



UNIVERSITI PUTRA MALAYSIA

**A RETROSPECTIVE STUDY ON HYPOCALCAEMIA CASES
PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI
PUTRA MALAYSIA FOR 2015 - 2019**

SHAZEM ASHRIL BIN SULAIMAN

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**A RETROSPECTIVE STUDY ON HYPOCALCAEMIA CASES PRESENTED
TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA
MALAYSIA FOR 2015 - 2019**

SHAZEM ASHRIL BIN SULAIMAN

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
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DECEMBER 2020

CERTIFICATION

It is hereby certified that I have read this project paper entitled “A RETROSPECTIVE STUDY ON HYPOCALCAEMIA CASES PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FOR 2015 - 2019 ” by Shazem Ashril Bin Sulaiman and in my opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 - Final year Project.

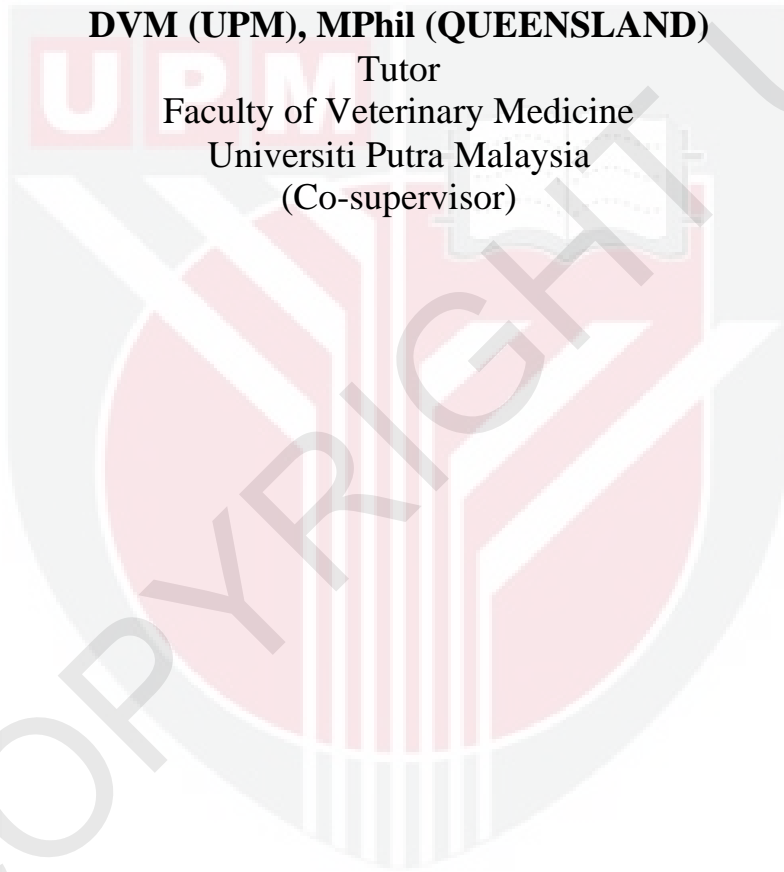


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DEDICATION

This project paper is dedicated to ALLAH SWT, who ease my journey and made everything possible.

To my family, Father and Mother whose affection, love, encouragement and prays are always there for me when I need.

To all the lecturers, staff and all my friend batch DVM21 who were involved directly or indirectly in this project.

Along with all hardworking and respected
SUPERVISOR

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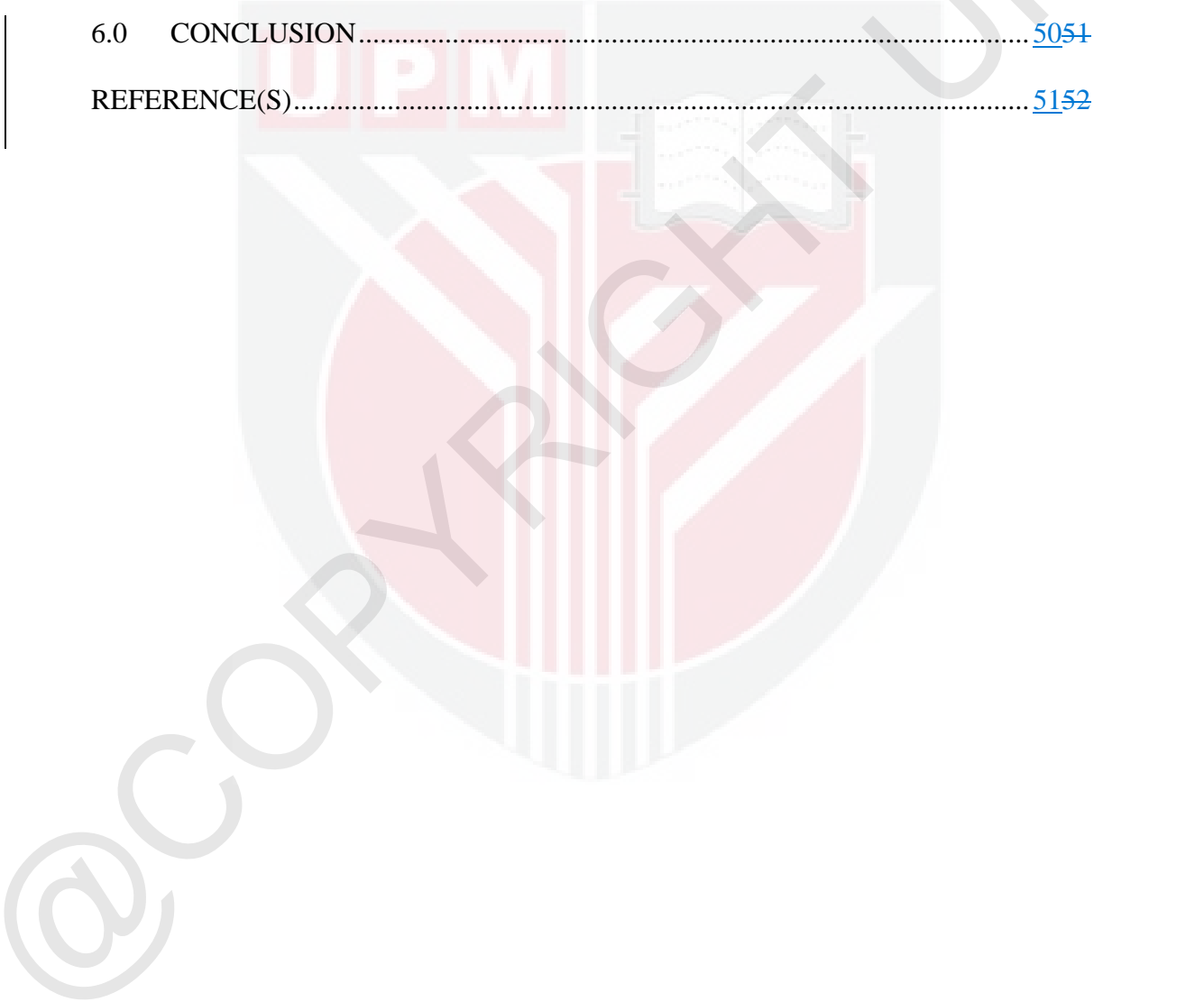
Words could not express my gratitude to all only Allah can repay all the efforts to everyone who helped me.

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LIST OF ABBREVIATIONS

ALT: Alanine aminotransferase

ALP: Alkaline phosphatase

GGT: Gamma-glutamyl transferase

CK: Creatine kinase

Ca: Calcium

PTH: Parathyroid hormone

tCa: Total calcium

Alb: Albumin

EDTA: Ethylenediaminetetraacetic acid

UVH: University Veterinary Hospital

UPM: Universiti Putra Malaysia

U/L: Unit per liter

ATP: Adenosine triphosphate

$\mu\text{mol/L}$: Micromole per liter

GFR: Glomerular filtration rate

PLE: Protein-losing enteropathy

GI: Gastrointestinal

Abstrak

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 - Projek Tahun Akhir

KAJIAN RETROSPEKTIF TERHADAP KES HIPOKALSEMIA YANG DIREKODKAN DI HOSPITAL VETERINAR UNIVERSITI, UNIVERSITI PUTRA MALAYSIA UNTUK TAHUN 2015 - 2019

oleh

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2020

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Satu kajian retrospektif telah dilakukan berdasarkan rekod yang terdapat di Makmal Hematologi dan Biokimia Klinikal, Jabatan Diagnosis Makmal Veterinar, Fakulti Perubatan Veterinar, UPM, selama lima tahun dari Januari 2015 hingga Disember 2019. Kajian ini bertujuan untuk menentukan kelaziman dan faktor risiko haiwan yang berkaitan dengan hipokalsemia. Sebanyak 254 kes hipokalsemia telah dikaji. Maklumat pesakit termasuk bilangan kes, spesies, keturunan, umur, kelainan klinikopatologi termasuk jumlah kalsium serum, fosforus serum, serum kreatinin, serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), serum gamma-glutamyl transferase (GGT) dan serum creatine kinase (CK) kepekatan), dan diagnosis akhir yang direkodkan telah dikaji. Kajian menunjukkan bahawa prevalensi hipokalsemia pada spesies caprine adalah 25% (65/260). Majoriti caprine adalah dewasa dan betina tetapi tidak ada hubungan yang signifikan antara usia dan jantina dengan status kalsium. Sebilangan besar caprine yang mengalami hipokalsemia didiagnosis dengan toksaemia

kehamilan (17%). Prevalensi hipokalsemia pada spesies leporin adalah 31% (58/187). Sebilangan besar leporin adalah dewasa dan betina tetapi tidak ada hubungan yang signifikan antara usia dan jantina dengan status kalsium. Sebilangan besar leporin yang mengalami hipokalsemia didiagnosis semasa pemeriksaan kesihatan biasa (47%). Kajian ini juga menunjukkan bahawa prevalensi hipokalsemia pada spesies sapi adalah 22% (40/178). Sebilangan besar lembu adalah dewasa dan betina tetapi tidak ada hubungan yang signifikan antara usia dan jantina dengan status kalsium. Sebilangan besar lembu hipokalsemia didiagnosis dengan serangan parasit darah (20%). Sebagai kesimpulan, kajian ini menunjukkan prevalensi pesakit hipokalsemia pada setiap spesies yang terlibat dan tidak ada hubungan yang signifikan dari kes-kes yang disertakan dengan faktor risiko.

Kata kunci: *hipokalsemia, kalsium, kreatin kinase, caprine, leporine*

Abstract

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 - Final Year Project.

A RETROSPECTIVE STUDY ON HYPOCALCAEMIA CASES PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FOR 2015 - 2019

by

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2020

Supervisor: Dr Mohd Mokrish Md. Ajat

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Dr Azalea Hani Othman

A retrospective study was performed based on records available at the Haematology and Clinical Biochemistry Laboratory, Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, UPM, for a period of five years from January 2015 to December 2019. This study aimed to determine the prevalence and risk factor of animals associated with hypocalcemia. A total of 254 hypocalcemia cases were reviewed. Patient's information including case number, species, breed, age, clinicopathologic abnormalities inclusive of total serum calcium, serum phosphorus, serum creatinine, serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), serum gamma-glutamyl transferase (GGT) and serum creatine kinase (CK concentrations), and final diagnosis recorded were reviewed. The study showed that the prevalence of hypocalcemia in caprine species was 25% (65/260). The majority of caprine were adult and female but there is no significant association between the age and gender with calcium status. Most of the hypocalcemic caprine were diagnosed with

pregnancy toxemia (17%). The prevalence of hypocalcemia in leporine species was 31% (58/187). Most of the leporine were adult and female but there is no significant association between the age and gender with calcium status. The majority of the hypocalcemic leporine were diagnosed during regular health screening (47%). The study also showed that the prevalence of hypocalcemia in bovine species was 22% (40/178). Majority of the bovine were adult and female but there is no significant association between the age and gender with calcium status. Most of the hypocalcemic bovine were diagnosed with blood parasite infestation (20%). In conclusion, this study showed the prevalence of hypocalcemia patients in each species involved and there is no significant association of the cases presented with any risk factors.

Keywords: *hypocalcemia, calcium, creatine kinase, caprine, leporine*

1.0 INTRODUCTION

Calcium (Ca) plays a central role in animals homeostasis including muscle contraction, blood clotting, enzyme production, neural excitability, hormone secretion and cell adhesion (Rosol et al., 1995). In terms of its structural role, the primary function of calcium is in the formation of hard structures such as bone and teeth.

About 99% of calcium in the body resides in the skeleton and is deposited as hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Most skeletal calcium is poorly exchangeable and is considered readily accessible to less than 1% (Schenck et al., 2006). Almost all non-skeletal calcium is located extracellularly in plasma or serum, with a small but biologically important quantity located intracellularly (Holowaychuk, 2013). Calcium is distributed within the plasma or extracellularly in three forms which are the physiologically active ionized form (50%), the chelated form complexed with lactate, citrate and bicarbonate (10%) and the protein-bound form (40%) (Dhupa & Proulx, 1998). Most intracellular calcium is sequestered in organelles or bound to cellular membranes or proteins (Schenck et al., 2006).

Ionized calcium is a biologically active form and concentrations of ionized calcium in the blood are closely regulated by the concerted actions of parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ (calcitriol) and calcitonin (Coady et al., 2019). The main target organs affected by calcium regulating hormones are intestines, kidneys, and bone. These interactions allow calcium to be retained in the extracellular fluid by renal reabsorption, increased intestinal calcium transport from diet and internal redistribution of calcium from the bone (Schenck et al., 2006).

Hypocalcaemia occurs when calcium mobilization of the bone decreased, skeletal calcium accretion increased or urinary loss of calcium increased. Furthermore, hypocalcaemia occurs due to decreased in gastrointestinal absorption of calcium, calcium intracellularly diffused or as a result of a combination of these mechanisms (Schenck et al., 2006). Hypocalcaemia can be observed in a number of clinical conditions, and typically occurs when intravascular loss of calcium occurs at a rate faster than can be replenished by kidney reabsorption, bone release or intestinal absorption (Dhupa & Proulx, 1998). There are three general classifications of hypocalcemia, impaired parathyroid hormone secretion or action, impaired vitamin D synthesis or action as with renal failure, and calcium chelation or precipitation, respectively (Dhupa & Proulx, 1998). Hypoparathyroidism, chronic kidney disease (CKD), acute pancreatitis, and eclampsia are frequently documented causes of hypocalcemia (Coady et al., 2019).

Parathyroid hormone is the key regulator of calcium and phosphorus levels whereby calcium receptors regulate the synthesis and release of PTH on the chief cells of the parathyroid gland, providing a rapid increase in serum calcium in response to hypocalcaemia (Bonczynski, 2007). PTH is primarily responsible for the perfect, minute-to-minute regulation of serum calcium by controlling the reabsorption of renal ionized calcium. Renal stimulation of the synthesis of 1,25-dihydrovitamin D₃ (calcitriol) and osteoclastic bone resorption takes more time but has the ability to produce a greater amount of calcium (Rosol et al., 1995). After days or weeks of hypocalcaemia, hypertrophy and hyperplasia of the chief cells of the parathyroid gland, with a greater proportion of the chief cells synthesizing and secreting PTH, will result in the increase secretion of PTH (Holowaychuk, 2013). Severe hypocalcemia may

induce prolong secretion of excessive PTH by parathyroid gland that can cause excessive bone resorption and finally osteoporosis.

Hypocalcaemia diagnosis can be performed through standard tests including complete blood count, biochemistry profile, and urinalysis that will provide useful information on blood calcium levels and information on potential causes of hypocalcaemia in animals (German, 2010).

The purpose of this retrospective study is to determine the occurrence percentage (%) of cases associated with hypocalcaemia and hyperparathyroidism in animals that were presented to University Veterinary Hospital, Universiti Putra Malaysia through clinicopathologic and radiographic findings between 2015 to 2019. At present, there is limited published studies investigating the incidence of hypocalcaemia in general population of animal and species prevalence associated with hypocalcemia and hyperparathyroidism in University Veterinary Hospital, Universiti Putra Malaysia.

2.0 LITERATURE REVIEW

2.1 Hormonal Regulation of Calcium Metabolism

Calcium metabolism plays an integral role in many physiological processes such as normal nerve and muscle activity, contraction of the heart muscle, stability and bonding of the cell membrane, coagulation and to a limited extent structural integrity of the bones and teeth (Stillion & Ritt, 2009). The extracellular calcium concentration is continuously challenged by deficient calcium absorption from bowel, decreased calcium resorption from bone and by calcium loss mainly through the kidney while both the foetus and the mammary gland also withdraw calcium from the maternal plasma during pregnancy and lactation (Schoenmakers et al., 1999). Normal homeostasis of calcium regulates the action of three hormones which are PTH, calcitriol and calcitonin that control calcium movement between the extracellular fluid, the gastrointestinal tract, the kidneys and the bones (Stillion & Ritt, 2009). Normal homeostatic control mechanisms normally maintain serum calcium concentration within a limited range and ensure a sufficient intracellular supply of calcium (Schenck et al., 2006).

2.1.1 Parathyroid Hormone

Parathyroid hormone (PTH) consist of 84 amino acids peptide formed by the parathyroid gland's principal cells (Rosol et al., 1995). PTH synthesis and release is regulated by calcium receptors on the parathyroid gland's main cells, providing rapid

serum calcium elevation in response to hypocalcemia (Bonczynski, 2007). Interaction of serum calcium ion with its chief cell receptor results in an inverse sigmoidal relationship between serum calcium ion concentrations and PTH concentrations and the sigmoidal relationship between serum calcium ion and PTH secretion helps the chief cells to respond quickly to serum calcium reduction (Rosol et al., 1995). Almost 10 percent decrease in serum calcium concentration results in an almost maximum response of PTH secretory (Schenck et al., 2006).

PTH is the key hormone involved in the minute to minute fine regulation of calcium concentration in the blood by conducting its biological actions directly by affecting the activity of target cell primarily in the bone and kidney and indirectly in the intestine to maintain plasma calcium at an appropriate concentration to ensure the optimal functioning of a wide range of body cells (Schenck et al., 2006). In the bone, PTH increases the differentiation of macrophage precursors into osteoclasts and since osteoclasts do not have direct receptors for PTH. Osteoblasts are stimulated in response to PTH release that increases metabolic activity and number in osteoclasts. This will significantly increase bone resorption and thus calcium levels (Bonczynski, 2007). Parathyroid hormone activity on the kidney involves increasing calcium transport proteins in the distal tubule, triggering a particular adenylate cyclase involving reabsorption in the distal tubule, and increasing absorption capacity in the thick ascending Henle loop by raising the number of calcium-sodium exchangers (Dhupa & Proulx, 1998). In addition, PTH stimulates the renal synthesis of 1,25-Dihydroxyvitamin D₃, which will stimulates the active absorption of calcium in the intestine and serves as a permissive factor for PTH-mediated bone resorption. 1,25-Dihydroxyvitamin D₃ will also influences osteoclasts recruitment from the

osteoprogenitor cell lineage (Schoenmakers et al., 1999). The end result of these action is an increase in the concentration of serum calcium which inhibits further release of PTH by negative feedback (Eckermann, 2008).

The PTH secretion rate is inhibited by negative feedback effects of a high serum ionised calcium concentration and a longer loop feedback provided by calcitriol. (Toverud & Boass, 1980). High serum and intracellular calcium concentrations inhibit PTH secretion via increased in arachidonic acid (Schenck et al., 2006). Inhibition of PTH synthesis by 1,25-Dihydroxyvitamin D₃ completes an essential endocrine feedback loop between the parathyroid chief cells and the renal epithelial cells since PTH stimulates 1,25-Dihydroxyvitamin D₃ renal production (Rosol et al., 1995). The plasma calcium concentration then decreases to normal, both within minutes, as a result of increased calcium excretion in the urine and cessation of bone resorption, and 1,25-Dihydroxyvitamin D₃ production decreased over a longer period (Schoenmakers et al., 1999).

2.1.2 Vitamin D

Vitamin D as cholecalciferol is either ingested or formed by 7-dehydrocholesterol in skin as a result of ultraviolet light exposure (Dhupa & Proulx, 1998). Dogs and cats inefficiently photosynthesized vitamin D in their skin and are therefore dependent on vitamin D in their diet (Schenck et al., 2006). Calciferol is hydroxylated in the liver to form 25-hydroxyvitamin D (calcidiol), which is then hydroxylated to 1,25-Dihydroxyvitamin D₃ (calcitriol) in the kidney (Schenck et al.,

2006). The active form of Vitamin D, 1,25-Dihydroxyvitamin D₃, is generated by α-1-hydroxylation of 25-hydroxyvitamin D of the epithelial cells of the proximal convoluted tubules throughout the kidney (Rosol et al., 1995). 1,25-Dihydroxyvitamin D₃ is known to be the hormonal type of vitamin D due to several factors which are it is produced and secreted by an endocrine gland, its circulating level is strictly regulated and it is circulated to distant sites where it operates (Toverud & Boass, 1980).

Hydroxylation of 25-hydroxyvitamin D to 1,25-Dihydroxyvitamin D₃ is regulated by low plasma concentrations of calcium or phosphorus and elevated PTH concentrations, as well as by calcitonin, insulin-like growth factor-1 IGF-1 and growth hormone (Schoenmakers et al., 1999). In response to hypocalcemia, PTH is secreted from parathyroid glands and activates the production and secretion of active form of vitamin D or calcitriol in the kidney (Stillion & Ritt, 2009).

The primary functions of 1,25-Dihydroxyvitamin D₃ in calcium control includes stimulation of intestinal calcium absorption, inhibition of PTH synthesis by decreasing PTH mRNA transcription, promotion of osteoclastic bone resorption and negative feedback on its own synthesis in renal epithelial cells (Rosol et al., 1995). Calcitriol works directly on the intestinal cells to increase the active absorption of calcium from the digestive tract and increase the calcium-binding protein in enterocytes, thus increasing the concentration of calcium in the plasma (Dhupa & Proulx, 1998). Calcitriol is also essential for normal bone resorption since it facilitates the differentiation of monocytic hematopoietic precursors in the bone marrow into osteoclasts which the relationship between calcitriol and osteoclasts explains the dependency of PTH on calcitriol for optimal bone resorption (Schenck et al., 2006). It

is also necessary to work with PTH to enhance the absorption of phosphorus and calcium from glomerular filtrate in the kidney into blood (Stillion & Ritt, 2009). Renal calcitriol synthesis is inhibited by hyperphosphatemia, hypercalcemia, and renal diseases accompanied by loss of renal tubular mass (Rosol et al., 1995). It also exerts negative feedback on the kidneys to prevent the formation of more calcitriol and on the parathyroid glands to minimise PTH synthesis and secretion when the serum calcium level is high (Stillion & Ritt, 2009).

2.1.3 Calcitonin

Calcitonin is a 32-amino acid polypeptide hormone that is synthesised by C cells in the thyroid gland (Schenck et al., 2006). Calcitonin is released rapidly by C cells in response to small elevation in serum ionic calcium concentration (Garel, 1987). Calcitonin released is also stimulated by estradiol, glucagon, and gastrointestinal hormones, including gastrin, cholecystokinin, and secretin (Eckermann, 2008). Chronic hypercalcemia results in chronic rises in serum calcitonin concentrations and C-cell hyperplasia with no biological significance in relation to the regulation of serum calcium (Rosol et al., 1995).

Calcitonin receptors are found in the plasma membranes of the kidney, and the hormone activates adenylate cyclase in the bones and kidneys (Garel, 1987). It works on bone tissue to raise serum calcium levels by stimulating osteoblasts to increase calcium absorption and deposition in the skeleton and inhibiting osteoclasts to decrease bone resorption of calcium (Stillion & Ritt, 2009). The effects of calcitonin in the bone

are transitory, which has restricted the effectiveness of calcitonin as a treatment for hypercalcemia (Schenck et al., 2006). Calcitonin causes reduced gastrin and gastric acid secretion in the gastrointestinal tract, thus decreasing the absorption of calcium from gastrointestinal tract (Eckermann, 2008). At high doses, calcitonin can promote the excretion of urinary calcium (Schenck et al., 2006). The end result of all these mechanisms is reduced in serum calcium concentration level. Calcitonin synthesis is inhibited by 1,25-Dihydroxyvitamin D3 and probably by several other, yet unknown factors (Schoenmakers et al., 1999).

2.2 Hyperparathyroidism

2.2.1 Parathyroid Glands

Parathyroid glands are found in all vertebrates (Capen, 1987). Most animal species consist of external and internal glands in the anterior cervical zone (Tomsa et al., 1999). Parathyroid glands are extremely sensitive to fluctuations in ionised calcium, particularly when ionised calcium is low (Parker et al., 2015). Branch of the thyroid artery and vein provide the majority of the vascular supply of the parathyroid glands (Bonczynski, 2007).

2.2.2 Nutritional Secondary Hyperparathyroidism

Nutritional secondary hyperparathyroidism is a widespread disease in domestic animals (Bas et al., 2005). Nutritional secondary hyperparathyroidism caused by long-term deficiency of calcium, phosphorus or vitamin D3 in the diet, or by imbalance in the last two nutrients, induces excessive development of parathyroid hormone (Toyoda et al., 2004). Nutritional secondary hyperparathyroidism can also occur when there is severe gastrointestinal disorder, which decreases the absorption of calcium and vitamin D (Schenck et al., 2006). Exotic animals may be at higher risk for the development of nutritional secondary hyperparathyroidism, as dietary requirements are not always known (Tomsa et al., 1999).

In the present analysis, both the decreased plasma calcium:phosphorus ratio and the mean 1,25-Dihydroxyvitamin D3 concentration confirmed as an etiologic diagnosis of nutritional secondary hyperparathyroidism (Tangredi & Krook, 1999). The imbalance causes hypocalcemia, which increases the secretion of PTH by the parathyroid gland (Moarrabi et al., 2008). The development of parathyroid hyperplasia is caused by an increase in the rate of proliferation of parathyroid cells influenced by hypocalcemia and hyperphosphatemia (Bas et al., 2005). Continued intake of the deficient diet retains the hyperparathyroid condition and induces gradual skeletal demineralization and consequent clinical signs (Schenck et al., 2006).

Nutritional secondary hyperparathyroidism is mainly a disease of young growing animals due to their increased demand for calcium in bone growth and their insufficient calcium reserves (Tomsa et al., 1999). Clinical symptoms associated with hypocalcemia included tremor and seizure disorder while long bones and vertebrae are frequently affected, resulting in painful ambulation, limb deformity and paresis. Diffuse

osteopenia, bone deformities and fractures can be identified by radiographs (Parker et al., 2015). The symptoms are less severe in adults, but generalised osteopenia and skeletal pain are often seen, and alveolar bone resorption can cause loosening and loss of teeth (Moarrabi et al., 2008).

2.2.3 Renal Secondary Hyperparathyroidism

Secondary hyperparathyroidism is a metabolic syndrome characterised by an elevated but not autonomous production rate of parathyroid hormone and this condition occurs typically in chronic renal failure (Potts et al., 1969). Effective diagnosis of renal secondary hyperparathyroidism is made by recording an elevated concentration of PTH in an animal with chronic renal failure (Parker et al., 2015). Parathyroid hyperplasia and hyperparathyroidism can occur secondary to chronic renal failure as the kidney play a key role in the metabolism of parathyroid hormone (PTH) and vitamin D (Stillion & Ritt, 2009). Retention of phosphorus and reduced 1,25-Dihydroxyvitamin D₃ are the two key factors responsible for the development of renal secondary hyperparathyroidism in chronic renal failure (Slatopolsky & Delmez, 1996).

Secondary hyperparathyroidism in chronic renal disease tends to begin with the first step of nephron destruction and to progress in severity with the progression of nephron failure (Slatopolsky et al., 1972). Chronic renal failure animals have a gradual depletion of nephron and a reduced glomerular filtration rate (GFR) which contributes to an increase in serum concentration of substances that are usually removed by kidney from the blood, including phosphate (Stillion & Ritt, 2009). Unexcreted phosphate will

result in a finite rise in serum phosphate concentration, the ionised serum calcium concentration will then decrease reciprocally, and the latter will act as a trigger for increased PTH secretion (Slatopolsky et al., 1971). Phosphorus was therefore believed to mediate the pathogenesis of parathyroid hyperplasia and secondary hyperparathyroidism indirectly (Denda et al., 1996). As phosphorus regulates the synthesis of 1,25-Dihydroxyvitamin D₃ by altering the enzyme 1- α -hydroxylase, the effect of phosphorus retention may be mediated by a decrease in the synthesis of 1,25-Dihydroxyvitamin D₃ (Stillion & Ritt, 2009).

The pathogenesis of secondary renal hyperparathyroidism is complex, but it has recently discovered that 1,25-Dihydroxyvitamin D₃ (calcitriol) plays a significant role in this disorder (Rosol et al., 1995). Animals with advanced chronic renal failure reduced circulating concentrations of 1,25-Dihydroxyvitamin D₃ due to decreased renal synthesis caused by the inhibitory effect of hyperphosphatemia on 25-hydroxyvitamin D 1- α -hydroxylase and progressive loss of renal epithelial cells (Parker et al., 2015). Low level of 1,25-Dihydroxyvitamin D₃ contribute to impaired intestinal calcium absorption and skeletal resistance to PTH action, which decrease serum ionised calcium concentration and promotes indirect PTH secretion (Denda et al., 1996). Since 1,25-Dihydroxyvitamin D₃ acts directly to decrease PTH mRNA expression, increase expression of the Vitamin D receptor, and control the set point of chief cell responsiveness to negative feedback by the serum calcium concentration, a decrease in 1,25-Dihydroxyvitamin D₃ contributes to a further increase in PTH levels (Stillion & Ritt, 2009). Decreased circulating levels of 1,25-Dihydroxyvitamin D₃ in animals with chronic renal failure results in chief cell hyperplasia and secondary hyperparathyroidism (Rosol et al., 1995).

2.3 Measurement of Calcium Level

Calcium circulates in the blood in three types that are protein bound (50%), chelated (10%) and ionized form (40%) (Zaloga, 1992). The ionized form is the active fraction of calcium and is the form that is physiologically regulated (Bourke & Delaney, 1993). Traditionally, serum total calcium (tCa) has been used to determine the calcium status of small animals, even though ionized calcium is the biologically active and closely regulated form (Holowaychuk, 2013). Although total calcium levels may be appropriate to diagnose hypocalcaemia, the measurement of ionized calcium is probably more indicative of the calcium balance as it is physiologically active (Dhupa & Proulx, 1998).

Total calcium is affected by factors that modify protein-bound and chelated calcium, regardless of ionized calcium (Zaloga, 1992). Alterations in protein (mostly albumin) concentrations affect the total calcium value as the proportion of serum calcium bound to protein, hence the total calcium (tCa) concentration may be falsely low following low albumin (Alb) concentrations (Sharp et al., 2009). Correction factors for decreased albumin can be used but are marginal in determining the true calcium balance (Dhupa & Proulx, 1998). Acidosis reduces calcium binding to albumin while alkalosis will enhance the binding of calcium to albumin, so changes in acid-based status can also alter total and ionized calcium values (Zaloga, 1992). Total calcium as a calcium status measure resulted in overestimation of hypocalcaemia and underestimation of normocalcaemia, whereas the use of total calcium adjusted albumin

or total protein resulted in overestimation of normocalcaemia and underestimation of hypocalcaemia (Sharp et al., 2009). Fastened serum or heparinized plasma samples may be used to test total calcium however, other coagulants including citrate or ethylenediaminetetraacetic acid (EDTA) complex with calcium should be avoided (Holowaychuk, 2013).

Measurement of ionized calcium is the gold standard for the determination of calcium status because it is the active form of calcium which is not bound to albumin or complexed to other ions (Sharp et al., 2009). Ionized calcium should always be assessed to accurately measure calcium status, particularly in patients with critical illness, acid-base disturbances, protein concentration changes, or kidney or parathyroid disease (Holowaychuk, 2013). Ionized blood or plasma calcium concentration can be analysed using a calcium specific ion electrode system that minimises contamination by other ions, proteins, hemolysis, and lipemia (Rosol et al., 1995). Serum can be used to measure ionized calcium concentrations, but preferably it should be collected anaerobically to reliably determine the physiological values of circulating calcium (Zaloga, 1992). Aerobic collection of serum allows air to be diluted and CO_2 to be decreased, which increases the pH of the sample and reduces protein binding, thereby reducing ionized calcium concentrations (Bourke & Delaney, 1993). It is most essential to measure the biologically active form of calcium which is ionized calcium, but this is not always feasible because of the need for special instruments and proper sample handling (Rosol et al., 1995).

3.0 MATERIALS AND METHODS

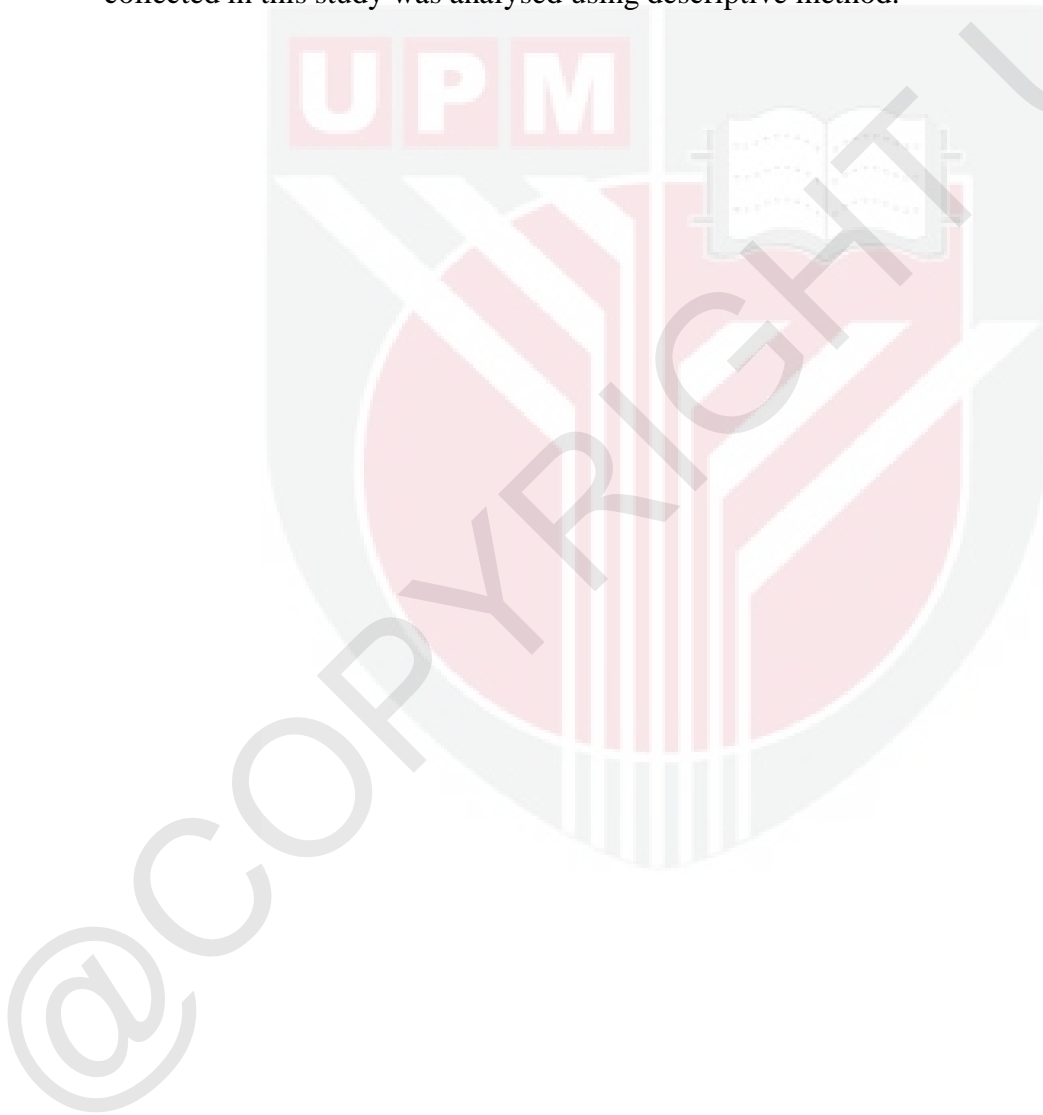
3.1 Source of Data

A retrospective study was performed based on the records available at the Haematology and Clinical Biochemistry Laboratory, Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, UPM, for a period of 5 years from January 2015 to December 2019. The clinical pathology database was searched for blood and biochemistry profiles of hypocalcaemic and non-hypocalcaemic patients. Hypocalcaemia was defined when the serum calcium concentration is lower than normal and the reference range for each species is varied. Two hundred and fifty-four hypocalcaemic patients were identified for the period of 5 years. From the records, case numbers, patient's ID, signalment inclusive of species, breed, age, gender and neuter status, clinicopathologic abnormalities (total serum calcium, serum phosphorus, serum creatinine, serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), serum gamma-glutamyl transferase (GGT) and serum creatine kinase (CK) concentrations), and final diagnosis were recorded for the hypocalcaemic patients.

Radiographic records of seventy-six patients out of 254 are available and were reviewed for any signs of diffuse or generalized osteoporosis. The radiographic signs that are suggestive of osteoporosis are reduced in bone opacity of the spine and long bones and cortices may appear abnormally thin. Data that were missing and not applicable were recorded as not available.

3.2 Data Tabulation and Statistical Analysis

All data recorded were tabulated in Microsoft Excel spreadsheet and transferred to IBM Statistical Package for the Social Sciences software version 23.0 to further statistical analysis to include Kolmogorov-Smirnov test, Mann-Whitney U test and Chi-Square analysis. Standard significance at value of $p < 0.05$ was used. Data collected in this study was analysed using descriptive method.



4.0 RESULTS

4.1 Prevalence of Hypocalcaemia

From January 2015 to December 2019, a total of 1865 patients presented to UVH, UPM have been tested for blood calcium level analysis. 254 cases have been identified to be associated with hypocalcaemia. The occurrence of cases was from 24 to 65 cases per species. The prevalence of species with hypocalcaemia in UVH are as in Table 1.

Table 1: Prevalence of Hypocalcaemia in animals presented to UVH, UPM from 2015 to 2019

Species	Number of animals		Prevalence (%)
	diagnosed with hypocalcaemia	tested for calcium level	
Caprine	65	260	25
Leporine	58	187	31
Bovine	40	178	22
Feline	35	572	6
Canine	24	621	4

4.2 Species of Patients

From the 254 cases, sixty-five cases (25.5%) identified were Caprine species and fifty-eight cases (22.8%) were of Leporine species. These two species are the highest identified species associated with hypocalcaemia cases presented to UVH, UPM. Furthermore, a total of 40 cases (15.7%) were Bovine species and another 35 cases (13.7%) presented to UVH, UPM that related to hypocalcaemia were Feline species. Another 24 cases (9.4%) out of 254 were Canine species and 12 cases (4.7%) were

Ovine species. The rest of the cases associated with hypocalcaemia include species of Deer, Otter, Iguana, Rodent, Stingray, Tiger, Turtle, Equine, Ferret, Goose, Leopard, Primate and Shark which contribute only 1% or less each (Figure 1).

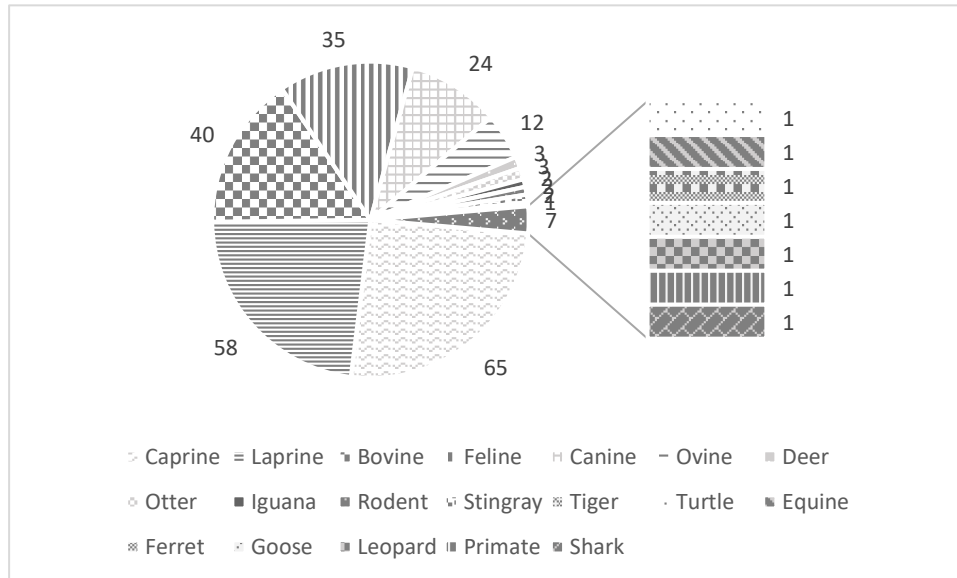


Figure 1: Species of patients (n=254) with hypocalcaemia

4.3 Gender and Neuter status

Among the 254 cases associated with hypocalcaemia, there are 97 male animals (38%) and 157 female animals (62%). In addition, 16 of the 97 male animals and 11 of the 157 females animals were neutered (Figure 2).

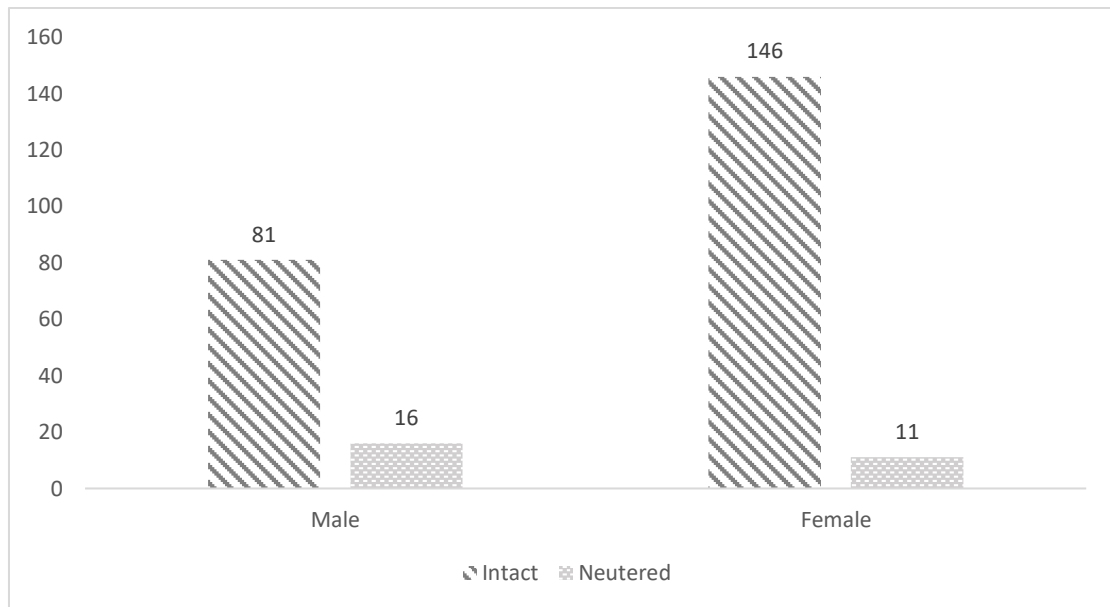


Figure 2: Gender and Neuter status of patients (n=254) with hypocalcaemia

4.4 Age Group

Age group of hypocalcaemia animals in this study are classified into three categories which are young, adult, and geriatric. The majority of animals diagnosed with hypocalcaemia were adult animals (n=154, 61%). This followed by young animals (n=74, 29%) and the least fall in the categories of geriatric animals (n=8, 3%) as reviewed in the Figure 3. There are a total of 18 animals (7%) with age not stated.

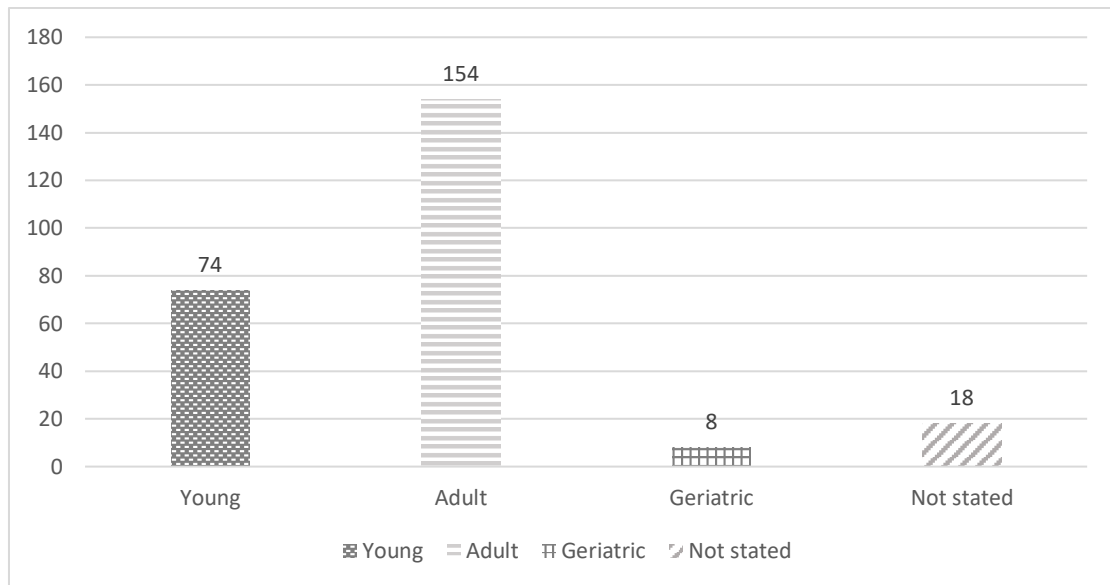


Figure 3: Age group of patients (n=254) with hypocalcaemia

4.5 Caprine

4.5.1 Age of Caprine

Age group of hypocalcaemia caprine in this study were classified into three categories; Young, Adult, and Geriatric. The majority of animals diagnosed with hypocalcaemia were adult animals (n=49, 75%) while the rest were young animals (n=16, 25%). The mean age of the affected caprine was 3.17 ± 0.248 years (range, 0.3-8.0 years) while the median was 3.0 years. There is no significant association between age and the calcium status ($P > 0.05$).

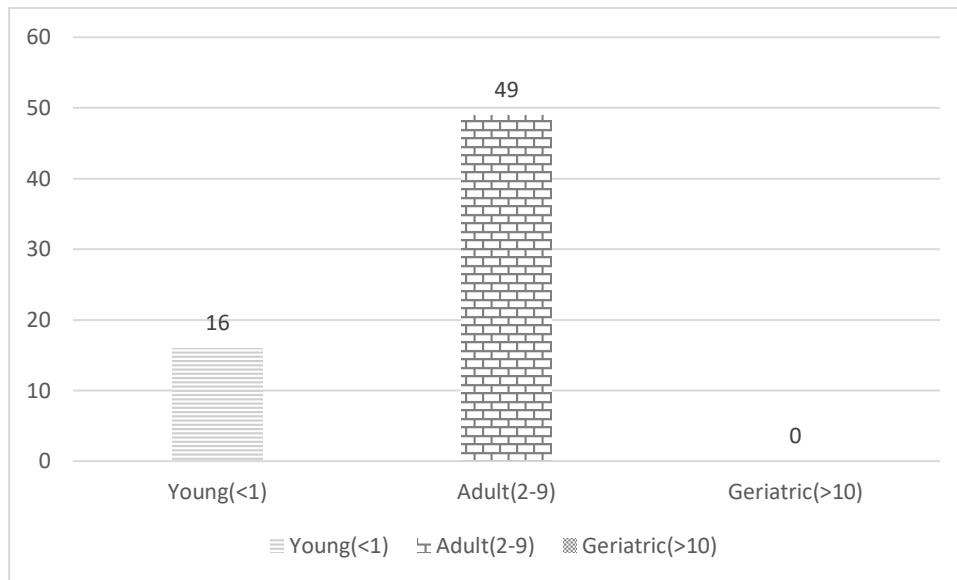


Figure 4: Age group of caprine (n=65) with hypocalcaemia

4.5.2 Gender of Caprine

Among the 65 bovine cases associated with hypocalcaemia, there are 19 male animals (29%) and 46 female animals (71%). There is no significant association between the gender and calcium status ($P>0.05$).

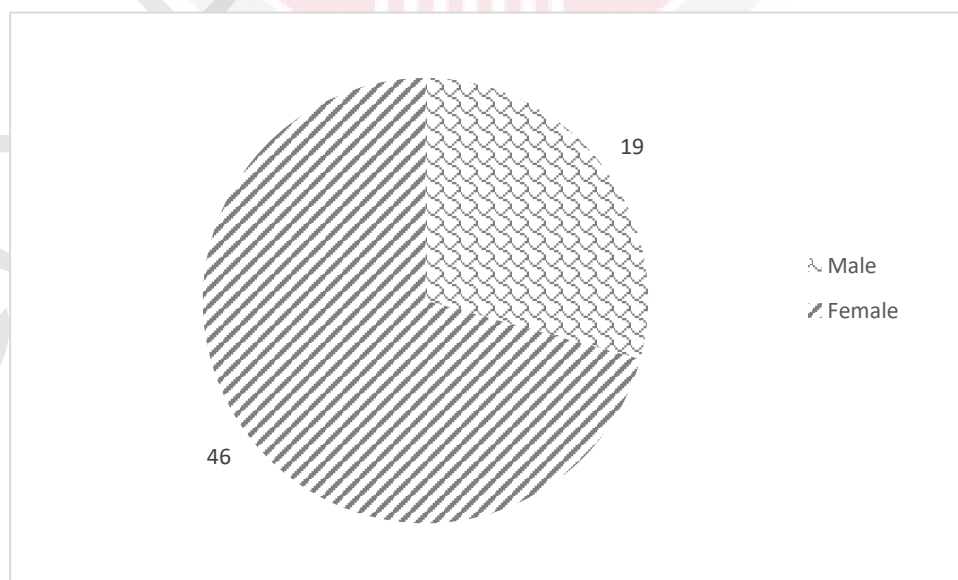


Figure 5: Gender of caprine (n=65) with hypocalcaemia

4.5.3 Clinicopathological Findings

Serum biochemistry profiles inclusive of inorganic phosphate, creatinine, ALT, AP(ALP), GGT, and CK(CPK) was reviewed and evaluated. It showed that 58% of the hypocalcaemia caprine had lower serum creatinine level with the mean concentration of 176.4 μ mol/L. In addition, 39% of the caprine showed increased serum GGT concentration with the mean concentration of 49.0U/L and 64% had increased serum CK(CPK) level with mean concentration of 546.2U/L.

Table 2: Clinicopathological findings in caprine with Hypocalcaemia

Variable	N	Mean	Median	Range	Caprine Ref.Range	Compared to Reference Range		
						Higher n(%)	Within n(%)	Lower n(%)
Inorganic Phosphate	12	2.167	2.020	0.400-4.710	1.6-2.3	5 (42%)	3 (25%)	4 (33%)
Creatinine	45	176.4	72.00	11.00-1633	88-160	10 (22%)	9 (20%)	26 (58%)
ALT	3	359.6	15.0	14.00-1050		1 (33%)	2 (67%)	0
AP(ALP)	2	128.0	128.0	123.0-133.0	90-200	0	2 (100%)	0
GGT	49	49.0	45.00	11.00-116.0	30-50	19 (39%)	20 (41%)	10 (20%)
CK(CPK)	33	546.2	367.0	8.00-2964	100-200	21 (64%)	8 (24%)	4 (12%)

4.5.4 Diagnosis in Caprine with Hypocalcaemia

Pregnancy toxemia was diagnosed in 11 caprines (17%) while another 7 of the caprines (11%) was diagnosed with parasitic gastro-enteritis. In addition, hypocalcaemia was diagnosed in 5 caprines (8%) and both clinical mastitis and blood parasite infestation was diagnosed in 3 caprines (5%) each. 2 of the caprines (3%) was

diagnosed with gangrenous mastitis and another 2 (3%) with myositis. Hypocalcaemia was observed in 2 of the caprines (3%) during health screening while another 2 caprines (3%) was diagnosed with mammary gland tumour. In addition, malnutrition, metritis and pneumonia was diagnosed in 2 caprines (3%) each. The rest of the caprines were diagnosed with various types of diseases which contribute only 2% each (Table 1).

Table 3: Diagnosis group in caprine (n=65) with hypocalcaemia

Diagnosis Group	n
Pregnancy Toxaemia	11
Parasitic Gastro-Enteritis	7
Hypocalcaemia	5
Clinical Mastitis	3
Blood Parasite infestation	3
Gangrenous Mastitis	2
Health screening	2
Myositis	2
Malnutrition	2
Metritis	2
Pneumonia	2
Bronchopneumonia	1
Brucellosis	1
Capture Myopathy	1
Dystocia and Retained placenta	1
Health screening	1
Intra-uterine Disease	1
Ketosis	1
Liver Disease	1
Lower Urinary Tract Disease	1
Mange problem	1
Mycoplasmosis	1
Orchitis	1
Osteomalacia	1
Peritonitis	1
Post C-Section	1
Post Partum Complication	1
Post-Uroabdomen Lavage	1
Post Perineal Urethrostomy evaluation	1
Septic Arthritis	1
Traumatic Injury	1
Urolithiasis	1
Vaginal Prolapse	1
Vitamin D Deficiency	1
Wasting Disease	1

4.6 Leporine

4.6.1 Age of Leporine

Age group of hypocalcaemia leporine in this study were classified into three categories; Young, Adult, and Senior. The majority of animals diagnosed with hypocalcaemia were adult animals (n=31, 53%) while the rest were young animals (n=24, 42%) and senior animals (n=3, 5%). The mean age of the affected leporine was 2.95 ± 0.360 years (range, 0.3-10.0 years) while the median was 1.5 years. There is no significant association between age and the calcium status ($P>0.05$).

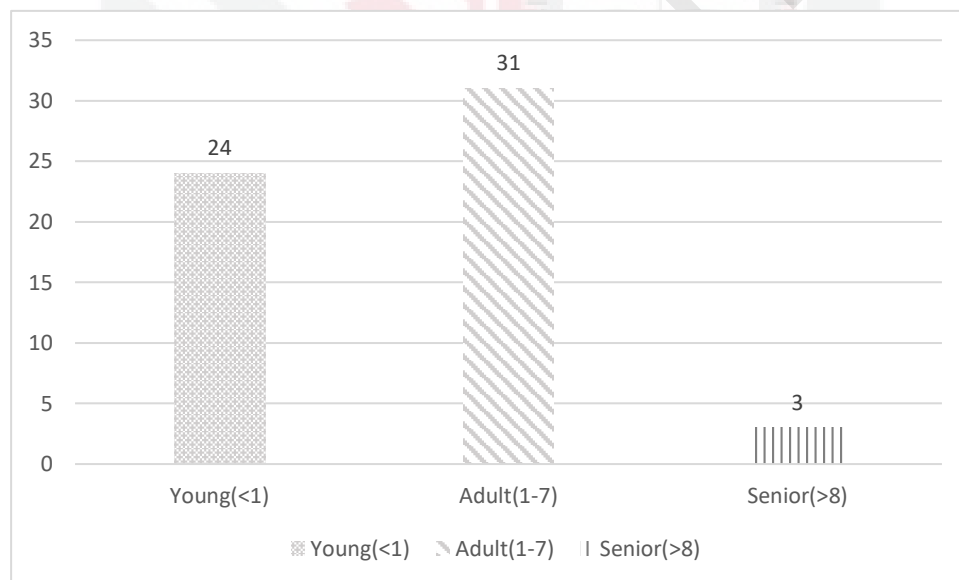


Figure 6: Age group of leporine (n=58) with hypocalcaemia

4.6.2 Gender of Leporine

Among the 58 leporine cases associated with hypocalcaemia, there are 21 male animals (36%) and 37 female animals (64%). There is no significant association between the gender and calcium status ($P>0.05$).

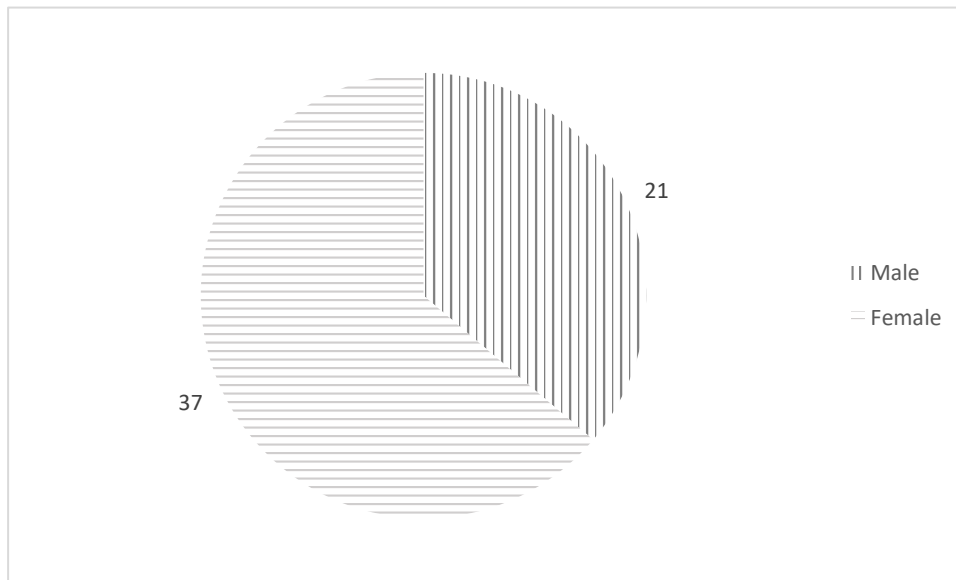


Figure 7: Gender of leporine (n=58) with hypocalcaemia

4.6.3 Clinicopathological Findings

Serum biochemistry profiles inclusive of inorganic phosphate, creatinine, ALT, AP(ALP), GGT, and CK(CPK) was reviewed and evaluated. It showed that only 9% of the hypocalcaemia leporine had lower serum creatinine level with the mean concentration of 96.67 μ mol/L. In addition, 23% of the leporine showed increased serum ALT concentration with the mean concentration of 76.75U/L and 93% had increased serum CK(CPK) level with mean concentration of 1641U/L.

Table 4: Clinicopathological findings in leporine with Hypocalcaemia

Variable	N	Mean	Median	Range	Laprine Ref.Range	Compared to Reference Range		
						Higher n(%)	Within n(%)	Lower n(%)
Inorganic Phosphate	44	1.477	1.300	0.640-3.50	0.74-2.22	3 (7%)	38 (86%)	3 (7%)
Creatinine	57	96.67	90.00	20.00-332.0	44-234	1 (2%)	51 (89%)	5 (9%)
ALT	44	76.75	51.00	5.800-692.8	45-80	10 (23%)	18 (41%)	16 (36%)

AP(ALP)	6	50.33	44.50	14.00-91.00	12-96	0	6 (100%)	0
GGT	40	15.48	6.500	1.00-181.0	0-7	16 (40%)	24 (60%)	0
CK(CPK)	30	1641	893.0	247.0-15069	140-372	28 (93%)	2 (7%)	0

4.6.4 Diagnosis in Leporine with Hypocalcaemia

Hypocalcaemia was observed in 27 of the leporines (47%) during health screening while another 4 leporines (7%) were diagnosed with chronic kidney disease. In addition, both bacterial infection and fracture was also diagnosed in 4 other hypocalcaemic leporines (7%) each. Tumour was diagnosed in 4 leporines (7%) and malnutrition was diagnosed in 3 leporines (5%). The rest were diagnosed with dystocia, encephalitozoon cuniculi, fibrosarcoma, glaucoma, left hindlimb splayleg, malocclusion of incisor teeth, metastasis carcinoma, neurological deficits, osteomyelitis, metabolic bone disease and babesiosis which contribute only 2% each (Table 1).

Table 5: Diagnosis group in leporine (n=58) with hypocalcaemia

Diagnosis Group	n
Health Screening	27
Chronic Kidney Disease	4
Bacterial Infection	4
Fracture	4
Abscess/Tumour	4
Malnutrition	3
Muscle Disease	1
Dystocia	1
Encephalitozoon cuniculi	1
Fibrosarcoma	1
Glaucoma	1
Left Hindlimb Splayleg	1
Malocclusion of Incisor Teeth	1
Metastasis Carcinoma	1
Neurological Deficits	1
Osteomyelitis	1
Metabolic Bone Disease	1
Babesia	1

4.7 Bovine

4.7.1 Age of Bovine

Age group of hypocalcaemia bovine in this study were classified into three categories; young, adult, and geriatric. The majority of animals diagnosed with hypocalcaemia were adult animals (n=28, 70%) while the rest were young animals (n=12, 30%). The mean age of the affected bovine was 2.91 ± 0.416 years (range, 0.2-12.0 years) while the median was 2.0 years. There is no significant association between age and the calcium status ($P>0.05$).

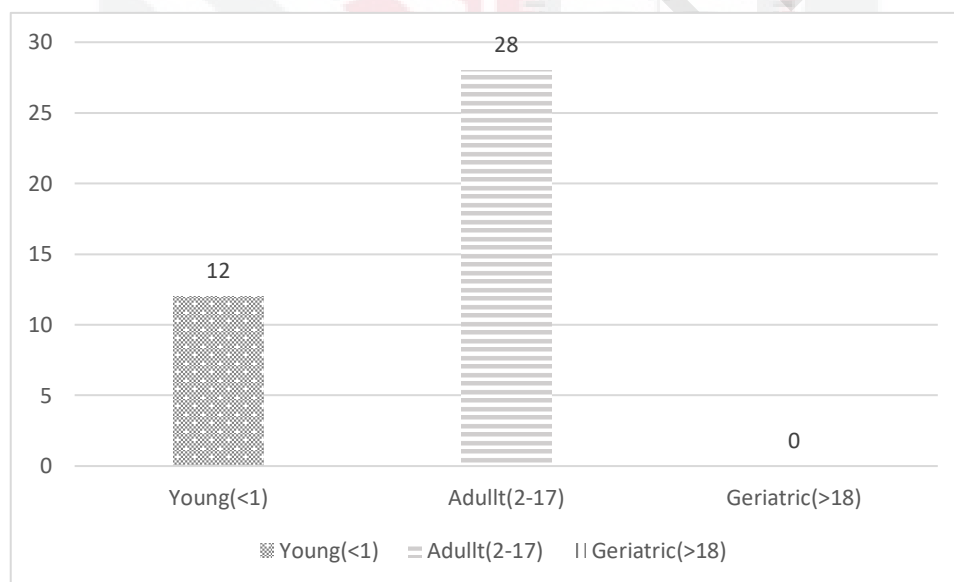


Figure 8: Age group of bovine (n=40) with hypocalcaemia

4.7.2 Gender of Bovine

Among the 40 bovine cases associated with hypocalcaemia, there are 11 male animals (28%) and 29 female animals (72%). There is no significant association between the gender and calcium status ($P>0.05$).

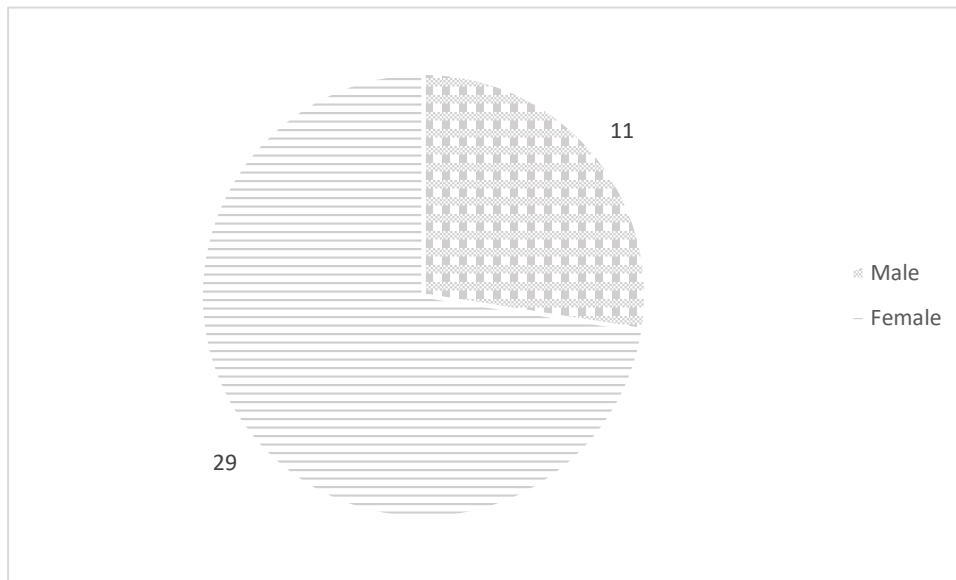


Figure 9: Gender of bovine (n=40) with hypocalcaemia

4.7.3 Clinicopathological Findings

Serum biochemistry profiles inclusive of inorganic phosphate, creatinine, ALT, AP(ALP), GGT, and CK(CPK) was reviewed and evaluated. It showed that 15% of the hypocalcaemia bovine had increased serum creatinine level with the mean concentration of 100.2 μ mol/L. In addition, 30% of the bovine showed increased serum GGT concentration with the mean concentration of 55.74U/L and 88% had increased serum CK(CPK) level with mean concentration of 1451U/L.

Table 6: Clinicopathological findings in bovine with Hypocalcaemia

Variable	N	Mean	Median	Range	Bovine Ref.Range	Compared to Reference Range		
						Higher n(%)	Within n(%)	Lower n(%)
Inorganic Phosphate	13	1.844	1.700	1.2-2.57	1.6-2.9	0	10 (78%)	3 (22%)
Creatinine	20	100.2	77.50	22.00-256.0	<176	3 (15%)	17 (85%)	0
ALT	6	13.45	12.20	7.000-21.80	5-15	2 (33%)	4 (67%)	0

AP(ALP)	1	75.00	75.00	75.00-75.00	50-100	0	1 (100%)	0
GGT	23	55.74	17.00	7.000-467.0	<25	7 (30%)	16 (70%)	0
CK(CPK)	17	1451	1177	140.0-5036	50-200	15 (88%)	2 (12%)	0

4.7.4 Diagnosis in Bovine with Hypocalcaemia

Blood parasite infestation was diagnosed in 8 bovines (20%) while another 8 of the bovines (20%) was undergoing health screening. In addition, malnutrition was diagnosed in 4 bovines (10%) and 3 bovines (7.5%) was diagnosed with muscle disease. 2 of the bovines (5%) was diagnosed with Johne's disease and another 2 (5%) with parasitic gastro-enteritis. The rest were diagnosed with bloodborne disease, clinical mastitis, cutaneous myiasis, listeriosis, metritis, myositis, peritonitis, retained placenta, right abomasal displacement, salmonellosis, traumatic injury, vaginal prolapse, and uterine prolapse which contribute only 2.5% each (Table 1).

Table 7: Diagnosis group in bovine (n=40) with hypocalcaemia

Diagnosis Group	n
Blood Parasite infestation	8
Health Screening	8
Malnutrition	4
Johne's Disease	2
Muscle Disease	3
Parasitic Gastro-Enteritis	2
Bloodborne Disease	1
Clinical Mastitis	1
Lameness with cutaneous myiasis	1
Listeriosis	1
Metritis	1
Myositis	1
Peritonitis	1
Retained Placenta	1
Right abomasal displacement	1
Salmonellosis	1
Traumatic injury	1
Vaginal Prolapse	1
Uterine Prolapse	1

4.8 Feline

4.8.1 Age of Feline

Age group of hypocalcaemia feline in this study were classified into five categories; Junior, Adult, Mature, Senior, and Geriatric. The majority of animals diagnosed with hypocalcaemia were adult animals (n=13, 38%) while the rest were junior animals (n=11, 31%) and mature animals (n=6, 17%). Furthermore, there were (n=4, 11%) of senior animals and the least was geriatric animals (n=1, 3%) The mean age of the affected feline was 5.20 ± 0.640 years (range, 0.5-14.0 years) while the median was 5.0 years. There is no significant association between age and the calcium status ($P > 0.05$).

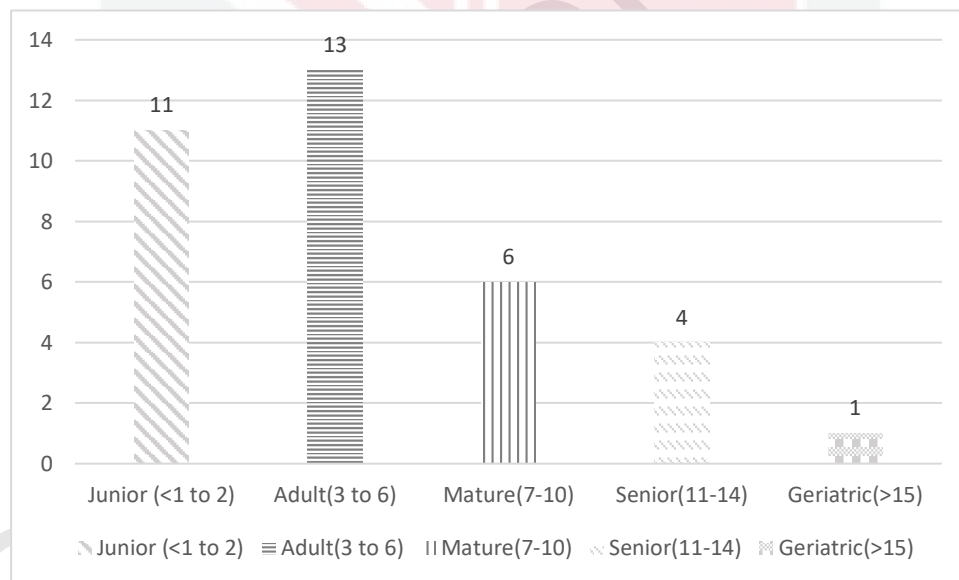


Figure 10: Age group of feline (n=35) with hypocalcaemia

4.8.2 Gender of Feline

Among the 35 feline cases associated with hypocalcaemia, there are 18 male animals (51%) and 17 female animals (49%). There is no significant association between the gender and calcium status ($P>0.05$).

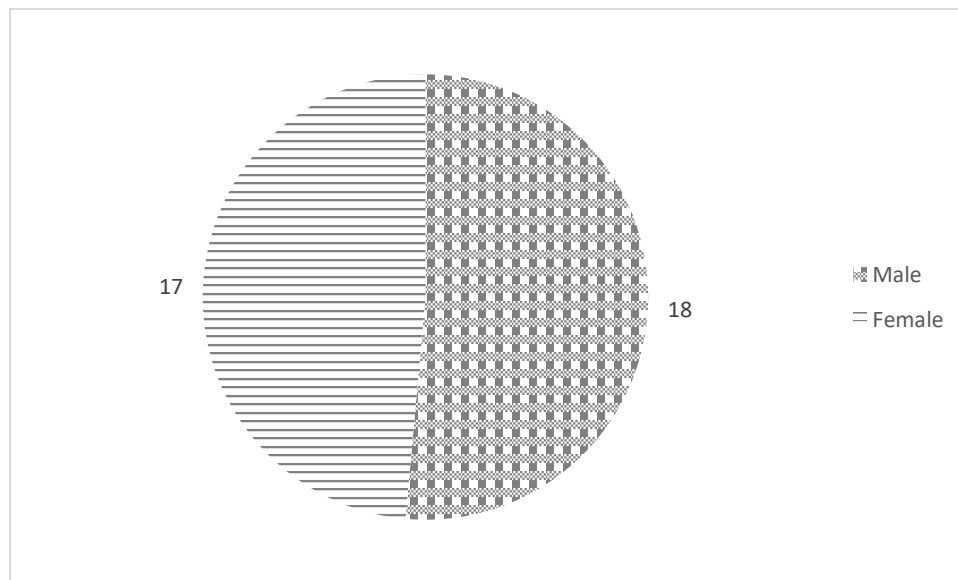


Figure 11: Gender of feline (n=35) with hypocalcaemia

4.8.3 Clinicopathological Findings

Serum biochemistry profiles inclusive of inorganic phosphate, creatinine, ALT, AP(ALP), GGT, and CK(CPK) was reviewed and evaluated. It showed that 44% of the hypocalcaemia feline had increased serum inorganic phosphate level with the mean concentration of 3.49mmol/L. In addition, 41% of the feline showed increased serum creatinine concentration with the mean concentration of 492.2 μ mol/L and only 9% had increased serum ALT level with mean concentration of 52.99U/L.

Table 8: Clinicopathological findings in feline with Hypocalcaemia

Variable	N	Mean	Median	Range	Feline Ref.Range	Compared to Reference Range

						Higher n(%)	Within n(%)	Lower n(%)
Inorganic Phosphate	27	3.497	2.710	0.970-9.600	1.1-2.8	12 (44%)	14 (52%)	1 (4%)
Creatinine	34	492.2	140.5	52.00-1796	60-193	14 (41%)	19 (56%)	1 (3%)
ALT	32	52.99	49.00	18.50-154.0	10-90	3 (9%)	29 (91%)	0
AP(ALP)	21	38.81	16.00	1.00-442.0	<80	1 (5%)	20 (95%)	0
GGT	7	2.857	3.000	2.00-3.00	<6.0	0	7 (100%)	0
CK(CPK)	2	5397	5397	101.0-10692	<300	1 (50%)	1 (50%)	0

4.8.4 Diagnosis in Feline with Hypocalcaemia

Chronic kidney disease was diagnosed in 5 felines (14%) while another 5 of the felines (14%) was diagnosed with obstructive Flutd. In addition, non-obstructive Flutd was diagnosed in 3 felines (9%) while traumatic injury, feline infectious peritonitis, and tumour was all diagnosed in 2 felines (6%) each. The rest were diagnosed with anorexia, bone deformity, cardiomegaly, circulatory disorder, eclampsia, electrolyte imbalance, bacterial infection, liver disease, poisoning, fracture, recurrent faucitis, septicaemia, severe gastritis, severe pancreatitis, and spinal injury which contribute only 3% each (Table 1).

Table 9: Diagnosis group in feline (n=35) with hypocalcaemia

Diagnosis Group	n
Chronic Kidney Disease	5
Obstructive Flutd	5
Non-Obstructive Flutd	3
Traumatic Injury	2
Feline Infectious Peritonitis	2
Tumour	2
Anorexia	1
Bone Deformity	1
Cardiomegaly, Pulmonary oedema	1
Circulatory Disorder	1
Eclampsia	1
Electrolyte Imbalance	1
Health Screening	1
Infected Right 1st to 4th Mammary Gland	1
Liver Disease	1

Poisoning	1
Fracture	1
Recurrent Fauritis	1
Septicaemia	1
Severe Gastritis	1
Severe Pancreatitis	1
Spinal Injury	1

4.9 Canine

4.9.1 Age of Canine

Age group of hypocalcaemia canine in this study were classified into five categories; Puppy, Young Adult, Mature, Senior, and Geriatric. The majority of animals diagnosed with hypocalcaemia were mature animals (n=12, 54%) while the rest were young adult animals (n=8, 33%) and senior animals (n=4, 13%). The mean age of the affected canine was 8.0 ± 0.395 years (range, 4.0-12.0 years) while the median was 8.0 years. There is no significant association between age and the calcium status ($P > 0.05$).

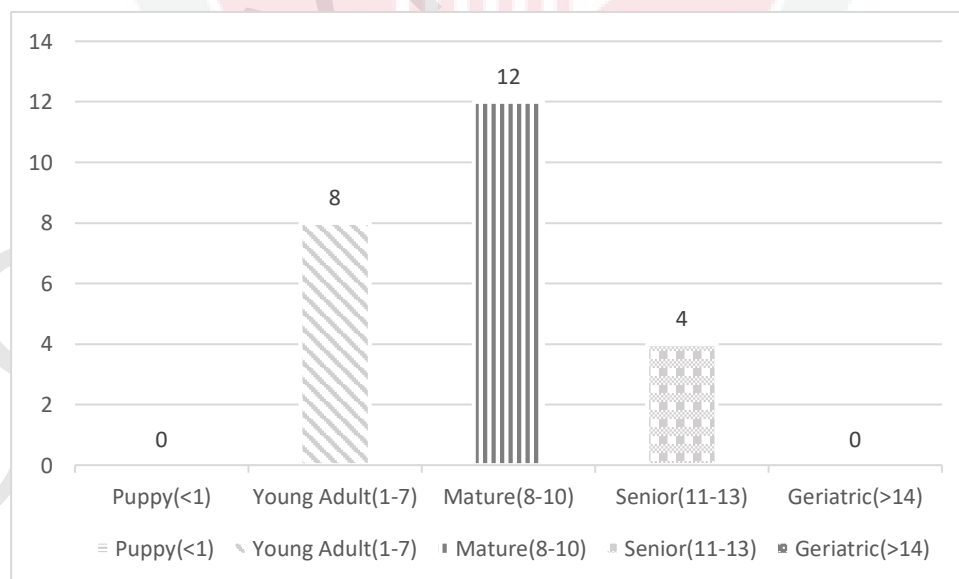


Figure 12: Age group of canine (n=24) with hypocalcaemia

4.9.2 Gender of Canine

Among the 24 canine cases associated with hypocalcaemia, there are 11 male animals (46%) and 13 female animals (54%). There is no significant association between the gender and calcium status ($P>0.05$).

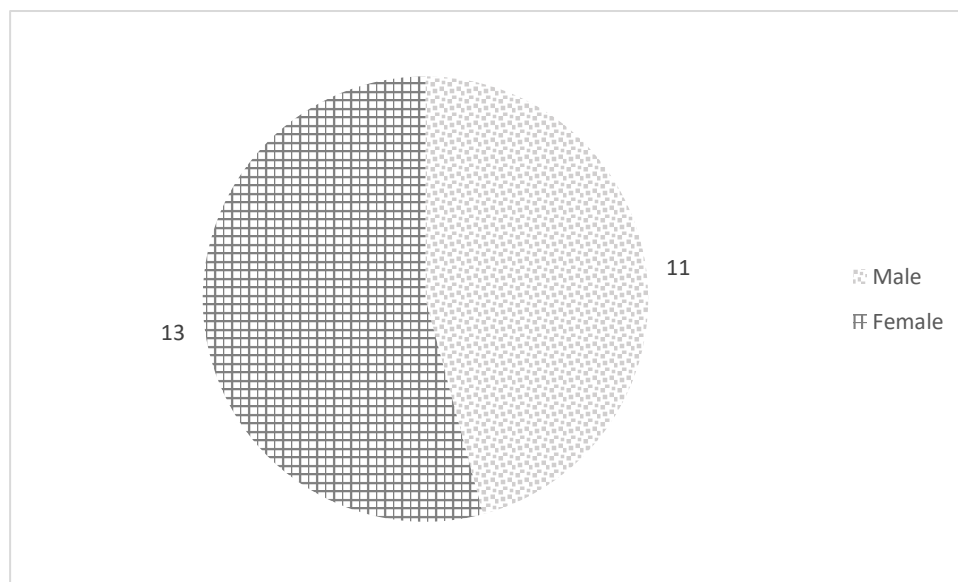


Figure 13: Gender of canine (n=24) with hypocalcaemia

4.9.3 Clinicopathological Findings

Serum biochemistry profiles inclusive of inorganic phosphate, creatinine, ALT, AP(ALP), GGT, and CK(CPK) was reviewed and evaluated. It showed that 57% of the hypocalcaemia canine had lower serum creatinine level with the mean concentration of $155.9\mu\text{mol/L}$. In addition, 18% of the canine showed increased serum ALT concentration with the mean concentration of 89.12U/L and 40% had increased serum AP(ALP) level with mean concentration of 151.8U/L .

Table 10: Clinicopathological findings in canine with Hypocalcaemia

Variable	N	Mean	Median	Range	Canine Ref.Range	Compared to Reference Range		
						Higher n(%)	Within n(%)	Lower n(%)
Inorganic Phosphate	7	1.804	1.200	0.650-5.580	0.8-2.5	1 (14%)	5 (72%)	1 (14%)
Creatinine	21	155.9	80.00	51.00-1495	88-176	2 (10%)	7 (33%)	12 (57%)
ALT	22	89.12	40.00	1.00-535.6	5-90	4 (18%)	17 (77%)	1 (5%)
AP(ALP)	20	151.8	70.50	19.00-558.0	40-100	8 (40%)	4 (20%)	8 (40%)
GGT	7	27.29	5.000	2.00-163.0	<6.0	3 (43%)	4 (57%)	0
CK(CPK)	1	346.0	346.0	346.0-346.0	<300	1 (100%)	0	0

4.9.4 Diagnosis in Canine with Hypocalcaemia

Hypocalcaemia was observed in 3 of the canines (12.5%) during health screening while another 3 canines (12.5%) was diagnosed with mammary gland tumour. In addition, protein losing enteropathy was also diagnosed in 3 other canines (12.5%) and both lymphoma and blood parasite infestation was diagnosed in 2 canines (8.3%) each. The rest were diagnosed with chronic kidney disease, electrolyte imbalance, immune-mediated hemolytic anaemia, inflammatory bowel disease, intra-abdominal mass, liver disease, lymphangiectasia, rectal leiomyoma, urolithiasis, and Wobbler Syndrome which contribute only 4.17% each (Table 1).

Table 11: Diagnosis group in canine (n=24) with hypocalcaemia

Diagnosis Group	n
Health Screening	3
Mammary Gland Tumour	3
Protein Losing Enteropathy	3
Lymphoma	2
Blood parasite infestation	2
Chronic Diarrhea	1
Chronic Kidney Disease	1
Electrolyte Imbalance	1
Immune-Mediated Hemolytic Anaemia	1
Inflammatory Bowel Disease	1
Intra-Abdominal Mass	1
Liver Disease	1
Lymphangiectasia	1

Rectal Leiomyoma	1
Urolithiasis	1
Wobbler Syndrome	1

4.10 Radiograph

3 out of 76 available radiography records of hypocalcaemia animals were showing signs of osteoporosis. A leporine was showing mild to moderately poor bone density of the spine (Figure 14) with diagnosis of metabolic bone disease and a feline was showing reduce bone density and lordosis of the spine (Figure 15) with diagnosis of bone deformity. Furthermore, a caprine was showing thinning of bone cortex with increase in radiolucency in bone medulla area of the radius bone (Figure 16) with diagnosis of Vitamin D deficiency.

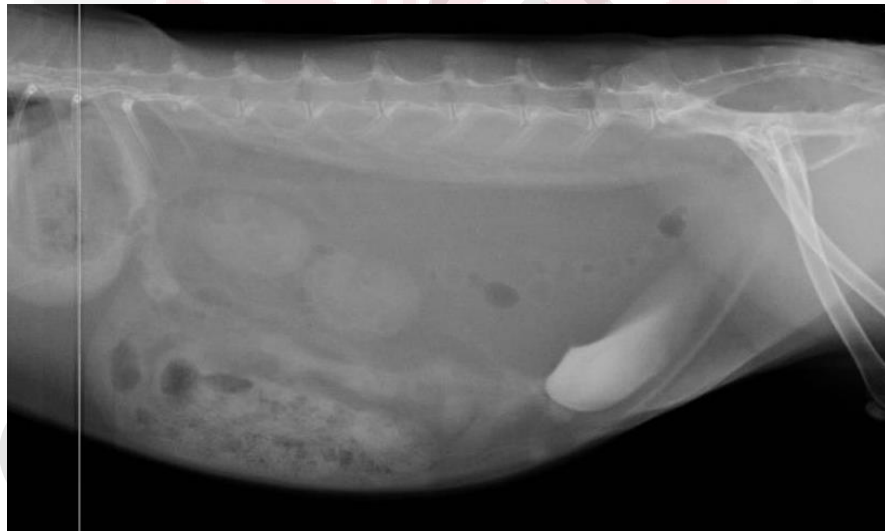


Figure 14: Mild to moderately poor bone density of spine of a leporine

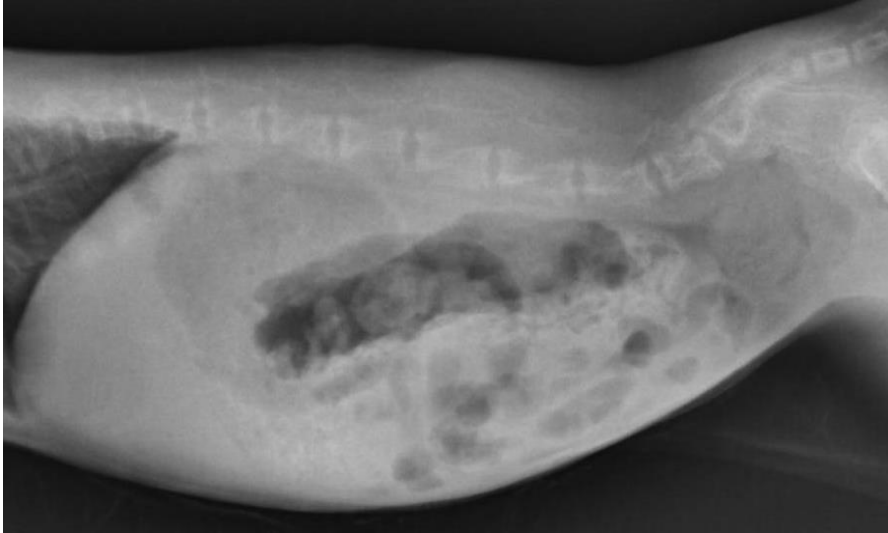


Figure 15: Reduce bone density and lordosis of the spine of a feline

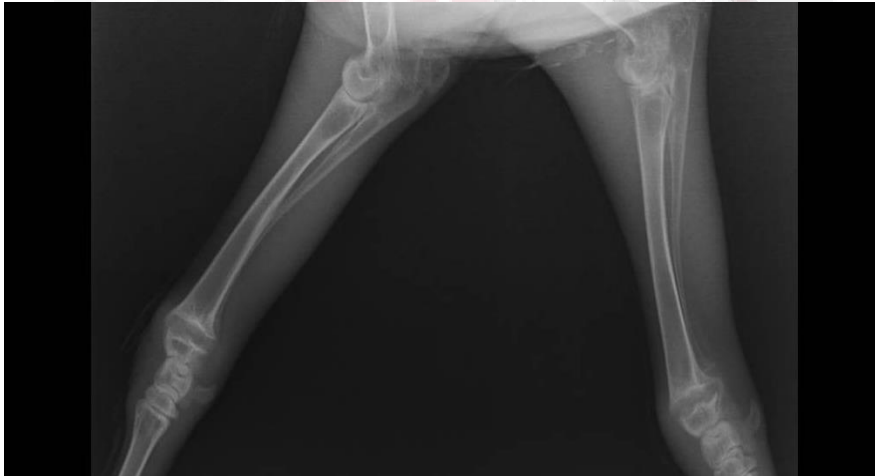


Figure 16: Thinning of bone cortex with increase in radiolucency in bone medulla area of the radius
bone of a caprine

5.0 DISCUSSIONS

The results from this study indicates that more adult (n=49) than young (n=16) caprines had hypocalcaemia. The current study supports previous findings by Quader et al. (2017) that reported older animals were found commonly affected by hypocalcaemia. This may be related to different physiologic capabilities of older and young animals to regulate calcium. The ability of absorption and mobilization of stored calcium is reduced in older animals; thus, these animals are more susceptible to the condition (Brozos, 2011).

There are more female animals (n=46) than male animal (n=19,29%). Higher number of cases in the female and older goat in the present study corresponds to the study performed by Quader (2017). Female caprines are prone to suffer from hypocalcaemia because of high requirement of calcium during certain stages of their life. Female goats are potentially vulnerable to all three manifestations of hypocalcaemia that are during late pregnancy, immediately following calving, and near the time of peak lactation (Saun, 2010).

Furthermore, for clinicopathological findings it shows that 26 of the hypocalcaemia caprine had lower serum creatinine level but the mean concentration was 176.4 μ mol/L that is above reference range while 21 caprine had increased serum CK(CPK) level with mean concentration of 546.2U/L which is above reference range. The low serum creatinine level observed in 26 caprine may result from reduced of muscle mass from malnourished individuals, or high GFR as in pregnancy as suggested by Charat et al., (2016). Low serum creatinine level also may due to typical clinical

signs showed by caprine with hypocalcaemia which are recumbency and lethargy that result in less muscle contraction and metabolism. Creatinine is a by-product of muscle contraction that is normally excreted by the kidney (Sakha et al., 2008).

High serum CK(CPK) level in 21 hypocalcaemic caprine may indicate damage to the muscle due to recumbency, muscle injury or because of catabolism of the muscle to produce energy. Remarkable changes in the CK enzymes concentration are mostly related to skeletal muscles and cardiac muscle, and the damages to them (Fakour, 2020). As most of the caprine with high serum CK(CPK) level was diagnose with pregnancy toxaemia, elevation of CK(CPK) may due to muscle damage because of recumbency which is clinical sign typically show in goat with pregnancy toxaemia or due to catabolism of the muscle to produce ATP because of the negative energy balance.

The data reported here suggest that pregnancy toxaemia is the most frequent disorder associated with hypocalcaemia in caprine. Pregnancy toxaemia, also known as “twin-lamb” disease, is a metabolic condition of pregnant small ruminants caused by an irregular metabolism of carbohydrates and fats arising at the final stage of pregnancy (Quadir et al., 2017). During the last stage of gestation, the growing fetus also require an increasing amount of calcium in the circulation for the skeletal development and since the ewes that carry twins are at the higher risk of developing pregnancy toxaemia and at the same time are in even greater need of calcium than ewes with only one fetus. Decreased concentrations of serum calcium in sheep and goats with pregnancy toxaemia have been reported in Sigurdsson (1991), Brozos et al., (2011), and Hefnawy et al. (2011), which confirms the concept of the importance of calcium in the metabolism of goats at the end of gestation and its possible involvement in pregnancy

toxaemia pathogenesis (Souto et al., 2019). Hypocalcaemia can co-exist with pregnancy toxaemia (Brozos, 2011).

The data showed that most of leporine diagnosed with hypocalcaemia were adult animals (n=31) and young animals (n=24) with the mean age of 2.9 ± 0.36 years. The age of the rabbit may not play a role in hypocalcaemia because they require high concentration of calcium in the body throughout their life due to continual tooth eruption. Most mammals make 1 or 2 sets of teeth in a lifetime, and rabbits' continual tooth eruption plays a role in their long-term calcium requirements (Redrobe, 2002).

There are more female leporine (n=37) than male leporine (n=21) reported in this study. Higher number of cases in the female rabbit in the present study corresponds to the study performed by Meredith and Benato (2017) that reported female rabbits are predisposed to hypocalcaemia especially pregnant or lactating does. Pregnancy toxemia, lactation, low dietary level of calcium and vitamin D, and unbalanced Ca:P are the major cause of hypocalcaemia in rabbits (Meredith, 2017). Two of the main causes of hypocalcaemia are only affecting female rabbits hence explain the higher number of cases of hypocalcaemia in female rabbits.

For clinicopathological findings in the leporine with hypocalcaemia, majority of the parameters were within the normal range except for CK(CPK) level. 28 of the hypocalcaemic leporine had increased in serum CK(CPK) level with the mean concentration of 1641U/L which is above the reference range. Among those leporine, 25 of them was diagnosed during regular health screening which may be incidental finding and most probably related to the diet or management of the rabbits. High serum

CK(CPK) level may indicate damage to the muscle and this may be due to nutritional muscular dystrophy which according to Peter (2016) is associated with hypovitaminosis E and is characterized by degeneration and necrosis of skeletal muscle myofibers. Deficiency of Vitamin E in the diet may cause the condition and it is related to degradation of Vitamin E content in the feed due to prolonged storage by owners or manufacturers. Diet not protected from light or temperature extremes and prolonged storage of food adversely affects the quality of vitamin E (Varga, 2018).

Hypocalcaemia was observed in 27 of the leporine (47%) during health screening which is an incidental finding that may be due to deficiency in the diet because those leporine were managed by the same owner. Rabbits were found to be selective feeders, and the most frequently rejected ingredients being pellets and whole grain (Harcourt-Brown, 1996). The owner may have provided the feed to rabbits as mixed rations or known as muesli-style mixes rather than pelleted feed. Harcourt-Brown (1996) demonstrated that rejection of the pellets and whole grain from the food will reduce a rabbit's calcium intake to below the minimum dietary requirement. According to Lowe (2011), alfalfa hay a moderate protein source rich in fiber, calcium, carotene and vitamin E is a common ingredient in rabbit diets, however in the author's experience it may be rejected if it is fed as part of a mixed ration. The fact that vitamins and minerals are added to the mix ration, which is the ingredient most frequently rejected by rabbits may be the cause of calcium and vitamin E deficiency in diet

The result from this study shows the majority of bovine diagnosed with hypocalcaemia were adult bovine (n=28) and the rest were young bovine (n=12). This

can be explained by decreasing ability of bovine to regulate calcium in response to the decreased calcium level in the blood with increasing age. Advancing age results in a decrease in the capacity to mobilise calcium from bone stores and a decrease in the active transport of calcium in the intestine, as well as a decrease in production of 1,25-Dihydroxyvitamin D₃ (Horst, 1997). The greater efficiency of calcium absorption in the young animal may be a partial reason for the low incidence of hypocalcaemia in young cattle (Boda and Cole, 1995).

Among the 40 bovine cases associated with hypocalcaemia, there are more female animals (n=29) than male animals (n=11). The cow's metabolism is often under severe stress when compared with bulls as she transitions to lactation and pregnancy specifically. Pregnancy and particularly lactation tend to lower the amount of calcium in the blood as a result of the transfer of calcium to the foetal skeleton and milk (Boda and Cole, 1995). In addition, in female animals, there are fewer osteoclasts exist to respond to parathyroid hormone due to decreased calcium level in blood, which could delay the ability of bone resorption to contribute calcium to the plasma calcium pool. Moreover, the presence of 1,25-Dihydroxyvitamin D₃ increases the maturation of monocytes to macrophages, which are thought to be direct precursors to osteoclasts, and there are published reports of a decrease in the number of monocytes at parturition in cows, which could eventually result in a decrease in osteoclasts formation (Horst, 1986).

For clinicopathological findings in the bovine with hypocalcaemia, majority of the parameters were within the normal range except for GGT and CK(CPK) concentration. It showed that 7 of the bovine showed increased serum GGT concentration with the mean concentration of 55.74U/L that is above reference range.

Liver enzymes AST and GGT are measures of liver function, and elevated enzyme activity reflects liver injury (González & Silva, 2006). This may be due to the production of Very low density lipoprotein (VLDL) in ruminants is lower when compared with other species, and low levels of VLDL during hypocalcaemia could contribute to an increase in the incidence of fatty liver. Fatty liver leads to hepatocyte degeneration and leakage of enzymes into the cytoplasm, such as aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) (Paiano et al., 2020). In addition, 3 of the bovine with increased serum GGT concentration were diagnosed with blood parasite infestation and this could explain the rise in GGT level. Infected cattle with blood parasite reported a substantial rise in AST, GGT, hypoproteinemia, hypoalbuminemia and decreased A/G ratio (Hussein et al., 2007). Alternatively, elevated liver enzymes during a blood parasite infection may result from RBC lyses or hyperbilirubinemia or liver damage and lesions caused by a parasite during blood proliferation that indirectly results in hepatic dysfunction (Nasreldin et al., 2020).

Furthermore, 15 bovine had increased CK(CPK) level with mean concentration of 1451U/L that is above reference range. Most of the bovine with increased CK(CPK) level was diagnosed with muscle disease or traumatic injury. Cattle has increased serum CK activity with a number of muscle injuries and diseases, and large animal species have also often increased serum CK activity due to extended recumbent or pressure necrosis (Hoffmann and Solter, 2008). CK activity is predominantly used in domestic species as a marker for trauma-related skeletal muscle injury, nutritional myopathy, exercise-induced muscle injury or congenital myopathy (Hoffmann and Solter, 2008). This finding is correspond to study performed by Basbugan et al., (2015) who

reported persistent rise in CK activity due to muscular myopathy that occurs in hypocalcaemia.

The data shows that blood parasite infestation (n=8) was the most commonly diagnosed in bovine with hypocalcaemia. In blood parasite infection, hypocalcaemia may be due to hypoproteinemia, decreased food intake, intestinal malfunction and kidney damage, and decreased phosphorus levels due to diarrhoea and renal failure (Khan et al., 2010). Moreover blood parasite infection could cause a decrease in serum albumin and total protein level which could lead to a decrease in serum total calcium concentration. Concentrations of serum total protein, albumin and globulin in infected cattle have decreased in the current study (Khan et al., 2010). Alterations in protein (mostly albumin) concentrations affect the total calcium value as the proportion of serum calcium bound to protein, hence the total calcium (tCa) concentration may be falsely low following low albumin (Alb) concentrations (Sharp et al., 2009).

The majority of feline diagnosed with hypocalcaemia were adult animals (n=13) and junior animals (n=11) with the mean age of the affected feline was 5.20 ± 0.640 years (range, 0.5-14.0 years). Age does not play a major role in the development of hypocalcaemia in feline and this can be shown by the wide range of age of feline affected with hypocalcaemia. In fact, commercial diet available in the stores may provide adequate calcium content for all age groups. For both calcium and phosphorus, the concentration examined also exceeded the minimum concentrations declared on the food label claim when translated to a calorie basis (Summers et al., 2019). Apart from that, the underlying diseases that play a major role in the development of hypocalcaemia in feline that are going to be discussed in the diagnosis group.

In addition, it showed that 12 of the hypocalcaemia feline had increased serum inorganic phosphate level with the mean concentration of 3.49mmol/L that is above the reference range. 8 of the feline with increased serum inorganic phosphate level were diagnosed with renal disease. Since the kidney are the primary route of phosphorus excretion, decreasing renal function results in the accumulation of phosphorus and its implications (Polzin, 2011). Hyperphosphatemia may occur secondary to decreased in glomerular filtration rate in kidney disease, which in turn may result in hypocalcemia due to increased phosphate binding (Coady et al., 2019).

Then, 14 of the feline showed increased serum creatinine concentration with the mean concentration of 492.2 μ mol/L that is above the reference range. Creatinine is created as a waste product of muscle metabolism and excreted through the kidney. A significant decreased in GFR will cause creatinine retention, but non-renal factor, especially dehydration and changes in muscle mass will also have an effect on serum creatinine (Cannon, 2016). 7 of the feline with increased serum creatinine level were diagnosed with feline lower urinary tract disease and another 3 with chronic kidney disease. Consistently elevated serum creatinine concentrations likely reflected underlying renal insufficiency, whereas in others, high creatinine concentrations may have been due to chronic ureterolithiasis (Horowitz et al., 2013).

The study shows that chronic kidney disease (n=5) and obstructive feline lower urinary tract disease (n=5) were the most commonly diagnosed disease in feline with hypocalcaemia. Current study corresponds to previous study by Coady et al., (2019) who reported kidney injury was the most common pathologic cause of hypocalcaemia

in feline, accounting for 21% of cases, of which 62% were diagnosed as chronic kidney disease and 38% were diagnosed as acute kidney disease. Coady et al., (2019) also reported that urethral obstruction accounted for 15.1% of cases.

Hypocalcaemia in patients with renal disease is due to an abrupt decrease in the glomerular filtration rate, resulting in a rapid rise in serum phosphorus concentration resulting in reciprocal secondary hypocalcaemia (Holowaychuk, 2013). Animals with chronic kidney disease reduced circulating concentrations of 1,25-Dihydroxyvitamin D₃ due to decreased renal synthesis caused by the inhibitory effect of hyperphosphatemia on 25-hydroxyvitamin D 1- α -hydroxylase and progressive loss of renal epithelial cells which will lead to hypocalcaemia (Parker et al., 2015). Hypocalcaemia in urethral obstruction can be due to phosphate retention secondary to obstruction, PTH resistance, or acid-base modification (Coady et al., 2019).

The majority of canine diagnosed with hypocalcaemia were mature animals (n=12) while the rest were young adult animals (n=8) and senior animals (n=4). The mean age of the affected canine was 8.0 ± 0.395 years (range, 4.0-12.0 years). Most of the canine affected are of adult canine as adult dogs require more calcium respectively, due to the fact that older dogs are unable to up-regulate calcium absorption efficiently in case of reduce calcium concentration in blood. In the period after weaning until 1 year of age, the impact of passive absorption to total absorption of calcium reduces and the active absorption of 1,25-Dihydroxyvitamin D₃-dependent calcium increases (Tryfonidou et al., 2002). It has been shown that calcium is absorbed more efficiently and preserved in young growing dogs than in their older counterparts (Tryfonidou et al., 2002).

For clinicopathological findings in the canine with hypocalcaemia, it showed that 12 of the hypocalcaemia canine had lower serum creatinine level with the mean concentration of $155.9\mu\text{mol/L}$ that is within the reference range. A lot of factors could contribute to the low creatinine level in the blood. Factors such as age and breed have an impact on plasma creatinine concentration. Creatinine trends lower in small breeds of dogs, based on body weight (Misbach et al., 2014). Young dogs have lower creatinine level than adult dogs, possibly due to lower in muscle mass (Rosset et al., 2012, Rørtveit et al., 2015). Creatinine was found to be reduced in some cachectic dogs and glucocorticoids may also cause a mild decrease of creatinine in normal dogs (Braun, Lefebvre, & Watson, 2003).

In addition, 8 canine had increased serum AP(ALP) level with mean concentration of 151.8U/L that is above the reference range. Alkaline phosphatase (ALP) is mainly an indication of cholestatic liver disease and it also increases with extreme bone loss or due to steroid induction (Latimer et al., 2003). Canine with increased serum ALP level in this study were diagnosed with diverse diseases but it is believed that increased of ALP in the blood is due to hepatic injury or bone turnover due to hypocalcaemia. Changes in the serum ALP level are mainly due to hepatic (L-ALP) and bone (B-ALP) isoenzymes, since intestinal, renal, and placental isoenzymes have extremely short half-lives (Kinoshita et al., 2016).

Furthermore, both mammary gland tumour and protein-losing enteropathy were diagnosed in 3 canine with hypocalcaemia each which were the most common diagnosed disease in canine with hypocalcaemia in this study. Mammary gland tumour

usually will cause increase in blood calcium level in the dog which is contraindicated with this study. Neoplasia is the most common cause of hypercalcaemia in dogs (Raposo-Ferreira et al., 2016). The most common cancer associated with high calcium and low phosphorus is the cancer of breast (Nathaniel et al., 2010). Hypercalcaemia and hyperphosphatemia seen in mammary tumour patients can be due to bone osteolysis enhancement and reabsorption and excretion of calcium and phosphate ions (Dash & Singh, 2018).

Hypocalcaemia is a common finding in dogs with protein-losing enteropathy due to several mechanisms discovered. Protein-losing enteropathy (PLE) is a condition associated with excessive loss of albumin by gastrointestinal mucosa (Dossin & Lavoué, 2011). Decreased serum total calcium concentration is a typical finding in dogs with protein-losing enteropathy (PLE) and is typically due to hypoalbuminemia and decreased protein-bound, inactive fraction of serum calcium (Kimmel, Waddell, & Michel, 2000). Hypocalcaemia in canine PLE is possibly due to a combination of low intestinal absorption and increased calcium and magnesium leakage in the GI lumen, insufficient PTH secretion, calcitriol deficiency due to decreased intestinal absorption of lipid-soluble vitamins (A, D, E and K) and decreased 1 α -hydroxylation by the kidney (Dossin & Lavoué, 2011).

Finally, 4 out of 76 available radiography records of hypocalcaemia animals were showing signs of osteoporosis which consist of 2 caprine, 1 leporine and 1 feline species. Osteoporosis is a lesion, not a particular disorder, which occurs when bone resorption chronically exceeds bone formation. Osteoporosis can be caused by a number of factors that either increase bone resorption and decrease bone formation

(Uhl, 2018). Osteoporosis can be diagnosed with radiography imaging which showing common features of osteoporosis. In general, the typical characteristics of osteoporosis on radiography include thinned cortices, endosteal reabsorption and a reduction in the amount of trabeculae (Patel et al., 2015). With radiography imaging, the changes of the bone are most prominent in the axial skeleton, ends of long bones and trabecular bones of the juxta-articular appendicular skeleton (Patel et al., 2015).

Chronic hypocalcaemia is the major cause of osteoporosis in animals with various of underlying factors. One of the factors may be calcium deficient diet which could cause significant reduction of calcium level in the blood. The low-calcium diet induced a reduction of serum calcium level, which stimulated the synthesis of hormones by parathyroid glands that in turn increased bone resorption, leading to osteoporosis (Jowsey & Raisz, 1968). Both cortical thickness and trabecular bone volume were decreased in animals fed with calcium-deficient diet and bone resorption was increased as demonstrated by an increased number of osteoclasts in metaphyseal spongiosa (Weinreb et al., 1991).

Furthermore, insufficiency of Vitamin D will also lead to osteoporosis. Vitamin D deficiency will causes prolonged secondary hyperparathyroidism, which can lead to reduced bone density and thinning of cortical bone arising from a net loss of cortical bone on the endosteal surface (Veldurthyn et al., 1991). Hypovitaminosis D has detrimental effects on calcium metabolism, osteoblastic activity, matrix ossification, bone remodelling and bone density (Christodoulou et al., 2013).



6.0 CONCLUSION

From this retrospective study, we can conclude that the prevalence of hypocalcaemia is reported to be 31% in leporine, 25% in caprine, 22% in bovine, 6% in feline and 4% in canine in UVH,UPM. There is no significant association can be found between age and gender to the calcium status of all species. Furthermore, the main diagnosis of hypocalcemia in each species are different from each other. The

main diagnosis of hypocalcemia in caprine was pregnancy toxemia, majority of hypocalcemic leporine were diagnose during health screening and blood parasite infestation was most commonly diagnosed in bovine with hypocalcemia. Finally, the main diagnosis of feline with hypocalcemia was chronic kidney disease and protein losing enteropathy was the most commonly diagnosed in canine with hypocalcemia.



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