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Determinants for milk fever

An epidemiological study of Swedish dairy cows

Sofia Daresjö

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***Supervisor**: Ulf Emanuelson, Department of Clinical Sciences*

***Assistant Supervisor**: Cecilia Kronqvist, Department of Animal Nutrition and Management*

***Examiner**: Karin Alvåsen, Department of Clinical Sciences*

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SUMMARY

Milk fever is a worldwide disease, seen mainly in dairy cows. At the onset of lactation the cow is not prepared for the sudden calcium requirement, which causes a drop in the blood calcium and the cow develops hypocalcaemia. Calcium is needed for the correct function of both nerves and muscles in the body. A cow suffering from milk fever can show a variety of clinical signs, such as being recumbent, having a reduced appetite and having cold extremities. In the worst cases of milk fever the cow can develop a comatose state and die. To cope with the loss of calcium the cow has several mechanisms in the body involving the kidneys, intestines and bones. These include increased absorption of calcium from the kidneys and intestines and a resorption of calcium from the bones.

Milk fever has been studied for many decades and several risk factors have been identified, *e.g.* body condition score around calving, diet around calving, age, breed and production. The aim of this study was to analyze suspected and previously stated risk factors and their association with the incidence of milk fever. Data was collected through the milk recording scheme managed by VÄXA Sverige. All herds studied were selected based on the milking year 2015/16 (September-August) and had at least 40 calvings, 20% Swedish Holstein breed and 20% Swedish Red breed. Information about incidence of milk fever and the risk factors in these herds was retrieved from 2006/07 through 2015/16. The risk factors studied were breed, parity, previous milk fever cases, previous lactation length, previous dry period length, calving season, previous calving interval and information about milk production (ECM, fat, protein). A decreasing trend of the incidence of milk fever was observed throughout the years. All of the studied factors had an association with the incidence of milk fever. High risk cows were of Holstein breed, were of high parity, had suffered from milk fever in the previous lactation, had a long previous lactation length, had a dry period length of 70-89 days, calved in spring, had a long calving interval and had a high milk production. The determinant with the strongest association was parity, showing that cows of higher parity have clearly increased odds of developing milk fever. A sixth or higher parity cow had an odds ratio of 277.84 of developing milk fever compared to a first parity cow. The second most important determinant was milk fever in the previous lactation (OR=9.74). Several major factors, which previously have been shown to have an effect on milk fever incidence, could not be studied due to limitations in the available information. Furthermore, only univariable analyses were done although it is known that several of the studied determinants have an effect on each other.

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INTRODUCTION

Milk fever is a complex metabolic disease caused by an imbalance in the calcium (Ca) homeostasis at the onset of lactation putting the cow in a hypocalcaemic state. The hypocalcaemia is caused by the sudden production of large amounts of milk, draining the animal of its available Ca. In the beginning of lactation the body of the cow is not prepared for the Ca loss so the resorption of Ca from the bones and the absorption of Ca from the intestines cannot make up for the loss (Goff & Horst, 1993). Calcium is essential for the correct function of both nerves and muscles in the body. Therefore a cow suffering from milk fever becomes recumbent, has a reduced appetite and can develop a comatose state and even die (Constable *et al.*, 2017). There are several risk factors for a cow developing this condition, such as age, body condition score and mineral composition of diet around calving (Goff, 2000). Milk fever can in most cases be treated successfully with an intravenous infusion of Ca (Goff, 1999). Though the treatment is fairly simple and usually successful it is important to remember that milk fever, also called parturient paresis, is considered a gateway disease for other problems. It has been shown that a cow suffering from milk fever has a larger risk of secondary problems for example mastitis and reproductive disorders such as retained placenta, metritis and dystocia increasing the future risk of culling (Curtis *et al.*, 1985; Erb *et al.*, 1985).

The incidence of milk fever in Sweden in the milk recording year of 2017/18 was reported to be 2.1% (VÄXA, 2018). Comparatively a 2.8 % incidence of milk fever was reported by United States Department of Agriculture. The report was based on data provided by dairy producers from the year of 2014 (USDA, 2018). In Norway a milk fever incidence of 3.5% was reported during 2018 (TINE, 2019). Considering the potential consequences of milk fever it is important to understand the physiology behind it and factors affecting its incidence.

The objective of this epidemiological study was to study the incidence of milk fever and associated risk factors in Swedish dairy herds, both at the level of the individual cow and the herd. Furthermore, the goal was to get a deep understanding of the Ca homeostasis and the pathophysiology of milk fever.

LITERATURE REVIEW

Hypocalcaemia and milk fever

Milk fever is a severe form of hypocalcaemia when clinical signs are observed. The cut off blood Ca value for clinical hypocalcaemia is <1.4 mmol/L. When the blood Ca value is between 1.4 and 2.0 mmol/L it is classified as subclinical hypocalcaemia and the clinical signs associated with milk fever are not seen (Roche, 2003). Calcium has several important roles in the body. One of them is keeping cell membrane stability. A reduction of Ca in the body leads to nerve cells being more excitable. Calcium is essential for muscle contractility and a reduced Ca level can thus impair muscle contractility. Furthermore Ca is needed for the release of acetylcholine in the neuromuscular junctions, and without Ca the signaling is inhibited (Constable *et al.*, 2017). The disruptions on a molecular and cellular level cause clinical signs such as the cow being less willing to move, having a decreased feed intake and muscle tremors. As the hypocalcaemia progresses clinical signs such as recumbency, a dry muzzle, a low body temperature, increased heart rate and a weaker pulse are seen. If left untreated the cow can develop a comatose stage, collapse and die (Constable *et al.*, 2017).

Calcium homeostasis

A large amount of Ca is required for the daily milk production of a dairy cow. The blood Ca of a cow is around 8.5-10 mg/dl (2.1-2.5 mmol/L), which is equal to approximately 3 grams in the total blood volume of a 600 kg cow. The total extracellular Ca content will be around 8-9 grams. It is not uncommon that a cow produces milk that requires 20-30 grams of Ca per day. To be able to keep the blood Ca at stable levels the animal must replace the Ca lost to the milk by increasing the absorption of Ca from the intestines and the kidneys or by mobilizing it from the reserves in the body, *i.e.* the bones (Goff, 2014; Goff, 2000). Approximately 42-48% of the blood Ca is in ionized form and is considered the biologically active portion. Depending on the pH of the blood the percentage shifts. When the blood is more acidic the ionized Ca is closer to 48% while a more alkaline pH makes the available Ca nearer 42% (Goff, 2014).

Parathyroid hormone

The regulation of Ca absorption and mobilization is regulated by parathyroid hormone (PTH). The hormone is produced in the parathyroid glands and its secretion is stimulated by low blood Ca concentrations. Parathyroid hormone stimulates the reabsorption of Ca in the kidneys, the mobilization of Ca from the bones and increases the uptake of Ca from the intestinal tract (Goff, 2014).

Parathyroid hormone is a peptide that binds to receptors of the cells in its target tissues, primarily osteoblasts, osteocytes and renal tubular epithelial cells. The PTH-receptors are G-protein-linked and activate an intracellular signal cascade when PTH binds to them. The G-protein has alfa, beta and gamma components. The G-alfa unit is in its inactive state bound to guanosine diphosphate (GDP). When activated the GDP is exchanged to a magnesium bound guanosine triphosphate (Mg^{2+} GTP) and the alfa-unit dissociates from the PTH-receptor and links to an adenylate cyclase protein in the cell membrane. Together they convert a magnesium adenosine triphosphate (Mg^{2+} ATP) into a cyclic adenosine monophosphate (cAMP). The cAMP has the role of a second messenger and continues the signaling pathway in the cell to

finally activate a mechanism in the target cells which works to restore the Ca levels in the body (Goff, 2014).

Renal Ca reabsorption

When bound to the renal tubular epithelial cells PTH stimulates the reabsorption of Ca from proximal renal tubular fluids. Though this system is efficient for small Ca deficiencies the renal reabsorption is not large enough to compensate the Ca losses of milk producing dairy cows since only small amounts of Ca are lost in the urine (Goff, 2014).

Bone Ca resorption

A large part of the Ca in the body of the animal is a part of the skeleton. The Ca in the bones is stored in two forms. The major part is bound to the collagen matrix in hydroxyapatite crystals, generally made up of ten calcium (Ca^{2+}), six phosphate (PO_4^{3-}) and two hydroxyl ions (OH^-). The Ca in these crystals can only be liberated through the enzymatic degradation by osteoclasts (Goff, 2014). The osteoclasts do not have receptors for PTH and need to be activated by cytokines released by osteoblasts. The release of these cytokines occurs when PTH binds to osteoblasts (Hoorn & Zietse, 2013). The differentiation of osteoclastic progenitor cells into osteoclasts is stimulated by PTH. The complete osteoclastic activation takes several days (Goff, 2014).

Apart from the hydroxyapatite crystals, a smaller, though critical amount of Ca is stored in the bone fluid inside the canaliculi and lancunae of the bone. When PTH binds to the receptors on the osteocytes in this compartment, Ca is pumped from the bone fluid to the extracellular fluid (Goff, 2014).

Intestinal absorption

Even though the resorption of Ca from the bones can raise the blood Ca a good amount, absorption of Ca in the gastrointestinal tract is essential. The absorption in the intestines can be transcellular or paracellular (Goff, 2014).

The transcellular absorption is regulated by vitamin D. The vitamin D required is either absorbed in the gastro-intestinal tract in form of vitamin D_2 from forages or produced as vitamin D_3 in the skin of the animal when exposed to ultraviolet light (Nelson *et al.*, 2016). The vitamin D has to be converted into a hormonal version to be able to work as a signal molecule. After being either absorbed through the feed or synthetized in the skin, vitamin D travels to the liver through the blood stream where it is hydroxylated into 25-OH vitamin D. To become the hormonal version it needs to be hydroxylated a second time in the kidney. The kidney hydroxylates it to 1,25-dihydroxy vitamin D (1,25$(OH)_2$ vitamin D) when stimulated by PTH. Once the kidney has converted it into 1,25$(OH)_2$ vitamin D, it is released into the blood stream and works as a steroid hormone (Goff, 2014). When binding to a vitamin D receptor (VDR) in the nucleus of a target cell, 1,25-$(OH)_2$ vitamin D stimulates an upregulation of the genes transcribing the proteins needed for calcium transport. The VDRs exist in the majority of the enterocytes along the gastro-intestinal tract. The transcellular absorption is divided into three steps, which are all believed to be vitamin D-dependent. The first step of the absorption is the entry of Ca in the enterocyte from the intestinal lumen. The production of transport proteins that allow the passage of Ca through the apical membrane is stimulated by 1,25-$(OH)_2$ vitamin

D. Since the concentration of Ca is higher in the intestinal lumen than inside the cell no active transport is required as the Ca travels down the concentration gradient. When the Ca has entered the cell it is bound to a protein called calbindin-D_{9k} which transports the Ca to the basolateral membrane of the enterocyte. Calbindin is also vitamin D-dependent. Once the Ca is transported to the basolateral membrane it needs to be actively pumped out of the cell since the concentration of Ca is higher in the blood. The vitamin D regulated transport protein, Ca-ATPase, uses ATP while transporting Ca out of the cell (Christakos, 2012).

Calcium is additionally absorbed from the intestinal lumen paracellularly. This passive absorption only occurs when the concentration of soluble Ca is greater in the intestinal lumen than in the extracellular fluid. The soluble Ca passes in between the enterocytes, across the tight junctions (Christakos *et al.*, 2011). The paracellular Ca absorption mechanism is not considered to be vitamin D dependent, although there are studies that suggest that vitamin D might have a role in both the transcellular and paracellular Ca absorption. Further research needs to be done based on this hypothesis (Christakos, 2012).

Phosphorus homeostasis

Phosphorus (P) is an important part of the body being a component of phospholipids, phosphoproteins, nucleic acids and adenosine triphosphate (ATP). Phosphorus is the second major element of bone apart from Ca (Goff, 2000). The phosphorus homeostasis is, similarly to the Ca homeostasis, controlled by PTH and vitamin D. When phosphorus is high it has an inhibitory effect on the renal hydroxylation of 25-OH vitamin D and therefore a negative effect on the mechanisms controlling the Ca homeostasis (Goff, 2014). For this reason it is important to understand the mechanisms behind phosphorus regulation since it can have a secondary effect on the Ca homeostasis. The concentration of inorganic P is 4-8 mg/dL (1.3-2.6 mmol/L), which equals approximately 1-2 g of P in the total blood plasma of an adult 500 kg cow. In the extracellular compartments the amount of inorganic P is approximately 4-7 g. Similar to Ca, a large amount P is lost when the cow is growing a fetus and producing milk. Approximately 1 g of P is lost for the production of one liter of milk. To control the phosphorus levels in the body of the cow there are well developed mechanisms (Goff, 2000).

Intestinal absorption

The major phosphorus intake happens in the small intestine. Similar to the Ca absorption there is both an active and a passive absorption through the intestinal wall. Unlike the Ca absorption the passive transport stands for the major P absorption and requires the P levels of the luminal content to be high (Horst, 1986). The active absorption mechanism for phosphorus is similarly to Ca absorption stimulated by 1,25-dihydroxyvitamin D. Although PTH is needed to stimulate the hydroxylation of 25-hydroxyvitamin D to 1,25-$(OH)_2$ vitamin D in the kidney for the Ca homeostasis, PTH does not need to be involved in the phosphorus homeostasis. This is because low blood phosphorus directly stimulates the hydroxylation producing 1,25-$(OH)_2$ vitamin D in the kidney without involvement of PTH (Tanaka & Deluca, 1973).

Phosphorus in saliva

A large amount of phosphorus, around 30-90 grams, is excreted in the saliva every day. The purpose of this is to buffer the ruminal pH and provide the microbes with phosphorus needed

for cellulose digestion (Goff, 2000). The salivary P mixes with the ingested material in the rumen and is partly reabsorbed in the small intestine together with the dietary P (Horst, 1986).

The phosphorus concentration of the saliva increases under the influence of PTH. Therefore when PTH levels in the blood are high the phosphorus secretion through saliva increases (Wright *et al.*, 1984). For this reason it is not uncommon that cows suffering from hypocalcaemia develop a secondary hypophosphatemia. The amount of P lost through the saliva also depends on the daily rumination length since longer periods of mastication leads to a larger salivary secretion (Goff, 2000).

Bone phosphorus

In a state of hypocalcaemia bone is resorbed to liberate Ca. As earlier described the degradation of the hydroxypaptite crystals not only liberates Ca, but also phosphate ions. The osteoclastic degradation is secondarily stimulated by PTH (Goff, 2014). Since PTH secretion is not triggered by hypophosphatemia the liberation of the skeletal phosphorus needs to be triggered by a hypocalcaemia and PTH secretion (Goff, 2000).

Hypophosphatemia

Cattle fed a diet containing marginal levels of phosphorus for a long period of time will risk suffering from chronic hypophosphatemia with blood phosphorus levels of 0.6-1.1 mmol/L. A chronic hypophosphatemia causes a reduced growth, inappetence and a reduced milk production. In the time surrounding calving, cows are in the risk of suffering from acute hypophosphatemia with blood phosphorus levels dropping below 0.3 mmol/L. Clinical signs include recumbent cows though they are alert and will continue to eat if feed is offered. It is not uncommon that the lack of phosphorus is concurrent with hypocalcaemia or hypomagnesemia (Goff, 2000).

Risk factors for milk fever

Milk production

A high milk production is a risk factor for parturient paresis (Bendixen *et al.*, 1987a). Since every liter of milk requires a certain amount of Ca, a larger production leads to a greater Ca loss, increasing the risk of milk fever (Horst *et al.*, 1997). Saborio-Montero *et al.* (2017) studied 68,870 cows from 126 Costa Rican dairy herds and showed that cows that had a 305-day corrected milk yield that was 1,500 kg higher than the mean breed production had higher odds of developing milk fever (OR=2.39). Chiwome *et al.* (2017) studied the incidence of milk fever in a Zimbabwean dairy farm with 417 Holstein and 181 Jersey cows. In both breeds a higher milk production caused a significantly increased risk of milk fever. Holstein cows with a 305-day production >9,149 liters had a milk fever incidence of 10.49% compared to a 2.62% incidence in cows of the same breed producing 7,543-9,149 liters of milk. In Jersey cows the incidence in the highest producing cows (>6,114 L 305-d lactation) was 27.02% compared to cows producing 4,828-6,114 L of milk that had an incidence of 9.86%. The 305-day milk yield in this project was not based on energy corrected milk (ECM) and the exact production numbers can therefore not be used for comparison. The study clearly shows that a high production equals an increased risk of cows suffering from milk fever. Although it is important to point out that

the statistics were not corrected for age which could be linked to higher production and potentially higher milk fever incidence.

Age

Increasing age has been shown to be a risk factor for hypocalcaemia and milk fever (Thompson *et al.*, 1983; Dohoo *et al.*, 1984; Curtis *et al.*, 1985). One of the reasons for this is that multiparous cows generally have a higher milk production (Horst *et al.*, 1997). Gröhn *et al.* (1989) calculated the odds ratio for parity numbers 2, 3-4, 5-6, >6 and got the result of 1.0, 9.7, 27.4 and 31.3 respectively, showing that age and increased parities clearly augments the risk for parturient paresis. What is important to remember is that Gröhn *et al.* (1989) corrected their statistical model to individually evaluate every examined risk factor after accounting for all other factors. Higher parity and a larger milk production were thus evaluated separately, and the results showed that not only the greater milk production is the reason behind the higher milk fever incidence with increasing parity.

Another theory behind the reason of older cows being more prone to develop milk fever is that they have a lower number of active cells in the bone, making older animals less effective in mobilizing Ca from the skeleton (Goff, 2000). In other species a change in the Ca homeostasis has been observed. It has been shown that ageing rats had a downregulation of PTH receptors in the renal tissues (Hanai *et al.*, 1990). Furthermore the amount of $1,25(OH)_2$ receptors in the intestine of rats have been studied showing that young rats had a significantly higher number of receptors and a larger potential of upregulating these when stimulated by the vitamin D (Horst *et al.*, 1990). These studies show a decreased tissue responsivity to PTH in ageing animals which possibly could be the case in cattle as well.

Breed

There are studies that report a difference in milk fever incidence between cow breeds. One study found that Jersey cows were at 4.96 times higher odds of suffering from milk fever than Holstein cows, and that Jersey-Holstein mixed breed cows had a 2.44 times higher odds of suffering from milk fever compared to a purebred Holstein (Roche & Berry, 2006). In a case-control study it was shown that Jersey cows had a clearly higher risk (OR=5.35) of suffering from hypocalcaemia than Holstein cows of the same age (OR<1). Each breed was compared to the mixed pool group of the other breeds (Erb & Martin, 1978). In yet another study it was shown that Jersey cows had an OR=3.04 compared to the Holstein that had an OR=1.61 of suffering from milk fever (brown Swiss was used as reference breed, OR=1) (Saborio-Montero *et al.*, 2017). Chiwome *et al.* (2017) similarly observed a significantly ($P < 0.05$) higher incidence of milk fever in Jersey cows (14.78%) compared to Holstein cows (4.82%). The reason for Jersey cows being more prone to develop milk fever remains unknown. Though Goff (2000) mentions that preliminary data from their laboratory suggests that Jersey cows have less receptors for $1,25(OH)_2$ vitamin D in their intestines than Holstein cows.

The Ca content in milk is either in form of free calcium salts or bound to micellar structures as a part of the milk protein casein. Approximately two thirds of the Ca content in milk is bound to casein (Holt, 2004). The majority of the protein in milk is in form of casein. The amount of casein has been seen to differ between breeds. One study showed that Holstein cows have an

average casein content of 2.53% whereas Jersey cows have an average of 3.39% (Cerbulis & Farrell, 1975). Cerbulis and Farrell (1976) studied the total Ca concentration in the milk of 151 cows and compared the concentrations between breeds. They observed that Jersey cows had the highest Ca concentration with a mean of 1.46 g/L compared to the overall average of 1.25 g/L. The higher casein content and therefore Ca content of the milk could potentially be a reason why Jersey cows are more prone to develop milk fever.

Bendixen *et al.* (1987a) compared the incidence of milk fever in Swedish Red breed (SRB) and Swedish Holstein breed (SH), the two most common dairy cow breeds in Sweden at that time. They observed that SRB had a significantly higher risk of developing milk fever than the SH breed, with a risk ratio of 1.4. However, VÄXA (2018) reported a milk fever incidence of 1.7% in SRB and 2.5% in SH in the milking year of 2017/18 (September-August).

Body condition scores

Cows are under constant changes in their metabolism when switching between milk production, pregnancy and dry periods. Depending on what stage they are in they require a different amount of energy. To balance the energy requirements they need to mobilize and manage their body reserves. By controlling the feed given, the farmers can regulate the cows' reserves. To be able to judge the requirements of a certain cow, body condition scoring (BCS) has become a popular tool (Roche *et al.*, 2004). There are several different body condition scoring systems used around the world. The scoring used in the United States and Ireland are based on a five point scale while the Australian system is based on an eight point scale and the New Zealand scale is ten point based. Further differences are that in the New Zealand and Ireland scoring systems palpation is included while the others are solely visual. What all scales have in common is that the lowest score on the scale equals emaciation and the highest score indicates obesity. The body condition score (BCS) is based on evaluation of certain anatomical parts such as the spinal column, the ribs, the spinosus processes, the tuber sacrale, tuber ischia, the tail head and the thigh region (Roche *et al.*, 2004).

Depending on what BCS scale is used, the target body condition score at calving will differ. In Sweden the five point scale is most commonly used and a BCS of 3.5 is recommended at calving (VÄXA, 2017). In New Zealand the target BCS is 5.0/10 for mixed age cows and 5.5/10 for first and second calvers (Dairy NZ, 2011). In Australia the target BCS is between 4.5 and 5.5 on their eight point scale (Dairy Australia, 2013).

One of the risk factors for dairy cows suffering from hypocalcaemia and milk fever is a high BCS. One study showed that a cow with a high BCS (defined as 4 or higher on a 5 point scale) had a 3.3 times higher risk of suffering from milk fever than a cow with a BCS between 2 and 4 on the five point scale (Heuer *et al.*, 1999). Another study showed that cows with a pre-partal high BCS (5 out of a 10 point scale) had a higher initial milk production and at the same time a higher negative energy balance than BCS 4 cows (Roche *et al.*, 2015). Not only the high BCS cows have an increased risk of milk fever. It has been shown that both cows with a high BCS (>3.5/5) or a low BCS (<2.5/5) have an increased risk of developing milk fever (Roche & Berry, 2006).

The reason for a high BCS causing a larger risk for cows developing parturient paresis has not been established. The higher milk yield in the first couple of days after calving causes a higher Ca loss, which is one of the theories behind the increased risk in high BCS cows (Roche *et al.*, 2015). Furthermore it has been observed that cows with a high BCS around calving have a lower dry matter intake (DMI) postpartum than cows calving with a more suitable, lower BCS. A lower dry matter intake is equal to a lower intake of nutrients such as calcium, causing these cows to be more prone to develop severe hypocalcaemia (Broster & Broster, 1998; Matthews *et al.*, 2012).

The gain or loss of BCS is correlated to energy intake and energy output, and there are several factors that affect the energy output and requirement. Gallo *et al.* (1996) found that the loss of BCS during lactation was higher in multiparous cows and high yielding cows. Similar findings regarding higher parity cows having a greater BCS loss was observed by Waltner *et al.* (1993). Though the loss of BCS during the lactational period happens when the major risk period for milk fever has passed, the change in BCS has the potential of affecting the subsequent lactation. As mentioned above, it has been shown that a high BCS around calving is a predisposing factor for parturient paresis, though it is important that the cow does not lose too much BCS during the dry period. Chebel *et al.* (2018) found that a loss of BCS during the dry period was a risk factor for health disorders, *e.g.* milk fever, and reduced productivity in the subsequent lactation. The authors point out that it is important that cows are dried off at a BCS of 3.25 or lower (out of a 5 point scale) to avoid that cows with a high BCS at dry-off need to lose condition during the dry period to have an optimal BCS at calving. The breed of the cow has also been shown to have an effect on the BCS. Kadarmideen and Wegmann (2003) found that an increase of the percentage of Holstein genes in the dairy cow caused a general decrease in BCS. Another study showed that purebred Holstein had a generally lower BCS than Holstein crossed with Norwegian Red. It was also observed that the Norwegian Red breed had a smaller variation in their BCS throughout the lactation period (Rinell & Heringstad, 2018). Friggens and Badsberg (2007) similarly showed that Danish Red breed had a smaller change in BCS during the lactation compared to both Danish Holstein and Jersey breed. The Danish Red breed had a generally higher BCS than Holstein and Jersey.

Season of calving

Roche and Berry (2006) studied several climatic situations and their potential effect on the incidence of milk fever. The authors were interested in the climate around calving and because of New Zealand's seasonal spring calving the winter months were mainly studied. Roche and Berry's interest in examining this was based on previous findings of Simesen (1974), stating climatic conditions as a potential risk factor for metabolic disorders. Simesen observed an increased risk of parturient paresis in case of precipitation during winter. Roche and Berry (2006) similarly found that there was an increase of milk fever cases in periods of rainfall. The reason behind this is not clear, though one theory is that it could be caused by a reduced DMI in these periods. Simesen's study was based in southeast Norway where there is a cold and dry inland climate. Dohoo *et al.* (1984) studied the occurrence of milk fever in 2,008 Holstein-Friesan cows over a 2.5 year period, totaling 2,875 lactations based in Ontario, Canada. The authors could not find a certain time of year when there was an increased risk of milk fever. These findings are in line with a previous study by Erb and Martin (1978) who also could not

find any seasonality for hypocalcaemia. Because of conflicting results, it is not clear whether seasons and weather have an impact on the incidence of parturient paresis. Saborio-Montero *et al.* (2017) analyzed the occurrence of milk fever in Costa Rican dairy cows in relation to month of the year. They found that cows had higher odds of suffering from milk fever in both May and July and the lowest risk in December. The reason behind this was not established but the authors theorized that it could be caused by a change of minerals in the grass at this time of year or the fact that rain season begins in these months and could affect the DMI. It is important to note that the climatic conditions in Costa Rica differ a lot from the climates in the other studies on milk fever and climate.

Dry period length

The dry period is the period between the end of the lactation and the subsequent calving. During this period the cow does not produce milk and the udder is given a chance to rest between lactations to enable the optimal udder health and production in the next lactation. The current recommendation in Sweden is a dry period length of around two months (VÄXA, 2017). Saborio-Montero *et al.* (2017) studied the incidence of milk fever related to the length of the previous dry period. They used a DPL of one month or less as reference category and observed increased odds of milk fever when the DPL was longer than one month. All categories (2, 3, 4, 5, >6 months) compared to the reference showed increased odds for parturient paresis. Andrée O'Hara *et al.* (2019) similarly studied different dry period lengths in relation to incidence of parturient paresis, but could not find any significant differences between the DPL groups (30-39, 40-49, 50-59, 60-69, 70-79 and 80-89 days).

Previous case of milk fever

It has been reported that cows that have suffered from milk fever in previous lactations are at greater risk of developing milk fever in the current lactation. In a review article, Erb and Gröhn (1988) found a 2 to 5 times higher chance of a cow developing milk fever if she had already undergone illness in a previous lactation. Roche and Berry (2006) found that the odds of parturient paresis were 2.2 times higher in cows that had previously suffered from milk fever. Saborio-Montero *et al.* (2017) similarly found that previous milk fever increased the risk of this disease in the next lactation. These cows were seen to be 2.35 times more likely to develop parturient paresis.

Previous lactation length

Some studies suggest that the previous lactation length has an effect on the incidence of milk fever or hypocalcaemia. Saborio-Montero *et al.* (2017) studied this but could not find a statistical association between the two. Valldecabres *et al.* (2019) studied subclinical hypocalcaemia in 598 Jersey purebred and 218 Jersey-Holstein crossbred cows. They identified the previous lactations length as a cow level factor affecting the incidence of subclinical hypocalcaemia. The authors observed that cows with a previous lactation length shorter than the 25th percentile (285 days) had a lower risk of developing subclinical hypocalcaemia. Even though this study was not focused on clinical hypocalcaemia it is interesting since it suggests that the previous lactation length has an effect on the Ca homeostasis in the subsequent lactation and could be a potential risk factor for milk fever.

Calving interval

The calving interval is the length between two calvings, the combination of the lactational period and the dry period. Since both the lactational length and the DPL have been discussed and thought of as potential risk factors or determinants for milk fever it is interesting to look at the combination of both of them. Saborio-Montero *et al.* (2017) studied the calving interval length in relation to incidence of parturient paresis but could not see a statistical association between the two. Valldecabres *et al.* (2019) could not find a link between subclinical hypocalcaemia and calving interval.

Hypomagnesemia

Hypomagnesemia is considered a risk factor for parturient paresis. One of the reasons for this is that a lack of magnesium causes a reduced tissue sensitivity to PTH (Rude, 1998). The theory behind this is that the signaling cascade in the cell activated by PTH requires magnesium ions in several steps. A lack of magnesium inhibits this signaling, dulling the tissue response to PTH (Rude, 1998). Van de Braak *et al.* (1987) compared the blood Ca levels around partus in cows fed a diet with low (0.2% Mg of the DM) and high (0.85% Mg of the DM) content of Mg. The Ca concentrations observed had a trend to be lower in the groups fed the low Mg diet, though it was only statistically significant 100-160 hours pre-partum. Though not statistically significant a higher incidence of milk fever was seen in the low Mg fed group (2/9 cows), compared to the high fed group where no cases were seen. Contreras *et al.* (1982) concluded that the Ca mobilization rate was significantly ($P<0.005$) slower in hypomagnesic steers (0.21 mmol/min) compared to normomagnesic ones (0.32 mmol/min). They studied the Ca mobilization in cows as well, which also showed a significant ($P<0.02$) difference between the hypomagnesic (0.26 mmol/min) and the normomagnesic ones (0.41 mmol/min).

Prevention of milk fever

Dietary cation-anion difference and its effect on Ca homeostasis

It has for a long time been suggested that a difference in the cation-anion concentration in the diet can affect the risk of milk fever in dairy cows. The theory behind calculating a cation-anion difference of the feed is based on the strong ion difference theory (Horst *et al.*, 1997). The strong ion difference theory is based on two principles. The first being that in any given solution the amount of cations will equal the amount of anions to equal each other's positive and negative charge. The second being the equation $[H^+] \times [OH^-] = 1 \times 10^{14}$, meaning that the product of the concentration of hydrogen and hydroxyl ions will equal the dissociation constant of water (K_w) (Stewart, 1983). The theory is founded on these two principles being true simultaneously. Therefore a change in ions in the blood, which are not H^+ or OH^-, will lead to a compensatory change in the concentration of hydroxyl and hydrogen ions to keep the total charge of anions and cations equal. Since the blood pH is defined as the negative logarithm of the concentration of hydrogen atoms the change in the concentration of H^+ will lead to a change in pH (Stewart, 1983).

The strong ion difference is based on the difference of the non-metabolizable dietary cations and anions absorbed into the blood stream. Even though all ions in the blood have an effect on the strong ion difference there are certain major dietary ions that have the largest impact. The cations being Na^+, K^+, Ca^{2+} and Mg^{2+} and the anions being Cl^-, S^{2-} and PO_4^{3-}. There are organic

acids that can have an effect on the strong ion difference, for example volatile fatty acids (VFAs), but since the liver rapidly metabolizes them their effect is negligible. There are situations when organic acids do have an effect on the pH, for instance when a cow suffers from metabolic acidosis with a build-up of lactic acid. The standard equation used for calculating the dietary cation-anion difference (DCAD) is $(Na^{+}+K^{+})–(Cl^{-}+S^{2-})$ and is measured in milliequivalents (mEq) (Goff & Horst, 2003).

Cows fed a diet with a high dietary cation-anion difference (DCAD) had a higher incidence of milk fever than cows fed a low DCAD diet (Ender *et al.*, 1971). Goff (2014) stated that a high DCAD diet in the period around partus (2 weeks pre- until 2 days post-partum) significantly increased the risk of the cow suffering from hypocalcaemia and milk fever, compared to a low DCAD diet. The cause of this, as explained by the authors, is that the metabolic alkalosis caused by a high DCAD diet reduces the tissue sensitivity to PTH. Because of the central role of PTH has in the Ca homeostasis the cow has a lesser ability of reversing a hypocalcaemic state. The theory of the authors is that the alkalosis causes a change in the tertiary structure of the G-linked protein receptor of PTH leading to a reduced response to the PTH secretion.

It has been shown that mechanisms following PTH stimulation, such as osteoclastic bone resorption and 1,25-$(OH)_2$ vitamin D-production, were enhanced in cows fed a low DCAD diet leading to a lower incidence of milk fever. The high DCAD diet group had a 26% incidence of milk fever, while the low DCAD diet group only had a milk fever incidence of 4% (Goff *et al.*, 1991). However Moore *et al.* (2000) did not see an increased osteoclastic bone resorption when feeding a low DCAD diet. Numerous other studies exist confirming the fact that a low DCAD prepartal diet reduces the postpartal hypocalcaemia and the risk of developing milk fever in dairy cows (Gaynor *et al.*, 1989; Moore *et al.*, 2000; Razzaghi *et al.*, 2012).

Potassium

As mentioned above, low DCAD diets have resulted in a reduced risk of milk fever. Potassium (K) is one of the cations contributing to a high DCAD diet and therefore increasing the risk of milk fever. Potassium is the cation that is the most common in the feedstuffs fed to cows (Horst *et al.*, 1997). Goff and Horst (1997) studied the effect of potassium on the incidence of milk fever and showed that high levels of potassium in the feed significantly increased the occurrence of milk fever. None of the cows fed the 1.1% K diet suffered from milk fever, while 36% of the cows fed the 2.1% diet and 80% of the cows fed the 3.1% diet did.

Pre-partum low Ca-diet

Several studies have reported that a low Ca diet before calving leads to higher Ca levels postpartum and a lower incidence of milk fever. Goings *et al.* (1974) compared twelve cows fed a low Ca diet (8 g/day) with 13 cows fed a control diet (either 52 g or 41 g Ca/day). None of the cows fed the low Ca diet suffered from milk fever, although five cows in the control group were treated for parturient paresis. Green *et al.* (1981) compared the effect of a low (8 g/day) and a high (80 g/day) Ca diet. Prepartum the Ca and P levels in the blood were lower in the group fed a low Ca diet. Peripartum the Ca blood levels of the group fed the low Ca diet were higher than the group fed the high Ca diet. Trends of higher hydroxyproline (indicating higher Ca bone resorption) and higher 1,25$(OH)_2$ vitamin D were seen in the group fed a low

Ca diet. The theory behind these results is that the low pre-partal Ca diet upregulates the Ca metabolism before calving avoiding the lag phase of Ca metabolism the first days postpartum when the Ca requirements are high. This diet is strictly before calving. Post-partum a sufficient Ca amount in the feed is required. Though Green *et al.* (1981) did observe an effect of a low pre-partal Ca diet, Kronqvist (2011) did not see a difference in milk fever incidence in groups fed different pre-partal Ca diets.

Ca:P Ratio

The Ca homeostasis is affected by many factors and it has been suggested that the ratio between Ca and P in the diet is linked to the incidence of parturient paresis. Though several studies show statistically significant results many of them are contradictive and it is hard to conclude whether the ratio matters or if the total Ca and P is the important factor. Gardner and Park (1973) saw a milk fever incidence of 2% in 97 cows fed a Ca:P ratio of 2.3:1, while the incidence was 19% in a 1.8:1 ratio, 8% in 1.5:1 ratio and 13% in a 1.7:1 ratio. Beitz *et al.* (1974) showed a higher incidence of milk fever in 12 cows fed a 2.3:1 Ca:P (75%) diet than in 14 cows fed a 1.1:1 Ca:P diet (50%).

Jorgensen (1974) discusses the usage of an optimal Ca:P balance in the pre-/peri-partal diet to reduce the risk of milk fever. When reviewing multiple studies he did not find a particular ratio that would be preferable. His conclusion was that to help maintain an adequate Ca and phosphorus homeostasis the cow should be fed enough P for her requirements. No more than 100-125 grams of Ca per day should be given to the cow because this, according to the author, causes a failure/downregulation of the Ca homeostasis.

Peri-partal Ca addition

Since the Ca need increases around calving a preventive measure for hypocalcaemia and milk fever is oral addition of Ca one or two days pre- or post-partum (Horst *et al.*, 1997). When distributing Ca orally the goal is to achieve a high concentration of Ca in the gastrointestinal tract to increase the passive Ca-transport (Goff, 1999). Several formulas of oral Ca exist and they each have advantages and disadvantages (Horst *et al.*, 1997). Goff and Horst (1997) compared several Ca preparations and their effects on blood Ca. The two most efficient preparations were calcium chloride ($CaCl_2$) and calcium propionate. Calcium chloride was absorbed most efficiently and resulted in a higher increase in plasma Ca than calcium propionate. The negative effect of the $CaCl_2$ is that it has an anionic effect and in large quantities it can cause a metabolic acidosis. Another problem with $CaCl_2$ is that it is not very palatable. Calcium propionate on the other hand does not pose the same risk of acidosis and is more palatable. Its disadvantage is that it has to be ingested in large quantities of water (800 ml for 50 g of Ca) because of its low solubility (Goff & Horst, 1993). Calcium chloride does not only carry the risk of causing a metabolic acidosis. Since it is highly caustic it has been shown that it can cause severe necrosis in the mucosa in the forestomaches. The necrotic effect depends on the preparation and the amount administered (Wentink & van den Ingh, 1992).

Pehrson *et al.* (1998) compared the effect of $CaCl_2$ and calcium propionate as a preventive measure for milk fever. The study showed that both treatments led to a lowered incidence of

milk fever. The authors concluded that calcium propionate is the favorable treatment choice since there are not the same reported negative effects as for $CaCl_2$ salts.

Vitamin D supplementation

When the importance of vitamin D for the Ca homeostasis was discovered there were theories that an addition of vitamin D could prevent milk fever. However it has been shown that the administration of exogenous vitamin D has the opposite effect. Because of its inhibitory effect on the production of endogenous vitamin D the exogenous vitamin D resulted in episodes of hypocalcaemia and milk fever later in the lactation (Horst *et al.*, 1997). Though in systems where dairy cows are kept inside a large portion of the year and not exposed to sunlight supplementation is required to replace the lack of production of vitamin D in the skin (Nelson *et al.*, 2016).

Treatment of milk fever

The most effective treatment for milk fever is supplying Ca salts intravenously. The most common Ca salt is calcium borogluconate. The standard commercial solutions contain 8.5-11.5 grams of Ca/500 mL and the most effective dosage is considered to be 2 grams/100 kg of BW. Usually the preparations also contain magnesium, phosphorus and dextrose. When administering Ca intravenously it is important to not do it too fast because a rapid infusion can lead to arrythmias and cardiac arrest. To avoid this it is recommended to administer Ca at a rate of 1 gram/minute. If not given intravenously most Ca solutions can be injected subcutaneously, though the uptake is not as guaranteed since the blood flow in the peripheral tissues can vary. There are certain specific Ca solutions that can be given intramuscularly. The limitation of this type of administration is that only 0.5-1.0 grams of Ca should be injected at one spot to avoid tissue necrosis, requiring 6-10 injections. The injections can impact the meat quality, why intramuscular injections are not common. Oral administration of Ca can be an effective preventive measure against milk fever, but is not recommended for the treatment of clinical cases (Goff, 2008).

Effects of milk fever

Ketosis

Curtis *et al.* (1985) showed that milk fever was the second strongest disease factor leading to ketosis with an OR=23.6. Bendixen *et al.* (1987b) did an epidemiological study on both SRB and SH, and found an increased risk of cows suffering from ketosis if previously exposed to milk fever in SRB (RR=1.3). Swedish Holstein breed did not have a significant link between parturient paresis and ketosis. Although simultaneous exposure to parturient paresis and retained placenta increased the risk of ketosis in both breeds (RR=1.8 for SRB and RR=2.8 for SH). Gröhn *et al.* (1989) conducted a large epidemiological study on 41,989 multiparous cows with ketosis showing that parturient paresis is a predisposing factor (OR=1.6). Though there are several studies linking milk fever and ketosis it is more probable that parturient paresis is a risk factor for ketosis and not the other way around. The connection between parturient paresis and ketosis is most likely caused by the decreased feed intake of a cow suffering from milk fever leading to a negative energy balance (Curtis *et al.*, 1985; Gröhn *et al.*, 1989).

Uterine and reproductive disorders

Erb *et al.* (1985) found that milk fever increased the risk of several reproductive disorders. The OR of a cow suffering from dystocia requiring vet assistance was 4.2 when concurrently affected by milk fever. Parturient paresis was also seen to be a risk factor for cows developing metritis (metritis, endometritis or pyometra) with an OR of 1.6. A previously hypocalcaemic cow was also showed to have increased odds of having a retained placenta (OR=2.0). Milk fever can indirectly increase the risk of culling because cows suffering from dystocia, metritis or retained placenta have an increased risk of poor reproductive performance (Erb *et al.*, 1985). Curtis *et al.* (1985) also observed an increased risk of vet-assisted dystocia (OR=7.2) and retained placenta (OR=4.0) in cows previously diseased with milk fever.

Milk yield

It has been discussed that the milk yield can be affected after an episode of milk fever. Rajala-Schultz *et al.* (1999) examined the milk production in cows after recovering from milk fever. They found that cows affected by milk fever had a lower milk production the first 4 to 6 weeks after calving, with 1.1-2.9 kg per day. The loss of milk was calculated by comparing the production of the milk fever cow with a healthy cow or to the milk production of the same cow more than 8 weeks after the milk fever diagnosis. Though there is a loss in the first couple of weeks it is important to point out that the cows affected by milk fever in general had a higher milk production than non-affected cows, with 1.1-1.7 kg per day. One theory behind this is that high producing cows are more likely to develop milk fever.

Milk production in Sweden

During most of the year cows in Sweden are kept inside in barn systems and therefore not directly affected by the weather. Though during the summer months milk producers are required, by law, to keep the cows on pasture during at least 6 hours per day. Depending on where in Sweden the farm is the pasture period is between two and four months (VÄXA, 2017).

The two most common dairy cow breeds in Sweden are Swedish Red breed (SRB) and Swedish Holstein breed (SH). In the milking record year of 2017/18 56.3% of the reported cows were SH and 34.5% were SRB (VÄXA, 2019). Table 1 represents the average yearly milk production by the individual dairy cow in Sweden in the years 2016 through 2018. Statistics were collected from the Swedish Board of Agriculture (Jordbruksverket, 2018). It is important to point out that the milk quantities are not energy corrected. The production in Sweden is high compared to the average production in for example New Zealand where the average production was 4,151 liters per cow and year in the milking year of 2017/18 (LIC & Dairy NZ, 2018). Though the Swedish production is slightly lower than that of the USA where an average production of 10,394 kg of milk per cow and year was reported in 2017 (USDA, 2019).

Table 1. *Milk production of the Swedish dairy cow, years 2016-2018 (Jordbruksverket, 2018)*

	2016	2017	2018
1,000 tons of milk delivered[a]	2,862.23	2,816.66	2,760.23
Number of dairy cows	330,833	322,010	319,387
Average milk production in kg/cow and year	**8,652**	**8,747**	**8,642**

[a]*Delivered to the dairy company*

MATERIAL AND METHODS

Data collection

Data was collected from the Swedish official milk recording scheme managed by VÄXA Sverige. Herds with at least 40 calvings during the year 2015/16 (September of 2015 through August of 2016) and that had 20% of SH and 20% SRB were included in the data set. Individual cow data of these herds was retrieved for the years of 2006/07 through 2015/16. The years were defined as September through August the following year. Variables were grouped as individual cow factors or management related factors. Individual cow factors consisted of age, parity, breed, incidence of milk fever and incidence of milk fever in the previous lactation. Furthermore individual milk production was included; amount of ECM produced in the previous lactation, amount of fat and protein produced in the previous lactation, herd-breed averages for ECM/fat/protein production and protein production in the last test milking in the previous lactation. Management related factors included season of calving, calving interval, previous lactation length and previous dry period length (DPL).

Data coding

Milk fever was coded as 0, 1; milk fever not present (0) or present (1) during the 8 days pre and post calving. Breeds were coded as 1, 2 or 9; purebred SRB (1), purebred SH (2) and the miscellaneous other breeds and mixed breeds (9). Parities were coded as 1, 2, 3, 4, 5, ≥6; first (1), second (2), third (3), fourth (4), fifth (5) or sixth or above (≥6). The calving dates were divided into four seasons coded 1, 2, 3 or 4; March-May (1), June-August (2), September-November (3) or December-February (4). Milk fever in the previous lactation was coded as 0, 1; milk fever not present in previous lactation (0) or milk fever present in previous lactation (1). The dry period length was coded as 0, 1, 2, 3, 4 or 5; <30 days (0), 30-49 days (1), 50-69 days (2), 70-89 days (3) and 90-109 (4) or 110-129 (5). The data was divided into three groups based on the length of the previous lactation. Each group contained a third of the data and the limits were based on the division of groups; <296 days (1), 296-335 days (2), >335 days (3). The data was divided into three groups depending on the previous calving interval. Each group containing a third of the data and limits based on the division of groups; <359 days (1), 359-401 days (2), >401 days (3). The data was divided into three groups depending on the difference in production of kg ECM in the previous lactation compared with the herd-breed average. Each group containing a third of the data and limits based on division of groups; <-664 kg (1), -664-<700 kg (2), ≥700 kg (3). The data was divided into three groups depending on the difference in production of kg fat plus kg protein in the previous lactation compared with the herd-breed average. Each group containing a third of the data and limits based on division of groups; <-49 kg (1), -49-<52 kg (2), ≥52 kg (3). The data was divided into three groups depending on the

difference in production of kg fat in the previous lactation compared with the herd-breed average. Each group containing a third of the data and limits based on division of groups; <-28 kg (1), -28-<28 kg (2), ≥28 kg (3). The data was divided into three groups depending on the difference in production of kg protein in the previous lactation compared with the herd-breed average. Each group containing a third of the data and limits based on division of groups; <-22 kg (1), -22-<23 kg (2), ≥23 kg (3). The data was divided into three groups depending on the protein percentage of the milk in the last test milking in the previous lactation. Each group containing a third of the data and limits based on the division of these groups; <3.7% (1), 3.7-<4.1% (2), ≥4.1% (3).

Modelling technique

A causal diagram was created to model the hypotheses for potential determinants of milk fever. Arrows were drawn between different determinants where links could be expected. Each arrow representing a hypothesis. The hypothesis model used in this study is represented in Figure 1. All paths where a correlation could be expected were drawn, even though the data did not exist to test the hypotheses.

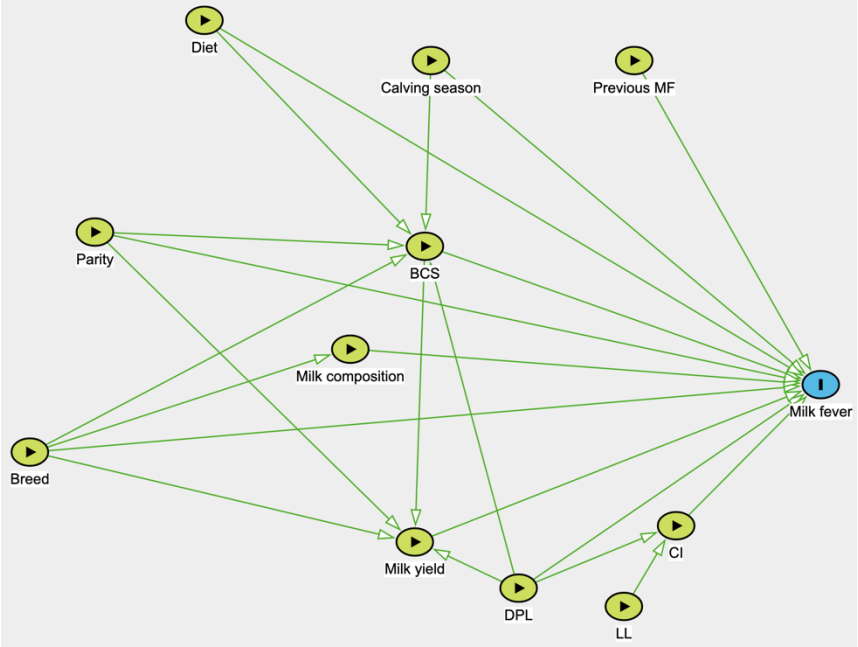

Figure 1. *Causal diagram of potential determinants of milk fever. Each arrow to the outcome (milk fever) representing an individual hypothesis. BCS = Body condition score, DPL = Dry period length, MF = Milk fever. LL = Previous lactation length, CI = Previous calving interval.*

Statistical analysis

All statistical analyses were performed using Minitab 18. The proportion function was used to calculate incidences and their confidence intervals (CI) of milk fever in the different years and for the studied risk factors. Frequencies of milk fever were tabulated for each potential risk factor and associations between risk factors and milk fever were subsequently analyzed using binomial logistic regressions. Odds ratios (OR) and their CI:s were calculated for each category of each risk factor.

RESULTS

General statistics

Herd size characteristics of the included farms are presented in Table 2. What is most striking with these numbers is that the maximum herd size almost doubled over the course of the studied ten years. Apart from the maximum herd size increasing, the average herd size increased with almost 50% over the studied years.

Table 2. *Average, largest (Max) and smallest (Min) number of calvings per year in the herds studied from 2006/07 through 2015/16*

Year	Average	Min	Max
2006/07	85	41	487
2007/08	87	41	527
2008/09	93	41	507
2009/10	99	42	547
2010/11	102	41	717
2011/12	106	41	770
2012/13	110	42	711
2013/14	114	41	740
2014/15	116	41	785
2015/16	115	41	826

The breed composition throughout the years in our sample is presented in Figure 2. As showed SRB was the most common breed until the final control year, where SH became the dominating one.

Figure 1. *Breed representation, number of cows, in the studied herds from 2006/07 through 2015/16. The mixed group consists of all mixed breed cows and purebred cows of other breeds than SRB or SH.*

Figure 3A shows the average kg ECM produced by each breed in the years studied. Figure 3B shows the average total kg of fat and protein produced by each breed group in the years studied. As shown SH have a generally higher total production of both quantity of milk and of fat and protein than both SRB and the mixed breed group. There is a similar increase in production seen over the years in all three breeds. Swedish Holstein cows produced between 4-7.5% more than the mixed breed group and 4.5-8.5% more than the SRB group over the years.

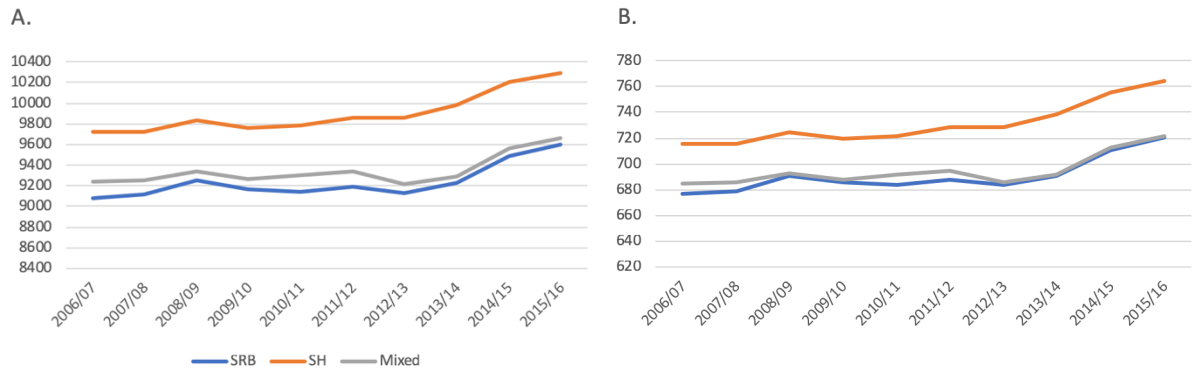

Figure 3. *Average kg ECM (A) and average total kg of fat and protein (B) produced by breed from 2006/07 through 2015/16.*

Milk fever over the years

Figure 4 is a boxplot representing the herd incidences of milk fever in the studied dairy herds in the years of 2006/07 through 2015/16. The maximum incidences varied between 13 and 23%. For all years, the minimum prevalence was 0%. The median values of milk fever varied between 2.06 and 3.12%.

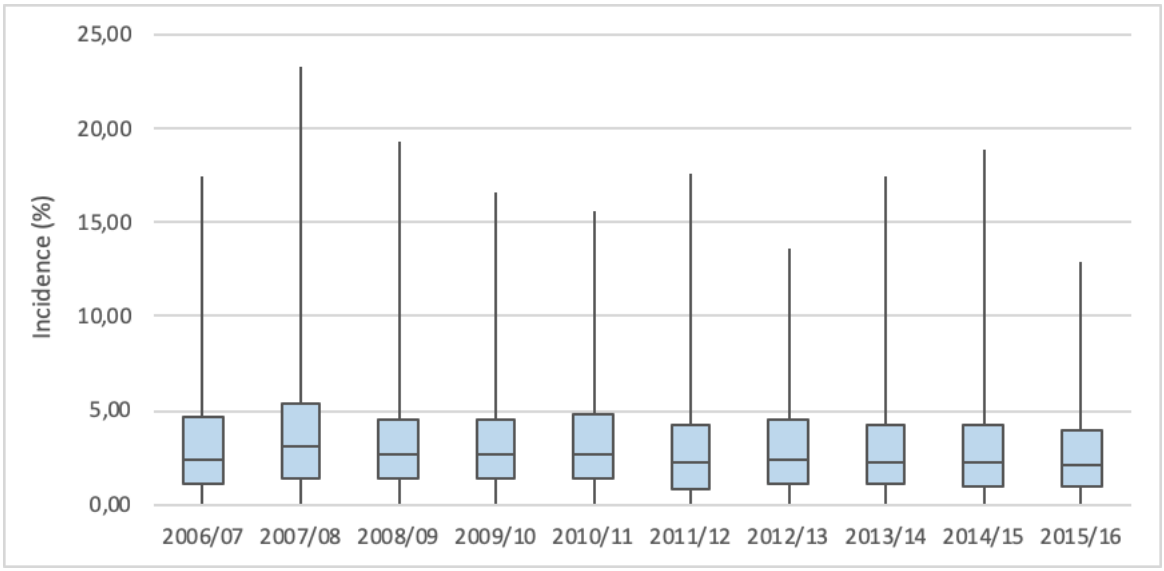

Figure 4. *Within-herd incidence of milk fever in the studied Swedish dairy herds in the control years of 2006/07 through 2015/16. Number of herds per year varied between 388-455.*

Results from the analyses of milk fever incidence over the years 2006/07 until 2015/16 are presented in Table 3. A general trend of a decreasing incidence is seen over the years. The odds ratios for milk fever are significantly lower in 2014/15 ($p<0.05$) and 2015/16 ($p<0.001$) compared to the year of 2006/07. No other years have a statistically significant lower incidence of milk fever compared to 2006/07.

Table 3. *Milk fever incidence in the control years of 2006/07-2015/16.*

Year	Number of calvings	Milk fever cases	Incidence (%)	95% CI[a] incidence	OR[b]	95% CI OR	P-value
2006/07	32878	969	2.95	(2.77; 3.14)			
2007/08	36833	1264	3.43	(3.25; 3.62)	1.17	(1.07; 1.27)	<0.001
2008/09	38715	1216	3.14	(2.97; 3.32)	1.07	(0.98; 1.16)	0.133
2009/10	42591	1273	2.99	(2.83; 3.16)	1.01	(0.93; 1.10)	0.738
2010/11	44311	1396	3.15	(2.99; 3.32)	1.07	(0.99; 1.16)	0.105
2011/12	45899	1278	2.78	(2.64; 2.94)	0.94	(0.87; 1.03)	0.176
2012/13	48934	1445	2.95	(2.80; 3.11)	1.00	(0.92; 1.09)	0.962
2013/14	50773	1414	2.78	(2.64; 2.93)	0.94	(0.87; 1.02)	0.168
2014/15	51846	1380	2.66	(2.52; 2.80)	0.90	(0.83; 0.98)	0.014
2015/16	52433	1327	2.53	(2.40; 2.67)	0.86	(0.79; 0,93)	<0.001
Total	445213	12962	2.91	(2.86; 2.96)			

[a]*CI = Confidence Interval.* [b]*OR = Odds ratio for milk fever compared to 2006/07.*

Cow level factors

Results from the breed analyses showed significantly ($p<0.001$) increased odds of SH being affected with milk fever compared to SRB. No increased odds of the mixed breed group was seen compared to SRB (Table 4). Analysis of parities showed a clearly increased odds of milk fever in higher parity cows. All parities, second or above, had a significantly ($p<0.001$) higher odds of developing milk fever compared to first parity cows (Table 4). Cows from the parity group of ≥6 had an OR of 18.8 ($p<0.001$) of developing MF compared to second parity cows. In Table 4 the results from the analysis of the effect on the incidence of milk fever if the cow was affected by milk fever in her previous lactation are presented. There is a clear statistically significant ($p<0.001$) higher odds of a cow suffering from milk fever if she was affected by this in her previous lactation.

Table 4. *Incidence of milk fever and odds ratios depending on breed, parity and milk fever in previous lactation*

Risk factor	Milk fever cases	Number of calvings	Incidence (%)	95% CI[a] incidence	OR[b]	95% CI OR	P-value
Breed							<0.001
SRB	4149	180008	2.30	(2.24; 2.38)	1		
SH	5168	147653	3.50	(3.41; 3.60)	1.54	(1.47; 1.60)	<0.001
Mixed	1350	57302	2.36	(2.23: 2.48)	1.02	(0.96; 1.09)	0.479
Missing[d]		*60571*					
Parity							<0.001
1	98	164108	0.06	(0.05; 0.07)	1		
2	1071	122465	0.87	(0.82; 0.93)	14.77	(12.00; 18.16)	<0.001
3	3272	80177	4.08	(3.95; 4.22)	71.20	(58.23; 87.07)	<0.001
4	4001	44404	9.01	(8.75; 9.28)	165.73	(135.59; 202.56)	<0.001
5	2608	20630	12.64	(12.19; 13.10)	242.19	(197.84; 296.48)	<0.001
≥6	1912	13429	14.24	(13.65; 14.84)	277.84	(226.60; 340.67)	<0.001
Missing[d]		*321*					
Previous MF[c]							<0.001
No	10605	260613	4.07	(3.99; 4.15)	1		
Yes	1572	5377	29.24	(28.02; 30.47)	9.74	(9.16; 10.36)	<0.001
Missing[d]		*179544*					

[a]CI = Confidence Interval. [b]OR = Odds ratio for milk fever. [c]Previous MF = Milk fever in the previous lactation. [d]Missing = Number of calvings where information about the risk factor was lacking.

Management related factors

Results from analyses of factors affecting the incidence of milk fever, classed as management related factors are presented in Table 5 and 6. The previous lactation length had an effect on incidence of milk fever ($p<0.001$). The lowest incidence of milk fever was observed in the group with the shortest lactation length, <296 days. Increasing the lactation length increased the odds of milk fever. The highest odds were seen with a lactation length >335 days (Table 5). Analysis of different dry period lengths showed that there was significant difference of milk fever incidence between the groups ($p<0.001$). The lowest incidence of milk fever was observed in the group with the shortest DPL (<30 days). The highest odds of developing milk fever and the highest incidence of milk fever was seen in cows that had a dry period length of 70-89 days. Both cows with a longer or shorter DPL than 70-89 days showed a lower incidence of and lower odds of developing milk fever (Table 5).

Table 5. *Incidence of milk fever in current lactation and odds ratios depending on previous lactation length and dry period length*

Risk factor	Milk fever cases	Number of calvings	Incidence (%)	95% CI[a] incidence	OR[b]	95% CI OR	P-value
Previous LL[c]							<0.001
<296	2417	60274	4.01	(3.85; 4.17)	1		
296-335	2938	61295	4.79	(4.63; 4.97)	1.21	(1.14; 1.27)	<0.001
>335	3252	61318	5.30	(5.13; 5.48)	1.34	(1.27; 1.41)	<0.001
Missing[e]		*262647*					
Previous DPL[d]							<0.001
<30	34	1621	2.10	(1.46; 2.92)	1		
30-49	1042	25227	4.13	(3.89; 4.38)	2.01	(1.42; 2,84)	<0.001
50-69	5122	106356	4.82	(4.69; 4.95)	2.36	(1.68; 3.32)	<0.001
70-89	1596	31092	5.13	(4.89; 5.38)	2.53	(1.79; 3.56)	<0.001
90-109	491	10108	4.86	(4.45; 5.29)	2.38	(1.68; 3.39)	<0.001
110-129	170	4455	3.82	(3.27; 4.42)	1.85	(1.28; 2.69)	0.001
Missing[e]		*266675*					

[a]CI = Confidence Interval. [b]OR = Odds ratio for milk fever. [c]Previous LL = Previous lactation length in days. [d]Previous DPL = Previous dry period length in days. [e]Missing = Number of calvings where information about the risk factor was lacking.

Table 6 presents the results from the analysis of the effect of calving season on the incidence of milk fever ($p<0.001$). The lowest incidence was seen in September-November. March-May was the season with the highest incidence and OR for milk fever, followed by December-February and then June-August. Furthermore the previous calving interval was analyzed to find a potential correlation with milk fever incidence (Table 6). Cows with a calving interval <359 days had the lowest incidence of milk fever. Compared to this group the highest odds of developing milk fever were seen in the group of >400 days. The 359-400 day group showed higher odds of developing milk fever than the <359 day group, though a lower odds than >400 days.

Table 6. *Incidence of milk fever in current lactation and odds ratios depending on calving season and previous calving interval*

Risk factor		Milk fever cases	Number of calvings	Incidence (%)	95% CI[a] incidence	OR[b]	95% CI OR	P-value
Season[c]								<0.001
	1	2841	89404	3.18	(3.06; 3.29)	1.18	(1.12; 1.24)	<0.001
	2	3240	113523	2.85	(2.76; 2.95)	1.06	(1.01; 1.11)	0.023
	3	3306	122448	2.70	(2.61; 2.79)	1		
	4	3575	119838	2.98	(2.89; 3.08)	1.11	(1.06; 1.16)	<0.001
Missing[e]			*321*					
Previous CI[d]								<0.001
	<359	3027	76028	3.98	(3.84; 4.12)	1		
	359-401	3735	77545	4.82	(4.67; 4.97)	1.22	(1.16; 1.28)	<0.001
	>401	4198	77278	5.43	(5.27; 5.59)	1.39	(1.32; 1.45)	<0.001
Missing[e]			*214683*					

[a]CI = Confidence Interval. [b]OR = Odds ratio for milk fever. [c]Season = Season of calving divided into four groups; 1=March-May, 2=June-August, 3=September-November, 4=December-February. [d]Previous CI = Previous calving interval in days. [e]Missing = Number of calvings where information about the risk factor was lacking.

Milk production and composition

The results from the analyses on milk production and composition related to milk fever are presented in Table 7. The difference to the herd-breed average of total kg ECM, kg fat, kg protein and kg of fat and protein combined produced, all showed a statistically significant ($p<0.001$) effect on milk fever incidence. It was clear for all factors that a higher production caused higher incidence and higher OR for milk fever. What was similar for all factors was that a production clearly lower than the average reduced the incidence and OR of milk fever. A statistically significant association between protein content and milk fever incidence was observed ($p<0.001$). The lowest incidence of milk fever was observed in the group producing milk with a protein content >4.1%. Cows producing milk with a protein content <3.7% were observed to have a higher incidence milk fever and higher odds of developing parturient paresis. No statistically significant increased odds of milk fever could be observed in the middle group producing milk with a protein content between 3.7 and <4.1%.

Table 7. *Incidence of milk fever in current lactation and odds ratios depending on milk production and composition in previous lactation*

Risk factor	Milk fever cases	Number of calvings	Incidence (%)	95% CI[a] incidence	OR[b]	95% CI OR	P-value
DECM[c]							<0.001
<-644	1640	74293	2.21	(2.10; 2.32)	1		
-664-<700	3015	74311	4.06	(3.92; 4.20)	1.87	(1.76; 1.99)	<0.001
≥700	5951	74541	7.98	(7.79; 8.18)	3.84	(3.64; 4.06)	<0.001
Missing[h]		*222389*					
DFP[d]							<0.001
<-49	1671	74295	2.25	(2.14; 2.36)	1		
-49-<52	3043	74302	4.10	(3.95; 4.24)	1.86	(1.75; 1.97)	<0.001
≥52	5892	74542	7.90	(7.71; 8.10)	3.73	(3.53; 3.94)	<0.001
Missing[h]		*222395*					
DF[e]							<0.001
<-28	1736	74296	2.34	(2.23; 2.45)	1		
-28-<29	3041	74306	4.09	(3.95; 4.24)	1.78	(1.68; 1.89)	<0.001
≥29	5829	74543	7.82	(7.63; 8.01)	3.55	(3.36; 3.74)	<0.001
Missing[h]		*222389*					
DP[f]							<0.001
<-22	1689	74294	2.27	(2.17; 2.38)	1		
-22-<23	3104	74314	4.18	(4.03; 4.32)	1.87	(1.76; 1.99)	<0.001
≥23	5813	74537	7.80	(7.61; 8.00)	3.64	(3.44; 3.84)	<0.001
Missing[h]		*222389*					
Protein in LM[g]							0.001
<3.7	2971	59198	5.02	(4.84; 5.20)	1.08	(1.03; 1.14)	0.001
3-7-<4.1	4269	91858	4.65	(4.51; 4.79)	1.00	(0.96; 1.05)	0.993
4.1-	3628	78081	4.65	(4.50; 4.80)	1		
Missing[h]		*216397*					

[a]CI = Confidence Interval. [b]OR = Odds ratio for milk fever. [c]DECM = Difference in kg ECM production from the average production during the previous lactation. [d]DFP = Difference in total fat and protein (kg) production from the average production during the previous lactation. [e]DF = Difference in total fat (kg) production from the average production during the previous lactation.[f]DP = Difference in total protein (kg) production from the average production during the previous lactation. [g]Protein in LM = Protein (%) in last test milking in previous lactation. [h]Missing = Number of calvings where information about the risk factor was lacking.

DISCUSSION

Milk fever over the years

During the studied years a reduction of the milk fever incidence was observed. As seen in Table 2 the average herd size has increased over the time studied. In general a bigger herd means a possibility of less time spent per cow. While the average herd size increases, the milk fever incidence decreases. This could indicate that it is not the time spent on the individual cow that lowers the milk fever incidence, but the general management of the whole herd. If the theory of better management is the cause for the reduction of milk fever cases the question is why the management has become better. One reason could be the increasing knowledge about milk fever and its risk factors. Another factor could be that as the herds get bigger the farmer needs more knowledge to manage a large group of cows and therefore is better at managing a herd or working with preventive measures. Furthermore bigger herds have a better possibility of grouping cows with different, for example, dietary needs compared to smaller herds where the small amount of cows hinders grouping.

Cow level factors

This study found that SH had a higher incidence of milk fever and have higher odds of developing paresis than SRB. The higher incidence observed in SH is consistent with the report by VÄXA (2018). The cause for this is most likely the fact that SH have a higher production of both ECM, protein and fat than SRB (Figure 3A & 3B). As stated above, about two thirds of the Ca content in milk is bound to casein. With casein being the major milk protein, a higher protein production equals a higher Ca requirement. Calcium also exists in form of free Ca salts in the milk, therefore a higher ECM production similarly equals a higher Ca loss. Though higher incidence has been observed for SH compared to SRB in the last years, this has not always been the case. As previously mentioned, Bendixen *et al.* (1987a) found that SRB had a higher incidence of milk fever when compared to SH, which indicates interesting changes in the breeds over the last decades.

Similar to previous findings (Thompson *et al.*, 1983; Dohoo *et al.*, 1984; Curtis *et al.*, 1985; Gröhn *et al.*, 1989) this study found that increasing parity had a link with a higher milk fever incidence. It is clear that multiparous cows have increased odds of developing milk fever compared to first parity cows. Furthermore it is observed that the incidence is not the same between primiparous and all multiparous cows, an increase in incidence is seen with increased parity even between multiparous cows. When comparing the group of cows in their sixth parity or higher with the second parity group an OR of 18.8 was calculated. In this study, each risk factor was not evaluated separately, which is important to point out since higher parity cows have been shown to have a higher milk production. Therefore we cannot say that it is the age that causes the higher incidence of milk fever, it could in fact be caused by the higher production. Although it is not clear whether the milk production increases for every parity as much as the milk fever incidence does. This could indicate that more factors than the production itself causes the higher incidence of milk fever in older cows. Furthermore it is important to remember that Gröhn *et al.* (1989) corrected their analyses for age and production and still observed an increased incidence of milk fever, indicating age as an individual risk factor.

This study found that cows that had been affected with parturient paresis in their previous lactation had higher odds of developing milk fever in their subsequent lactation (OR=9.74). These results are in line with former studies by Erb and Gröhn (1988), Roche and Berry (2006) and Saborio-Montero *et al.* (2017). Though there are risk factors than can be altered between the years, such as BCS at calving, multiple risk factors cannot be changed. For example the breed of the cow, which has been shown to have an effect on milk fever, does not change. Similarly the unavoidable increasing parity of the cow is linked with higher incidence of parturient paresis. Though these are factors that cannot be altered, the information can be useful for the farmer. Knowing which cows are at increased risk of milk fever is useful for preventive work. This can also be a factor for the farmer to have in mind when culling cows in the herd.

Management related factors

The previous lactation length has been shown to have an effect on incidence of subclinical hypocalcaemia in the subsequent lactation (Valldecabres *et al.*, 2019), though when connection between previous lactation length and clinical hypocalcaemia was studied, no link was found (Saborio-Montero *et al.*, 2017). Our study showed that cows with a previous lactation length shorter than the 33rd percentile (<296 days) had the lowest incidence and odds of developing milk fever in the subsequent lactation. With longer lactation length a higher incidence was observed. The highest risk group consisted of cows with a lactational length higher than the 67th percentile (>335 days), with an odds ratio of 1.34 compared to the lowest incidence group. One cause for this could be that the cows that had a longer previous lactation used more of their bodily Ca and therefore, if not restored properly before the subsequent lactation, are at higher risk of a Ca imbalance. Another reason could be that higher producing cows are dried off later since their production in late lactation is higher than the average cow and that the farmer therefore choses to elongate their lactation. Again our statistical analyses did not correct for other factors and the actual risk factor could be the higher production and not the actual lactation length.

This study found that the highest incidence of parturient paresis was observed in cows with a dry period length of 70-89 days. Cows with dry period lengths that were both longer and shorter than 70-89 days showed lower odds of milk fever. The lowest incidence and OR was observed in cows with a DPL <30 days. Sorensen and Enevoldsen (1991) compared DPLs of 4, 7 and 10 weeks and studied the subsequent lactation yield. They found that a shortening of the DPL to 4 weeks resulted in a decrease in milk production of, on average, 2.8 kg of 4% fat corrected milk (FCM)/day the first 84 days. A lower subsequent milk production and therefore a lower Ca requirement could be the explanation for the low incidence of milk fever in the DPL group of <30 days. Furthermore Sorensen and Enevoldsen (1991) found that a 10 week dry period increased the milk production in the subsequent lactation by 0.4 kg 4% FCM/day compared to a DPL of 7 weeks. This higher production and concurrently Ca requirement could explain the higher odds ratio of cows developing parturient paresis when the DPL is 70-89 days (around 10 weeks). Morrow *et al.* (1979) stated that a longer dry period length causes the Ca homeostasis to be more inactive than when the DPL is shorter. This could be another cause for shorter DPLs being favorable when considering milk fever incidence. No clear reason exists why a DPL >89 days is better than 70-89 days. Possibly this could be because the cow has a longer time to restore its Ca pools. Although the results from this study point towards a shorter

DPL being preferable when considering odds for milk fever, a short DPL has negative effects on other aspects such as subsequent lactation yield (Andrée O'Hara *et al.*, 2019).

Season of calving has been discussed to be a factor that could potentially affect the milk fever incidence, although only a few studies have been done and show conflicting results. The only real weather condition that has been concluded to be a risk factor is precipitation (Roche & Berry, 2006; Simesen, 1974). This study found that the highest odds of cows developing milk fever was when calving in March-May, followed by December-February and June-August. The lowest incidence was observed in September-November. As previously mentioned cows in Sweden are kept inside the majority of the year, though they are required to be outside parts of the year in the spring and summer. Depending on where in Sweden the farm is situated the cows are let out during different months. Usually the earliest the cows are let out is in April. During the spring the weather is still unstable and there is usually quite a bit of rain. The cause for a higher incidence of milk fever during these months could be caused by stressors such as cows not being used to being outside and being affected by weather such as rain. The climate is usually better in the summer months (June-August) compared to spring but the direct exposure to the weather (precipitation, wind, sun) could be the cause for slightly higher odds of paresis in the summer months. The higher incidence of milk fever observed in December-February, compared to September-November, could potentially be caused by the cooler temperatures that could act as a stressor. Another theory is that the higher incidence of milk fever observed during the winter months is caused by a lack of vitamin D since these are the months with the lowest sun exposure. However, farmers usually supplement this vitamin in animals kept indoors. Furthermore it is important to note that the differences in OR between the seasons are small and that the statistics did not evaluate seasons independently from the other potential risk factors.

Calving interval length is another factor that has been suspected to affect milk fever occurrence. Although Saborio-Montero *et al.* (2017) studied the calving interval in relation to milk fever but could not find a connection between the two. Valldecabres *et al.* (2019) studied calving interval in relation to subclinical hypocalcaemia could not find a relation either. However this study found that a longer calving interval increases the risk of milk fever. The highest risk was when the calving interval was >401 days (OR=1.39) compared to a calving interval of <359 days. Since the calving interval is the combination of lactation length and dry period length the information found from studying these as separate risk factors is useful. As above described, a longer previous lactation length increased the odds of milk fever in the subsequent lactation, the highest risk group having a lactation length >335 days. The DPL that showed the highest incidence and odds ratio for milk fever was 70-89 days. Combining these groups gives a calving interval of >400 days.

Milk composition

When analyzing the difference to the herd-breed average of produced ECM, fat, protein and fat and protein combined, this study showed that all cows producing higher than the 67th percentile had clearly higher odds of developing milk fever. All cows producing lower than the 33rd percentile showed to have a clearly lower incidence and odds ratio of developing parturient paresis. These findings are in line with previous findings by Bendixen *et al.* (1987a), Chiwome

et al. (2017) and Saborio-Montero *et al.* (2017). As discussed above both a higher ECM and protein production leads to a higher Ca consumption and requirement, making the cow more prone to develop milk fever. Though our statistics show that a higher fat production is a risk factor for milk fever, this most likely is caused by the fact that the higher milk production causes a higher fat production. As previously mentioned, univariable binomial logistic regression was used for the statistics, why each factor, such as fat production, was not evaluated separately.

Though the analysis of total protein production in the previous lactation showed that cows with a high total protein production had a higher risk of developing milk fever, the analysis of the protein percentage at the last test-milking in the previous lactation showed some surprising results. The cows with a protein production lower than the 33rd percentile (<3.7%) showed to have the highest incidence of milk fever. This could possibly be explained by the fact that SH are known to be the dairy cow breed with the lowest protein percentage (VÄXA, 2019). Since test-day protein percentage was not expressed in relation to the breed average, in contrast to the lactation yields, the breed effect could be an explanation. Another theory is that the cows with the lowest percentage of protein, and thus lowest percentage of Ca, in the end of lactation are the ones that have used most of their Ca storages and are therefore at greater risk of a Ca imbalance in their coming lactation.

Limitations with this study

Milk fever in this study was defined as cases that were treated and recorded by a veterinarian, therefore there are a couple of factors that could alter the true incidence. First of all the veterinarian can affect the incidence by forgetting to report a case or misdiagnosing a case since there are several other conditions where the symptoms can remind of milk fever. Secondly only the cases where a veterinarian was contacted were recorded. If a cow only has a mild form of milk fever the farmer might try to treat it on his/her own or a farmer might decide to kill a cow with severe milk fever and not bother contacting a veterinarian.

We had no information on possible preventive measures administered by the farmer, *e.g.* oral administration of Ca around calving. This could potentially cause a variation in the true incidence of milk fever. For example a farmer might supply all cows of third parity or higher with Ca before calving, which could lower the true incidence of milk fever in these groups. Another example could be that the farmer has better possibilities of administering Ca pre partum to cows when they are indoors and therefore the milk fever incidence varies over the year and is not linked to the actual season but the variation in management.

Since the data used for this study did not contain information about all potential risk factors drawn in Figure 1, not all factors that could have an effect on the incidence of milk fever could be evaluated. Both body condition score at calving and diet around calving have been shown to be major factors influencing the milk fever incidence. Information about these factors would have been desirable to make this study more complete.

Furthermore a limitation with this study is that only univariable binomial logistic regressions were used, which causes the problem of not being able to exclude the effect of the factors on each other.

CONCLUSION

The multifactorial etiology of milk fever makes it a hard disease to control. This study showed that cow level factors such as breed, parity, previous milk fever cases and production have an effect on milk fever incidence. Management level aspects, such as previous lactation length, previous dry period length, calving season and previous calving interval also had an impact on the occurrence of milk fever. Therefore all these factors need to be taken into consideration when trying to prevent milk fever in dairy cows. However, it is important to remember that the diet and body condition score around calving are considered to have a major impact on milk fever incidence and that the results from this study are solely a piece of a larger puzzle.

POPULAR SCIENCE SUMMARY

Milk fever, also called parturient paresis, is a metabolic disease observed mainly in dairy cows in the days around calving. At the onset of lactation, after calving, the sudden production of large amounts of milk requires a lot of calcium. The cow is not prepared for this huge calcium loss and therefore develops a state of hypocalcemia, *i.e.* its calcium levels are too low. The cow can restore its calcium levels by absorbing more calcium from the intestines and the kidneys and by freeing calcium stored in the bones, though these mechanisms can take a couple of days before they reach their maximal potential. Therefore cows can develop milk fever before these mechanisms are fully working. Calcium is a mineral that is essential for the function of both muscles and nerves. Therefore clinical signs such as the cow being unable to stand up, having a low body temperature and a loss of appetite can be seen. Milk fever is a serious disease and left untreated it can lead to death.

The main treatment for milk fever is by administering a calcium solution into the blood stream. Usually the cows respond well but in some cases the cows do not recover, despite of treatment. Though the treatment is fairly simple and most cows recover, studies have shown that a cow previously affected by milk fever, is more likely to develop reproductive disorders in the future. Furthermore milk fever has been shown to have a negative effect on the milk yield. These factors have an economical effect, why it is important to try to lower the incidence of milk fever. Milk fever affects approximately 2% of Swedish dairy cows every year.

There are certain preventive measures, such as administering oral calcium around calving, that have been shown to lower the milk fever incidence. Though to be able to prevent milk fever successfully, information about risk factors are essential. There have been many studies done on milk fever over the decades and multiple factors have shown to be able to affect its occurrence. The aim of this study was to evaluate previously determined and suspected risk factors for milk fever and their effect on the incidence. The data for this study was collected through information registered by farmers and veterinarians through VÄXA Sverige. The herds studied were selected based on the milking year of 2015/16 (September 2015-August 2016). The requirements for the herds were that they needed to have at least 40 calvings during the year and 20% of Swedish Holstein breed and 20% of Swedish Red breed. Information about these herds was then retrieved from milking years 2006/07 through 2015/16. Milk fever incidence in the herds was recorded for these years. The risk factors studied were breed, parity, previous milk fever cases, previous lactation length, previous dry period length, calving season, previous calving interval and information about milk production (total milk production, fat and protein). Each risk factor was analyzed in relation to the incidence of milk fever and the odds of a cow suffering from milk fever in the different groups of risk factors were determined.

A general trend of decreasing incidence of milk fever was observed over the studied years. The reason for this is not clear, though a theory is that a generally better management and more information about risk factors have led to this change. This study concluded that all the factors analyzed had a statistically significant effect on the incidence of milk fever. High risk cows were of Holstein breed, were of high parity, had suffered from milk fever in the previous lactation, had a long previous lactation length, had a dry period length of 70-89 days, calved in spring, had a long calving interval and had a high milk production. Though many risk factors

were included in this study there was a lack of information about body condition and diet, which are two important aspects that affect milk fever incidence. Furthermore no information about preventive measures was available. All these aspects show limitations with this study.

The many factors affecting the incidence of milk fever makes it a hard disease to control. Though this study covered multiple risk factors it is solely a piece of a large puzzle. A similar study would be desirable with the inclusion of factors such as body condition score and diet.

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