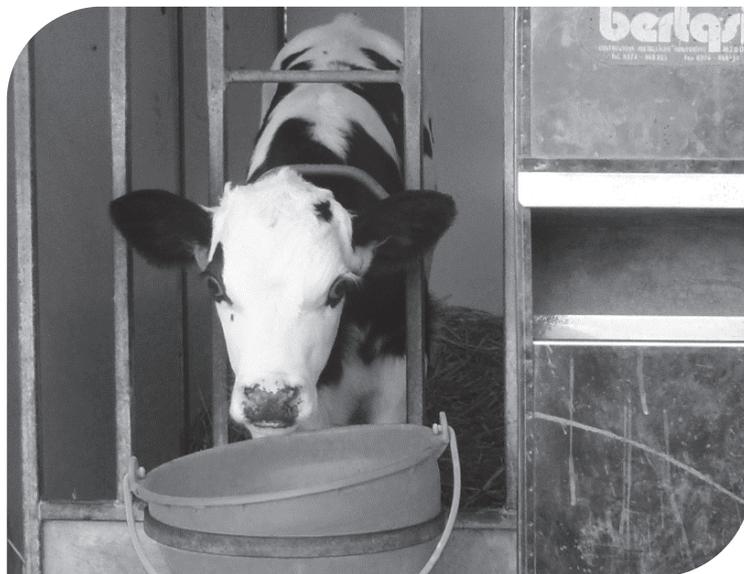
- Gulliksen, S. M., K. I. Lie, L. Sølvørød, and O. Østerås. 2008. Risk factors associated with colostrum quality in Norwegian dairy cows. *J. Dairy Sci.* 91:704–712. doi:10.3168/jds.2007-0450.
- Hudson, S. J., and M. M. Mullord. 1977. Investigations of maternal bonding in dairy cattle. *Appl. Anim. Behav. Sci.* 3:271–276. doi:10.1016/0304-3762(77)90008-6.
- Jaster, E. H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. *J. Dairy Sci.* 88:296–302. doi:10.3168/jds.S0022-0302(05)72687-4.
- Jensen, M. B. 2011. The early behaviour of cow and calf in an individual calving pen. *Appl. Anim. Behav. Sci.* 134:92–99. doi:10.1016/j.applanim.2011.06.017.
- Kälber, T., and K. Barth. 2014. Practical implications of suckling systems for dairy calves in organic production systems - a review. *Appl. Agric. Forestry Res.* 64:45–58. doi:10.3220/LBF_2014_45-58.
- Lidfors, L. M. 1996. Behavioural effects of separating the dairy calf immediately or 4 days post-partum. *Appl. Anim. Behav. Sci.* 49:269–283. doi:10.1016/0168-1591(96)01053-2.
- Maunsell, F., and G. A. Donovan. 2008. Biosecurity and Risk Management for Dairy Replacements. *Vet. Clin. North. Am. Food. Anim. Pract.* 24:155–190. doi:10.1016/j.cvfa.2007.10.007.
- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North. Am. Food. Anim. Pract.* 20:593–603. doi:10.1016/j.cvfa.2004.06.005.
- Morin, D. E., G. C. McCoy, and W. L. Hurley. 1997. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in Holstein bull calves. *J Dairy Sci.* 80:747–753. doi:10.3168/jds.S0022-0302(97)75994-0.
- Morin, D. E., P. D. Constable, F. P. Maunsell, and G. C. McCoy. 2001. Factors associated with colostral specific gravity in dairy cows. *J. Dairy Sci.* 84:937–943. doi:10.3168/jds.S0022-0302(01)74551-1.
- Murray, C. F., and K. E. Leslie. 2013. Newborn calf vitality: Risk factors, characteristics, assessment, resulting outcomes and strategies for improvement. *Vet. J.* 198:322–328. doi:10.1016/j.tvjl.2013.06.007.

- Petrie, L. 1984. Maximising the absorption of colostral immunoglobulins in the newborn dairy calf. *Vet. Rec.* 114:157–163. doi:10.1136/vr.114.7.157.
- Quigley, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. *J. Dairy Sci.* 81:2779–2790. doi:10.3168/jds.S0022-0302(98)75836-9.
- Quigley, J. D., K. R. Martin, D. A. Bemis, L. N. D. Potgieter, C. R. Reinemeyer, B. W. Rohrbach, H. H. Dowlen, and K. C. Lamar. 1995. Effects of housing and colostrum feeding on serum immunoglobulins, growth, and fecal scores of Jersey calves. *J. Dairy Sci.* 78:893–901. doi:10.3168/jds.S0022-0302(95)76703-0.
- Rajala, P., and H. Castrén. 1995. Serum immunoglobulin concentrations and health of dairy calves in two management systems from birth to 12 weeks of age. *J. Dairy Sci.* 78:2737–2744. doi:10.3168/jds.S0022-0302(95)76904-1.
- Stěhulová, I., L. Lidfors, and M. Špínka. 2008. Response of dairy cows and calves to early separation: effect of calf age and visual and auditory contact after separation. *Appl. Anim. Behav. Sci.* 110:144–165. doi:10.1016/j.applanim.2007.03.028.
- Svensson, C., K. Lundborg, U. Emanuelson, and S.-O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Prev. Vet. Med.* 58:179–197. doi:10.1016/S0167-5877(03)00046-1.
- Tóthová, C., O. Nagy, H. Seidel, and G. Kováč. 2013. Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. *Comp. Clin. Path.* 22:15–20. doi:10.1007/s00580-011-1363-8.
- Trotz-Williams, L. A., S. W. Martin, K. E. Leslie, T. Duffield, D. V. Nycham, and A. S. Peregrine. 2007. Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Prev. Vet. Med.* 82:12–28. doi:10.1016/j.prevetmed.2007.05.003.
- Tyler, J. W., D. D. Hancock, J. G. Thorne, C. C. Gay, and J. M. Gay. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. *J. Vet. Intern. Med.* 13:335–337.
- Vogels, Z., G. M. Chuck, and J. M. Morton. 2013. Failure of transfer of passive immunity and agammaglobulinaemia in calves in south-west Victorian dairy herds: prevalence and risk factors. *Aust. Vet. J.* 91:150–158. doi:10.1111/avj.12025.

- Weary, D. M., and B. Chua. 2000. Effects of early separation on the dairy cow and calf 1. Separation at 6 h, 1 day and 4 days after birth. *Appl. Anim. Behav. Sci.* 69:177–188. doi:10.1016/S0168-1591(00)00128-3.
- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive Transfer of Colostral Immunoglobulins in Calves. *J. Vet. Intern. Med.* 14:596–577. doi:10.1111/j.1939-1676.2000.tb02278.x.
- Wells, S. J., L. P. Garber, and G. W. Hill. 1996. Health status of preweaned dairy heifers in the United States. *Prev. Vet. Med.* 29:185–199. doi:10.1016/S0167-5877(96)01078-1.
- Windeyer M. C., K. E. Leslie, S. M. Godden, D. C. Hodgins, K. D. Lissemore, and S. J. LeBlanc. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med.* 113:231–240. doi:10.1016/j.prevetmed.2013.10.019.

CHAPTER 5

Passive immunity and health in dairy calves fed maternal colostrum or a commercial colostrum supplement



CHAPTER 5

Passive immunity and health in dairy calves fed maternal colostrum or a commercial colostrum supplement

I. Lora^{1,2}, F. Gottardo², B. Contiero², A. L. Stefani³, and A. Barberio³

¹*PhD Course in Animal and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

²*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

³*Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD), Italy*

ABSTRACT

The use of commercial colostrum supplements (**CS**) or replacers is becoming increasingly common in dairy farming, due to the high prevalence of failure of transfer of passive immunity (**FTPI**) in dairy calves. However, the actual efficacy of such commercial products is often not proved. The aim of this study was to evaluate on field the effectiveness of a commercial CS in prevention of FTPI and reduction of calf disease and mortality occurrence. The study was carried out between 2015 and 2016 in two Italian dairy farms with a high prevalence of FTPI (50.0%). Newborn female Holstein calves were alternatively fed maternal colostrum (C group, n = 35) or maternal colostrum and a single dose of CS (T group, n = 35). Maternal colostrum was provided according to the farmers' habits, and CS was administered within 6 h of birth according to the label instructions. Blood sampling was performed on each calf between 1 and 5 d of age, and a sample of the first colostrum meal was collected too. Individual data about calves and colostrum management were recorded, and calves were monitored during the first month of life for disease and mortality occurrence. Blood and colostrum samples were analyzed by the same electrophoretic method in order to assess the immunoglobulin (**Ig**) concentration. The statistical analysis aimed at first excluding baseline differences between C and T groups. The effect of the CS administration on calf serum Ig concentration was then investigated considering also the effect of the individual calf colostrum management (delivery time, amount and quality the first colostrum meal), which was expressed as a score assigned to each calf. No differences were found in calf

serum Ig concentration between T and C groups, nor in the percentage of calves with FTPI ($P > 0.05$). Percentage of calf disease, age at disease onset, days of illness, percentage of diseased calves treated with antibiotics, and mortality rate were also similar for both the treatment groups ($P < 0.05$). The CS tested in this study failed to prevent FTPI in calves and to reduce calf disease and mortality occurrence.

INTRODUCTION

Failure of transfer of passive immunity (**FTPI**) in calves is usually defined when calf serum immunoglobulin (**Ig**) concentration is lower than 10.0 g/L at 48 h of age (Godden, 2008). This condition is determined by improper colostrum provision, which lead to a poor transfer of maternal Ig to the calf, and which is associated to an increased risk of calf morbidity and mortality (Donovan et al., 1998; Berge et al., 2005; Furman-Fratczak et al., 2011). To avoid FTPI, the newborn calf should consume at least 4.0 L of good quality colostrum (i.e., with Ig concentration ≥ 50.0 g/L) within 6 h of birth (Weaver et al., 2000; McGuirk and Collins, 2004; Godden, 2008). Despite the wide knowledge on risk factors associated with FTPI and the proposal of several practical indications to dairy producers, FTPI still remains a main issue of young stock rearing in dairy farming, with prevalences ranging from 20.0% to 40.0% (Jaster, 2005; Beam et al., 2009; Vogels et al., 2013). In dairy practice, the administration of a commercial colostrum supplement (**CS**) or replacer (**CR**) soon after birth could help in achieving the successful transfer of passive immunity in calves. Colostrum supplements and replacers are usually colostrum- or plasma-derived products that are characterized by a high Ig content aiming at the prevention of FTPI. Colostrum replacers are formulated to completely replace maternal colostrum (e.g. when it is not available at all or it is not suitable for biosecurity reasons), so their nutrient composition is similar to that of bovine colostrum, and the Ig contents is at least of 100 g/dose (Quigley, 2002; Swan et al., 2007). On the other hand, CS are generally added with particular nutrients (e.g. Vitamin E), but they usually provide less than 100 g of Ig/dose because their purpose is to offer an extra source of Ig in addition to maternal colostrum (e.g. in case of colostrum of poor quality or available in a small amount) (Quigley, 2002). The variety of CS and CR available in trade is wide, but it appears from literature that some of them could not be effective in FTPI prevention when administered according to the label instructions (Santoro et al., 2004; Smith and Foster,

2007; Fidler et al., 2011). Particularly, the effectiveness of CS in the dairy practice could be influenced by several external factors, such as variability in maternal colostrum characteristics and in calf management. The aim of this study was to test, on field and under condition of high FTPI prevalence, the effectiveness of a commercial CS in FTPI prevention and, consequently, in reduction of neonatal calf disease occurrence.

MATERIALS AND METHODS

The study was carried out from January to April 2015 and from October 2015 to April 2016 in two Italian dairy farms. The selected farms reared 93 and 123 Italian Holstein cows on loose housing system, and were characterized by the same high prevalence of FTPI in newborn calves (50.0%), as found in a previous study addressed to evaluate the prevalence of FTPI in a group of farms in which they were included (Lora et al., under revision). The newborn calf management was similar in both farms: calves were born in a straw-bedded calving pen that housed no more than three cows at a time, and they were separated from dams immediately after birth. After navel disinfection, calves were provided their own dam colostrum by nipple-bottle, and were housed individually until 8 wk of age.

The CS tested in this study was a colostrum whey concentrate obtained from selected and controlled herds, free from infectious bovine rhinotracheitis, tuberculosis, and brucellosis, and vaccinated against calf neonatal diarrhea, as declared by the producer company. The CS was in ready-to-use form: it was liquid and packed in single dose bottle with bayonet cap that allowed the individual and hygienic administration to calves. A single dose (100 mL) of the CS contained 12.0 g of Ig. To conduct the study, the CS was provided to the farmers who were trained about the proper product storage (between 4°C and 20°C) and administration. Only female Holstein purebred calves that were separated from dams within 15 min of birth were included in the study, and they were alternately assigned to the control (**C**) or to the treated (**T**) group (n = 35 calves per each group). Newborn calves of both treatment groups (C and T) were fed colostrum of their dams according to the own farm practices. In addition to that, farmers provided calves of the T group with a single dose of CS within 6 h of birth, as indicated by the CS label instructions. For each calf included in the study, data about day and time of birth, time of CS provision (only for calves of the T group), and time and amount of the first colostrum

feeding were registered by the farmers in a report form. Moreover, farmers collected into a 100 mL tube and stored at -20°C a sample of the first colostrum meal given to each calf. Because both farms adopted a voluntary plan for the control of bovine viral diarrhea (BVD), the farm veterinarian routinely collected blood samples from calves between 1 and 5 d of age to identify persistently infected animals. Therefore, to avoid further calf manipulation and distress, calf serum Ig concentration was assessed on blood samples collected for the BVD control plan. Blood was withdrawn from calf jugular vein by a 10 mL Vacutainer® tube without anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). The person in charge to conduct the study visited the farms two times per week to take colostrum samples, gather information recorded by the farmers, and collect additional data about dam parity, occurrence of dystocia at birth, and calf health. Particularly, calves were monitored until 30 d of age to record age at disease and mortality occurrence, days of illness, and use of antibiotic treatments. Sick calves were treated according to the own farm therapeutic protocol.

Laboratory Analysis

During transportation to the laboratory, blood samples were refrigerated, whereas colostrum samples were maintained frozen. At the laboratory, within 2 h of collection, blood samples were centrifuged at $3076 \times g$ for 10 min at 20°C . Serum was transferred into 2 mL tubes and, as colostrum samples, it was stored at -20°C until the day of analysis. The electrophoretic method described by Tóthová et al. (2013) was used to determine the Ig concentrations in both blood serum and colostrum samples. To perform the analysis, blood serum and colostrum samples were thawed in water-bath at 20°C and at 37°C , respectively. Colostrum samples were then processed according to the method by Ceniti et al. (2016): 40 μL of a commercial rennet solution (Naturen, CHR Hansen, Hoersholm, Denmark) was added to 4.5 mL of each colostrum sample, in order to separate the albumin and globulin fractions from the casein fraction, and samples were incubated at 37°C for 5 min. The clot was then disaggregated using a plastic stick and samples were centrifuged at $3076 \times g$ for 15 min at 15°C . The colostrum whey (supernatant) was transferred into a new tube and it was added with distilled water in order to restore the initial volume of extraction (4.5 mL). The biuret method on an automated analyzer (Cobas C501, Roche Diagnostics, Mannheim, Germany) was used to determine total protein concentration

(g/L) of both blood serum and colostrum whey samples. The protein fractions (%) of each sample were then assessed by a semi-automated agarose gel system (Hydrasys LC Sebia, Bagno a Ripoli, FI, Italy) associated with Phoresis software, as described in Tóthová et al. (2013). Finally, Ig concentration (g/L) of each blood serum and colostrum whey sample was obtained using the total protein concentration (g/L) resulted by the biuret method to convert into absolute concentration (g/L) the percentage of Ig obtained from the electrophoretic analysis.

Statistical Analysis

At first, baseline differences between the two treatment groups regarding colostrum management (i.e., delivery time, amount, and quality of the first colostrum meal), dam parity, and occurrence of dystocia were screened by PROC LOGISTIC (SAS Institute Inc., Cary, NC), including the farm in the model and considering $P < 0.05$ as significant. The effect of CS administration on calf serum Ig concentration was then evaluated considering also the characteristics of the first colostrum meal provided to each calf. According to the method developed in a previous study (Lora et al., under revision), a colostrum management score that combined the effects of delivery time, amount and quality of the first colostrum meal was calculated for each calf included in the study, where higher scores corresponded to an overall better management of colostrum. Briefly, a single score from 0 to 3 was assigned to time after birth of the first colostrum meal (>5.5 h = score “0”; from 5.5 to 2.6 h = score “1”; from 2.5 to 1.1 h = score “2”; ≤ 1.0 h = score “3”), to amount of colostrum fed at the first meal (≤ 1.5 L = score “0”; from 1.6 to 2.0 L = score “1”; from 2.1 to 2.5 L = score “2”; >2.5 L = score “3”), and to quality of colostrum fed at the first meal (Ig concentration ≤ 49.4 g/L = score “0”; from 49.5 to 69.0 g/L = score “1”; from 69.1 to 87.6 g/L = score “2”; >87.6 g/L = score “3”). The colostrum management score was then generated for each calf by the following formula: [(score of time at the first colostrum meal) $\times 0.68$] + [(score of amount of colostrum fed) $\times 0.60$] + [(score of quality of colostrum fed) $\times 1.00$]. The effect of the treatment on calf serum Ig concentration was finally evaluated by PROC MIXED (SAS Institute Inc., Cary, NC), including colostrum management score as covariate and farm as random effect. The effect of treatment on other continuous variables (i.e., age at disease onset and days of illness) was tested by PROC MIXED (SAS Institute Inc., Cary, NC) considering farm as random

effect, whereas the effect of treatment on binary variables (i.e., FTPI - defined as serum Ig concentration <10.0 g/L -, disease and mortality occurrence, antibiotic treatments on sick calves) was tested by PROC LOGISTIC (SAS Institute Inc., Cary, NC) including the farm in the model. For all the statistical analyses performed, effects were considered significant for $P < 0.05$.

RESULTS AND DISCUSSION

The FTPI is one of the main issues of calf rearing in dairy farms since many years. Correct newborn calf management and colostrum administration require good expertise, labor and time consuming that frequently are not available in dairy practice. Therefore, the use of commercial CS or CR is becoming increasingly common in dairy farming, due to the several advantages that such products could offer, like ease and quickness of use, assurance of successful transfer of passive immunity in calves, and guarantee of being pathogen free. However, the efficacy of commercial colostrum products is not always proved, leading to potential risks to calf health and to economic losses for the farmers. The main advantages offered by the CS tested in this study were the ready-to-use form and the ease of application, which, differently from most of CS available in trade, did not need the addition of water. Because the study was conducted on field, the presence of baseline differences in calves between T and C groups was firstly excluded (Table 1).

Table 1. Description of study calves fed maternal colostrum (control group) or maternal colostrum and a commercial colostrum supplement (treated group) ($P > 0.05$)

Parameter	Treatment group	
	Control (n = 35)	Treated (n = 35)
Time after birth of the first colostrum meal (h) ¹	3.36 ± 0.63	3.74 ± 0.62
Amount of colostrum fed at the first meal (L) ¹	2.87 ± 0.29	2.39 ± 0.29
Colostrum immunoglobulin concentration (g/L) ¹	61.82 ± 12.20	65.54 ± 12.14
Primiparous dams (%)	45.7	57.14
Dystocia at birth (%)	11.4	8.6

¹Least squares means ± standard error.

Despite this, according to farmers' habits and practical needs, each calf received a different treatment in terms of delivery time, amount and quality of colostrum provided at the first meal. It is well known that a variation in one or more of those three factors (delivery time, amount and quality of colostrum) could strongly influence the level of serum Ig concentration in calves (Stott et al., 1979; Stott and Fellah, 1983; Godden, 2008).

Because the indication of the commercial product tested in this study is to be administered to calves as a supplementation of maternal colostrum, its efficacy could be affected by the variability in colostrum quality or in colostrum delivery practices. Therefore, the influence of individual calf colostrum management (measured as a score assigned to each calf) was considered for the evaluation of the effect of CS administration on calf serum Ig concentration. However, no differences were found in calf serum Ig concentration between treated and control calves, and neither in the percentage of calves that had FTPI, even if the latter seemed to be higher in the C group (Table 2).

Table 2. Differences in passive immunity and health status of study calves fed maternal colostrum (control group) or maternal colostrum and a commercial colostrum supplement (treated group) ($P > 0.05$)

Parameter	Treatment group	
	Control (n = 35)	Treated (n = 35)
Serum immunoglobulin concentration (g/L) ¹	11.89 ± 1.78	11.20 ± 1.76
FTPI (%) ²	57.1	42.9
Calf disease (%)	37.1	37.1
Age at disease onset (d) ¹	5.34 ± 3.38	4.88 ± 3.38
Days of illness ¹	4.43 ± 1.70	3.75 ± 1.71
Antibiotic treatment (%) ³	69.2	61.5
Calf mortality (%)	2.9	0.0

¹Least squares means ± standard error.

²FTPI = failure of transfer of passive immunity (serum immunoglobulin concentration <10.0 g/L).

³Parameter calculated considering only sick calves (n = 13 for both the control and the treatment group).

Other studies on different whey-derived CS reported no efficacy of the tested product when administered according to the label instructions. For example, Abel Francisco and Quigley (1993) found no differences in peak mean immunoglobulin concentrations of calves fed 2 L of maternal colostrum or 2 L of maternal colostrum added with a CS that provided about 14 g of Ig per calf. Similarly, Zaremba et al. (1993) reported no differences in serum Ig concentrations of calves fed 3 kg of colostrum or 3 kg of colostrum added with a CS that provided 9.6 g of Ig per calf. Even the CS tested by Mee et al. (1996) (30 g of Ig/dose) failed in enhancing calf immunity when diluted in 1 L of warm water and added to 1 L of pooled colostrum compared to calves fed 2 L of pooled colostrum. Morin et al. (1997) reported that the addition of a dried CS to low quality colostrum not only did not increase calf serum Ig concentration, but even reduced the efficiency of Ig absorption in calves, probably due to some substances in the product that

might have inhibited the intestinal absorption of Ig. It could be hypothesized that in the previous studies the failure of CS in improving the level of passive immunity in calves was due to the too low amount of Ig provided by the CS applied. About this, the study by Haines et al. (1990) highlighted that the commercially available CS often contain low Ig concentrations compared to those found in high quality fresh colostrum, and stated that if these products might have been of some benefit to hypogammaglobulinemic calves, they were unlikely to be completely effective in colostrum deprived calves. The Ig concentration of the CS tested in this study (12 g/100 mL) was similar to that of a high quality fresh colostrum (Dardillat et al., 1978). However, with a single dose administration, the actual extra provision of Ig to calves was probably low. In fact, based on the formula of the apparent efficiency of absorption (AEA) proposed by Quigley and Drewry (1998) {calf serum Ig concentration (g/L) = [Ig intake (g) × AEA] / calf serum volume (L)}, if the CS tested in this study had been applied to an average Holstein calf (BW = 40 kg, plasma volume = 8.0% of BW and AEA = 0.3), the serum Ig concentration would have been increased of about 1.1 g/L. It was probable, therefore, that the benefit of colostrum supplementation on calf serum Ig concentration was hardly detectable in this study, even considering the effect of the individual calf management of colostrum.

The aim of the tested CS was to improve calf immunity in order to reduce the occurrence and severity of neonatal calf diseases. All the calf that got sick during this study suffered from enteric disease (diarrhea), and two calves of each group of treatment showed also concomitant respiratory signs. Only one calf died during the study, due to enteric disease. The percentage of disease cases was the same in both the treatment groups (Table 2). Neither age at disease onset, days of illness, percentage of sick calves treated with antibiotics, and calf mortality differed between T and C groups (Table 2). A study by Furman-Fratczak et al. (2011) reported that higher serum Ig concentrations in newborn calves were associated with delayed disease onset and lower severity of disease cases. In the present study, however, the lack of difference in serum Ig concentration between the treatment groups was probably responsible for the lack of benefit on calf health of CS administration. Similar findings were reported by both Zaremba et al. (1993) and Mee et al. (1996), which did not find any differences in health and mortality rates of calves fed colostrum or colostrum plus a commercial CS.

Considering that the minimum mass of colostrum Ig required to ensure an adequate transfer of passive immunity in calves is of 150 g (Chigerwe et al., 2008), it could be hypothesized that at least 4 doses of the CS tested in this study should be needed to be administered per calf to provide a concrete Ig integration. However, further studies would be needed to prove the effectiveness of such a protocol of treatment, and anyway the cost-benefit ratio would considerably increase.

CONCLUSIONS

The CS tested in this study failed to prevent FTPI in calves. No differences were found in serum Ig concentration between treated and control calves, and no benefits of CS administration were observed on disease and mortality occurrence. Due to the wide variety of commercial colostrum products available in trade, farmers should be aware of CS and CR features before purchase them, in order to be able to choose the most suitable product for its own needs and the proper protocol of application.

REFERENCES

- Abel Francisco, S. F., and J. D. Quigley. 1993. Serum immunoglobulin concentrations after feeding maternal colostrum or maternal colostrum plus colostrum supplement to dairy calves. *Am. J. Vet. Res.* 54:1051–1054.
- Beam, A. L., J. E. Lombard, C. A. Koprak, L. P. Garber, A. L. Winter, J. A. Hicks, and J. L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *J. Dairy Sci.* 92:3973–3980. doi:10.3168/jds.2009-2225.
- Berge, A. C. B., P. Lindeque, D. A. Moore, and W. M. Sisco. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. *J. Dairy Sci.* 88:2166–2177. doi:10.3168/jds.S0022-0302(05)72892-7.
- Ceniti, C., F. Trimboli, G. Massimini, V. M. Morittu, T. Gigliotti, C. Cicino, and D. Britti. 2016. Valutazione della concentrazione delle immunoglobuline nel colostro ovino mediante strumento di routine per l'elettroforesi capillare. *Large Animal Review* 22, 33–36.

- Chigerwe, M., J. W. Tyler, L. G. Schultz, J. R. Middleton, B. J. Steevens, and J. N. Spain. 2008. Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. *Am. J. Vet. Res.* 69:1158–1163. doi:10.2460/ajvr.69.9.1158.
- Dardillat, J., G. Trillat, and P. Larvor P. 1978. Colostrum immunoglobulin concentration in cows: relationship with their calf mortality and with the colostrum quality of their female offspring. *Ann. Rech. Vet.* 9:375–384.
- Donovan, G. A., I. R. Dohoo, D. M. Montgomery, F. L. and Bennett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev. Vet. Med.* 34:31–46. doi:10.1016/S0167-5877(97)00060-3.
- Fidler, A. P., M. L. Alley, and G. W. Smith. 2011. Short communication: serum immunoglobulin G and total protein concentrations in dairy calves fed a colostrum-replacement product. *J. Dairy Sci.* 94:3609–3612. doi:10.3168/jds.2011-4358.
- Furman-Fratczak, K., A. Rzasa, and T. Stefaniak. 2011. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* 94:5536–5543. doi:10.3168/jds.2010-3253.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* 24:19–39. doi:10.1016/j.cvfa.2007.10.005.
- Haines, D. M., B. J. Chelack, and J. M. Naylor. 1990. Immunoglobulin concentrations in commercially available colostrum supplements for calves. *Can. Vet. J.* 31:36–37.
- Jaster, E. H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. *J. Dairy Sci.* 88:296–302. doi:10.3168/jds.S0022-0302(05)72687-4.
- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North Am. Food Anim. Pract.* 20:593–603. doi:10.1016/j.cvfa.2004.06.005.
- Mee, J. F., K. J. O'farrell, P. Reitsma, and R. Mehra. 1996. Effect of a whey protein concentrate used as a colostrum substitute or supplement on calf immunity, weight gain, and health. *J. Dairy Sci.* 79:88–94. doi:10.3168/jds.S0022-0302(96)76437-8
- Morin, D. E., G. C. McCoy, and W. L. Hurley. 1997. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on

- immunoglobulin G1 absorption in Holstein bull calves. *J. Dairy Sci.* 80:747–753. doi:10.3168/jds.S0022-0302(97)75994-0.
- Quigley, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. *J. Dairy Sci.* 81:2779–2790. doi:10.3168/jds.S0022-0302(98)75836-9.
- Quigley, J. 2002. Passive immunity in newborn calves. *Advances in Dairy Technology: proceedings of the Western Canadian Dairy Seminar.* 14:273–292.
- Santoro, H. M., P. S. Erickson, N. L. Whitehouse, A. M. McLaughlin, C. G. Schwab, and J. D. Quigley. 2004. Evaluation of a colostrum supplement, with or without trypsin inhibitor, and an egg protein milk replacer for dairy calves. *J. Dairy Sci.* 87:1739–1746. doi:10.3168/jds.S0022-0302(04)73328-7.
- Smith, G. W., and D. M. Foster. 2007. Short communication: absorption of protein and immunoglobulin G in calves fed a colostrum replacer. *J. Dairy Sci.* 90:2905–2908. doi:10.3168/jds.2006-682.
- Stott, G. H., and A. Fellah. 1983. Colostral immunoglobulin absorption linearly related to concentration for calves. *J. Dairy Sci.* 66:1319–1328. doi:10.3168/jds.S0022-0302(83)81941-9.
- Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. I. Period of absorption. *J. Dairy Sci.* 62:1632–1638. doi:10.3168/jds.S0022-0302(79)83472-4.
- Swan, H., S. Godden, R. Bey, S. Wells, J. Fetrow, and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. *J. Dairy Sci.* 90:3857–3866. doi:10.3168/jds.2007-0152.
- Tóthová, C., O. Nagy, H. Seidel, and G. Kováč. 2013. Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. *Comp. Clin. Path.* 22:15–20. doi:10.1007/s00580-011-1363-8.
- Vogels, Z., G. M. Chuck, and J. M. Morton. 2013. Failure of transfer of passive immunity and agammaglobulinaemia in calves in south-west Victorian dairy herds: prevalence and risk factors. *Aust. Vet. J.* 91:150–158. doi:10.1111/avj.12025.

- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:596–577. doi:10.1111/j.1939-1676.2000.tb02278.x.
- Zaremba, W., W. M. Guterbock, and C. A. Holmberg. 1993. Efficacy of a dried colostrum powder in the prevention of disease in neonatal Holstein calves. *J. Dairy Sci.* 76:831–836. doi:10.3168/jds.S0022-0302(93)77408-1.

CHAPTER 6

Association between passive immunity and occurrence of disease and mortality in calves of less than 30 days of age



CHAPTER 6

Association between passive immunity and occurrence of disease and mortality in calves of less than 30 days of age

I. Lora^{1,2}, F. Gottardo², B. Contiero², A. L. Stefani³, and A. Barberio³

¹*PhD Course in Animal and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

²*Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

³*Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD), Italy*

ABSTRACT

The first month of the calf life is the most critical period for disease and mortality occurrence. Even if failure of transfer of passive immunity (FTPI) is commonly recognized to be one of the main factors that affects calf health and survival, few studies investigated the association between FTPI and disease occurrence in calves. The aim of the present study was to evaluate, under field conditions, the association between FTPI and disease and mortality occurrence in calves of less than 30 d of age. During winters of 2014, 2015 and 2016, 78 calves of three Italian dairy farms were monitored for disease and mortality occurrence from 1 to 30 d of age. Blood sampling was performed on all calves at 1 to 5 d of age and fecal samples were collected from calves with clinical diarrhea that were not treated yet. Blood serum samples were analyzed by an electrophoretic method for the assessment of immunoglobulin (Ig) concentration, whereas fecal samples undergone to ELISA test for positivity to *Escherichia coli* K99, rotavirus, coronavirus, and *Cryptosporidium* spp. Only enteric diseases were observed in this study. Calves that suffered from disease (diarrhea) or died within the first month of life had lower serum Ig concentrations to those that remained healthy or survived ($P < 0.05$). Lower serum Ig concentrations were observed also in sick calves that were treated with antibiotics compared to those that were not treated ($P < 0.05$). The odds of disease and mortality occurrence was 24 and 11 times higher, respectively, for calves with FTPI (serum Ig concentration <10.0 g/L) compared to those with an adequate transfer of passive immunity ($P < 0.05$). Calves with adequate transfer of passive immunity had also

a delay in the age at disease onset of about 4 d compared to calves with FTPI. Even the risk of enteric infections by rotavirus and *Cryptosporidium* spp. was considerably higher for calves with FTPI. In this study, the level of passive immunity influenced the occurrence of enteric disease and mortality in calves of less than one month of age, confirming the importance of proper colostrum provision to calf health and to the reduction of antimicrobial use in dairy farming.

INTRODUCTION

The most critical period of dairy heifer rearing is the first month of the calf life, due to the high risk of disease and mortality occurrence (Sivula et al., 1996; Svensson et al., 2006). Diarrhea is the most typical health problem that affects calves in the first month of life, and it is mainly caused by infectious pathogens like *Escherichia coli*, rotavirus, coronavirus, and *Cryptosporidium parvum* (Maunsell and Donovan, 2008; McGuirk, 2008). If calves survive diarrhea, they will have lower growth rates and will be more susceptible to other diseases, especially respiratory, with possibility of long-term consequences (Curtis et al., 1988; Virtala et al., 1996; van der Fels-Klerx et al., 2002). For example, it is reported that heifers with history of respiratory disease during the first months of life had higher age at first calving and lower production in first lactation, along with a higher risk of early culling (Waltner-Toews et al., 1986; van der Fels-Klerx et al., 2002; Heinrichs and Heinrichs, 2011). Several factors can influence calf disease occurrence in the first months of life, but they can be summarized into three main managerial categories: colostrum provision, management of calf feeding and housing, and biosecurity practices (Bruning-Fann and Kaneene, 1992; van der Fels-Klerx et al., 2002; Maunsell and Donovan, 2008). Colostrum administration is the first factor affecting health in calf life. Because calves are born nearly devoid of antibodies, they depend almost entirely on colostrum consumption to obtain the immunity needed to be protected throughout the first months of life. Proper colostrum management practices should include the provision of at least 4 L of good quality colostrum (i.e., with immunoglobulin - **Ig** - concentration ≥ 50 g/L) within the first 6 h of life (Weaver et al., 2000; McGuirk and Collins, 2004; Godden, 2008). Inadequate colostrum provision could lead to failure of transfer of passive immunity (**FTPI**), which is defined as a low Ig concentration (< 10.0 g/L) in calf serum at 48 h of age, and which is recognized to be a main risk factor for calf

disease and mortality occurrence (Weaver et al., 2000; Godden, 2008; Maunsell and Donovan, 2008). According to that, a recent study by Raboisson et al. (2016) estimated that the total costs per dairy calf with FTPI could range from 52 € (in the best scenario) to 285 € (in the worst scenario), and those costs would be increased of about 50% in case of beef calves. The same study, which revised the literature about FTPI and its consequences to calf health and performances, pointed out that only few studies were published regarding the association between FTPI and calf health, and particularly concerning FTPI and diarrhea occurrence. Therefore, the aim of the present study was to investigate, under field conditions, the association between FTPI and occurrence of disease and mortality in calves of less than one month of age.

MATERIALS AND METHODS

The study was conducted on 78 calves born from November to February of 2014, 2015 and 2016 in three Italian Holstein dairy farms of the Veneto region (northeastern Italy). The farms reared 77, 105 and 142 lactating cows on loose housing system with cubicles. In the three farms, calves were included in the study irrespective of sex and genotype (Holstein purebred or crossbred). Cows calved in a calving pen, parturition was monitored by the farmer, and calf was separated from dam immediately after birth. Navel disinfection was performed and colostrum of the own dam was administered to the calf by nipple-bottle according to the own farm practices. Calves were housed in straw bedded single crates until 8 wk of age. The farms involved in this study adopted a voluntary plan for bovine viral diarrhea (**BVD**) prevention, which required blood sampling on newborn calves to identify persistently infected animals. Therefore, serum Ig concentration of calves included in the study was assessed on blood samples collected by the veterinarian responsible for the BVD control plan, without requiring additional calf manipulation or distress. Blood was collected from jugular vein of calves between 1 and 5 d of age by a 10 mL Vacutainer® tube without anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). Health of enrolled calves was monitored from birth to 30 d of age by the veterinarian in charge to carry out the study, who visited the farms twice a week to record data about calf disease and mortality occurrence, such as type of disease, cause of mortality, age at disease onset or mortality occurrence, days of illness, and use of antibiotic treatments on sick calves. In case of clinical diarrhea (i.e., profuse liquid feces)

at the time of the farm visit, and if the calf was not treated yet, the veterinarian collected a fecal sample from the rectum into a 100 mL tube. Sick calves were treated according to the therapeutic protocol of the own farm. Blood and fecal samples were transported to the laboratory at refrigeration temperature.

Laboratory Analysis

At the laboratory, blood samples were centrifuged at $3076 \times g$ for 10 min at 20°C within 2 h of collection and serum was transferred into 2 mL tubes. Serum and fecal samples were stored at -20°C until the day of the analysis. After thawing in water-bath at 20°C, serum samples were firstly analyzed by the biuret method on an automated analyzer (Cobas C501, Roche Diagnostics, Mannheim, Germany) to assess the total protein concentration (g/L). The electrophoretic method described by Tóthová et al. (2013) was then used to determine the protein fractions (%) of each serum sample, by a semi-automated agarose gel system (Hydrasys LC, Sebia, Bagno a Ripoli, FI, Italy) associated with Phoresis software. Finally, the Ig concentration (g/L) of each blood serum sample was calculated using the total protein concentration (g/L) obtained by the biuret method to convert the percentage of Ig obtained from the electrophoretic analysis into the absolute concentration (g/L).

After thawing at room temperature, fecal samples were submitted to ELISA test for antigens of *E. coli* K99, rotavirus, coronavirus, and *Cryptosporidium* spp. The “Rota-Corona-K99” kit and the “Cryptosporidium Ag test” kit (Idexx, Montpellier, France) were applied according to the label instructions, and the plates were read by spectrophotometer at a wavelength of 450 nm. Based on the sample to positive (S/P) percentage, which was calculated for each well, samples were considered positive to *E. coli* K99 if S/P $\geq 7\%$; to rotavirus if S/P $\geq 14\%$; to coronavirus if S/P $\geq 14\%$; and to *Cryptosporidium* spp. if S/P $\geq 20\%$.

Statistical Analysis

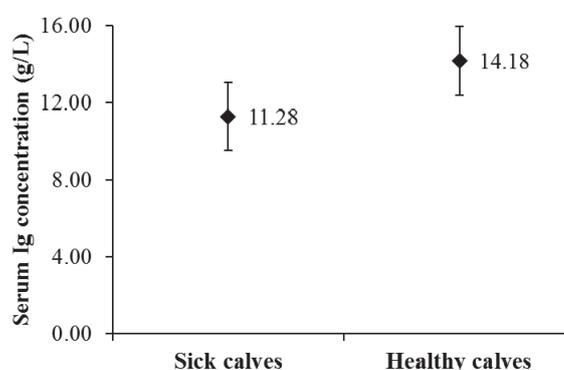
Differences in serum Ig concentration between sick and healthy calves, dead and survived calves, and sick calves treated with antibiotics or not were assessed by PROC MIXED (SAS Institute Inc., Cary, NC), considering the farm as random effect. The same procedure was used to test the effect of FTPI on the age at disease onset. Effect of FTPI

on disease and mortality occurrence, and on specific pathogen infections was investigated by PROC LOGISTIC (SAS Institute Inc., Cary, NC), including the farm in the model. Particularly, the effect of FTPI on specific pathogen infections was evaluated by considering only healthy calves and calves whose fecal samples resulted to be positive to the specific pathogen included in the model. Odds ratio and 95% CI were generated for the variables that resulted to be influenced by FTPI. For all the statistical analyses performed, the level of significance was set at $P < 0.05$.

RESULTS

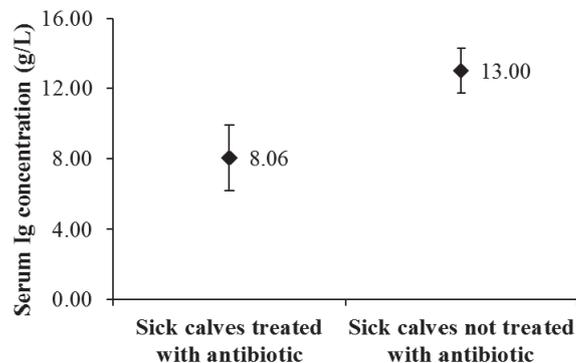
The overall mean (\pm SD) of serum Ig concentration in calves was 12.6 ± 6.2 g/L, with a minimum of 1.6 g/L and a maximum of 30.9 g/L. The overall percentage of calves with FTPI was 34.6%. Fifty per cent of the calves got sick during the first month of life: all of them suffered from enteric disease, whereas no cases of respiratory disease were observed. Fecal samples were collected from 28 out of the 39 sick calves (71.8%): 13 samples (46.4%) were positive only to *Cryptosporidium* spp., five samples (17.9%) were positive only to rotavirus, four samples (14.3%) were positive to more than one infectious pathogen (two to rotavirus and *Cryptosporidium* spp., one to rotavirus and coronavirus, and one to *E. coli*, rotavirus and *Cryptosporidium* spp.), and six samples (21.4%) were negative to all the tested pathogens. Overall mean (\pm SD) of age at disease onset was of 8.5 ± 5.3 d, with a minimum of 1 d and a maximum of 20 d of age. The overall percentage of sick calves that were treated with antibiotics was 25.0%. Five calves (6.4%) died within the first month of life. As showed in Figure 1, calves that suffered from diarrhea within 30 d of age had lower serum Ig concentrations than calves that never showed signs of disease in the same period ($P < 0.05$) (Figure 1).

Figure 1. Least squares means and standard errors of serum immunoglobulin (Ig) concentration in calves that suffered from disease (diarrhea) or not within 30 d of age ($P < 0.05$)



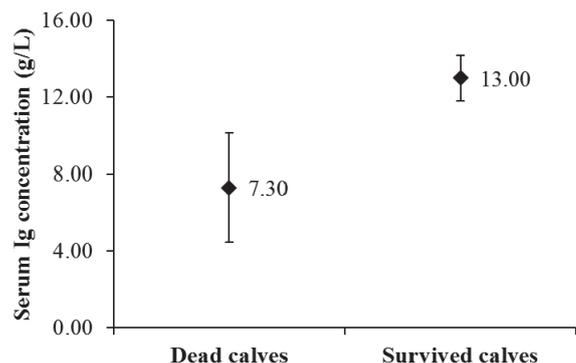
Similarly, sick calves that were treated with antibiotics had lower serum Ig concentrations compared to those that were not treated ($P < 0.05$) (Figure 2).

Figure 2. Least squares means and standard errors of serum immunoglobulin (Ig) concentration in calves of less than 30 d of age that suffered from disease (diarrhea) and were treated or not with antibiotics ($P < 0.05$)



Moreover, survived calves had serum Ig concentrations nearly two times higher than calves that died in the first month of life ($P < 0.05$) (Figure 3).

Figure 3. Least squares means and standard errors of serum immunoglobulin (Ig) concentration in calves of less than 30 d of age that died or survived ($P < 0.05$)



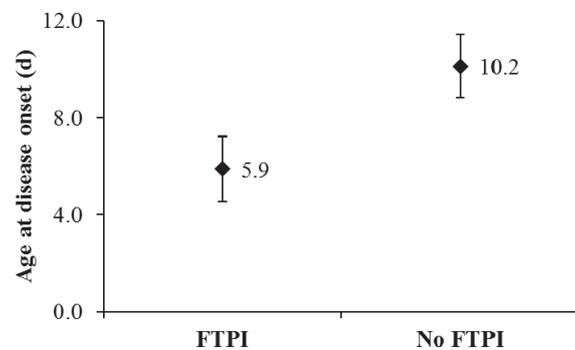
Failure of transfer of passive immunity resulted to be a considerable risk factor for the occurrence of disease ($P < 0.01$) and mortality ($P < 0.05$) in calves of less than 30 d of age, with risks 24 and 11 times higher, respectively, for calves with FTPI compared to those with a sufficient transfer of passive immunity (Table 1).

Table 1. Percentage, odds ratio (OR) and 95% CI of disease and mortality occurrence in calves of less than 30 d of age with or without failure of transfer of passive immunity (FTPI - serum immunoglobulin concentration < 10.0 g/L) ($P < 0.05$)

FTPI	Disease occurrence			Mortality occurrence		
	%	OR	95% CI	%	OR	95% CI
Absent	43.1 (22/51)	1.00	–	2.0 (1/51)	1.00	–
Present	63.0 (17/27)	24.12	2.52–231.09	14.8 (4/27)	10.59	1.01–110.78

Furthermore, the disease onset was delayed of about 4 d in calves with an adequate transfer of passive immunity compared to calves with FTPI ($P < 0.05$) (Figure 4).

Figure 4. Least squares means and standard errors of age at disease onset in calves of less than 30 d of age with or without failure of transfer of passive immunity (FTPI - serum immunoglobulin - Ig - concentration <10.0 g/L) ($P < 0.05$)



About specific pathogens responsible for calf diarrhea, FTPI resulted to be a predisposing factor for rotavirus and for *Cryptosporidium* spp. infections ($P < 0.05$), with increased risks of 12 and 9 times, respectively (Table 2).

Table 2. Percentage, odds ratio (OR) and 95% CI of rotavirus and *Cryptosporidium* spp. infections in calves of less than 30 d of age with or without failure of transfer of passive immunity (FTPI - serum immunoglobulin concentration <10.0 g/L) ($P < 0.05$)

FTPI	Rotavirus infection ¹			<i>Cryptosporidium</i> spp. infection ¹		
	%	OR	95% CI	%	OR	95% CI
Absent	14.7 (5/34)	1.00	–	25.6 (10/39)	1.00	–
Present	28.6 (4/14)	11.85	1.02–137.48	37.5 (6/16)	8.64	1.04–72.07

¹Only healthy calves and calves whose feces were positive to the specific pathogen included in the model were considered for the statistical analysis.

DISCUSSION

The percentage of calves with FTPI found in this study was slightly lower than those reported by Vogels et al. (2013) for Australian calves (38.0%) and by Trotz-Williams et al. (2008) for Canadian calves (37.1%), but it was considerably higher than that reported by Beam et al. (2009) for U.S. heifer calves (19.2%). Moreover, it was comparable to the percentage found by Cavirani et al. (2005) in Italian dairy calves, indicating that the level of passive immunity in Italian dairy calves was not improved in the last ten years. Even the percentage of calves that got sick in this study was considerably high, considering that the prevalence and the incidence risk for calf neonatal diarrhea in dairy herds is reported to be around 20 and 21%, respectively (Lundborg et al., 2005; Bartels et al., 2010; Windeyer et al., 2014). The absence of cases of respiratory

disease in calves of the present study was not unexpected, because diarrhea is reported to be the most common disease in calves of less than 30 d of age, whereas respiratory diseases are more typical of older calves (McGuirk, 2008). Probably due to the high percentage of calves that got sick, the mortality rate found in this study was greater than that reported by other authors, which ranged from 3.1 to 4.2% (Svensson et al., 2006; Raboisson et al., 2013; Windeyer et al., 2014). Not many studies are available in literature with the specific aim to investigate the relationship between calf immunization and disease occurrence, and only few studies were focused on the relationship between FTPI and enteric diseases in calves (Raboisson et al., 2016). However, in line with results of this study, McGuire et al. (1976) and Blom (1982) reported that low serum Ig concentrations were associated to disease and mortality occurrence in dairy calves, confirming the importance of a proper transfer of passive immunity to calf health. Moreover, several studies reported a higher risk of disease and mortality occurrence for calves with FTPI compared to those with an adequate level of serum Ig concentration (Donovan et al., 1998; Berge et al., 2005; Berge et al., 2009). The level of immune protection in calves could affect not only disease occurrence, but also disease severity. About that, Furman-Fratczak et al (2011) found that calves with higher levels of passive immunity suffered from milder forms of both enteric and respiratory diseases, whereas calves with serum Ig concentration >15.0 g/L at 30 to 60 h of life avoided respiratory tract infections at all. Even if no specific parameters were measured in this study to assess the severity of calf diarrhea (e.g. dehydration, body temperature, fecal scoring), the lower Ig concentration found in sick calves that needed antibiotic treatment compared to those that were not treated indicated reasonably a greater severity of the disease in treated calves. The latter result was in line with the study by Berge et al. (2009), which reported that FTPI lead to a higher risk of antibiotic treatments, and, together with Berge et al. (2005), pointed out that improvements in colostrum management practices are needed also in order to reduce the antimicrobial use in dairy farming. Results of the present study showed that the adequate transfer of passive immunity was associated also to a delayed onset of enteric diseases in calves. Davidson et al. (1981) found similar results about calf respiratory infections, reporting a delay of 5 to 7 d in disease onset for calves with higher serum Ig concentrations. Such a difference could be important to calf health, because it could not be excluded that a delay in the age at disease onset may enhance the possibility

of calf recovery. Among the major infectious agents that cause calf neonatal diarrhea (*E. coli* K99, rotavirus, coronavirus, and *Cryptosporidium* spp), rotavirus and *Cryptosporidium* spp. are considered to be the predominant ones (de la Fuente et al., 1999; Barrington et al., 2002; Meganck et al., 2015). According to the previous statement, in this study *Cryptosporidium* spp. and rotavirus resulted to be the main agents of calf diarrhea, whereas positivity to *E. coli* K99 and coronavirus were detected rarely and only in mixed infections. Outcomes of this study showed that FTPI was an important predisposing factor for specific infections by rotavirus and *Cryptosporidium* spp. However, even if there is evidence that specific passive immunity in calves could be protective against diarrhea caused by rotavirus (Kohara and Tsunemitsu, 2000), it is not clear yet if maternal immunity can act as protective factor against infections by *Cryptosporidium* spp. (Trotz-Williams et al., 2007). Further studies would be needed in order to better understand the relationship between passive immunity and specific enteropathogens infections in calves, particularly by *Cryptosporidium* spp.

CONCLUSIONS

This study showed that FTPI in calves of less than one month of age was associated with an increased risk of diarrhea and mortality occurrence. Moreover, low levels of passive immunity in calves were associated to younger age at disease onset and necessity of antibiotic treatment for recovery. Adequate transfer of passive immunity resulted to be protective also against infections by specific enteropathogens like rotavirus and *Cryptosporidium* spp. Ensuring an adequate transfer of maternal immunity to calves, through proper colostrum management practices, is important therefore to avoid severe enteric infections and to reduce antimicrobial use in dairy farming.

ACKNOWLEDGMENTS

This study was supported by the grants obtained from the Italian Ministry of Health for the research project (RC IZSVE 10/2011).

REFERENCES

- Barrington, G. M., J. M. Gay, and J. F. Evermann. 2002. Biosecurity for neonatal gastrointestinal diseases. *Vet. Clin. North Am. Food Anim. Pract.* 18:7–34. doi:10.1016/S0749-0720(02)00005-1.
- Bartels, C. J. M., M. Holzhauser, R. Jorritsma, W. A. J. M. Swart, and T. J. G. M. Lam. 2010. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Prev. Vet. Med.* 93:162–169. doi:10.1016/j.prevetmed.2009.09.020.
- Beam, A. L., J. E. Lombard, C. A. Koprak, L. P. Garber, A. L. Winter, J. A. Hicks, and J. L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *J. Dairy Sci.* 92:3973–3980. doi:10.3168/jds.2009-2225.
- Berge, A. C. B., P. Lindeque, D. A. Moore, and W. M. Sischo. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. *J. Dairy Sci.* 88:2166–2177. doi:10.3168/jds.S0022-0302(05)72892-7.
- Berge, A. C. B., T. E. Besser, D. A. Moore, and W. M. Sischo. 2009. Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of preweaned calves. *J. Dairy Sci.* 92:286–295. doi:10.3168/jds.2008-1433.
- Blom, J. Y. 1981. The relationship between serum immunoglobulin values and incidence of respiratory disease and enteritis in calves. *Nord. Vet. Med.* 34: 276–284.
- Bruning-Fann, C., and J. B. Kaneene. 1992. Environmental and management risk factors associated with morbidity and mortality in perinatal and pre-weaning calves: a review from an epidemiological perspective. *Veterinary Bulletin* 62: 399–413.
- Cavirani, S., S. Taddei, C. S. Cabassi, G. Donofrio, F. Toni, F. Ghidini, C. Piancastelli, E. Schiano, and C.F. Flammini. 2005. Deficit di IgG colostrali e di trasferimento di immunità passiva colostrale in allevamenti bovini da latte ad alta produzione. *Large Animal Review.* 11:17–21.
- Curtis, C. R., J. M. Scarlett, H. N. Erb, and M. E. White. 1988. Path model of individual-calf risk factors for calfhood morbidity and mortality in New York Holstein herds. *Prev. Vet. Med.* 6:43–62. doi:10.1016/0167-5877(88)90025-6.

- Davidson, J. N., S. P. Yancey, S. G. Campbell, and R. G. Warner. 1981. Relationship between serum immunoglobulin values and incidence of respiratory disease in calves. *J. Am. Vet. Med. Assoc.* 179:708–710.
- de la Fuente, R., M. Luzón, J. A. Ruiz-Santa-Quiteria, A. García, D. Cid, J. A. Orden, S. García, R. Sanz, and M. Gómez-Bautista. 1999. Cryptosporidium and concurrent infections with other major enteropathogens in 1 to 30-day-old diarrheic dairy calves in central Spain. *Veterinary Parasitology.* 80: 179–185. doi:10.1016/S0304-4017(98)00218-0.
- Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev. Vet. Med.* 34:31–46. doi:10.1016/S0167-5877(97)00060-3.
- Furman-Fratczak, K., A. Rzasa, and T. Stefaniak. 2011. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* 94:5536–5543. doi:10.3168/jds.2010-3253.
- Godden, S. 2008. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* 24:19–39. doi:10.1016/j.cvfa.2007.10.005.
- Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336–341. doi:10.3168/jds.2010-3170.
- Kohara, J., and H. Tsunemitsu. 2000. Correlation between maternal serum antibodies and protection against bovine rotavirus diarrhea in calves. *J. Vet. Med. Sci.* 62: 219–221. doi:10.1292/jvms.62.219.
- Lundborg, G. K., E. C. Svensson, and P. A. Oltenacu. 2005. Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0–90 days. *Prev. Vet. Med.* 68:123–143. doi:10.1016/j.prevetmed.2004.11.014.
- Maunsell, F., and G. A. Donovan. 2008. Biosecurity and Risk Management for Dairy Replacements. *Vet. Clin. North. Am. Food. Anim. Pract.* 24:155–190. doi:10.1016/j.cvfa.2007.10.007.
- McGuire, T. C., N. E. Pfeiffer, J. M. Weikel, and R. C. Bartsch. 1976. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. *J. Am. Vet. Med. Assoc.* 169:713–78.

- McGuirk, S. M. 2008. Disease management of dairy calves and heifers. *Vet. Clin. North. Am. Food. Anim. Pract.* 24:139–153. doi:10.1016/j.cvfa.2007.10.003.
- McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North. Am. Food. Anim. Pract.* 20:593–603. doi:10.1016/j.cvfa.2004.06.005.
- Meganck, V., G. Hoflack, and G. Opsomer. 2015. Evaluation of a protocol to reduce the incidence of neonatal calf diarrhoea on dairy herds. *Prev. Vet. Med.* 118:64–70. doi:10.1016/j.prevetmed.2014.11.007.
- Raboisson, D., P. Trillat, and C. Cahuzac. 2016. Failure of passive immune transfer in calves: a meta-analysis on the consequences and assessment of the economic impact. *PLoS One.* 11:e0150452. doi:10.1371/journal.pone.0150452.
- Raboisson, D., F. Delor, E. Cahuzac, C. Gendre, P. Sans, and G. Allaire. 2013. Perinatal, neonatal, and rearing period mortality of dairy calves and replacement heifers in France. *J. Dairy Sci.* 96:2913–2924. doi:10.3168/jds.2012-6010.
- Sivula, N. J., T. R. Ames, W. E. Marsh, and R. E. Werdin. 1996. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. *Preventive Veterinary Medicine.* 27:155–171.
- Svensson, C., A. Linder, and S. O. Olsson. 2006. Mortality in Swedish dairy calves and replacement heifers. *Journal of Dairy Science.* 89:4769–4777.
- Tóthová, C., O. Nagy, H. Seidel, and G. Kováč. 2013. Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. *Comp. Clin. Path.* 22:15–20. doi:10.1007/s00580-011-1363-8.
- Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. *J. Dairy Sci.* 91:3840–3849. doi:10.3168/jds.2007-0898.
- Trotz-Williams, L. A., S. W. Martin, K. E. Leslie, T. Duffield, D. V. Nydam, and A. S. Peregrine. 2007. Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Prev. Vet. Med.* 82:12–28. doi:10.1016/j.prevetmed.2007.05.003.
- van der Fels-Klerx, H. J., S.W. Martin, M. Nielen, and R. B. M. Huirne. 2002. Effects on productivity and risk factors of bovine respiratory disease in dairy heifers; a review

for the Netherlands. *Neth. J. Agric. Sci.* 50:27–45. doi:10.1016/S1573-5214(02)80002-1.

- Virtala, A.M. K., G. D. Mechor, Y. T. Grohn, and H. N. Erb. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. *J. Dairy Sci.* 79:104–1049. doi:10.3168/jds.S0022-0302(96)76457-3.
- Vogels, Z., G. M. Chuck, and J. M. Morton. 2013. Failure of transfer of passive immunity and agammaglobulinaemia in calves in south-west Victorian dairy herds: prevalence and risk factors. *Aust. Vet. J.* 91:150–158. doi:10.1111/avj.12025.
- Waltner-Toews, D., S. W. Martin, and A. H. Meek. 1986. The effect of early calfhood health status on survivorship and age at first calving. *Can. J. Vet. Res.* 50:314–317.
- Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:596–577. doi:10.1111/j.1939-1676.2000.tb02278.x.
- Windeyer M. C., K. E. Leslie, S. M. Godden, D. C. Hodgins, K. D. Lissemore, and S. J. LeBlanc. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med.* 113:231–240. doi:10.1016/j.prevetmed.2013.10.019.

CHAPTER 7

General conclusions



CHAPTER 7

General conclusions

Outcomes of this study revealed that the management of newborn calves is an issue in several dairy farms. High mortality rates of young stock indicated a welfare problem in nearly one third of the enrolled farms. The highest percentage of young stock mortality was recorded within the first 2 mo of life. As failure of transfer of passive immunity (FTPI) is reported to be one of the main factors that affects calf mortality in the first months of life, the prevalence of FTPI and management factors associated to it at the farm level were investigated in a sample of Italian dairy farms. Results showed severe deficiencies in colostrum management for dairy calves, with an overall prevalence of FTPI of 41.0%. The analysis of the management factors associated to FTPI made in this study could be useful in producing specific recommendations for dairy producers about the correct practices of colostrum provision to dairy calves. Particularly, in the dairy practice, the combined effect of delivery time, amount and quality of the first colostrum meal should be taken into account in order to reduce the prevalence of FTPI.

The common practice in intensive dairy farms of separating calf from dam soon after birth is adopted mainly for biosecurity reasons and to facilitate proper colostrum administration, but it represents a welfare issue for calf due to the lack of maternal cares. However, results of the study showed that allowing the calf to nurse colostrum from the dam was an effective practice in terms of successful transfer of passive immunity only if supported by farmer assistance, and it lead to a considerable separation distress for both calves and dams. Therefore, the early separation of calf from dam, associated with proper colostrum provision, resulted to be the most suitable practice in intensive dairy farms to maximize the transfer of passive immunity and minimize the calf-dam separation distress. Beside the correct immunization of the calves, proper hygienic management of housing facilities and of equipment for calf feeding is needed in order to avoid neonatal infections.

Due to the actual difficulties that may be encountered in dairy practice about colostrum management, the use of commercial colostrum supplements could help in reducing the prevalence of FTPI in calves. However, results of this thesis showed that the commercial product tested was not effective in FTPI prevention when administered according to the label instructions. Dairy producers should be therefore aware of the

composition and of the most suitable protocol of administration when choosing a commercial colostrum supplement.

In this thesis, a relationship was found between serum Ig concentration and occurrence of diarrhea and mortality in calves, confirming the importance of proper colostrum provision to calf health. Particularly, FTPI was associated to an earlier age at disease outbreak, which may lead to a reduced probability to recover for the calves. The evidence that diseased calves with higher serum Ig concentrations were not treated with antibiotics to recover put in light that reaching a good level of immunization in calves is important also to reduce the antimicrobial use in dairy farming.

In conclusion, greater awareness by farmers of the correct practices of colostrum management for newborn calves is needed in order to address the first step in the improvement of dairy calf welfare and to reduce the antimicrobial use in dairy farming.

Supplementary material



Supplementary material

Chapter 3: Factors associated with failure of transfer of passive immunity in dairy calves: combined effect of delivery time, amount and quality of the first colostrum meal

Supplementary Figure S1. Classification and Regression Tree analysis (C&RT) of the effects of time after birth (TIME), amount (AMOUNT) and immunoglobulin (Ig) concentration (Ig_COL) of the first colostrum meal on failure of transfer of passive immunity (FTPI - serum Ig concentration <10.0 g/L) in dairy calves

