



Clinical, Hematological and Some Serum Biochemical Alterations in Local Cows Affected with Milk Fever in Gogjalee Region, Mosul, Iraq



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MILK fever (Hypocalcemia) is a metabolic disorder that has serious economic impacts on health of dairy cattle and production. Current research endeavored to evaluate the clinical, hematological and some biochemical alterations in cows affected with milk fever in the Gogjalee region, Mosul in Iraq. This research was conducted on 30 local breed cows aged (three to seven years) from private farms diagnosed with postparturient paresis according to clinical signs and laboratory confirmations. Ten healthy cows served as control groups. Blood samples were taken from each animal for biochemical and hemogram analysis. Results showed typical clinical signs of milk fever with different stages of the disease. Significant ($P < 0.05$) increases in the body temperature, respiratory rates and significant ($P < 0.05$) decreases in heart rates in affected animals. Older ages (five to seven years) had considerably higher rate of 73.33% ($n=22$) than three to four-years-old with 26.66% ($n=8$). No significant changes were seen in the blood picture except in the total leukocytic count (TLC). Results revealed significant decrease of Calcium, Inorganic phosphorus, and significant increase of magnesium, haptoglobin (Hp), aspartate amino transferase (AST), alanine amino transferase (ALT), creatine kinase (CK), and no significant changes in total protein levels compared to the control groups. Our study indicates the prevalence of milk fever in local breed cows in Gogjalee region. A certain parameters (Ca, P and Mg), hepatic enzymes (ALT, AST, CK and Hp) are also useful and important in the diagnostic process for detection of milk fever.

Keywords: Milk fever, Clinical signs, Hematology, Biochemical.

Introduction

Milk fever, also known as parturient paresis is a production disease in high producing dairy cows, mostly occurring during the initial 48 hours of calving. It negatively affects the profitability of dairy production systems [1,2]. Hypocalcemia at the transitional period makes animals susceptible to several illnesses and constitutes a risk factor for higher mortality. In the field the incidence of clinical milk fever ranges from 0–10%, but may reach 25% of cows calving and some authors place it around 65% [3-5]. A decline in ionized calcium concentration in the extracellular space, such as plasma is the fundamental biochemical disturbance in milk fever and characterized by the

failure of homeostasis to sustain normal blood Ca level. Following calving body Ca demand is elevated to certain levels in daytime because of high colostrum and milk production subjecting the animals to risk of the development of milk fever especially when the majority of homeostatic mechanisms for Ca balance are not functioning as they should [6,7].

Cows are usually affected by several stressors during transition period due to increased demands of the fetus and onset of lactation, stressors include: increased body lipid mobilization, oxidative stress, and changes in endocrine and immune function which collectively increase the risk of the disease [7,8]. Animals react to these changes via a series of

physiological responses, one of which is the “acute phase response” as an element of the overall non-specific immune system and producing a range of proteins in the liver, including serum amyloid A (SAA) and Hp [9,10].

Hypocalcemia usually occurs in two forms: clinical and subclinical. The risk of hypocalcemia in dairy cows increases with age and number of lactations, from 25% in the first lactation to 54% in the fifth lactation [11]. Clinical manifestations of this disease comprise inappetence, tetany, difficulty of urination and defecation, lateral recumbency, and ultimate coma and demise if left untreated [7, 11]. Reduced Ca concentration in the organism results in a loss of appetite, contributing to a negative energy balance, increased fat mobilization and ketosis [12,13].

Clinical hypocalcemia in dairy cows significantly increases the susceptibility to other disease conditions such as retained placenta, abomasum displacement, dystocia and acetoneamia. It also affects muscle contraction causing teat sphincter inadequacy, which may lead to inflammation of the mammary gland and metritis [10,11,14].

Milk fever is economically significant in terms of the direct cost associated with the treatment of clinical cases and estimated losses in production. Cows afflicted by milk fever are also at risk of developing secondary metabolic disorders, and changes in the reproductive system. Removing affected cows from farms and/or lowering the productive life of a dairy cow add significantly to production costs [11,13,14,15,16].

The current study has focused on evaluating the clinical signs, hemogram and serum biochemistry profile in cows affected with milk fever in Gogjalee region, Mosul, Iraq.

Material and Methods

Animals and Study area

A total of 30 local breed cows (aged three to seven years) from private farms in Gogjalee region, Mosul, Iraq, were diagnosed as postparturient paresis according to history, clinical signs and laboratory confirmation. Other ten cows apparently healthy served as control group were used over a one-year period. Clinical signs and clinical parameters (body temperature, heart rate, respiratory rate, and rumen contraction) were documented through preformed clinical examination card.

Samples collection

Blood sampling (10 ml) was performed through coccygeal venipuncture from each animal before treatment and divided into 5ml with anticoagulant for hemogram analysis and other 5ml into sterile vacuum glass tubes (Vacutainer, Plymouth, china) without anticoagulant and centrifuged immediately at 3000 rpm for 10 min at room temperature for serum biochemical measurements in Diagnostic Laboratory of the Faculty of Veterinary Medicine. Serum was stored at -20°C until assay.

Laboratory analysis

Blood analysis including red blood cells count, hemoglobin concentration, packed cell volume (%), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count and TLC measurements were obtained with a Hematology analyzer (Mythic 18VET/France).

Serum was utilized for biochemical analysis, which included AST, ALT, CK, total protein and total serum calcium, magnesium, and inorganic phosphorus concentration was subjected to analysis employing commercial kits. Commercial reagent kits (Biolabo, France), based on spectrophotometric methods using the spectrophotometer BT 1000 (Biotecnica Italia). The steps of analyzing were adapted from BIOLABO manufactures. Serum Hp was measured with a commercial colorimetric kit (Phase HP kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. An automatic plate reader (BioTek® Elx800, USA) was employed in this study.

Statistical analysis

The results obtained were evaluated by assessing the mean values and standard deviations in each affected and healthy group of cows. The significant between ages analyzed using chi-square in IBM SPSS Statistics 19 (IBM, USA). The importance of variances in mean values between the groups was assessed with Student's t-test using IBM SPSS Statistics 19 (IBM, USA). Values of $p < 0.05$ and $p < 0.01$ were considered significant.

Results

The results of the study revealed that the affected cows showed different stages of milk fever. Generally clinical signs were loss of appetite, decrease ruminal contractions, cold mouth, dry muzzle, irritability and tremor of the

limbs muscles, inability to move, reeling and shaking head, grinding of teeth and lying prone for long periods with the head tilted to the wild. Other animals suffer from the inability to stand, have cold skin and a tendency to lie on the lateral side (Table 1).

The study also revealed that 16 (53.33%) of the affected cows showed first stage of the disease, and 12 (40%) and 2 (6.6%) showed the second and third stages of the disease respectively. (Table 2).

The results of the study showed that cows aged five to seven years had significantly ($P < 0.05$) highest infection rate of 73.33% ($n=22$), whereas animals aged three to four years accounted for 26.66% ($n=8$) of affection (Table 3).

Our study showed a significant ($P < 0.05$) increase in the body temperature, respiratory rates and significant ($P < 0.01$) decreases in heart rate and rumen contraction in affected animals compared with the control groups (Table 4).

The blood picture of animals infected with milk fever in this study showed significant ($P < 0.05$) elevation of TLC and no significant changes were recorded in other parameters in cows infected with milk fever in comparison with control group (Table 5).

The study also indicated significant ($P < 0.05$) decrease of serum calcium, inorganic phosphorus concentrations and significant ($P < 0.05$) increase of magnesium, Hp, AST, and CK and no significant change in total protein levels in infected animals compared with control group (Table 6).

TABLE 1. The clinical signs in cows affected with milk fever (n=30).

Clinical signs	Frequency	Percentage (%)
Loss of appetite	12	40
Decreased intestinal motility	6	46.66
Cold mouth	7	33.33
Dry muzzle	5	86.66
Excitation	13	73.33
Muscle tremors	11	40
Incoordination and trismus	6	64.66
Sternal recumbency	7	33.33
Unable to stand	5	86.66
Cold extremity and lateral recumbency	1	6.6

TABLE 2. Stages and frequencies of milk fever.

Stage	Frequency	Percentage (%)
Stage 1	16	53.33
Stage 2	12	40
stage 3	2	6.6

TABLE 3. Rate of milk fever according to the age of the animals.

Ages (years)	No of affected animals	Percentage %
3 - 4	8	26.66%*
5 - 7	22	73.33%

*Values significantly different ($P < 0.05$).

TABLE 4. Clinical parameters in animals infected with milk fever and in control group.

Parameter	Control group	Affected group
Body temperature (C°)	38.64 ±0.12	39.06 ±0.05*
Respiratory rate / min	26.00±1.70	32.40 ±1.52*
Heart beat rate / min	72.20 ±2.70	64.13 ± 1.58**
Rumen contraction / 5min	5.93 ±0.32	2.71 ± 0.30*

*Values significantly different (P < 0.05). **Values significantly different (P < 0.01).

TABLE 5. Blood parameters of cattle infected with milk fever and healthy group. Data are presented as mean ± standard error.

Parameters	Control group	Affected group
Erythrocytes, ×10 ⁶ µl	6.20±1.18	6.12±0.53
Packed cell volume, %	31.27 ± 2.11	32.25±5.53
Hemoglobin, g/ dL	10.30 ± 0.74	9.00±1.20
MCV(fl)(54.75±2.50	53.23±3.59
MCH(pg)(14.800±.66	14.46±0.84
MCHC (%)	28.16±1.25	27.00±0.55
Platelet counts, x10 ³ µl	260.00±35.79	303.50±44.54
Total leukocyte counts, x10 ³ µl	9.38±2.25	10.70±4.66*

*Values significantly different between infected cattle and healthy group (P<0.05).

TABLE 6. Biochemical parameters of cattle infected with milk fever and healthy group. Data are presented as mean ± standard error of mean.

Serum component	Control group	Affected group
Calcium (g/dL)	9.57± 0.13	5.46 ±0.24*
Phosphorus (g/dL)	6.73± 0.15	5.51±0.30*
Magnesium (g/dL)	1.84± 0.03	2.03±0.06*
Total protein (g/dL)	6.78±0.22	6.43±0.22
Haptoglobin (Hp g/L)	0.1 ± 0.01	1.7 ± 0. 12*
Aspartate aminotransferase (AST, U/L)	53.45 ± 18.76	92.61 ± 13.05*
Alanine transaminase (ALT, U/L)	34.53 ± 10.75	66.45 ± 10.24 *
Creatine kinase (CK, U/L)	1.2±0.1	2.3±0.3*

*Values significantly different (P < 0.01).

Discussion

This study provided an evaluation of the clinical, hematological and some biochemical (some minerals status and acute phase proteins response as well as some serum enzyme levels) in cows infected with parturient paresis.

Characteristic clinical signs of Milk fever recorded in the present study were: ataxia, loss of appetite, cold extremities, tachypnea,

increased rectal temperature, lateral and sternal recumbency, muscle tremor and excitation which may be the result of significant decrease of calcium and phosphorus concentration in the blood. Magnesium, like calcium reduces neuromuscular irritability and a drop in its concentration in the blood results in spontaneous muscle contractions or tetany. These results agree with earlier studies [17-19]. It should be noted that the seriousness of the clinical signs relies on the extent of decrease of serum calcium level, but, signs may

also be exacerbated when other minerals and tissue elements are changed such as Mg, P, Fe, Na, K and glucose. The failure of the cow to sustain normal serum calcium concentration is due to the failure to adapt adequately the mineral metabolism in responding to a higher calcium requirements. Dairy cows require approximately 20g of calcium daily at the end of the dry period. When colostrum is produced, the requirement rises to 30 to 70 g daily, based on milk yield. The mechanisms to reduce urinary calcium excretion, to enhance absorption of calcium from the gut, and to improve the release of calcium from bone tissue, on the other hand, needs approximately 48 h, which may result in inadequate calcium supply in this period [20,21].

Cows five to seven years old group have the highest affection rate compared to those aged two to four years. This could be due to the fact that milk fever is age-related and older animals, especially multiparous cows are more susceptible to disorder as their intestinal absorption and Ca bone resorption efficiency decrease with age. Moreover, management and vitamins deficiency may also be predisposing factors. This result is consistent with [7,22]. Previous studies documented that the risk of a cow developing milk fever rises with age, from the third lactation onwards, as dairy cows produce more milk, which requires a higher demand for calcium. Meanwhile, ageing causes a decreased ability to obtain calcium from bone stores and a thus a decline in the active transport of calcium in the intestine, besides negatively impacting production of 1,25(OH)₂D₃. Increasing age also leads to a decline in some of the 1,25(OH)₂D₃ receptors [23]. Martin-Tereso and Martens [24] revealed that hypocalcemia is the predominant reason for recumbency during the periparturient period, usually taking place 12–72 h after calving with a rise of 5% to 7.5% in multiparous cows.

Increase in respiratory and pulse rates in affected cows may be related to stress and the decrease in ruminal movements could be attributed to hypocalcemia which reduces rumen motility. These results agree with previous literatures [25,26].

Results of the current study also showed a significant decrease in serum calcium and phosphorus levels while the serum magnesium was substantially higher in affected animals compared to healthy control group, thus suggesting increased use of these macroelements by the mammary glands. Moreover, it is also

probable that lower feed intake prior to clinical signs of disease can have an effect on serum calcium levels. These observations are in accordance with many investigators [27-30]. It has been shown that the alterations in Ca metabolism caused by lactation are more substantial than parturition as there is loss of blood Ca to milk [3]. It is important to note that hypocalcemic cows have a tendency toward hypophosphatemia, due to the secretion of parathyroid hormone (PTH) in responding to low blood Ca, and increased urinary and salivary loss of phosphorus [31]. Otherwise, fetal inorganic phosphorus (iP) demand in late gestation, decreased absorption of salivary iP because of lowered ruminal motility, and higher tumor necrosis factor (TNF)- α level, as well as excessive cortisol secretion can cause hypophosphatemia [32, 33].

The results obtained in this study showed increased magnesium levels in typical hypocalcemia cases could be the result of heightened parathyroid hormone (PTH) level in responding to decrease of serum calcium level which in turn induces higher renal tubular reabsorption of Mg makes kidneys excrete lower levels of the excess dietary magnesium absorbed, besides the maintenance of physiological blood Mg levels is nearly totally dependent on a constant influx from nutrition. The result of this study is similar to what was previously mentioned by [34,35,36].

The blood picture of animals affected with milk fever in this study showed significant rise in the overall TLC but no significant changes were recorded in other parameters. Increase of TLC might be attributed to the antepartum rise in cortisol. This finding is in agreement with those of other researches [10,37].

Our results indicate significant increase of Hp levels in affected animals. The study analysis concurs with [38,39]. Generally, the critical phase response is an intricate and total initial defense mechanism of reactions triggered by trauma, infection, tissue impairment, inflammation, stress or neoplasia. Other possible reasons may be the fact that dairy cattle often develop pathologic conditions in the peripartum period. In contrast, Saini et al. [37] revealed that lactation and pregnancy in cattle seemed not to affect the Hp level in blood serum. Tóthová et al. [38] stated that at the time nearing parturition there are substantial alterations in the levels of acute phase proteins, and also in the

total protein metabolism of dairy cows, which suggest that the postparturient period is a crucial biological stage, during which there is maximum occurrence of metabolic illnesses.

Further serum biochemical analysis in the current study showed significant increase of aspartate amino transferase (ALT) and alanine transferase (ALT) in affected animals compared to control group, due possibly to the recumbency as Protracted recumbency induces ischemic muscle trauma and necrosis and raises in the serum muscle enzyme activity of CK, ALT and AST. This may be attributed to fatty liver changes. The results come in agreement with [40,41]. Stari and Zadnik [41] revealed that the recumbency of milk fever cows could have a negative effect on hepatic tissue and consequently result in a rise of activity of liver enzymes.

In conclusion it could be said that these results indicate that hypocalcemia is prevalent in dairy cows in the Gogjalee region. The results provided above are indications that around the time of parturition, important alterations occur in the levels of acute phase proteins. Biochemical blood tests facilitate the diagnosis. Some parameters (Ca, P and Mg) and hepatic enzymes (ALT, AST, CK and Hp) are also useful and important for the process of diagnosing the detection of milk fever.

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Conflicts of interest

The authors declare no conflict of interests of the manuscript.

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Ethical consideration

All Ethical consideration had been taken during dealing and sampling with study animals

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التغيرات السريرية والدموية والكيموحيوية في الأبقار المحلية المصابة بحمى الحليب في منطقة كوكجلي ، الموصل، العراق.

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¹ جامعة الموصل . كلية الطب البيطري . فرع الطب الوقائي والوقائي . الموصل . العراق.

² باحث مشارك . جامعة ولاية أوهايو . الولايات المتحدة الأمريكية.

حمى الحليب (نقص كالسيوم الدم) هي إحدى الأمراض الأيضية المهمة التي لها آثار اقتصادية سلبية على صحة وإنتاجية الماشية. هدفت الدراسة الحالية تقييم التغيرات السريرية والدموية والكيموحيوية في الأبقار المصابة بحمى الحليب في منطقة كوكجلي. الموصل-العراق. أجري هذا البحث على 30 رأس من الأبقار المحلية بعمر (3 إلى 7 سنوات) شخصت إصابتها بحمى الحليب بعد الولادة اعتماداً على تاريخ الحالة والعلامات السريرية والفحوصات المختبرية فضلاً عن عشرة إبقار سليمة ظاهرياً عدت كمجموعة سيطرة. تم أخذ عينات الدم من كل الحيوانات لأجراء التحاليل الدموية والكيموحيوية في المختبر. أظهرت نتائج الدراسة علامات سريرية نموذجية لحمى الحليب مع مراحل مختلفة من المرض. لوحظت زيادة معنوية ($P < 0.05$) في درجة حرارة الجسم. ومعدلات التنفس وانخفاض معنوي ($P < 0.05$) في معدل ضربات القلب في الحيوانات المصابة. كما كان معدل الإصابة في الأعمار (5 إلى 7 سنوات) أعلى معنويًا 73.33٪ من الأعمار (3 إلى 4 سنوات) 21.11٪. لم يلاحظ أي تغييرات كبيرة في الصورة الدموية ما عدا في العدد الكلي لكريات الدم البيض. أظهرت النتائج أيضاً انخفاضاً معنوياً للكالسيوم، الفسفور غير العضوي، وزيادة معنوية للمغنيسيوم، الهابتوكلوبين، خميرة الأسبارتات ناقلة الأمين، والألانين ناقلة الأمين، انزيم الكرياتينين، ولم يلاحظ تغييرات معنوية في مستوى البروتين الكلي مقارنة مع مجموعة السيطرة. استنتجت الدراسة الحالية أن مرض حمى الحليب منتشر في سلالات الأبقار المحلية في منطقة كوكجلي. بعض العلامات مثل (Ca و P و Mg) والإنزيمات الكبدية (ALT و AST و CK و Hp) يمكن أن تكون مفيدة ومهمة أيضاً في عملية التشخيص للكشف عن حمى الحليب.