Study on Epidemiology of Milk fever and Ketosis Affecting Transition Period Crossbred Dairy Cows in Selected Sites of Bale and West Arsi Zones

A Research Report

Submitted by

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DECLARATION

Researchers: We, the undersigned, solemnly declare that this RESEARCH REPORT entitled "*Study on Epidemiology of Milk fever and Ketosis Affecting Transition Period Crossbred Dairy Cows in Selected Sites of Bale and West Arsi Zones*", funded by and carried out in Madda Walabu University under the ARTTCE Vice President Office and the Research, Publication, Ethics & Extension Directorate. I assert that the statements made and conclusions drawn are the outcome of the research. I further declare that to the best of my knowledge and belief the project report does not contain part of any research work which has been previously conducted, published or submitted for the award of a degree/diploma/ certificate in Madda Walabu University or any other University. We are aware that we will be held accountable.

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ABSTRACT

Milk fever and ketosis are among the most prevalent transition period metabolic diseases that have direct impact on productive and reproductive performance of dairy cows. It constitutes one of the key health issues occurring in dairy cattle. A cross sectional study was conducted from November 2023 to June 2024 with objective of epidemiological study of milk fever and ketosis affecting transition period crossbred dairy cows in selected sites of Bale and West Arsi Zones. Purposive and simple random sampling techniques were employed to select study sites and study animals/households. Data were collected by semi-structured questionnaire, focus group discussion, key informant interview, personal observation and document review to assess the hypothesized potential risk factors. In addition, clinical examination/ observation, collection of blood samples and laboratory examination were undertaken. Accordingly, the results of the study showed that, out of total 527 dairy cattle evaluated, 37.57%(198) were found to be affected by milk fever, ketosis or both (mixed infections). The prevalence of rates of milk fever, ketosis and mixed infections were 13.66% (72), 17.27% (91) and 6.64%(35), respectively. A chi-square analysis revealed that prevalence of milk fever and ketosis was significantly associated with the hypothesized risk factors investigated such as age, parity, lactation stages, milk yield, herd size, and grazing patterns ($P<0.05$). Thus, older cows (> 5 years) (OR = 5.744), parity number (OR = 11.00), higher daily milk yield (> 15L/d) (OR = 3.52), purchased cows (OR = 7.343), history of previous transition period disorders (OR= 1.244) and zero grazing pattern (OR = 9.191) were more likely to be infected with the diseases than their counter parts were at higher risk of infection with milk fever and ketosis than their counter groups. The present epidemiological investigation indicated that milk fever and ketosis is among the major dairy cattle health problems in the area which warrants implementation of appropriate and integrated control and prevention programs to mitigate the disease impact through multidirectional approaches.

Key words*: Epidemiological, Milk fever, Ketosis, Transition, Dairy Cattle, Bale, West Arsi.*

ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
BCS	Body Condition Score
BHBA	Beta-hydroxybutyric acid
Ca	Calcium
CK	Clinical ketosis
CMT	California Mastitis Test
CNS	Central Nervous System
DA	Displaced abomasum
DCAD	Dietary Cation-Anion Difference
DHCC	Dihydroxycholecalciferol
DIM	Days in Milk
DMI	Dry Matter Intake
GH	Growth Hormone
HC	Hypocalceamia
IMIs	Intra-mammary infections
IGF	Insulin-like growth factor
LDA	Left Displaced Abomasum
mEq	milliequivalent mmol millimole
MF	Milk fever
Mg	Magnesium
NEB	Negative Energy Balance
NEFA	Non-esterified fatty acids
OAA	Oxaloacetic acid
PH	Parturient hypocalcaemia
PMN	Polymorphonuclear leukocytes
RFM	Retained fetal membranes
RP	Retained placenta
SCK	Sub-Clinical Ketosis
SCMF	Subclinical milk fever

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1. INTRODUCTION

In dairy cattle, the period between 3 weeks before and 3 weeks after parturition is called as transition period (Saed *et al*., 2020). It is one of the most critical physiological stage since most of the metabolic and infectious diseases occur during this period (Caixeta *et al*., 2015). It is very critical period for the dairy cow production cycle because it is associated with multiple changes including physical, physiological, hormonal/endocrine, immunological, nutritional/feed and drastic metabolic changes which can impact subsequent health, production and reproductive performance (Naher *et al*., 2020). It is moving from non-lactating to lactating state, a major drop in feed intake and feed changes as well as considerable fetal growth, mammary tissue remodelling and high nutritional demands (Seifi, 2011). The period is characterised by increased energy and calcium demands due to foetal growth, lactogenesis/ colostrum synthesis and milk production/beginning of lactation combined with a decline of dry matter intake (DMI) around parturition which leads to negative energy balance (NEB), increased lipid mobilization and a reduction in blood concentrations of calcium (Wankhade *et al*., 2017). Approximately, 75% of diseases in dairy cattle occur in the first month of post-partum and 50% of dairy cattle (one in two dairy cows in a herd) suffered from one or multiple metabolic disorders and infectious diseases in the transition period (Roche *et al*., 2013).

Among the transition period metabolic diseases, milk fever and ketosis are the most prevalent and economically important diseases that have huge impact on productive and reproductive performance of dairy cows (Azizi *et al*., 2022). Milk fever is a metabolic disease of high yielding transition dairy cows and caused by calcium deficiency in the body of the animal which is important for contraction of muscles (Hesam *et al*., 2018). It is characterised by low blood calcium level which interferes with muscle function throughout the body, causing general weakness, depression, recumbency and death (Thirunavukkarasu *et al*., 2010). Ketosis is a major metabolic disorder of dairy cows in early lactation, which develops when dairy cows fall into a condition of excessively negative energy balance (Kshandakar *et al*., 2017). It is manifested by high concentrations of the ketone bodies and concurrent low concentration of glucose in the blood, causing anorexia, depression, decreased in milk production, loss of body condition, nervous signs and death (Asl *et al*., 2014). Both the clinical and sub-clinical forms of metabolic diseases occur in dairy animals (McArt *et al*., 2012). The subclinical cases are more important because they are far more frequent, they cannot be easily diagnosed, and may impair the longevity and production of the cow. Subclinical disease incidences of metabolic diseases are more common than clinical cases (Eliana *et al*., 2018). Studies revealed that subclinical milk fever (SCMF) (hypocalcaemia) and subclinical ketosis (SCK) have been found to be higher than clinical hypocalcaemia and ketosis (Mulligan *et al*., 2006a). Recent studies indicated that the incidence of subclinical hypocalcaemia is 8-10 times greater than clinical hypocalcaemia (Hesam *et al*., 2018). Likewise, SCK has been reported to be higher than clinical ketosis (Garro *et al*., 2014).

There are various potential risk factors for the precipitation of milk fever and ketosis in the dairy cows (Neves *et al*., 2017). These include cow-level factors, farm/herd-level factors, management factors, management errors, and stressors (Oetzel, 2007). The cow level/the animal risk factors involved include the followings; breed, age, parity number, body condition score (BCS), stage of transition period, stage of lactation, daily milk yield, length of dry period and history of previous transition period disorders (Neves *et al*., 2017). Moreover, on the farm level, the potential risk factors include; type of the diet, mineral supplementation, housing criteria, grouping strategies, pen moves, and periodical monitoring of metabolic profile test were basically recorded (Oltenacu and Broom, 2010).

Dairy cows affected with clinical milk fever and clinical ketosis could be diagnosed in the field on the basis of disease history, clinical findings and response to therapy (Hunter, 2015). An understanding and day to day update of these metabolic diseases becomes essential and critical for successful venture of dairy farm (Zaidi, 2016). Metabolic profile test is most useful in the diagnosis and management of periparturient disease (Suthar *et al*., 2013). It is designed to monitor the metabolic health of cows in dairy herd in relation to management, nutrition, milk production, disease and aid in the diagnosis of metabolic disorders (Lager and Jordan, 2012). It does this by comparing the average concentration of blood constituents of a group of cows to the defined mean concentration values (Hunter, 2015). Metabolic profile test in conjunction with animal and facility evaluation, body condition scoring and ration evaluation is useful tool for nutritional evaluation in dairy herds as well as diagnosis and management of periparturient disease (Kaneko *et al*., 2008). Major efforts should be directed toward minimizing health problems that generally arise during the transition period. Therefore, a smooth transition is important for minimizing health problems and optimizing productivity and profitability for the forthcoming lactation (Rodríguez *et al*., 2017).

1.1. Statement of the Problem

Despite significant advances in management of dairy cattle, metabolic diseases continue to be a substantial challenge (Saed *et al*., 2020). A major challenge for dairy producers and veterinarians is to maintain a dairy cow health during the transition period (Berge and Vertenten, 2014). This continuing challenge is related to progressive improvement in dairy cow genetics for milk, improvements in production capacity challenge us to manage; the ever-increasing average milk yields of modern dairy cattle, and feeding dairy cows to allow them to adapt to the tremendous metabolic demands of high milk production (Roche *et al*., 2013). The sharp and tremendous increase in milk yield imposed great impact on metabolic capacities of dairy cows (Kshandakar *et al*., 2017).

Keeping transition cows healthy is one of the most important steps in maintaining good fertility and efficient milk yield (Zaidi, 2016). It is now recognized that defining and meeting the appropriate management and health requirements of the transition dairy cow can greatly impact animal health, production in the ensuing lactation, overall longevity, and animal well-being (Rodríguez *et al*., 2017). After all, cows are fragile mothers and should be treated with the best care, especially during transition period. The milk-secreting and future reproductive capability of the dairy cow is related to transition period events (Hernandez, 2013). Therefore, major efforts should be directed toward minimizing health problems that generally arise during transition period (Uyarlar *et al*., 2018).

Milk fever and ketosis are the most prevalent and economically important transition period metabolic disorders (Eliana *et al*., 2018). They occur at the most productive stage of lactation (Zaidi, 2016). The diseases inflict huge/massive economic losses directly or indirectly and affect the health and welfare of dairy cows, hence can be extremely costly and cause frustration to dairy farmers/producers (Uyarlar *et al*., 2018). The diseases cause impaired/severe reduction in milk production, reduced productivity and reproductive performance/poor fertility, predisposition/increased risks for developing other metabolic and infectious diseases and culling losses (Gantner, 2015; Azizi *et al*., 2022). Milk fever and ketosis can also be called the “gateway diseases” as it increases the risk of other diseases (Kshandakar *et al*., 2017). Cows that develop milk fever and ketosis are more prone to mastitis, retained placenta, metritis, left displaced abomasum (LDA), fatty liver, dystocia, udder edema, acidosis, laminitis, reduced feed intake, and lowered gluconeogenic capacity (Uyarlar *et al*., 2018).

The existing literatures revealed that high prevalence rates of milk fever and ketosis have been reported from crossbred transition period dairy cows from different parts of the world (Wankhade *et al*., 2017; Naher *et al*., 2020; Saed *et al*., 2020). Various investigators recorded prevalence of milk fever ranging from 3.8 to 48.04% and of ketosis from 5.21 to 53.33% in high-producing herds with SCMF and SCK found to be higher than clinical cases (Chiwome *et al*., 2017; Naher *et al*., 2020; Saed *et al*., 2020). The SCMF (48.04%) and SCK (53.33%) were recorded 10 times greater than clinical milk fever and clinical ketosis from Egypt and Bangladesh, respectively (Saed *et al*., 2020; Naher *et al*., 2020).

Milk fever and ketosis in Ethiopia have received little attention which occurs at a much higher rate than other diseases, yet it is the worst in terms of reduced productivity (APHRD, 2015). Moreover, the government, researchers, farmers and NGOs are focusing presently towards cross breeding practices to produce high milk yielding dairy cattle to meet ever increasing demands. However, along these practices farmers are not much aware of management of metabolic diseases such as milk fever and ketosis, particularly the subclinical cases which occurred at higher rates (up to 10x) than the clinical diseases (Naher *et al*., 2020).

Implementation of appropriate and effective prevention and control of milk fever and ketosis affecting dairy cattle requires comprehensive investigation, understanding and quantification of its epidemiological factors (Kshandakar *et al*., 2017). However, limited/no studies have been conducted so far in Ethiopia on epidemiology of the diseases (Anteneh *et al*., 2012; APHRD, 2015). Hence, no published research so far on epidemiology of metabolic diseases.

Therefore, taking all the above critical gaps into consideration, cross-sectional studies will be undertaken with the objectives of epidemiological investigations in to milk fever and ketosis affecting transition period crossbred dairy cows in selected sites of Bale and West Arsi zones to generate comprehensive epidemiological data that will facilitate the development of strategic effective interventions.

1.3. Objectives

This study was executed based on the following objectives:

1.3.1. General objective

- To study epidemiology of milk fever and ketosis affecting transition period crossbred dairy cows in selected sites of Bale and West Arsi Zones**.**

1.3.2. Specific objectives

- To determine prevalence of milk fever and ketosis affecting transition dairy cows.
- To assess potential risk factors contributing to occurrence of the diseases.

2. LITERATURE REVIEW

2.1. Transition Period Health and Associated Changes in Dairy Cows

The term "transition period/peri-parturient" stems from the word "parturition" and the prefix "peri," which literally but vaguely means "around" (Wankhade *et al*., 2017). This phase generally includes the dry period and the first 3 weeks after calving. The milk-secreting and future reproductive capability of the dairy cow is related to peri-parturient events (Caixeta *et al*., 2015). Parturition is one of the most critical stages of the reproductive cycle and is a period of significant death rate, as well as potentially severe debilitating injury to dam and newborn calf. Future efficiency of reproduction and milk yield can be affected adversely at this time (Amral-Phillips, 2014). Therefore, major efforts should be directed toward minimizing health problems that generally arise during the peri-parturient period (Rodríguez *et al*., 2017). Keeping cows healthy is one of the most important steps in maintaining good fertility and efficient milk yield. Healthy cows produce more milk, rebreed sooner, and have lower culling rates than their unhealthy herd-mates (Seifi, 2011). Healthy cows were first bred at about 70 days, had conception rates greater than 55%, had fewer than 100 days open or 382-day calving intervals, and were culled at rates less than 12% (Azizi *et al*., 2022). Good sanitation and a herd-health program designed to maintain healthy cows were essential to this achievement. Studies have shown that herd health programs return added profit to dairy producers, even though veterinary costs per cow increase (Galvão, 2012; Kshandakar *et al*., 2017).

As cows transition from pregnant nonlactating to lactating nonpregnant, numerous physical, physiological and endocrine changes occur and such changes can impact subsequent health, production and reproductive performance (Wankhade *et al*., 2017). It is one of the most critical physiological stage since most of the metabolic and infectious diseases occur during this period (Allen and Piantoni, 2013). Fetal dry weight increases exponentially during gestation, particularly during last trimester of pregnancy, hence during this period higher demand for energy, protein, calcium and other nutrients to fulfill the requirement (Mulligan *et al*., 2006a). Disturbance of one or multiple metabolic processes, related to regulation of a certain metabolite in the body fluids/plasma metabolites such as ketone bodies, calcium, or nonesterified fatty acids, is known as metabolic disease or disorder **(**Chapinal *et al*., 2011**).**

On the other hand, the occurrence of health problems during the transition period is a major risk factor for subsequent productive and reproductive performance (Neves *et al*., 2017).

There are many contributing causes of transition cow problems, but most of the problems are caused by three common pathways (Azizi *et al*., 2022); negative energy balance around calving and likely negative protein balance; hypocalcemia, both clinical and more importantly subclinical; immune suppression, and partly due to the first two, but also mediated by hormonal changes, stress, management errors, and other nutritional deficiencies (selenium, vitamin E) (Overton and Yasui, 2014). Intense selection for production has led to a reliance on body reserves to support early lactation (Gantner, 2015). Consequently, the commencement of lactation and some of the remainders of lactation are often in negative energy balance. This leads to an imbalance in hormones and metabolites giving rise to metabolic diseases. Dysfunction or imbalance in metabolic processes leads to disease (Buttchereit *et al*., 2012).

2.2. Metabolic Disorders and Associated Diseases Affecting Transition Period Dairy Cows

The transition period is very critical for dairy cows and it is period of time associated with multiple changes such as physical, physiological, hormonal and immunologic changes (Galvão, 2012). One in two dairy cows in a herd is affected by one or multiple metabolic disorders and infectious diseases. A major challenge for dairy producers and veterinarians is to maintain a dairy cow health during the transition/peri-parturient period (Eliana *et al*., 2018). It is a pivotal time in the production cycle of the cow, in which cattle are at high risk for the occurrence of most of production diseases. The transition to lactation underscores the importance of gluconeogenesis in ruminants as hypoglycemia, ketosis, and related metabolic disorders and infectious diseases are often observed (Chapinal *et al*., 2011).

Cows affected by milk fever are more prone to mastitis, retained placenta, metritis, left displaced abomasum (LDA), distocia, udder edema, and ketosis; cows affected by acidosis are more prone to laminitis, LDA, milk fever, mastitis, and fatty liver (Amral-Phillips, 2014). Those animals affected by retained placenta are more prone to metritis, LDA, and ketosis. Ketosis is accompanied by fatty liver and cows that develop fatty liver and ketosis have reduced feed intake, lower gluconeogenic capacity, lower milk production, and an increased risk for developing other metabolic and infectious diseases (Allen and Piantoni, 2013). In other words, ketosis and fatty liver are common findings in cows affected by milk fever, mastitis, laminitis, LDA, metritis, retained placenta and udder edema. In general, subclinical disease incidence is more common than clinical cases (Mulligan and Doherty, 2008). A subclinical case frequently goes unnoticed and is associated with significant economic losses that include increased clinical diseases risks, impaired milk production and reduced reproductive performance, and culling losses (Oltenacu and Broom, 2010).

2.2.1. Milk fever in dairy cows

The birth and early lactation are periods of too much stress for dairy cows due to the large metabolic challenges that occur in this period (Wankhade *et al*., 2017). During the last 2 weeks pre-calving, non-concentrated feed especially fiber consumption decreased and result in negative energy balance due to fetal development and decreased rumen motility, in the last days before calving, the balance of other nutrients such as protein, vitamins and minerals may also be compromised (Kshandakar *et al*., 2017). The depletion of calcium stores within the cells likely begins several days before calving (Ferneborg, 2010). But, the reduction in serum calcium concentrations usually occurs about 12 to 24 hours after calving **(**Mulligan *et al*., 2006a**).** The disease occurs just before 12 hours, during calving or even 24-48 hours after calving. Blood calcium concentrations remarkably decline in dairy cows around calving, with the lowest concentrations occurring about 12 to 24 hours after calving. To maintain calcium homeostasis after calving, at the start of lactation, calcium compensating mechanisms are activated **(**Ferneborg, 2010**)**. With the initiation of lactation and continued milk production, tremendous adaptations occur in the dairy cow because of the increased need for nutrients to support milk synthesis (Hesam *et al*., 2018).

Milk fever (hypocalcaemia) affects mature dairy cows just before, or soon after calving, when there is a sudden drop of blood calcium levels. According to DeGaris and Lean (2008), before calving, the approximate daily requirement (demand) for calcium for calcium (Ca^{++}) is about 30g per day, allowing 15g for fecal and urinary loss and the remaining 15g to be available for the fetal growth (gestating calf). When supplying calcium far in excess of the daily requirements at dry period, the passive transfer of calcium is sufficient to overcome the needs of the cow and the fetus (Mulligan *et al*., 2006b). Therefore, active hemostatic mechanisms of absorption and resorption of calcium become depressed. As a consequence, at calving when sudden massive demands for calcium occur, the cow is unable to rapidly return to hemostatic mechanisms and is susceptible to severe hypocalcemia until these mechanisms can be activated, which may take several days **(**Kshandakar *et al*., 2017). When intake and homeostatic mechanisms cannot meet the increased demands for calcium metabolism, the pathogenesis of hypocalcemia is initiated (Mulligan *et al*., 2006b).

Besides, the increased need for energy and amino acids for colostrum and afterward for milk synthesis, the requirement for calcium increases two- to three-fold over that required by the dairy cow before calving (DeGaris and Lean, 2008). Shortly before calving, a dairy cow deposits 8 to 10 g/d of calcium into her fetus, but when she calves, 20 to 30 g/d are secreted into colostrum and milk **(**Kshandakar *et al*., 2017). Thus, metabolic adaptations must take place to support the increased need for calcium. If they do not take place soon enough or of sufficient magnitude, the concentration of calcium in the blood drops below a critical threshold and clinical and subclinical hypocalcemia, or milk fever, can result (Hunter, 2015).

2.2.2. Ketosis in dairy cows

Ketosis is a metabolic disease characterized by the excessive increase of ketone bodies such as acetoacetate, BHBA and acetone concentrations in tissues and fluids (Berge and Vertenten, 2014). This disease is extremely important for dairy cattle production. The reason is that it is common; it develops insidiously (proceeding in a gradual, subtle way, but with harmful effects) and causes major economic losses (Galvão, 2012). Ketosis develops when the liver metabolism cannot respond adequately to the gluconeogenesis need which has increased due to the influence of the negative energy balance that occurs when the energy expenditure in the organism is higher than the dietary energy intake (Uyarlar *et al*., 2018). The oxaloacetate pool, one of the most important factors determining the capacity of gluconeogenesis in the liver, is depleted; fatty acids cannot be completely oxidized and are converted into ketone bodies and released into the blood (Suthar *et al*., 2013). Thus, the ketone level rises in all fluids such as blood, urine, milk, saliva and so on.

Milk production is the primary cause for the ketosis that leads to the exacerbation of the negative energy balance in dairy cows (Oetzel, 2007). Serving the respective needs for today's daily milk yields above 30 to 40 kg, lipolysis of adipose tissue compensates the prevailing NEB with detectable metabolic changes already during the periparturient period

(Uyarlar *et al*., 2018). Thus, susceptibility towards metabolic disorders depends on the metabolic adaptation at a very early stage of lactation (Oetzel, 2007).

Excessive release of NEFA from fat depots may overwhelm the capacity of the liver to use the fatty acids as a source of energy and converted to ketone bodies (Uyarlar *et al*., 2018). Feed intake is naturally depressed by about 20% around the time of calving (Seifi, 2011). Simultaneous with reduced feed intake, plasma NEFA and liver triglyceride concentrations are also increase (McArt *et al*., 2012). All factors exacerbating negative energy balance and depression of feed intake around calving increase the risk of ketosis, especially in over conditioned cows (Haq *et al*., 2016). Negative energy balance, high blood levels of NEFA and ketosis reduce the response capacity of white blood cells so that invading bacteria can compete the immune system of the cow (Seifi, 2011). Hypoglycemia alone is not likely to exacerbate periparturient immunosuppression, but hyperketonemia appears to have multiple negative effects on immune functions (Asl *et al*., 2014). Impaired immune functions around calving make cow more prone to infectious diseases (Haq *et al*., 2016).

2.4. Epidemiology and Risk Factors for Development of Milk Fever and Ketosis

With the onset of lactation, most lactating dairy cows enter a stage of negative calcium balance (Azizi *et al*., 2022). The cow will spend the next six to eight weeks of her lactation period increasing calcium intake by increasing calcium rich feed intake and improving intestinal calcium absorption (Mulligan *et al*., 2006b). Parturient paresis is a disease of high producing dairy cattle. It is estimated that 5 to 10% of cows are affected by this disease with some herds having prevalence as high as 25 to 30%. It occurs before calving, but most cases occur within the first 48 hours following calving (Buttchereit *et al*., 2012). Subclinical hypocalcaemia in which concentrations of calcium in the blood does not decline as severely affects about 50% of lactating dairy cows. According to Oetzel (2007), animals that are supplemented with minerals prior to calving have reduced risk of milk fever and the hypocalcaemia percentage of cows is reduced to about 15 to 25% **(**Lean *et al*., 2006**).** The risk factors for hypocalcaemia could be grouped into intrinsic risk factors, which are associated within the animal itself and extrinsic risk factors, which are outside of the animal's body which are commonly known as environmental factors (Neves *et al*., 2017).

According to Neves *et al*. (2017), the factors that contribute to occurrence of milk fever and which influence the incidence and severity of milk fever includes; parturition or number of parity (9% increased risk for each successive lactation), stage of lactation (first-lactation dairy cattle rarely develop milk fever because they produce less colostrum and because they can rapidly mobilize calcium from bone owing to the high osteoclastic activity in their growing skeleton), age (older cows are more sensitive than younger) (Naher *et al*., 2020). Parturient hypocalcemia (PH) is uncommon before the third parturition, and incidence is highest at the fifth or sixth parturition. Thus, cows are most likely to develop PH during their most productive years (Oltenacu and Broom, 2010). In addition, milk yield (cows with higher yield are more predisposed than cows with lower yield) (Naher *et al*., 2020), breed, body condition, length of dry period and diet composition (Neves *et al*., 2017). There is a breed difference in incidence of parturient hypocalcaemia; incidence is highest in the Jersey breed than Holstein Friesian, however exotic and cross breeds are more prone to hypocalcemia than local zebu breeds (Oltenacu and Broom, 2010; Buttchereit *et al*., 2012). Achieving the correct body condition score (BCS) at calving and drying-off is critical for the prevention of milk fever. It has been reported that dairy cows that are over-conditioned at calving are up to four times more likely to develop milk fever (Oetzel, 2007; Neves *et al*., 2017). Older cows and over-conditioned cows have been found to be at greater risk for experiencing milk fever, whereas primiparous cows are much less likely to have low blood calcium levels near calving (Oetzel, 2004). Despite being at low risk for clinical milk fever, prevalence of subclinical milk fever was estimated to be 25% in first lactation cows (DeGaris and Lean, 2008). Throughout later lactations, prevalence of subclinical milk fever was as high as 54% in fifth lactation cows (Reinhardt *et al*., 2011).

There are many risk factors for ketosis such as presence of disease condition (any disease in the perpartum period that causes anorexia in cow) (Azizi *et al*., 2022), breed, lactation, increasing parity and ketosis in the previous lactation, BCS at calving higher than 3.5 on a 5-point of scale, season of calving, ketosis is more likely in winter, and dry period length (Naher *et al*., 2020). Other herd-level management factors (housing type, grouping strategies and pen moves) and many cow-level factors highly risk for ketosis and also dry period length (Asl *et al*., 2011). Ketosis is seen in all parities (although it appears to be less common in primiparous animals) and does not appear to have a genetic predisposition, other than being associated with dairy breeds (Oltenacu and Broom, 2010; Asl *et al*., 2011).

2.5. Clinical Findings of Milk Fever and Ketosis Affecting Crossbred Transition Cows

Goff (2014) showed that many cases of milk fever do not externalize the clinical signs in animals (DeGaris and Lean, 2008). Hypocalcaemia can be clinical or subclinical based on whether an animal may or may not show clinical signs. Milk fever signs include staggering, inability to rise, muscular weakness, recumbency (laying down) and a subnormal temperature (Lean *et al*., 2006). Clinical milk fever (hypocalcaemia) is the most severe hypocalcaemia results in a cow that is unable to rise (from lying to stand position) and is the most easily recognized form of hypocalcaemia with blood Ca++ concentration less than 5 mg/dl (Goff, 2014). Subclinical hypocalcaemia results in less severe disturbances in blood calcium and does not have any outward signs of milk fever. During subclinical hypocalcaemia, blood calcium concentration ranges between 5.5 and 8.0 mg/dl (Hunter, 2015). The after-effects of hypocalcemia conditions are very costly, illustrating the importance of prevention. Clinical milk fever has very serious economic point of view because if not rapidly controlled may lead to loss of the affected animal; on the other hand, subclinical hypocalcaemia assumes a more insidious role leading to loss of production and fertility (Ferneborg, 2010). It is also stated that based on the degree of hypocalcaemia and time of occurrence the clinical sign of milk fever in dairy cattle around calving can be divided into three stages (Chapinal *et al*., 2011);

Stage I: Stage I milk fever is early signs without recumbency. It may go unnoticed because its signs are subtle and transient (Caixeta *et al*., 2015). Affected cattle may appear excitable, nervous, or weak. Some may shift their weight frequently and shuffle their hind feet (Saed *et al*., 2020).

Stage II (Sternal Recumbency): Cows in Stage II milk fever are down but not flat out on their side (Caixeta *et al*., 2015). They exhibit moderate to severe depression, partial paralysis and typically lie with their head turned into their flank (Lean *et al*., 2006). The clinical signs of stage II milk fever can last from 1 to 12 hours. This is frequently seen with lateral kink or S-shape neck curvature in which the cow tends to lie with her head tucked into her flank. Her temperature is subnormal, her muzzle dry, coldness of skin and extremities (Allen and Piantoni, 2013). The heart rate will be rapid exceeding 100 beats per minute, gastrointestinal atony predisposes to constipation and mild bloating; in addition, the animal exhibits incoordination when walking (Rodríguez *et al*., 2017).

Stage III (Lateral Recumbency): Stage III hypocalcemic cows are flat out on their side, completely paralyzed, typically bloated and are severely depressed (to the point of coma). They will die within a few hours without treatment (Goff, 2014). Generally, this stage is characterized by inability to stand and a progressive loss of consciousness leading to coma. There is a marked fall in temperature and heart sounds become nearly inaudible and the heart rate increases to 120 beats per minute or more. Cows will not survive for more than a few hours without treatment in this stage (Kshandakar *et al*., 2017).

The clinical signs of ketosis include a refusal to eat grain and concentrate feeds and a sudden drop in milk output (Garro *et al*., 2014). "Off feed," weight loss, decreased milk production, listlessness and other unusual signs. There is a sweet smell of acetone in the breath and milk. Sweet-smelling breath (caused by exhalation of ketone bodies), and may also result in reduced appetite and depression of intake, reduced milk yield, changes in behavior, and in cases of prolonged energy deficit, weight loss (Garro *et al*., 2014). The costs associated with ketosis include ketosis treatment, increased risk of other diseases, risk of poorer reproductive performance, and higher risk of culling in early lactation (Gordon et al., 2013). Some cows may exhibit nervous signs, which include excessive salivation, abnormal chewing movements, licking of walls, gates or metal bars, incoordination with apparent blindness and a degree of aggression (Berge and Vertenten, 2014). The nervous signs often only last for a few hours. The affected cows have fatty infiltration and degeneration of the liver. Cows with ketosis are at greater risk of developing retained foetal membranes, displacement of the abomasum and are more likely to have prolonged calving to conception intervals and lower fertility (Garro *et al*., 2014). Due to an impaired immune system, they are also more susceptible to certain types of mastitis (Berge and Vertenten, 2014).

2.6. Diagnosis of Milk Fever and Ketosis

Diagnosis of milk fever can be based on the history of the animal at birth, clinical signs, and response to intravenous calcium borogluconate solution (Oetzel, 2004). The occurrence of paresis and depression of consciousness in cows that have recently given birth to young are diagnostic signs for the disease (Berge and Vertenten, 2014). Basis of clinical diagnosis is in early stages cows appear unsteady when standing or walking (Kaneko *et al*., 2008). This leads to recumbency. Lower than normal body temperature. If untreated with calcium, can lead to death. The diagnosis is confirmed by laboratory examination of the blood and rapid characteristic response to treatment with calcium borogluconate (DeGaris and Lean, 2008). The commonly accepted gold standard for diagnosing health disorders (in particular metabolic diseases) is the analysis of key factors in the blood circulation (Oetzel, 2004; LeBlanc, 2010).

Periodic metabolic profile tests help to find and treat the diseases before their complication. Also, monitoring of cows on the day of calving for parturient hypocalcaemia can provide early detection of diet-induced problems in calcium homeostasis (Lager and Jordan, 2012). The most notable changes occurring in the blood are a decrease in blood calcium and blood phosphorus levels and an increase in blood magnesium levels (Oetzel, 2004). Basis of subclinical diagnosis is serum calcium <2.0 m*M* (Reinhardt *et al*., 2011). Low blood calcium concentration (< 2.0 mmol/L), immediately post-calving, is a risk indicator for subclinical hypocalcemia (Oetzel, 2004). Cows with serum calcium lower than 7.5 mg/dl are as considered as hypocalcaemic. Animals with serum calcium level of 5.5 to 7.5 mg/dl show sign of stage I hypocalcaemia (Hunter, 2015). Stage II hypocalcaemia seen with calcium levels of 3.5 to 6.5 mg/dl and stage III seen when calcium concentration falls to as low as 2 mg/dl (DeGaris and Lean, 2008).

The clinical diagnosis of ketosis is based on presence of risk factors (early lactation), clinical signs, and ketone bodies in blood, urine or milk (Berge and Vertenten, 2014). When a diagnosis of ketosis is made, a thorough physical examination should be performed, because ketosis frequently occurs concurrently with other peripartum diseases (Oetzel, 2007). Especially common concurrent diseases include displaced abomasum, retained fetal membranes, and metritis. Rabies and other CNS diseases are important differential diagnoses in cases exhibiting neurologic signs (Seifi, 2011; Uyarlar *et al*., 2018).

2.7. Management and Prevention of Milk Fever and Ketosis Affecting Dairy Cows

The biggest advance in dairy health in the last decades has been the paradigm shift from treatment of clinical illness to disease prevention (Allen and Piantoni, 2013; Roche *et al*., 2013). Health management has been defined as the promotion of health, improvement of productivity, and prevention of disease in cows within the economic framework of the owner and industry (Hernandez, 2013). Another major advance has been redefining disease more broadly, to include subclinical conditions (e.g., subclinical hypocalcemia, subclinical ketosis and subclinical mastitis) (Zaidi, 2016). Herd-based tests are now available for use in routine herd monitoring and for investigating dairy herds with metabolic subclinical problems (metabolic status and metabolic disease risk) (Herath *et al*., 2018). The transition period is the most crucial time period for the health of the dairy cow and expected production during the 305-day lactation cycle (Roche *et al*., 2013). Because the metabolic disorders are interrelated and can occur as a complex in one animal or in a herd, herd health management strategies designed to prevent these diseases are urgently needed (Berge and Vertenten, 2014).

Management of the transition period should focus on good feeding management practices, health of the animal, handling, sanitation, minimizing overcrowding (Roche *et al*., 2013). A balanced dry cow ration, daily exercise; maintaining a clean, dry, comfortable calving areas, and stress-free environment and proper sanitation (Goff, 2008) during the calving period should be implemented. The animal should be in optimal body condition of about 3.5 to 3.75 and should be given an adequate DCAD ration that is supplied by the dairy producer's nutritionist (Overton and Yasui, 2014). Management of the feed bunk should be in place to make sure the feed is fresh and accessible to the animal in order to prevent a reduction in dry matter intake. The dairy producer should closely monitor the close-up pen to be able to detect metabolic and infectious diseases and use proper treatment to decrease stress and increase immune response (Zaidi, 2016). Stress levels associated with cortisol can be caused by excessive pen moves, overcrowding, and rough handling of the animal (Hao *et al*., 2016). All of which affect the dry matter intake and ability of the animal's neutrophils to fight infection, allowing the animal to be more susceptible to reproductive and metabolic diseases (Mulligan *et al*., 2006b).

Overall, it is the dairy producer's responsibility to have a sufficient management program in place to treat and prevent the reproductive and metabolic diseases of the herd in order to optimize maximum productivity (Overton and Yasui, 2014). The management program should work closely with a nutritionist in order to supply an appropriate feeding program for the transition cow when she is close to calving and after parturition (Goff, 2008). It is also crucial for the dairy producer to implement a disease treatment and prevention program with their veterinarian and herd managers. After all, cows are fragile mothers and should be treated with the best care, especially during the transition period (Berge and Vertenten, 2014). The peri-parturient disorders can be prevented and/or reduced by careful management and cooperation between veterinarians, nutritionists and producers (Lean *et al*., 2006). Herd health programs as recommended by the veterinarian must include a way to avoid metabolic disorders and prevent or control infectious disease (Mulligan *et al*., 2006b). To maximize productivity and ensure successful reproduction, rations fed during this time need to be nutrient dense and allow for proper transitioning of the diet to the lactating cow ration (Haq *et al*., 2016).

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

The study was conducted in and around 3 selected sites of Bale and West Arsi zones, namely: Agarfa, Dodola and Shashemene. The three study areas were selected based on population size and abundance of dairy farms that constituted the known milk shed areas.

Agarfa district is located at 464 km south east of Addis Ababa. The area is situated at $6^{o}11'$ N and $40^{o}3'$ E with average altitude of 2350 masl. The lowest and highest altitude of the district is 1700m and 3000 masl, respectively. The average annual rainfall is 800ml whereas 760ml and 1200ml is the minimum and maximum annual rainfall recorded in the district, respectively. The mean annual temperature of the district is 17.5^{0}C. The minimum and maximum temperature is 10^{0}C and 25^{0}C respectively (AWAO, 2023). The dominant soil type is clay soil and slightly acidic (pH = 5.8). Agarfa district has huge potential for livestock production. Agricultural production system of the study area is mixed farming with dominant crop production. Agarfa district has two main agro-climate zones, namely, dega (bada) and woina dega (bada dare). Agriculture is the backbone of the economy of the district. It provides means of occupation and livelihood for almost all population of the district. Rearing and breeding of livestock plays important role in livelihoods of the community. There are about 229,206 bovine, 63,485 ovine, 15,674 caprine, 33,777 equines and 40,150 poultry in Agarfa district (AWAO, 2023).

Shashemene town is the capital city of west Arsi administrative zone of Oromia Regional State, Southern Ethiopia. It is used as capital town of the zone and Shashemene district. It is located at 250 km south of Addis Ababa. It is extended from 7011'09" to 7013'19" northing and 38035'02" to 38037'05" easting. Shashemene town is located in bada dare (weyina dega, temperate) agro-climatic zone ranging from 1900 to 1950m.a.s.l. Its climate is favorable for human settlement. Its annual average temperature ranges between 18 and 25^{0}C and has moderate annual rainfall ranging between 800 and 1300mm (SDAO, 2023). Agricultural production system in the vicinity of Shashemene town is mixed crop and livestock farming. Dairy farming using improved breeds is a common practice in the in around the town (SDAO, 2023). Livestock activities have some interdependence with the urban livelihood and make a substantial contribution to the urban economy. In the town, livestock extension package of dairy and beef farms development were well adapted. According to SDAO (2023), the livestock in and around the town is 21,345 cattle (predominantly dairy cattle), 8,931 sheep, 1,963 goats, 1,376 horses, 498 donkeys, 22 mules and 19,786 chicken.

Dodola woreda is located in West Arsi zone of the Oromia Region, southeastern Ethiopia. Dodola town is the administrative center of Dodola woreda. It has latitude and longitude of 06°59′N 39°11′E, with an elevation ranging from 2362 to 2493 meters above sea level. The mean annual temperature of the woreda is found between 15-20.6^{0}c in the central high land. However, there is a slight variation of temperature from months to month. On average, the woreda gets annual mean rainfall of maximum of 1419mm, and average of 1200mm. Agricultural production system of the study districts is mixed farming with crop and livestock production system (DWAO, 2023). Dodola woreda is divided into three agro-climate zones mainly due to variation in altitude. It is dominantly characterized by the cool/alpine and temperate (badda) agro – climatic zone which covers 1% and 91% of the woreda and the rest 8% covers by badda-dare. The district is potential rich particularly for farming practice as its agro climatic condition (dominates by cool (alpine) 91% and Badda dare (sub-tropical 8%) (DWAO, 2023). Dodola has high potentialities for livestock rearing. However, the society gives more emphasis to quantity than quality due to lack of awareness and animal health infrastructure. Therefore, the benefit that the society gets from their livestock is very low compared to their cost. The major livestock feeds in the woreda are common grazing land and crop residues. Dodola woreda possess huge livestock which comprises, 294,857 cattle, 58,298 sheep, 22,986 goats, 31,683 horses, 14,971 donkeys, 954 mules, and 19,849 chickens (DWAO, 2023).

3.2. Study Population and Animals

The study population were consisted of crossbred dairy cattle (Local Zebu-mainly Arsi-Bale breed × Holstein-Friesian/Jersey) targeted mainly for milk production in urban and peri-urban areas (towns and peri-towns) of selected districts. The cattle under the study were comprised of transition period crossbred dairy cows (Holstein-Friesian/Jersey crosses with indigenous Local Zebu- mainly Arsi-Bale breed) in the study settings (from urban/town and peri-urban/town farms).

3.3. Study Design

Cross-sectional study design was employed from November 2023 to June 2024 to conduct epidemiological investigations in to milk fever and ketosis affecting peri-parturient dairy cows in selected sites of Bale and West Arsi zones. Questionnaire survey, focus group discussion, key informant interview, personal observation and document review were conducted to assess potential risk factors. In addition, clinical examination/observation, collection and laboratory examination of blood samples from farms were undertaken to determine prevalence of major metabolic disorders affecting peri-parturient dairy cows. Moreover, potential risk factors contributing to occurrence of major metabolic diseases in the study sites were assessed.

3.4. Sample Size Determination and Sampling Methods

The sample size for the study was calculated using a method recommended by Thrusfield (2007):

$$n = \frac{1.96^2 \times Pex\,(1 - Pexp)}{d^2}$$

Where: n = required sample size,

Pexp = expected prevalence, and

d = desired absolute precision

To maximize the sample size (in order to improve accuracy), the proportion (P) was taken as 50%; the sample size is calculated using a method recommended by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Accordingly; the total numbers of sample required for this study were 384 blood samples**.** However, by taking in to consideration the non-response rate/non-responding households, number of sites investigated and to increase precision, a total of 527 serum samples from transition period dairy cows were sampled for the investigation.

The sampling was performed using a two-level approach (purposive and simple random sampling methods), selecting first a household/farm possessing dairy cows and then randomly selecting individual animals systematically inside each farm. Purposive sampling was employed to select 3 sites based on population size, dairying and milk production (major milk sheds). From those sites, a total of 6 kebeles were selected purposely based on population size, dairy activities and major milk suppliers to the towns. Prior to commencement of the study, a sampling frame of dairy farms were prepared for each site in collaboration with the relevant district livestock department to take sampling frame of transition period crossbred dairy cows synchronized by the district. Then, list of households possessing transition period crossbred dairy cows synchronized by the woredas were sorted with the help of agricultural development agents (sampling frame). Identification numbers were given for the sorted households from the farm list. Then, 527 transition period crossbred dairy cows were selected randomly for the investigation. In addition, proportionality of incorporating the sampled households (dairy cows) was applied as per the population size of each town and kebele.

3.5. Determination of Prevalence of Major Metabolic Diseases Affecting Transition Cows

3.5.1. Clinical/physical examination and systematic observation

Detailed clinical examination of the dairy cows under investigation were performed for the presence of milk fever and clinical ketosis following standard diagnostic definitions (Constable *et al*., 2016). The examination was directed to detect evidence of systemic illness (dullness, fever, tachycardia, appetite disturbances, ruminal stasis, and recumbency). Cows were assumed to be suffered from milk fever and clinical ketosis on the basis of clinical signs reported by Constable *et al*. (2016) following standard diagnostic definitions, and further confirmed by analysis of serum.

3.5.2. Collection and evaluation of blood samples

Blood samples (between 7 and 10 ml) were collected from jugular vein using a sterile needle and a vacutainer tube. The tubes were allowed to stand overnight (the blood samples were left at room temperature for a maximum of 12 hours for serum separation) and then centrifuged at 1000×g for 10 min. The serum samples were then be pipetted into sterile cryovials. All farm and individual animal identification codes were copied onto the cryovials to which serum decanted and the serum samples were transported in ice box containing preformed blocks of ice to a local laboratory and then, immediately kept frozen at -20ºC for subsequent biochemical analysis. Serum samples were analyzed for concentrations of metabolites. Cow with serum calcium lower than 7.5 mg/dl was considered as hypocalcaemic whereas ketosis was defined as blood β-hydroxybutyrate (BHBA) $\geq$ 1.2 mmol/L. Commercial kits were used for determination of BHB and serum calcium concentrations according to the manufacturer's instructions.

3.6. Assessment of Risk Factors that Played Roles for the Existence of the Diseases

3.6.1. Data collection techniques

Data collection tools such as questionnaire survey, focus group discussions, key informant interviews, field/farm observation, and secondary data gathering were used to collect data for the assessment.

3.6.1.1. Questionnaire survey

Questionnaire surveys were used in order to obtain information on risk factors (farm/herd level and individual level risk factors) associated with milk fever and clinical ketosis. The detailed questionnaires were pre-tested, checked for clarity prior to the interviews and adjusted by translating in to local language (Afan Oromo) and administered by the interviewer who speaks the same language (Afan Oromo) with the participant respondents and finally translated into English. The farmers were interviewed by trained enumerators with good knowledge of the local language using a semi-structured questionnaire. The semi-structured questionnaires were administered to 527 randomly selected study participants (households/farms possessing transition dairy cows) from whom the samples were collected for laboratory analysis. Respondents were briefed on the objectives of the study. Herd and individual animal level data were collected by a semi-structured questionnaire interview and direct observation of the farms.

3.6.1.2. Focus group discussions (FGD)

To complement the surveys, FGD was conduct with subject groups by collection of basic descriptive information at the survey sites. This is to acquire useful and detailed information, which is difficult to collect through the survey, and to cross-check and to triangulate the data collected from multiple sources as well as to overcome the limitations and biases that steam from using single method and thereby increasing the reliability of the finding. Thus, totally 6 FGD (each consists of 12 members) with 72 participants were formed based on their proximity to each other. FGD checklist was prepared to guide topics for open-ended discussion with group members.

3.6.1.3. Key informant interviews

Key informants (36 participants) who have good knowledge and experiences in cattle health management and production such as veterinarians, animal health and production experts, veterinary laboratory technicians, artificial insemination technicians, animal health extension workers/development agents, elders and model farmers were interviewed to probe further and to validate the findings of the study. Key informant interview guides were designed to collect data.

3.6.1.4. Field/farm observation

Field/personal observation of management/husbandry practices such as housing system (type, ventilation, floor type, drainage system, bedding, space/stocking density, sanitation/ hygiene of calving area, calf pen), hygiene of feeding and watering troughs, farm environment hygiene, feeding and overall management of transition dairy cows which can predispose animal to milk fever and clinical ketosis diseases were conducted.

3.6.1.5. Secondary data gathering

Use of documentary sources such as data recording from archive, clinical records, veterinary clinic case book/treatment records, records of diagnostic laboratory results, vaccination and drug returns, outbreak reports, etc, were undertaken. In addition, document content analysis/analysing written documents -both published and unpublished material, i.e., books, journals, reports, policy documents and maps, etc, were also used in this study.

3.7. Data Storage and Analysis

Data from farm and laboratory results were coded, entered and stored in Microsoft Excel spreadsheet to create a database and were then be imported/transferred to SPSS version 25 statistical software for processing and analysis. Descriptive statistics such as frequency, percentages, range, mean and standard deviation were computed to describe the relevant variables. The prevalence of the disease was determined as the proportion of affected animals out of the total animals examined. In addition, analytical statistics such as Chi-square ($\chi 2$) test and multiple logistic regression model were employed to determine the associations of hypothetical risk factors with the positive animals. Odds ratio (OR) was used to measure the degree (strength) of risk factors association with the disease occurrence indicated by 95% confidence intervals. A p-value < 0.05 was be considered statistically significant. Moreover, qualitative data such as unstructured observations, open-ended interviews, analysis of written documents, and focus groups transcripts were analyzed using drawing out themes and patterns and content analysis of notes taken during data collection.

4. RESULTS

4.1. General Characteristics of the Respondents and Study Farms

A total of 576 study participants/households possessing cross-bred dairy cows in transition period from the selected sites were requested to participate in the present study, 49 (8.5%) respondents declined to participate, resulting in a participation rate of 527 (91.5%).

According to the results of the study, most of the herd size investigated were small holders. The current evaluation disclosed that greater proportion of the study participants (61.86%) can read and write. According to the present study, relatively higher percentages of the farmers (56.36%) have no awareness on milk fever and ketosis disorders. Similarly, most of the farmers (66.22%) did not respond early to treat sick animal as well as did not practice proactive measures such as prevention and control of the diseases. However, only few of the farmers (11.20%) take part/participate in training and veterinary extension services. On the other hand, vast majority of study participants (76.74%) did not establish a valid veterinarian-client-patient relationship. Moreover, higher proportion of farm hygienic status, floor, ventilation system of the house and drainage/slope system of the house was poor. Furthermore, only 8.16% of the dairy farmers practice record keeping to review treatment regimen given and other managemental practices as presented in Table 1 below.

Table 1: General characteristics of the respondents and study farms

S/n	Variables	Category/level	Frequency	Percentages (%)
1.	Herd size	Small (≤ 5)	387	73.43
		Medium (> 5≤10)	109	20.68
		Large (> 10)	31	5.88
2.	Level of education	Literate	326	61.86
		Illiterate	201	38.14
3.	Awareness on milk fever & ketosis	Yes	297	56.36
		No	230	43.64
4.	Early response to sick animals	Yes	349	66.22
		No	178	33.78
5.	Training and veterinary extension services	Yes	59	11.20
		No	468	88.80
6.	Establish a valid veterinarian-client-patient relation	Yes	404	76.66
		No	123	23.34
7.	Hygienic status, ventilation, floor & drainage/slope system of the house	Poor	318	60.34
		Good	209	39.66
8.	Record keeping	Yes	43	8.16
		No	484	91.84

4.2. Determination of prevalence of milk fever and ketosis affecting dairy cattle

Based on the case history, clinical examination, and serum analysis; milk fever and ketosis were diagnosed. The result of the study showed that, out of the total blood samples collected, 37.57% (198) were found to be affected by milk fever, ketosis or both (mixed infections) as illustrated in Table 1. The prevalence of rates of milk fever, ketosis and mixed infections were 13.66% (72), 17.27% (91) and 6.64% (35), respectively.

Table 2: Prevalence of milk fever and ketosis in dairy cattle of study sites (n=527).

Type of disease	Samples tested	Samples positive	Prevalence (%)
Milk fever	233	72	13.66
Ketosis	169	91	17.27
Mixed infections	125	35	6.64
Overall	**527**	**198**	**37.57**

According to the study sites, the prevalence of the diseases was presented in Table 2. The prevalence of milk fever and ketosis was highest in Shashemene, 29.61% (62) followed by Dodola 18.34% (31) and Agarfa 15.20% (19).

Table 3: Prevalence of milk fever and ketosis in dairy cattle of each study sites (n=527).

Study sites	Samples tested	Samples positive	Prevalence (%)
Shashemene	233	62	29.61
Dodola	169	31	18.34
Agarfa	125	19	15.20
Overall	**527**	**198**	**37.57**

4.3. Assessment of Hypothesized Potential Risk Factors for Existence of the Diseases

4.3.1. Chi-square analysis of hypothesized risk factors association with the diseases

The evaluation of results of hypothesized potential risk factors that played roles for the existence of the disease are illustrated in table 3 below. Accordingly, a chi-square analysis revealed that prevalence of milk fever and ketosis were significantly associated with the hypothesized risk factors investigated such as age groups, parity number, daily milk yield, origin, history of previous transition period disorders, BCS, farm hygienic status, and grazing patterns ($P<0.05$).

However, association of prevalence of milk fever and ketosis with herd size of the dairy cattle was not statistically significant ($P>0.05$) as illustrated in Table 3 below.

Table 4: Association of hypothesized risk factors with prevalence of milk fever and ketosis.

S/n	Variables	Category	No. tested	Prevalence (%)	$\chi2$ (p-value)
1.	Age groups	≤ 5 years	178	44 (24.72)	
		> 5 years	349	154 (44.13)	18.928 (0.000)
2.	Parity	≤ 3 times	271	68 (25.09)	
		> 3 times	256	132 (51.56)	41.549 (0.000)
3.	Milk yield	Low producing < 15L/d	339	103 (30.38)	
		High producing ≥15L/d	188	95 (50.53)	20.933 (0.000)
4.	Herd size	Small (≤ 5)	387	140 (36.18)	
		Medium (> 5≤10)	109	44 (40.37)	
		Large (> 10)	31	14 (45.16)	1.446 (0.481)
5.	BCS	Poor	137	35 (25.55)	
		Medium/good	268	106 (39.55)	
		Over-conditioned	122	57 (46.72)	13.248 (0.001)
6.	Origin	Homebred	248	65 (26.21)	
		Purchased	279	133 (47.67)	25.780 (0.000)
7.	Previous history	Yes	164	82 (50.00)	
		No	363	116 (31.96)	15.681 (0.000)
8.	Farm hygienic status	Poor	315	133 (42.22)	
		Good	212	65 (30.66)	7.222 (0.008)
9.	Grazing patterns	Zero grazing	253	131 (51.79)	45.799 (0.000)
		Partial grazing	191	54 (28.27)	
		Free range & supplemented	83	13 (15.66)	

4.3.2. Univariable logistic regression analysis of risk factors association with diseases

Univariable logistic regression analysis of the effect of different putative risk factors on the prevalence of milk fever and ketosis is depicted in Table 4. Those variables which are significantly associated with prevalence of the diseases by chi-square analysis were also analysed by multivariable logistic regression to see the strength of the association between the factors and the diseases.

Accordingly, age groups, parity number, daily milk yield, history of previous transition period disorders and grazing patterns enhance the risk for bovine respiratory disease complex infection. Hence, older cows (> 5 years) (OR = 2.405, 95%CI: 1.611 - 3.591), parity number (OR = 3.306, 95%CI: 22.83 - 4.788), daily milk yield (OR = 2.341, 95%CI: 1.620 - 3.381), purchased cows (OR = 2.565, 95%CI: 1.775 - 3.705), over-conditioned cows (OR = 2.556, 95%CI: 1.514 - 4.313), history of previous transition period disorders (OR= 2.129, 95%CI: 1.460 - 3.105), poor farm hygienic status (OR = 1.653, 95%CI: 1.144 - 2.387) and zero grazing pattern (OR = 5.782, 95%CI: 3.045 - 10.979) were more likely to be infected with the diseases than their counter parts.

Table 5: Univariable logistic regression analysis of risk factors with the diseases.

Factors	**Label**	**Test results**		**COR (95%CI)**	**P value**
		No. tested	**No.positive(%)**		
Age groups	≤ 5 years	178	44 (24.72)	1	
	> 5 years	349	154 (44.13)	2.405 (1.611 - 3.591)	0.000
Parity	≤ 3 times	271	68 (25.09)	1	
	> 3 times	256	132 (51.56)	3.306 (22.83 - 4.788)	0.000
Milk yield	Low producing < 15L/d	339	103 (30.38)	1	
	High producing ≥15L/d	188	95 (50.53)	2.341 (1.620 - 3.381)	0.000
BCS	Poor	137	35 (25.55)	1	
	Medium/good	268	106 (39.55)	1.907 (1.209 - 3.007)	0.007
	Over-conditioned	122	57 (46.72)	2.556 (1.514 - 4.313)	0.000
Origin	Homebred	248	65 (26.21)	1	
	Purchased	279	133 (47.67)	2.565 (1.775 - 3.705)	0.000
Previous history	Yes	164	82 (50.00)	2.129 (1.460 - 3.105)	0.000
	No	363	109 (30.03)	1	
Farm hygienic status	Poor	315	133 (42.22)	1.653 (1.144 - 2.387)	0.007
	Good	212	65 (30.66)	1	
Grazing patterns	Zero grazing	253	131 (51.79)	5.782 (3.045 - 10.979)	0.000
	Partial grazing	191	54 (28.27)	2.122 (1.086 - 4.150)	0.028
	Free range & supplemented	83	13 (15.66)	1	

4.3.3. Multivariable logistic regression analysis of risk factors association with diseases

Multivariable logistic regression analysis of the effect of different putative risk factors on the prevalence of milk fever and ketosis is depicted in Table 5. Those variables which are significantly associated with prevalence of the diseases by chi-square analysis were also analysed by multivariable logistic regression to see the strength of the association between the factors and the diseases.

Accordingly, age groups, parity number, daily milk yield, history of previous transition period disorders and grazing patterns enhance the risk for bovine respiratory disease complex infection. Hence, older cows (> 5 years) (OR = 5.744, 95%CI: 2.087 - 15.810), parity number (OR = 11.00, 95%CI: 0.482 - 25.086), daily milk yield (OR = 3.52, 95%CI: 0.372 - 4.438), purchased cows (OR = 7.343, 95%CI: 0.000 - 9.743), history of previous transition period disorders (OR= 1.244, 95%CI: 0.452 - 3.424) and zero grazing pattern (OR = 9.191, 95%CI: 0.698 - 12.106) were more likely to be infected with the diseases than their counter parts.

Table 6: Multivariable logistic regression analysis of risk factors with the diseases.

Factors	**Label**	**Test results**		**Odds ratio**	**P value**
		No. tested	**No.positive(%)**	**AOR (95% CI)**	
Age groups	≤ 5 years	178	44 (24.72)	1	
	> 5 years	349	154 (44.13)	5.744 (2.087 - 15.810)	0.001
Parity	≤ 3 times	271	68 (25.09)	1	
	> 3 times	256	132 (51.56)	11.00 (0.482 - 25.086)	0.013
Milk yield	Low producing <15L/d	339	103 (30.38)	1	
	High producing ≥15L/d	188	95 (50.53)	3.52 (0.372 - 4.438)	0.091
BCS	Poor	137	35 (25.55)	1	
	Medium/good	268	106 (39.55)	0.409 (0.163 - 1.024)	0.056
	Over-conditioned	122	57 (46.72)	0.244 (0.076 - 0.779)	0.017
Origin	Homebred	248	65 (26.21)	1	
	Purchased	279	133 (47.67)	7.343 (0.000 - 9.743)	0.099
Previous history	Yes	164	82 (50.00)	1.244 (0.452 - 3.424)	0.672
	No	363	109 (30.03)	1	
Farm hygienic status	Poor	315	133 (42.22)	0.621 (0.0981 - 0.7491)	0.997
	Good	212	65 (30.66)	1	
Grazing patterns	Zero grazing	253	131 (51.79)	9.191 (0.698 - 12.106)	0.92
	Partial grazing	191	54 (28.27)	3.702 (1.658 - 8.262)	0.001
	Free range & supplemented	83	13 (15.66)	1	

5. DISCUSSION

The present assessment disclosed that prevalence of milk fever and ketosis affecting transition period cross-bred dairy cows was relatively high in the study sites. The result of the study showed that, out of the total blood samples collected, 37.57% (198) were found to be affected by milk fever, ketosis or both (mixed infections). The prevalence of rates of milk fever, ketosis and mixed infections were 13.66% (72), 17.27% (91) and 6.64% (35), respectively. The current finding is in agreement with previous reports made from different parts of (Chiwome *et al*., 2017; Naher *et al.*, 2020; Saed *et al*., 2020). However, the present findings are higher than the report of Buttchereit *et al*. (2012). On the other hand, the present result found positive in our study is lower than that of Neves *et al*. (2017). These differences in the results of the present study might be attributed to differences in ecological/ environmental conditions, where animals are raised, variations in the husbandry practices, season of the year, herd size, difference in sampling, study areas, the reaction of animals to different levels of stress, time of sampling and farm management (Abera *et al*., 2014).

The potential risk factors identified, that contribute to occurrence and existence of milk fever and ketosis and which influence the incidence and severity of the diseases, in this study include age, parity, milk yield, origin, grazing patterns and previous exposure of the cows to milk fever, ketosis or both.

The analysis of influence of age on the prevalence milk fever and ketosis showed higher prevalence of the disorders in older cows (> 5 years) than the younger ones (≤ 5 years). This finding is congruent with report of Hao *et al*. (2016) who showed that Older cows are affected by hypocalcaemia and ketosis more common and more severe than young cows. Age has a profound effect on susceptibility of dairy cows to hypocalcaemia and the risk of hypocalcaemia increases by approximately 9% per-lactation. In addition to increased milk production from the 3rd lactation resulting high calcium demand, ageing also results in a diminished ability to mobilize calcium from bone stores and a decline in the active transport of calcium in the intestine as well as impaired production of 1,25(OH)2D3 due to decrease number of 1,25(OH)2D3 receptors (Neves *et al*., 2017). The hypocalcemia at calving is age related and most marked in cows from 3rd to 7th parity and it is infrequent at the first parturition. age played a profound role in susceptibility of cows to such condition. Incidence of hypocalcaemia is mostly increased by age, where cows aged between five to ten years are the most commonly affected group (DeGaris and Lean, 2008). Both vitamin D and PTH are the chief players in Ca homeostasis mechanism. A decrease in the number of vitamin D receptors in the intestine has been reported with increasing age of the cows, as well as the PTH receptors in the target tissues (Oltenacu and Broom, 2010).

Evaluation of parities showed a clearly increased odds of milk fever and ketosis in multiparous cows (especially after the third parturition). All parities, fourth or above, had a significantly ($p<0.001$) higher odds of developing milk fever and ketosis compared to first parity cows. Similar to previous findings of Naher *et al*. (2020) this study found that increasing parity had a link with a higher incidence of milk fever and ketosis. Cows from the parity group of ≥ 6 had an OR of 18.8 ($p < 0.001$) of developing MF compared to second parity cows. It is clear that multiparous cows have increased odds of developing milk fever compared to first parity cows (Reinhardt *et al*., 2011). When comparing the group of cows in their sixth parity or higher with the second parity group an OR of 18.8 was calculated. Primiparous cows have a lower risk of suffering from SCHC while multiparous cows have an increased risk (Azizi *et al*., 2022). Multiparous cows, especially after the third parturition have an increased risk of suffering from severe SCHC. The age and parity-associated susceptibility might be related to calcium homeostatic mechanisms. With increasing age and parity, the hemostasis process is impeded and results in moderate to severe hypocalcaemia. It has been assumed that the number of vitamin D receptors in intestines decline with increasing age (Saed *et al*., 2020). In addition, as animals age increase, the number of receptors for PTH on target tissue decline. Likewise, the condition of increased incidence of hypocalcaemia with increased the parity times is related to depletion of resources of Ca homeostasis. Previous studies recorded that hypocalcaemia and ketosis occurred predominantly from the third to seventh parities (Suthar *et al*., 2013; Naher *et al*., 2020).

The occurrence of milk fever and ketosis was significantly associated with milk yield. The current report is concords with results by Naher *et al*. (2020) and Saed *et al*. (2020) who revealed association of incidence of hypocalcaemia and ketosis with higher daily milk production. Many studies have linked the incidence of hypocalcaemia and the high milk production in dairy cows (DeGaris and Lean, 2008). One possible explanation for this is that cows with high milk production will produce more colostrum, so the Ca loss will be higher. The sharp and tremendous increase in milk yield in last decades has imposed great impact on metabolic capacities of dairy cows (Kshandakar *et al*., 2017). Metabolic disease incidence typically escalates as milk production rises (Berge and Vertenten, 2014). This continuing challenge is related to progressive improvement in dairy cow genetics and the ever-increasing average milk yields of modern dairy cattle. Improvements in production capacity challenge us to manage and feed cows to allow them to adapt to the tremendous metabolic demands of high milk production (Roche *et al*., 2013; Saed *et al*., 2020).

Our finding of more infected cows among purchased cattle than homebred cattle is consistent with reports by Oltenacu and Broom (2010) and Buttchereit *et al*. (2012). These results may reflect lack of transparency in the transfer of animals between farms. Lack of screening cows for metabolic disorders such as milk fever and ketosis might be attributed for the higher prevalence of the diseases. Thus, purchasing cows of without screening and using those cows with a history of metabolic disorders for breeding purposes are likely to be important contributors to the prevailing high prevalence of metabolic diseases in cattle throughout Ethiopia (Abera *et al*., 2014).

The results from the analysis of previous exposure of cows to milk fever and ketosis revealed higher odds of infection in cows suffering from the disorders in previous lactation. The current investigation agrees with the reports of Roche and Berry (2006) and Saborio-Montero *et al*. (2017). They found that cows that had been affected with parturient paresis and ketosis in their previous lactation had higher odds of developing the the diseases in their subsequent lactation (OR=9.74). It has been established that the risk of milk fever and ketosis is increased if an animal was diagnosed with ketosis in the previous lactation (Azizi *et al*., 2022). Thus, it would be expected that an animal that was sub-clinically ketotic in the previous lactation would have a longer lactation period before becoming pregnant. Previous lactation days open could thus be acting as an indicator or confounder of the relationship between ketosis in successive lactations (Naher *et al*., 2020).

The current investigation disclosed that prevalence of milk fever and ketosis in dairy cows significantly varied with BCS ($p < 0.05$). Over-conditioned cows are significantly affected with milk fever and ketosis than their counter parts. The evaluation is in harmony with analysis by Roche *et al*. (2015) who recorded higher BCS (> 3.5) had higher prevalence of SCHC than the lower BCS (3 - 3.25). This finding supports that the higher BCS (≥ 3.75 out of 5.0) at calving developed up to 4 times more hypocalcemia in dairy cows. Higher BCS at calving have a higher calcium output in milk and also results in decreased feed intake during gestation period due to reduced appetite in critical period and around calving which predisposes them to hypocalcaemia and ketosis (Buttchereit *et al*., 2012). The higher BCS is usually associated with a higher milk yield and thus a higher calcium output, making it more prone to hypocalcemia. Furthermore, over-conditioned cows have a reduced feed intake at peri-calving period compared to thinner cows, and thus a reduced intake of Ca will predispose for the development of such condition (Oetzel, 2007). Older and over-conditioned cows have been reported to be at greater risk for experiencing MF, whereas primiparous cows are much less likely to have low blood calcium levels near calving (Naher *et al*., 2020).

Furthermore, the present epidemiological study of milk fever and ketosis in dairy cows disclosed that zero grazing (indoors housing system) was significantly associated with the disorders on the farm level. This result is in line with former study by Asl *et al*. (2011) and Azizi *et al*. (2022). Management factors (housing type, grouping strategies and pen moves) and many cow-level factors highly risk for milk fever and ketosis (Asl *et al*., 2011). The solar radiation in indoors and/or outdoors hosing system is essential requirement for normal production of vitamin D3 from the 7-dehydrocholesterol in skin (Oltenacu and Broom, 2010). The active metabolite of vitamin D3 is an essential mediator in the mechanism of calcium homeostasis in the body, which increases calcium absorption from the intestine and stimulates calcium re-absorption by kidneys (Neves *et al*., 2017).

6. CONCLUSIONS AND RECOMMENDATIONS

The present finding disclosed relatively higher prevalence of milk fever and ketosis disorders affecting transition period crossbred dairy cows in the study sites. The high prevalence of milk fever and ketosis recorded in this study should be viewed as a potential health risk to the transition cows. Milk fever and ketosis have detrimental effects on cow health, productivity and reproduction as well as predisposes to other diseases and disorders. Age, parity, milk yield, origin, previous exposure and grazing patterns were potential risk factors identified that contribute to occurrence, severity and existence of milk fever and ketosis in the study sites.

Based on the above conclusion, the following recommendations were forwarded:

- Great attention should be given for management, efficient balanced ration/application of the NRC recommendations as feeding system; diagnosis, periodic monitoring and screening of the metabolic profile test during risk stage could help for early detection and control of the diseases.
- It should be taken in to consideration that cow level risk factors plays crucial role for the occurrence of hypocalcemia and ketosis, which makes the issue of cows selection criteria applicable to build up control strategies, with the aim of reducing the potential risks of transition period disorders and improving dairy cattle industry in study sites and beyond.
- Great attention should be given to availability of the drugs (such as calcium borogluconate) to treat the cases and must be insured among governmental and non-governmental veterinary clinics and in the market at reasonable costs throughout the year.
- The owners of dairy farms and private animal owners should be aware of the disease and prepare themselves on how to manage peripartum cows to limit effects of the diseases on the dairy cows and to prevent economic loss in the study sites and beyond.
- The high prevalence of milk fever and ketosis was recorded in this study should be viewed as a potential health risk to the transition cows that requires further research.
- Further detailed epidemiological investigation and risk factors study is needed for each disorders in dairy cattle so as to design appropriate control and preventive measures at farm as well as national level.

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11. APPENDIX

11.1. Questionnaires

Remark: The personal profile obtained from respondents with regard to the theme will be kept confidential and will not have any consequence on the respondent in any ways. The information is needed only for the research purpose. Therefore, please give correct answers to the following questions.

Instructions for enumerators:

- Make brief introduction before starting any question, introduce you to the farmers, greet them in local ways and make clear the objective of the study.
- Please fill the interview schedule according to the farmers reply (do not put your own feeling).
- Please ask each question clearly and patiently until the farmer gets your points.
- Please do not use technical terms and do not forget local units.
- During the process write answers on the space provided.
- Prove that all the questions are asked and the interview schedule format is properly completed.

I. General Information

1. Name of interviewee (optional)____________________Age______Sex_____________

2. Date of interview____

3. Adress: Zone____________ district/woreda/town____________Kebele_____ Tele_____

4. Cattle ID______________________

5. Occupation

A. Farmer C. Non-government employee (NGO)

B. Government employee D. Self-business

6. What is your age? i) 15-35 years ii) 36- 55 years iii) Above 55 years

7. Marital status i) Single ii) Married iii) Divorced, Separated, Widowed

8. Educational level A. Illiterate B. Literate/read and write

C) Elementary school D) High school graduate E) Professional

If professional: A) related to animal production B) unrelated to animal production

9. Family income source (Rank): A. Crop sale B. Animal product sale C. Animal sale D. Others

10. Production system A. Pastoral B. Agro-pastoral C. mixed crop livestock farming

11. Household classification A. Rural B. Urban C. Peri-urban

12. Herd size: Cattle___Cows___Male calves___Bulls___Female calves___ Heifers___

12.1. How many of them are cross bred cattle? ______

13. The farm (livestock) as a source of income: A) primary B) secondary

14. Is livestock production is the major activity on your farm? A. Yes B. No

15. Are you practicing dairy cattle rearing as the only way of livelihood? A. Yes B. No

16. System of production: A. Extensive B. Semi intensive C. Intensive

17. Farm size: A. Small B. Medium C. Large

18. Livestock reared in the area in order of importance?

A. Cattle B. Sheep C. Goat D. Horse E. Donkey F. Mule G. camel

19. Major farming activity and rank:

A. Livestock production B. Crop production C. Mixed type

II. Farm and individual Management Practices

1. Dairy production system

1.1. What are your major livestock activities?

A) Dairy production B) Small ruminant production C) Chicken production

1.2. If dairy production as a source of income, is it a?

A) primary B) secondary/side line activity

1.3. How long have you engaged in dairy production? ___________________________

1.4. Dairy farm location A) Urban B) Peri-urban

1.5. Management systems (level of grazing) of dairy cattle production:

A) zero grazing/confinement C) partial grazing

B) free/total grazing D) others (specify) ____

1.6. Herd size and composition

Herd composition		local	Cross	Total
Calves (<6m)	Male			
	Female			
Calves (6-12m)	Male			
	Female			
Heifers				
Lactating cows				
Dry cows				
Bull				
Bullock				
Total herd size				

2. Farm management data

2.1. Cattle caretaker (attendant):

2.1.1. Ownership A) owner (family member) B) hired

2.1.2. Sex A) male B) female

2.1.3. Experience A) <= 5 years B) >5 years

2.2. Comingling with different age groups: A) Yes B) No

2.3. Source of replacement stock: A) Own herd B) purchased

2.4. Availability of water A) Adequate B) inadequate

2.5. How cattle were watered?

A. Animals go to water B. Water is fetched (provided) C. Both

2.6. Source of water: A. Bore hole B. Dam (pond) C. River D. Water well E. Spring F. Municipal (piped) G. Public water source

2.7. Distance to farthest water point:

A. At household B. <1Km C. 1-5Km D. 6-10Km F. >10Km

2.8. Frequency of watering A. Freely available/ad libitum B. Once a day C. Twice a day

2.9. Prevention from extremes of temperatures (very cold coupled with wetness/very hot with high moisture/damp and humid conditions): A) Yes B) No

2.10. Prevention from dust: A) Yes B) No

2.11. Transition/periparturient care for dairy cows A) Yes B) No

2.12. Awareness of health care of transition period cows A) Yes B) No

2.13. What is the type of the diet given to the dairy cows?

A) Preformulated ration considering the national research council recommendation nutrient requirements B) Total mixed ration feeding system B) Others if any ____________

2.14. Feed supplements to transition period cows a) yes b) no, if no, why____

2.14.1. If yes, what type of supplementary feed/supplementation regime:

A) roughage (crop residue) B) concentrates

C) minerals (salts), vitamins E) Others (specify if any) _______

2.15. Is there any feed change at time of transition period? A) Yes B) No

2.15.1. If yes, how could you change the feeds a) Abrupt/rapidly b) Gradually

2.16. Length of dry period ____

3) Housing management systems

3.1. House: A) Kraal B) Stall (shed) C. yard D. others (specify)_____

3.2. Housing type: A) Indoor/confinement B) Outdoor/hutch

3.3. Calf/calving/cows pen/use of pen: A. Yes B. No

3.3.1. If "Yes": A) Separate pen B) Together with other cattle in the barn C) Other___

3.3.1.1. If separate pen: A) Individual pen B) Group pen

3.3.1.2. If group pen, number of calves/cows kept /pen_____________________

3.3.2. Location of the calf pen A) In cow shed B) In separate pen/shed (cubicle)

3.3.3. Ways of cleaning calving pen:

A. Flushing with water B. Disinfection with detergent C. Others (specify if any)_____

3.3.4. Frequency of calving/calf pen cleaning?

A) once daily B) twice a day C) Others (specify)____________

3.5. Bedding A) Present B) Absent; If the bedding is present,

3.5.1. What is the hygienic status of the bedding? A) Good B) Bad/poor

3.5.2. What is the bedding material? A) Straw B) Stalk C) Sand

3.5.3. How frequently changed the bedding material?

A) > Once/week B) Once/week C) < Once/week

3.6. Ventilation system of the house: A) Good B) Bad/poor C) No ventilation at all

3.7. Floor of the house: A) Soil B) concrete

3.7.1. Floor condition of the house: A) Good B) High mud and manure with moisture/damp

3.7.2. Drainage/slope system of the house: A) Good B) Bad/poor C) No at all

3.7.3. Herd density/ideal spacing (4.0 m^2/cow): A) High B) Low

3.8. Hygienic status of the farm environment (based on waste disposal, drainage, ventilation and physical appearance of the coat of an animal): A) Clean/good B) Not clean/poor

4. Experience on cattle health problems, prevention and control of the problems

4.1. Major health problem for the farm ___________________________________

4.2. Disease or disease syndrome responsible for sickness and death of cattle in order of importance. A. ___________, B. ____________, C. ______________, D._______________

4.4. Number of cattle in the farm sick and dead due to metabolic health problems__________

4.5. Measures taken to treat sick animals____

4.6. Measures taken to prevent metabolic disease problems____________________________

4.7. Veterinary and client relationships: A. Yes B. No

4.8. Periodical monitoring of metabolic profile test: A. Yes B. No

III. The Individual Animal Biodata and Metabolic disorder Health Problems

1. Age: A) Calf B) Heifer C) Adult
2. Parity: A) Single B) Multiple
3. Breed: A) Cross B) Local
4. Body condition score: A) Poor B) Medium C) Good
5. Origin of individual animal: A) Homebred B) Purchased
6. Stage of transition period:

A) 3 weeks prior expected day of delivery

B) At calving till 3 weeks post-partum

7. Milk yield (liter/day): A) < 5.0 B) 05-10 C) 10-15 D) > 15.0
8. Lactation stages: A) < 1 week B) > 1 week
9. Parity: A) Primiparous B) Multiparous