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Faculty of Veterinary Medicine and Animal Science

Use of preventive measures against milk fever in Punjab, Pakistan

Syed Ahad Raza Zaidi

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Syed Ahad Raza Zaidi

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

Examiner: Kjell Holtenius, SLU, Department of Animal Nutrition and Management

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Abstract

Milk fever or hypocalcaemia is widely acknowledged disease of dairy animals at the time of parturition. The study was aimed to investigate preventive strategies against milk fever by small and large farmers in Punjab, Pakistan from the year 2011-2013. For the sample 212 farmers consented to be part of this study, a questionnaire survey was used as a tool for data collection. The sample was categorized as small (less than 10) and large herd size and data was divided into "no milk fever" and any milk fever" groups, then analyzed through chi square and logistic regression by Statistical Analysis Software (SAS). This study concluded that farmers in Punjab, Pakistan focused on few milk fever preventive methods i.e. administration of Vitamin D, milking strategy, oral prophylaxis (oral calcium intake) and feeding strategy. From these methods; oral prophylaxis came out as the most widely used strategy among farmers, but it was highly used in both risk groups with or without milk fever therefore statistically it didn't impacted any significant effect. Milking strategy as preventive measure; showed the significant higher use among farmers with no milk fever ($p = 0.001$). Statistical analysis also presented, use of Vitamin D as prophylactic method; was used more in herds with no milk fever. Logistic regression of other risk factors revealed that herds with buffalos have a tendency for higher risk of having milk fever ($p<0.09$)

Keywords: Milk fever in Pakistan; Prevention strategies; Use of Vitamin D; Restricted milking; Oral prophylaxis; Feeding strategy; Dairy animals; Questionnaire survey

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Author

Table of Contents

List of Tables

List of Figures

Introduction

Dairy Farming in Pakistan

Pakistan stands fourth in milk production in the world, after the US, China and India (FAO, 2013). The dairy sector of Pakistan has a considerable role in the national economy, the value of milk products is more than that of the wheat and cotton sectors of Pakistan combined (Zia, 2006). Milk production in Pakistan was estimated by 2013/14 to be approximately 50.990 billion liters (Finance Division, 2013-14). Dairy sector is mainly comprised of small scale rural and peri-urban producers which produce approximately 95% of the total milk, having two to three milking animals for their household (Social Science Institute NARC, 2003). Around eight million farming households are connected with dairy sector throughout the country (Pakistan Dairy Development Company, 2006).

Buffaloes and cows are the major milk producing animals in Pakistan, around 62% of the milk production is from buffaloes while 34% is from cows (Government of Pakistan, 2009). Almost 80% of milk producers are in rural areas while farmers in peri urban areas account for 15%. Some 5% farmers are from urban areas of the country. Punjab and Sindh provinces are the major milk producers particularly areas of upper Punjab and central Punjab contributes to the major part of country's milk production. Annual productions of both the provinces were 25.62 and 9.35 billion liters respectively (Pakistan Dairy Development Company, 2006).

Farmer's Awareness regarding Diseases

Pakistan is a developing country and proper knowledge of prophylactic measures to prevent several metabolic diseases, like milk fever also termed as clinical hypocalcaemia, is lacking to the farmers. Milk fever holds a very significant place in diseases of dairy animal's periparturient period (Charbonneau, et al., 2006). Milk fever occurs in cows when the calcium level decreases to below the normal concentration (DeGaris & Lean, 2008). Knowledge about latest developments and research in preventing milk fever is not easily available to farmers. Farmers are mostly illiterate and mostly rely on the advice of livestock assistants and local veterinarians. Knowledge about effect of calcium in prepartum or postpartum use in diet is very limited, both among the farmers and the technical staff of The Livestock Department (Sarwar, et al., 2000).

There are a variety of signs at onset of milk fever but due to resemblance of other diseases it gives a doubtful picture of the disease, therefore diagnosis of milk fever can be a bit difficult (Thilsing-Hansen, et al., 2003). Milk fever accounts for heavy economic losses particularly for the small scale farmers which are the major part of the dairy industry in the country. These economic losses comprise of treatment expenses, veterinarians fees, loss of production and in severe cases loss of the animal (Fikadu, et al., 2016).

The objective of this study was to visit dairy farmers in Pakistan to investigate which methods that are used for the prevention of milk fever.

Research Question

Which preventive measures are commonly used against milk fever by farmers with small and large herd size, and to what extent are they used?

Hypothesis

The study is based on following hypothesis

1. Farmers are using preventive measures against milk fever in various ways
2. Different preventive measures are used in the herds with cases of milk fever compared to herds with no cases of milk fever.

Review of Literature

Efficiency; defined as having best possible output with least input, is the key for business endurance. Cows with minimum disease occurrence, good reproductive performance and maximum milk production are favorable for the dairy farmer, and to achieve that; efficient ways for controlling different management problems and diseases is needed (Van Saun, n.d.).

Importance of Nutrition towards Disease Resistance

One of the vital and handy management factors which influence production is nutrition. Inappropriate nutrition during prepartum and postpartum has negative effects on the reproductive performance of dairy cows. Several clinical studies have proved importance of energy and protein balance in the feed of dairy cows during transition period on reproductive performance (Buttler & Smith, 1989) (Pushpakumara, et al., 2003).

Reproductive performance can also be influenced by vitamin and mineral composition of diet (Smith & Akinbamijo, 2000). Nutrition has an influence on reproductive performance and in addition it affects the reproduction potential by mediating prevalence of periparturient diseases (Van Saun, n.d.). The transition period is very critical in nature because of its relation with periparturient diseases and is considered very important by veterinarians. Considering losses from disease, loss from reduction in milk production is the major financial loss. Economic losses associated with periparturient diseases comprise of discarded milk, high labor costs, veterinary fees and medications and early culling. Cows with disease free calving have higher conception rates as compared to cows having any parturient disease (Loeffler, et al., 1999). In most of the farms considering distinctive heat detection rates this lowering of conception rate significantly affect pregnancy rates; reduction of conception rates are remarkably reducing pregnancy rates. A survey study shows that more than 50% of lactations are affected with at least one periparturient disease (Bigras-Poulin et al., 1990 as cited in Van Saun, n.d).

Milk fever in cows leads to four times higher risk of having retained placenta and 2.3 times higher risk of left displaced abomasum. Incidence of retained placenta results into higher risk of ketosis (16.4 times) ((Curtis, et al., 1985), (Correa, et al., 1993)). In a study of large commercial dairy herds cows with milk fever have higher risks for reproductive disorders i.e. dystocia 2.6 times, ketosis 2.4 times, left displaced abomasum 2.3 times. Higher risk factor of metritis and retained placenta can be linked with dystocia (Correa, et al., 1993). Fairly higher production in lactations and better reproductive performance can be achieved if a cow remains free of diseases during the transition period (Van Saun, n.d.).

Hypocalcaemia/ Milk Fever

The symptoms of hypocalcaemia/milk fever (decrease in calcium level) are dullness, relaxation, less appetite and more inclination towards resting. In addition, cold horns and ears, unusual resting postures, dullness and paralysis of hind quarters can also be noticeable symptoms. At the time of clinical symptoms animals can have a low surface temperature, risk of coma and groaning respiration. Moreover in a few cases animals can be unable to stand (Ender, et al., 1971).

The factors involved in hypocalcaemia can be identified while understanding its causes. Among many contributing factors the two most important are metabolic alkalosis and hypomagnesaemia which can damage the calcium homeostatic mechanisms to the extent where hypocalcaemia can occur (Goff, 2006). The level of blood calcium decreases in almost every cow during first few days postpartum. The risk of developing milk fever increases with age and heifers usually do not suffer from milk fever (Horst, et al., 1990). In USA, around 6%

of dairy cattle are affected with milk fever. Normal blood calcium level which is between 2.25-2.5mmol/l is maintained by homeostatic mechanisms. At the time of lactation onset this mechanism fails and calcium level may fall below 1.25 mmol/l (Goff, 2000). Decreased level of blood calcium affects muscle and nerve functions. Intravenous treatments with calcium keep the animal alive until homeostatic mechanism of calcium in intestines and bones is adapted. (Bačić, et al., 2007). Preventing decrease in magnesium levels of blood at calving can be achieved by feeding 0.35-0.40% magnesium in rations given prepartum. Further feeding 40-50g phosphorous/cow/day can meet the requirement; however downer cow syndrome can occur if given less than 25g (COX, 1998 as cited in Bacic, et al., 2007). Feeding more than 80gm of phosphorous per day will induce milk fever (Bačić, et al., 2007).

Many essential biological processes in the body, like blood coagulation, muscle contraction, and hormone release requires calcium ions as they play a very important role in the body also calcium is vital for structural components of skeleton (Rosol & Capen, 1997). Thus plasma concentration should remain at a narrow range, as it is of much importance. A very small amount of calcium (0.1%) in the body exist in extracellular fluid, 99% is stored in the bones, while remaining is found in the endoplasmic reticulum and plasma membrane.

The plasma membrane and endoplasmic reticulum contains the isolated remaining deposit (Sjaastad, et al., 2003). Production of colostrum at the time of calving requires large amount of Ca and at this time when cows are unable to compensate for this drastic increased requirement leads to hypocalcaemia. The most dramatic type of hypocalcaemia is clinical milk fever and it might be lethal for the animal (Charbonneau, et al., 2006).

Cows which are treated for milk fever and showed good response to treatment; still have possibilities of reduction of milk production by 14 % in next lactation (Block, 1984). Cows affected by milk fever are usually cured by single dose of IV administration of calcium compounds yet around 25% of cows relapse and repeated treatments are required to cure them (Mullen, 1975 as cited in (Oetzel, 2010)). A serious aspect of the situation is that cows with sternal recumbency are in most cases either submitted for disposal or die due to recumbency in Pakistan. Serum levels of calcium are also observed to be significantly lower in cows with uterine prolapse (Risco, et al., 1984). Involution of uterus and cervix during milk fever may explain the relation between milk fever and uterine prolapse (Oetzel, 2010). Different periparturient diseases might be linked with cystic ovaries, as no direct relation has been known yet. There are only theoretical connections between hypocalcaemia and reproductive disorders that are based on some field studies (Beede, et al., 1992).

Calcium Homeostasis

Decrease of blood calcium level at the beginning of lactation can be a serious life threat, to avoid this animal should start absorbing more calcium from the bones and diet to substitute calcium lost in milk. Resorption of calcium from bone is potentially harmful for bones, this leads to lactational osteoporosis and loss of 09-13 % of skeletal calcium in cows which is revocable in later lactation. Prime objective during that time is to keep optimum level of calcium in blood. Parathyroid hormone regularizes the mobilization of calcium from bone and is produced whenever there is decrease of calcium level in blood (Goff, 2006). Reabsorption of calcium from renal tubules is also increased by parathyroid hormone, but a very small quantity of calcium is recovered by this process. 1, 25 dihydroxyvitamin D is another hormone which is necessary to increase intestinal absorption of calcium. Rise in blood parathyroid hormone level stimulate production of 1, 25 dihydroxyvitamin D from vitamin D and induces increase of intestinal calcium absorption. Onset of milk fever is the result of a condition where body is unable to extract enough calcium from bones and digesta/ diet to cope with the loss of calcium in milk. The homeostasis of calcium is interrupted by several nutritional factors which results in milk fever (Goff, 2006).

Approximately 4 mmol/l free calcium concentration while 30 mmol/l is total calcium is present in milk of cow (Sjaastad, et al., 2003). Colostrum contains higher concentration of calcium which is around 42-58mmol/L (Goff, 2000). Blood calcium level is expected to be at lowest level during the 12 to 24 hours postpartum. Parturition and hypocalcaemia leads to an immunosuppression through blunting of the calcium signals in immune cells (Kimura, et al., 2006).

Measures to prevent milk fever

There are many methods that can be used to treat and prevent milk fever. These principles can be applied at different times, such as late pregnancy, after calving. Thilsing-Hansen et al. (2002) reviewed some used control principles for the prevention i.e. acidifying diets; around calving oral drenching with calcium; managing vitamin D and its metabolites and analogues intake and administration before calving; low calcium diet; control of body condition, dietary magnesium & carbohydrate intake; prepartum milking; short dry period and less milking in early lactation (Thilsing-Hansen, et al., 2002). Some of the preventive methods are discussed below.

Dietary cation–anion reduction

According to Goff (2006) Cation-Anion level in diet is one major factor to affect electrical charge of blood. Na, K, Ca and Mg are some of those cations present in feed and contain positive charge while Cl, SO_4 and PO_4 are key anions found in feed and bear negative charge. Cations and anions present in the diet if absorbed in the blood will only change the electrical charge of the blood. Fairly negligible impact is seen by absorption of trace elements from diets. Large amounts of volatile fatty acids are produced by the rumen microbiota. The acids are absorbed through the ruminal epithelium. They have insignificant effects on acid-base balance. Acid-base balance of blood is determined by the difference of cation and anions absorbed from diet, it also determines the pH level of blood. mEq/kg DM term is usually used to describe cation-anion difference of the diet. Dietary Cation- Anion Difference (DCAD) of

Na, K, Cl, SO_4 is calculated by the formula as follows: (DCAD) = (mEq Na^+ +mEq K^+) − (mEq Cl^- +mEq S^{2-}) (Goff, 2006). The equation above is found useful but presence of Ca, Mg and P in the diet and their absorption also influence pH level of blood. When evaluating relative acidifying activity of chloride vs. sulphate, it is observed that Sulfates is only 0.6 as acidifying as Cl. (Oetzel et al., 1991; Tucker et al., 1991; Goff et al., 2004 as cited in (Goff, 2006)). A number of equations for calculation of DCAD in dairy animal diets have been published. First of them was (Na + K) − (Cl + S) (Ender et al., 1971). Most recent equation proposed by Goff was (Na + K) – (Cl +0.6 S); which is considered as most accurate for calculating blood pH and excess of standard base from composition of minerals in diet (Goff, et al., 2004).

Complex DCAD equation could comprise of Ca, Mg, P and Ammonium also because of its contribution towards cationic content of blood. (Constable, 1999). Unfortunately there is not experimental data available which will provide actual coefficients of absorption for these cations fed to dry cows. DCAD equation is not a necessary element for formulation of amount of minerals in rations for prepartum dairy cows because other than K and Cl, the rate of inclusion can be fixed for other macrominerals; however for dietary manipulation of acid base status DCAD equations gives a theoretical base. (Goff, 2006).

Charbonneau (2006) mention that Dishington (1975) stated that occurrence of clinical milk fever can be reduced by giving feed mixture of chloride and sulfate salts before calving. Further studies also confirmed that decreasing the dietary cation-anion balance prepartum decreases the chances of both clinical and subclinical hypocalcaemia.

Compensated metabolic acidosis is associated with lowered DCAD, which is proved by reduced plasma bicarbonate, lower urinary pH, and higher urinary net acid excretion. (Charbonneau, et al., 2006).

A study by Huber et al., (1981) shows less number of cases with clinical milk fever & subclinical hypocalcaemia in cows which are given anionic salts prepartum. Higher pregnancy rates, less services per pregnancy, and decreases number of days to first estrous were also observed in those cows. All of this was a result of decreased number of periparturient diseases which are linked with clinical milk fever and reproductive inefficiency. Additional intake of dry matter during early lactation might also be an explanation of this positive effect. Huber et al., (1981) observed presence of subclinical hypocalcaemia in 50% of control cows, but 19% of cows which were receiving anionic salts were diagnosed of having subclinical hypocalcaemia.

Functioning of smooth muscles especially GI tract muscles need sufficient amount of calcium in the body (Huber et al., 1981, as cited in (Oetzel, 2010)). Hence lowering of DCAD is very significant both biologically and statistically for reduction of clinical milk fever.

Alfa alfa and other legumes contain high level of potassium in their tissues, hence removing potassium from the diet pose a big problem. Anions are added to decrease metabolic alkalosis and producing milk metabolic acidosis HCl is mostly used anion source (Goff & Horst, 1997). Schonewille et al. (1999) stated that in the state of acidosis; calcium excreted in the urine can be retained when there is an increased demand of calcium. During EDTA challenge[1], the amount of calcium which is retained from the urinary system is not sufficient for the cow. Some other mechanisms must be utilized during the state, e.g. during acidosis there is an increased receptivity of tissues towards parathyroid hormone (Goff, et al., 1991). Enhanced calcium absorption from intestines and reabsorption from bones are results of better receptiveness of parathyroid hormone which increases active vitamin D production (Charbonneau, et al., 2006).

Stimulation of PTH secretion by Ca deficient diet prepartum

Cows fall into negative calcium balance when they are fed diets with a lower calcium level than they require. This drop of blood calcium level stimulates PTH and osteoclastic bone

[1] EDTA Challenge is administration of a chelating agent making calcium unavailable

resorption and production of 1, 25 dihydroxyvitamin D by renal tubules (Green, et al., 1981). Osteoclasts in the cow at the time of parturition will then be fairly active and in large numbers which easily replace calcium lost in milk production from bone. Dietary calcium is efficiently utilized due to enterocytes stimulation by 1,25 dihydroxyvitamin D and milk fever is prevented (Green, et al., 1981). NRC, 2000 stated the requirement of calcium for cows in terms of absorbable calcium; because calcium amount and absorption varies in diets. If a cow is given true low calcium diet and provided significantly low absorbable calcium than the amount of calcium required by the cow PTH will be stimulated (Green, et al., 1981).

Diets with low amount of calcium are found to be more practical in combination with grazing (<4gCa/Kg DM) to fulfil the calcium requirement of dairy cow. e.g. a cow weighing 600 kg; which consumes 13 kg of DM should be provided with a diet which contains less than 1.5 g of absorbable Ca/kg DM; if the requirement is lower than 20g available Ca/day. (Sanchez, 2003 as cited in (Goff, 2006)). During the dry period, a traditional way of preventing hypocalcaemia is to give calcium restricted feed (Green et al., 1981). Diets with a low level of calcium help promoting active absorption of calcium from intestine and reabsorption of calcium from bones; ultimately cows being able to reply to the sudden increase in calcium outflow occurring at the time of parturition suddenly (Oetzel, 2010).

Supplementing Vitamin D

Feed and absorption in the skin are recognized as the main source of vitamin D. 25-hydroxyvitamin D is the major form of vitamin D which circulated in blood and is converted to 1, 25-dihydroxyvitamin D in kidneys. 1.25-dihydroxyvitamin D regulates homeostasis of P & Ca with the help of PTH by increasing the absorption of P and Ca in intestines. It is also important for the effect of PTH on bones (Rosol & Capen, 1989).

Vitamin D and its metabolites are found useful in milk fever prevention when used through injections or implanted under the skin. 24F-1, 25-dihydroxyvitamin D pellets when implanted 7 days before calving in the cows in a study by Goff and Horst (1990) resulted in milk fever incidence of 9% comparing to 80% in control group of cows. Significant difference in calcium plasma levels were observed between the groups. Plasma calcium concentration was higher post and pre calving in cows given implants. Vitamin D injections and implants both have been found effective but this can be accompanied with few problems with timing, toxicity and delayed milk fever ((Goff & Horst, 1990); (Thilsing-Hansen, et al., 2002)). Administering Vitamin D or its analogues, intramuscularly or by other ways to an animal requires strict observation on timing for administration. Minimum 24h and maximum 6 days before calving is the crucial time for administering Vitamin D or its metabolites to prevent milk fever (Goff & Horst, 1990). Parturition dates usually deviate from expected day in cows which can be a problem. In such cases few more injections have to be given to cow which can be a risk of getting hypocalcaemia; consequently 48-72h post injection, plasma concentration of 1, 25-dihydroxyvitamin D might increase suddenly (Goff & Horst, 1990). Vitamin D implants are better option than injection to prevent milk fever as they release vitamin D at slow and constant rate prepartum.

Vitamin D administration may lead to delayed milk fever, usually ocurring 1 to 2 weeks postpartum. Exogenous treatment with Vitamin D metabolites might inhibit production of endogenous 1, 25-dihydroxyvitamin D which could be a predisposing factor for milk fever after clearing of the exogenous 1, 25-dihydroxyvitamin D. Therefore, a gradual decrease of exogenous 1, 25-dihydroxyvitamin D might solve the problem (Goff & Horst, 1990); (Thilsing-Hansen, et al., 2002).To supplement the dry cow with app. 20,000 to 30,000 IU Vitamin D per day through the feed is an efficient method for treatment (Littledike & Horst, 1980). It was also recommended in previous literature that to control milk fever, feeding enormous doses e.g. 10 million units of Vitamin D 10- 14 days before calving can help (Littledike & Horst, 1980). Feeding of vitamin D helps in prevention of milk fever by an increased intestinal Ca absorption. But the problem is that the amount of Vitamin D that can be effective in the prevention of milk fever can also cause irreparable metastatic calcification of soft tissues. In some cases lower dose of vitamin D become a cause of milk fever in cows because as a result of the treatment, the higher levels of 25-OH D and 1,25-dihydroxyvitamin D reduced PTH secretion and renal production of endogenous 1,25-dihydroxyvitamin. When this exogenous sources of vitamin D was cleared from the body; which helped in retaining higher intestinal Ca absorption rates; these animals become hypocalcaemic. In some cases after calving the capability to increase endogenous production of 1, 25-dihydroxyvitamin D takes approximately 1 week (Littledike & Horst, 1980). Rather than using Vitamin D, usage of 1,25-dihydroxyvitamin D and its analogues as the treatment can be more effective and harmless, but the complications related to timings of administration will remain there (Bar et al., 1985; Goff et al., 1988 as cited in (Goff, 2006)). After calving, by slow withdrawal of exogenous hormone over a few days can minimize the problems of supression of renal 1,25-dihydroxyvitamin D production (Goff & Horst, 1990).

Supplementing Oral Ca at calving

Administration of calcium in the fresh cows is more likely to be called a treatment for hypocalcaemia than a preventive measure (Goff, 1999). A cow's capability to consume calcium across intestinal cell is insufficient to maintain blood calcium concentration; this become the main reason behind the oral supplementation of calcium. This is possible by passive diffusion of calcium across the intestinal tract, not across intestinal epithelial cells; by dosing the animal very large amounts of soluble calcium. Doses of Calcium between 50 and 125 g Calcium gives best results (Goff & Horst, 1993). Calcium chloride can be used but it can be caustic in some cases. If a cow is already getting an acidogenic diet, large doses of calcium chloride can result in uncompensated metabolic acidosis in the cow, whereas calcium propionate is not acidogenic and is less harmful to tissues ((Goff & Horst, 1993) & (Pehrson, et al., 1998)).

A calcium dose given at calving and again 24 hours later can be helpful in controlling hypocalcaemia. Per oral soluble calcium products prior to parturition directly increases the amount of available calcium in cows (Plasma free calcium). Large and repeated dose can be toxic, 250 g calcium in a soluble form is life threatening for the animal (Goff, 2006).

Calcium in Diet

To prevent hypocalcaemia a low calcium feed is normally suggested, but Ender et al. (1971) believes that calcium feeding is not as important as DCAD on calcium homeostasis around calving. To cope with the challenges of lactation after calving a cow is forced to activate her calcium mobilizing systems before calving. ((Goff, 2008), (Kitchura, et al., 1982)). On the other hand, a high calcium level of feed can also be useful (Oetzel, et al., 1988). Calcium feeding of high levels after calving will increase calcium stores through passive absorption and therefore protect against milk fever (DeGaris & Lean, 2008).

A meta-analysis for the risk of milk fever performed by Lean et al. (2006) resulted in an equation that gives a bell curve showing decreased risk for diets having low or high calcium levels (Lean, et al., 2006). Oetzel 1991 found 1.16% calcium levels of DM gave highest chances of milk fever. (Oetzel, 1991).

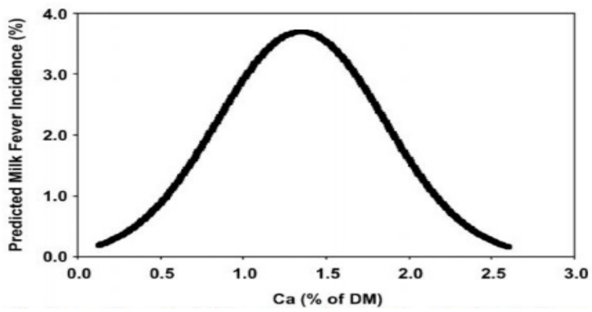

Figure 5: Predicted milk fever risk with different feeding levels of calcium (Lean, et al., 2006)

Time of exposure of calcium is another factor for risk of milk fever in addition to calcium feeding. Diet with high calcium level for short exposure given before parturition significantly increased milk fever risk. Similarly low calcium diet for long exposure time given before calving increased milk fever chances. Dietary calcium 1.1-1.3% of dry matter with long exposure gave highest incidences of milk fever (DeGaris & Lean, 2008).

During early lactation, reduced motility of GI tract leads to diminished intake of dry matter (Beede, et al., 1992). Negative energy balance occurs if intake of dry matter is decreased due to hypocalcaemia. This negative energy balance will affect reproductive performance of the animal.

Higher content of calcium in diet (>100g) will decrease DM intake and performance of animal however there is no described optimum level of prepartum dietary calcium. Animal given calcium concentration of less than 15g, fed 10 day prior to calving will result in decreased milk fever incidences. (Bačić, et al., 2007).

It is important to note that the cow should be moved to high calcium diet after calving. Now there are two techniques which have been adopted to minimize the availability of dietary calcium for absorption. One technique is the implantation of zeolite into the ration which binds to calcium and force it to be passed out in the feces (Thilsing-Hansen, et al., 2002). The method is not manageable because a heavy amount of zeolite must be given each day, whereas the effects of large amount of zeolite on P and trace mineral absorption are not clear (Thilsing-Hansen, et al., 2002). The specificity of zeolite for calcium can be possibly increased by modifying it chemically; which may permit its use. The other technique is the incorporation of vegetable oils, which fix calcium to make an insoluble soap avoiding absorption of dietary calcium. Both the techniques have been used effectively in cattle fed diets that contain 30-50 g calcium per day (Goff, 2006).

Milking Strategy

Incidents of clinical hypocalcaemia can be decreased by prepartum milking which has been shown by some studies (Greene, et al., 1988), however in other studies, prepartum milking has not shown any significant effects on the incidents (Smith & Blosser, 1947: Eaton et al., 1949 as cited in (Feneborg, 2010). According to Greene et al. (1988) prepartum milked cows showed 6.1% incidents of hypocalcaemia where as 11.4% cases could be seen in postpartum milked cows.

This study shows that higher peak and milk yield postpartum could be seen in the cows who yielded more before the day of calving than in the cows who yielded less before calving, but generally there was no significant effect on lactation milk yield due to prepartum milking (Greene, et al., 1988). It is also observed that cows with lower milk yield the day before calving had an increased risk of developing hypocalcaemia (Smith & Blosser, 1947 as cited in (Feneborg, 2010).

In the study by Salgado et al 2014, partial milking postpartum at first and second milking have no effect on prevention of milk fever (Salgado-Hernández, et al., 2014). Similarly Hoard's Dairyman in their book also quoted that partial milking does not show any significant effect in milk fever prevention (Hoard's Dairyman, 1993). However partial or delayed milking postpartum causes lower milk production and pressure maintenance in the udder which prevent hypocalcaemia as mentioned by Merck Veterinary manual (Merck Veterinary Manual, 2012). Therefore there is a need of further studies to find out the beneficial effect of postpartum milking strategy in decreasing the disease occurrence (Carbonneau, et al., 2012)

Effect of Breed, Season and milk yield on incidence of milk fever:

There are several studies which suggests that some breeds of animals are more vulnerable to milk fever than others, particularly Swedish Red and White, Channel Island, and Jersey cattle (Horst, et al., 1997). Lean, et al. (2006) also writes that Jersey cows in comparison to Holstein-Friesian cows are at 2.25 times greater risk of milk fever (Lean, et al., 2006). Roche & Berry (2006) in their studies found very significant effect of breed on occurrence of milk fever i.e. 4 times higher chances of milk fever in Jersey cows comparing Holstein-Friesian cows (Roche & Berry, 2006)

Seasons and environmental conditions have also found to be influencing factors for occurrence of milk fever and other metabolic diseases. Horst, et al. (1997) mentioned that Damgaard (1975) reported a strong relationship between sun-spot activity and incidence of milk fever, he suggests that the variations in Ultraviolet radiations may affect the endocrine glands of cattle (Horst, et al., 1997). Other climatic factors like average evaporation rate, difference between min & max temperatures, minimum grass temperature and average rainfall during last 48 hrs prepartum are considered to be effective on occurrence of milk fever during the study by Roche & Berry (2006). They found that milk fever incidence was increased during cold weather, increased evaporation, wet weather and when there is large difference between max and min temperatures. Roche & Berry (2006) also found that incidence of milk fever was 82% more often in cows when rainfall was between 0.95 mm and 3.374 during 48 hrs prepartum; as compared to no rainfall (Roche & Berry, 2006).

The occurrence of milk fever can also be affected by high milk yield, as Fikadu, et al (2016) writes that during the periparturient period, high milk producing cows are most vulnerable to metabolic diseases (Fikadu, et al., 2016). Also Hansen, et al (2007) found that high milk yielding cows are at more risk of having milk fever (Hansen, et al., 2007). The reason is that during this period the animal is trying to maintain calcium homeostasis because of physical and pathological factors (Fikadu, et al., 2016). Fleischer (2001) in his study found a correlation between milk yield and milk fever also with other diseases like retained placenta, ketosis and mastitis (Fleischer, et al., 2001).

Material and Methods

Selection of Farmers

In Pakistan, being a developing country, there is lack of centralized database systems in all fields of life. Hence The Livestock Department also holds no centralized database and no registration of farmers with the department/database exists.

Local veterinary hospitals have records from the surrounding areas of farmers that have gone through veterinary treatment at the hospital. Milk collecting companies also maintain records of farmers from their collection areas. Farmers were visited along with local veterinarians and milk company’s advisors where they have some appointments or any other treatments. Some of them were randomly selected from the local veterinary hospital records, milk companies and from Pak Dairy Info Guide, from the area of upper Punjab where most of the dairy farming exists. In total 223 farmers were visited out of them only 11 did not show willingness to be part of the survey. A geographical map as shown in figure 3 shows the plain land areas with river flowing in Punjab and Sindh provinces where most of the farming exists. Other provinces can be seen as hilly and mountainous. Cattle population in the

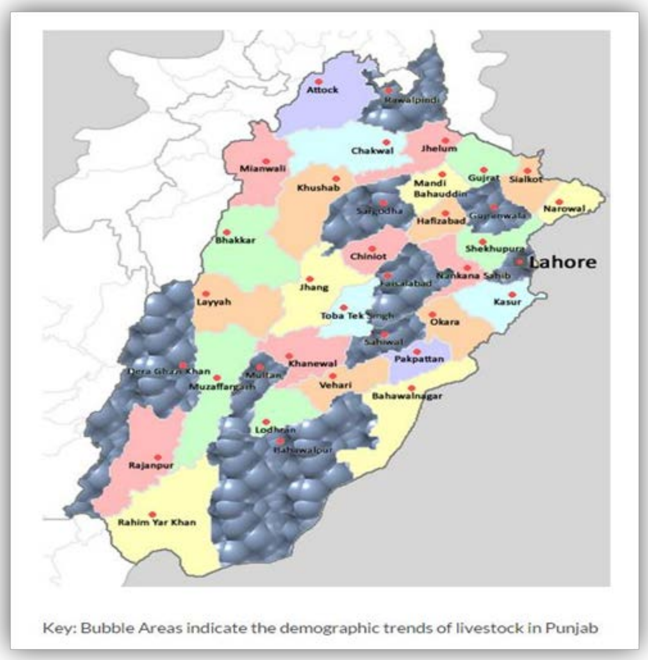

Figure 6: Visual Mapping of Demographic and Production Trends of Punjab (Malik, 2014)

Punjab province contributes total 57% of total population in the country (Finance Division, 2012). Whereas major population in Punjab exists in the central Punjab and upper Punjab, Bahawalpur, Faisalabad and Dera Ghazi Khan Divisions as the demographic distribution of cattle population shown in the figure 2.

Figure 7: Map of Pakistan (Pakistan Map, n.d.)

Most of the farmers are rather uneducated or very little educated and have their milking animals for fulfilling the milk and dairy products requirements of their household (e.g. drinking milk, making tea and yogurt etc.). Around 50% of farmers are very poor and hold only 1-2 animals as per census of 2006 (Statistics Division, 2006).

Herd Size	Percentage of Total	
	Pakistan	Punjab
1 – 2	43	47
3 – 4	28	29
5 – 6	13	12
Above 6	16	12

Source: Pakistan Livestock Census, 2006

Figure 8: Population Pattern of Cattle (Statistics Division, 2006)

There is, however, an increasing trend of building dairy farms at a commercial level. As mentioned above farmers were randomly selected from available data and contacted through their given contact numbers; they showed very high willingness except few, towards answering the questions and fully cooperated during the survey.

Questionnaire

The questionnaire was designed to be very simple and easy to understand and translated into Urdu (National Language of Pakistan) for better understanding of local farmers. Farmers were questioned on door to door visit to the farmers in different villages during February to July 2014. A total of 212 farmers responded about their practices for prevention of milk fever. Questions were asked in their local language for farmer's better understanding, relating to the years 2011-2013 and the method used to prevent milk fever during that period, and also general management of their animals.

The questionnaire contained 3 parts

1. About general information; type of animal, breed, age, current condition and milk production.
2. About general farm management, feeding, concentrates, prophylactic measures for the disease, ticks, flies etc.
3. Detailed question about milk fever prevalence, type of preventive measures and farmers perception of the effectiveness etc.

Questions from the thirds section which was related to milk fever prevalence were selected and statistically analyzed for the research.

Statistical Analysis

Descriptive analysis of the answers obtained from the questionnaire survey was done at first. Similar answers were counted and calculated per question basis. At the time of survey prevalence of milk fever was questioned of frequency of occurrence i.e. often, sometimes, rarely and no occurrence. During data compilation first portion was narrowed down and merged in to one section; as milk occurrence yes / no, for the easy interpretation in statistical software. Data accumulated after counting was submitted for statistical analysis in SAS software.

Two types of statistical tests were performed on the data.

1) Chi square test

2) Logistic regression

Answers from different ways of preventive measures of milk fever were examined with frequency analysis of associations in SAS along with their chi square values. P-values of 0.05 from chi square test and logistic regression were considered for their significance level, similarly P-value of range 0.05 to 0.1 was considered to describe tendency level. Parameters of milk fever cases were (Yes, No) for milk fever occurrence in years 2011-2013, (Complete, partially) for Restricted milking, (Yes, No) for Use of Vit. D, (Yes, No) for Per Oral Prophylaxis, (Yes, No) for feeding strategy. Herd size was often quite small, therefore herds with less than 10 animals was represented by 50 % of the farmers (106 out of 212). So to check the validity of the result with high variation statistical analysis was also done on the basis of herd size.

1) Farms with herds less than 10 animals
2) Farms more than 10 animals

Logistic regression method was also performed for general parameters including milk yield (divided into upper half, lower half), herd size (4 quartiles), type of species (Cows, Buffaloes) with the dependent variable milk fever (yes/no).

Results

Inquiries related to milk fever from the questionnaire were statistically analyzed in SAS. From the total sample 31% of farmers had herd size of 1-5 animals, 19% of 6-9 animals, 26% of 10-16 animals and remaining 24% were having herd size ranging 17-250 animals. Among the total herds 50% comprised of cows, whereas 29% were buffaloes and 21% were mixed (Both cows and buffaloes). The average milk yield calculated in the study was 11.7 liters. As mentioned in materials and methods the occurrence of milk fever (rarely 78%, sometime 11% & often 11%) was labelled as 'Any case of milk fever'.

Following Table 1 show comparison of the results from four types of preventive measures (Milking strategy, Vit. D administration, oral prophylaxis and feeding strategy {High calcium diet, low calcium diet and others}) adopted by the farmers to prevent milk fever.

Table 1: Analysis of preventive strategies using Chi Square Test in SAS

All Herds (212)			
	Any Case of Milk Fever	**No Milk Fever**	**Chi Square Test (P-value)**
Milking (Complete)	51.1 %	22%	
Partially	48.9%	78.1 %	0.0001
Vit. D (Yes)	13%	30%	
No	86%	70%	0.0039
Oral Prophylaxis (PO)	90.7 %	94.5%	
No	9.3%	5.5 %	0.3238
Feeding strategy (Yes)	59 %	75.3%	
No	41 %	24.7%	0.0180

Analysis for the preventive methods

All Herds

1. Statistical analysis of milking practice either complete or partial reveals that herds with no milk fever milked partially in a significantly larger extent compared to herds that had experienced milk fever
2. Use of Vitamin D is more common in the herds with no cases of milk fever.

Herds with more than 10 animals

Considering the difference in the number of animals between herds, the study separately analyzed herds with more than 10 animals to find out any significant differences due to herd size. The result showed no significant differences in the results which were anticipated because of the herd size.

Logistic Regression for the prevalence of disease

The results of general parameters i.e. herd size, milk yield and type of species were analyzed with basic logistic regression. The variables used for types of Species (Cow, buffalo or both), milk yield (upper half or lower half), and herd size divided into 4 quartiles. The highest herd size (quartile 4), "cows" and the highest milk yields are kept as baseline for the comparisons (Not mentioned in the table).

Table 2: Logistic regression of likelihood parameters

Analysis Of Maximum Likelihood Parameter Estimates				
Parameter		**OR (95% Confidence limits)**		**P- Value**
Herd size	1-5	1.2	7.2	0.022
Herd size	6-9	0.3	2.8	
Herd size	10-16	0.5	3.6	
Herd size	17-250	1	1	
Milk Yield (upper half)	1-10	0.4	1.3	0.264

Milk Yield (lower half)	11-20	1	1	
Species	Both	0.1	0.7	0.017
Species	Buffalo	0.3	1.1	
Species	Cow	1	1	

Note 1: The Scale parameter was held fixed

1. The Wald statistics for type 3 analysis shows that the effect of herd size was significant (p= 0.022) as the smallest herd (1-5) had a lower possibility to have had milk fever.
2. It also seems like the herds that has both buffaloes and cows have a higher risk of having milk fever compared to the herds with cows.
3. Also the herds with buffaloes have a tendency for higher risk of having milk fever cases (p<0.09) data not shown.

Discussion

The study aimed to explore the methods of preventing milk fever in dairy herds from Pakistan; which reveals the difference in the prevalence of milk fever associated with using different strategies. The study was conducted in Punjab considering that the province is known for agriculture and dairy production in Pakistan; 212 farms were randomly visited from Faisalabad; Jhang; Sargodha; Lahore; Sheikhupura; Mianwali; Bahawalpur and Multan region of central and upper Punjab. According to the livestock census of 2006 (latest) in Pakistan, around 50% of the farmers had only 1-2 animals, however the difference in the sample of the study is because it only included peri-urban farms of Punjab province. The criteria for the inclusion was that the areas have more and contemporary dairy farming which reflect the national herd characteristics. Also while selecting the sample kept in mind the traveling and living cost to visit the remote areas in these areas. As mentioned in the material and method section selection of a representative sample was a challenge and farmers who responded positively were surveyed. However, the research has a limitation that it couldn’t include all farms of these areas because of large number of farms, time and other constraints. A large sample study can show different results; thus, the results of this study cannot be generalized for the whole province. The dairy farms are mostly located in remote villages which are underdeveloped and have no transport infrastructure; which makes the outreach more difficult.

There are very few large scale dairy farms in Pakistan; most of the farmers are low income and have a very small herd size i.e. less than 10 animals (Statistics Division, 2006). The hygienic condition of these small farms and health condition of animals are very poor. Animals are poor fed and suffer malnutrition. Most of the farmers are uneducated and have less awareness about the health, hygiene and feeding requirements of animals resulting in very low milk production i.e. avg. 11.7 L found during the study. Consequently the prevalence rate of diseases is high among those animals; to treat these diseases farmers use conventional home remedies as they can’t afford the cost of required veterinary treatments which worsen the situation. Local veterinarians and farm advisors in Pakistan usually advise farmers about different preventive measures against milk fever. Several prophylactic measures are used, particularly in the case of milk fever these farmers mostly rely on per oral calcium supplementation which they consider more as a home remedy rather than a scientifically documented preventive method because of poor knowledge. Goff and Horst (1993) in their study also stated that use of calcium orally in slight deficit of calcium can be very effective and prevent from relapse. Metabolic status of these animals remain poor due to poor feeding; the situation get more complicated during the pregnancy. Which becomes a cause of milk fever in periparturient animals.

This was the situation which was observed and discussed on the field visit; however, all farmers participated in the research were well aware of the benefits of preventing milk fever and making an active effort in this regard. The use of per oral calcium is found as the common preventing strategy in combination with other methods which is quite similar with Hansen et al. (2007) study done with Danish herds. Though Hansen et al. (2007) found that Danish milk producers have equal focus on body condition scoring and reduced milking which is not in the case of Pakistan as body condition scoring are seldom practiced by farmers.

The results of the study shows that the occurrence of milk fever is low in the small herd size; however, it should kept in mind that a small herd might have the same theoretical risk as a larger one, but still not get milk fever as often because it has fewer cows at risk. This difference is thus not necessarily an effect of an actual difference in risks but more likely an effect of the design of the questionnaire and study. There may be many possible reasons in the favor of results; i.e. the small herds are easily manageable; identification of occurrence of disease is quick and also it was found that the farmers use conventional methods in which one is the frequent feeding of oral calcium which is favorable for preventing the disease. In Indian sub-continent and in few parts of Africa, buffaloes are also reared for dairy purposes and in Pakistan most of the farmers have a combination of both buffaloes and cows in their dairy farms. The research also finds out that herds with both buffaloes and cows have higher risk of milk fever compared to herds with only cows.

As for the prevalence of disease the herd size remain significant in the results; the second part of the research which aimed to look at the preventive measures used against milk fever also needs this consideration. Thus keeping in mind the huge difference in the size of herd (i.e. some farmers have more than 200 animals and some have less than 5); also 50% of the herds in sample was having less than 10 animals; it was decided to separately analyze the larger herd sample to see whether it effects the results or not. In the use of preventive strategies in both small and big farmers; very few differences were observed. An important point is that having less resources and awareness about modern research and technologies;

small farmers are giving the same results in preventing of milk fever comparing to big farmers. The partial milking strategy came out as the significant parameter in preventing milk fever; however small farmers are doing partial milking as an old practice which in their opinion is good for animal without knowing its result for preventing milk fever. The awareness about modern research for administration of Vitamin D as a prophylactic measure against milk fever is not very common amongst farmers. Also Kashfi et al., (2007) concluded in their study that administration of vitamin D and its analogues during the prepartum period gives better absorption of Ca from intestines and bones and thus it prevent milk fever (Kashfi, et al., 2012). Many of the veterinarians in Pakistan are not prescribing the use of Vitamin D particularly serving in remote areas; hence its less use comparing to other preventive strategies, is evident from the results of the study. It was felt that there is a dire need to aware the small and big farmers with latest researches and methods of preventing milk fever. Having milk fever in herds is giving financial losses to the farmers and can have fatal consequences for the animals.

Conclusion

Milk fever is seen as one of the major diseases which cause economic and other losses for Pakistani farmers. The study concludes that farmers in Pakistan are using various preventive measures to prevent milk fever in their herds. The most common preventive measures includes prepartum milking strategy i.e. partial or complete, per oral calcium therapy around calving, feeding strategies (low and high calcium diets etc.) use of vitamin D prepartum etc. The study finds out that herds with no milk fever were more often partially milked postpartum compared to those who had had milk fever during the previous years. Moreover it was observed that the herds with least cases of milk fever more often used vitamin D.

The results also revealed that herds with only cows have less probability of having milk fever than the herds with both buffaloes and cows. Farmers in Pakistan have less awareness about the latest techniques and researches in preventing milk fever; so practicing more traditional than modern methods.

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Appendix

Questionnaire

Part 1:

Animal: Buffalo/Cow/Both No. of Animals: ____________________

No. of lactating animals: ________________Age: ____________ Breed: ____________________

Weight: _______________

*Body Condition Score (Avg): 1_____, 2______, 3______, 4_______, 5_______

Current Status: Dry/ Lactating/ Pregnant Number of Lactations: _____________

Age at First Calving (Avg): _________ Present Parturition: ___________________

Avg. Milk Production (Previous lactations wise): 1_________, 2_________, 3_________, 4__________

Reproductive Disorder: If any ____

Any other disease problem present concomitantly: ____

Part 2:

Vaccination: Y/N Deworming: Y/N Product Name/Composition: ______________________

Animal Kept by owner from previous: ________________Days/Months

Feeding System: Stall base/Field Grazing/Combination

If grazed: Type of grazing area: Mountainous/Desert/River/Plain

Types of fodder: ____

Amount of Fodder (Per Day): _______________ Concentrates used: Y/N

Types of concentrates: _______________________ Amount of Concentrates: _________________

Salts/Herbal stomach powder used: Y/N Frequency of Use: _____________________________

Ingredients: ____

Water source at farm/home: Tap water/Hand pump/_____________________________

During Grazing: Pond/Canal/Commercial water trough

Tick control measure: Y/N Method: ___________________________________

Fly/Mosquito control measure: Y/N Method: ___________________________________

Types of Housing: Shed/Fence/Open Type of Bedding: Cut straw/ sand/concrete floor

Part 3: Results from the questions related to Milk fever

Question	Answers	Number of Answers
Do you think milk fever as economical problem in your herd?	Yes	212
	No	0
Any case of milk fever recorded in herd?	Yes	139
	No	73
How often you faced milk fever cases in your herd?	Often	15
	Sometimes	15
	Rarely	109
Are you using commercial calcium mixtures for dry cows?	Yes	194
	No	18
If yes, when during dry period?	Complete dry period	87
	First part	3
	Last part	104
What is your milking strategy for first two days after calving?	Complete emptying of udder	87
	partly emptying of udder	125
Type of commercial calcium containing product	Chloride	31
	Propionate	99
	Carbonate	58
	Acetate	0
	Other	14
Ways to prevent milk fever in herd	Per oral feeding of calcium product	38
	IV administration	0
	Feeding strategies	7
	Combination	2

	restricted milking	157
How many of the cows are actively prevented from milk fever	All None Some, based on age Some, based on other criteria	75 0 9 126
Reason for selecting any of above method	Easiness Efficacy Price Combination	140 6 27 34
If using feeding strategies, which method is used	High calcium diet Low calcium diet for dry cow any other	54 82 1
Did you use Vit.D metabolites prepartum?	Yes No	41 171
How many of the cows are given vitamin D metabolites prepartum?	All None Some, based on age Some, based on other criteria	4 0 1 36
How much effective, you found this method?	No effect at all some were prevented all of them	1 17 23