EVALUATION OF DENTAL FLUOROSIS AND FLUORIDE CONCENTRATION IN RUMINANT FEEDS, TISSUES AND PRODUCTS IN NAKURU COUNTY, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN ANIMAL PRODUCTION OF UNIVERSITY OF ELDORET

NOVEMBER, 2019

DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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DEDICATION

This work is dedicated to my wife, Violet, who constantly encouraged and supported me, my son, Jeremy and my daughters, Gloria and Sheryl, all who endured with patience, my constant absence and prayed for my success. They will remain my heroes. To my parents, the late Mr. Benard Asembo and Mrs. Julian Asembo for educating me despite the limited resources at their disposal.

ABSTRACT

The adverse effects linked to fluorosis in both human and livestock is irreversible. This problem has elicited global reactions and actions among the public health professionals. Multiple studies have provided evidence that fluorosis disrupts both teeth enamel and skeletal formation, others have associated it with reproductive defects in livestock. In Kenya, there is still scanty information regarding effects of fluorosis in livestock production and productivity. The present study was designed to assess the prevalence of dental fluorosis in livestock and map out the severity of teeth mottling, assess the fluoride concentration in livestock feeds sources, tissues, faeces and milk in Nakuru County. A cross-sectional study involving on–site epidemiological clinical examination of the Cattle and Sheep for dental fluorosis was conducted in Gilgil, Njoro, Egerton, Naivasha and Nakuru areas of the Nakuru County. Grading was done according to Dean Index of Classification. In this method, the defects were classified as normal (grade 0.0), questionable (grade 0.5), very mild (grade 1.0), mild (grade 2.0), moderate (grade 3.0) and severe (grade 4.0). A total of 549 livestock were sampled (242 Cattle and 307 Sheep). The study was based on Randomized Block Design. This was followed by collection of samples of feeds, drinking water, hooves, faecal and milk for estimation of fluoride levels. The sample were then prepared and analysed following standard laboratoty procedures. The estimation of fluoride concentration was determined using Ion Selective Electrode. The data was statistically analyzed using SPSS, version 23 to determine the prevalence rate, mean and standard deviation. The results were used to compare the percentage dental fluorosis between the regions, livestock species, breeds and different age cohorts. Fluoride concentration in tissues, water, feeds, products and faecal samples were used to determine the main sources of fluoride exposure to the livestock in the County. The results showed variations in dental fluorosis affecting livestock between regions. This confirmed presence of significant levels of fluoride that do affect animal dentition. The study findings showed that 45% of livestock had mild cases of dental fluorosis, followed by 31% of very mild cases and 14% questionable cases. Moderate and severe cases were found at 10%.

The mean fluoride concentration of drinking water from the five regions; Egerton, Gilgil, Naivasha, Nakuru and Njoro were 2.75 mg/l ± 0.064, 0.36 mg/l ± 0.259, 5.25 mg/l ± 1.36, 2.27 mg/l ± 0.24 and 0.25 mg/l ± 0.010 respectively. In feeds, it was 21.60 mg/kg ± 0.007, 26.88 mg/kg ± 0.004, 21.84 mg/kg ± 0.002, 22.70 mg/kg ± 0.009 and 23.12 mg/kg ± 0.001. In milk it was 0.081 mg/l ± 0.004, 0.079 mg/l ± 0.006, 0.086 mg/l ± 0.012, 0.147 mg/l ± 0.09 and 0.107 mg/l ± 0.40. In hooves, 13.12 mg/kg ± 0.15, 16.06 mg/kg ± 0.16, 11.74 mg/kg ± 0.26, 15.45 mg/kg ± 0.11, and 10.10 mg/kg ± 0.18. In faecal samples it was 17.78 mg/kg ± 3.523, 14.06 mg/kg ± 3.152, 18.58 mg/kg ± 7.244, 15.72 mg/kg ± 6.107, 18.38 mg/kg ± 6.007 respectively. There was significant difference ($p>0.05$) in fluoride concentration between milk and drinking water among the five regions. However, there was no statistical significant difference ($p<0.05$) in feeds, hooves and faecal samples. It was further established that most animals were still at early stages and are likely to progress to higher scales of dental fluorosis. Nonetheless, it is desirable to maintain surveillance on the possible sources of fluoride toxicity in ruminants so as to devise mitigation measures that will reduce dental fluorosis in ruminants. Average fluoride concentration in water was 2.75mg/l, in feeds was 23.25mg/kg and in milk was 0.1mg/l hence the ingestion of water, feeds and milk were the main contributors to fluorosis in livestock.

Key words: Dental fluorosis, Drinking water, Milk, Faeces, Hooves, Feeds,

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LIST OF ABBREVIATIONS AAND ACRONYMS

ISE	Ion Selective Electrode
WHO	World Health Organization of the United Nations
TISAB	Total ionic Strength Adjustment Buffer
ANOVA	Analysis of Variance
HMSO	Hexamethyldisiloxane
F	Fluoride
DF	Dental Fluorosis
GDP	Gross Domestic Product.
IGAD	Inter Governmental Agency for Development.
ICPALD	International Center for Pastoral Areas and Livestock Development
RVIL	Regional Veterinary Investigation Laboratory
Govt.	Government
PPM	Parts Per Million
PPB	Parts Per Billion

ACKNOWLEDGEMENTS

I sincerely wish to thank the Almighty God for good health and wisdom during the study. Successful completion of this thesis has been realized through valuable help from many people who tolerated my many questions and inquiries seeking answers that resulted in writing of this thesis. I humbly express my sincere gratitude to livestock farmers and extension officers from County Government of Nakuru, Regional Veterinary Investigation Laboratory (RVIL), Nakuru, University of Eldoret and Flowered Project who made this study possible. I thank the Department of Animal Science and Management and of the Chemistry and Biochemistry, both of the University of Eldoret, for allowing me unlimited access to their research facilities. I thank the technical staff in the two departments for the enabling environment they provided during my research work. Special thanks go to Dr. Paul Odhiambo of RVIL Nakuru for his kind support and encouragement during the period of data collection. Sincere gratitude also goes to Gem Sub County (Siaya County) administrative staff for their moral support throughout the course of this work. Gratitude is extended to farmers that provided valuable information, accepted their animals to be handled and even allowing taking of research samples that formed the basis for the data for this study. Special thanks go to my family members for their patience, prayers and encouragement.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Fluoride toxicity is one of the critical issues that adversely affect livestock industry and human health globally (Borgnino *et al.,* 2013; Samal *et al.,* 2016; Roy *et al.,* 2018). Even though optimal fluoride levels in the diet are vital for development of healthy bone and teeth, excessive exposure of livestock to fluoride results in developmental defects in both teeth and skeletal tissues (Sharma *et al.,* 2013). The excessive fluoride exposure gives rise to an irreversible teeth disorder known as dental fluorosis; a condition characterized by teeth staining and mottling at formative stages (Kanduti *et al.,* 2016). Animals exposed to elevated fluoride levels suffer dental disfigurement linked to poor assimilation of calcium leading to incomplete development of teeth enamel and excessive pitting and wear of erupted teeth (Choubisa, 2015). In addition, the consequences of over-exposure to fluoride are also magnified in skeleton tissue formations (Ulemale *et al.,* 2010). They manifest principally through hardening and elevation of bone density, thinning and reduction of bone mass and softening of bones through demineralization. The result is bone outgrowths around damaged joints and abnormal thickening of bone tissues, a condition known as skeletal fluorosis (Sharma *et al.,* 2013). In some cases, fluoride overexposure has been linked to functional disruption of thyroid glands and to brain and blood sugar regulations (Panda *et al.,* 2015). Livestock inflicted with this corrosive agent have difficulties in feeding and locomotion, which inevitably affects their growth, reproduction and productivity cycles (Roy *et al.,* 2018). The adverse ripple effects are felt almost immediately amongst the dairy sectors (Ulemale *et al.,* 2010) where milk is produced and processed for human consumption.

Third world countries like Kenya whose economies and food security lean heavily on primary livestock production (Herrero *et al.,* 2013; Njarui, 2016) face the greater challenges

According to (Ranjan and Ranjan, 2015a), fluorides ingested by livestock through their diets get excreted primarily through sweat, urine and faeces. Substantial amounts undergo deposition in eggs and milk, while certain amounts are retained in the body through absorption into vital body organs.

Nakuru County in Kenya is one of the fluoride deposit areas along the Eastern Rift Valley. It has fluoridated natural soils, geological rock types and waters (Wambu and Muthakia, 2011). The usual pathways of livestock and human exposure to excessive fluoride from the environment include geological degradation of fluoride bearing rocks, fluoride solubilization into soil water (Ranjan and Ranjan, 2015b), assimilation into agricultural food samples and seepage into drinking water (Parlikar and Mokashi, 2013). This shows that the unabated consumption of contaminated animal and crop products could exponentially increase public health risks in catastrophic proportions.

Some countries have tried to engage in community awareness campaigns, enacting policy measure to regulate environmental pollution from industries (Ranjan and Ranjan, 2015b) and use of organic manure in agricultural farms. Various researchers have also recommended constant monitoring of fluoride level in water sources and utilizing treated water for agricultural and domestic consumption (Jacintha *et al.,* 2016), alongside health risk assessments (Erdal and Buchanan, 2005). Nonetheless, there is an urgent need to relook at the fluoride problem with a view to devising new and more efficient strategies since these previous initiatives have not yielded results in desired proportions. In Kenya, for instance, more concern has been on water directly

consumed by humans. Possibilities of the role played by fluoride enriched livestock tissues and products along the food chain have received very limited attention.

This study was designed to assess the fluoride concentration levels of fluoride livestock feeds, in tissues, in products and in faeces and to assess the extent of its impact on livestock dentition reared in Nakuru County of Kenya. The methodology employed involved on farm-epidemiological survey to evaluate the prevalence of dental fluorosis in Cattle and Sheep of different ages, breeds, sex and weight reared in Nakuru County. The Dean, (1942) was used as template to compare and score the degree of teeth staining and mottling. Simultaneously, samples of livestock feeds, products, tissues and faeces were obtained for laboratory analysis to determine the fluoride concentration levels.

The results obtained depicted widespread dental fluorosis among ruminant livestock in Nakuru County. It was clear that fluoride-contaminated water remains the major source of fluoride ingestion by farm animals in these areas. It is hoped that these findings significantly contribute to the current understanding of the fluoride problem in this areas and form a basis for designing intervention strategies to mitigate human risks and reduce the disease burden linked to fluorosis among the affected communities and livestock.

1.2 Statement of the problem

Nakuru County has been classified as a high fluoride region. Natural geographical and climatic factors that contribute to the occurrence and distribution of excessive fluorides in these areas have extensively been discussed in literature (Kahama *et al*., 1997). Soils, water bodies and vegetation associated within Nakuru region habour high fluorides levels. The livelihoods supported by these natural resources are

therefore constantly faced with imminent threats of fluoride toxicity and the underlying consequences.

Fluoride toxicity poses a significant threat to livestock population in Nakuru County. Many challenges concerned with breeding and development defects can be traced to fluoride toxicity (Kanduti *et al.*, 2016). Some of the notable destructive effects include memory loss, teeth mottling and wearing off, impaired immunity and stillbirths. Others include male sterility, enteritis and hormonal imbalance. In chronic cases, animals suffer depression and imminent death is inevitable (Johansen, 2013).

Not much work has been done to try to explain fluoride toxicity and its effects in livestock in Kenya. The information generated will assist in developing mitigation measures and to create community awareness to both livestock keepers and general population.

1.3 Justification of the study

Nakuru County is one of the major livestock rearing zones in Kenya (Nakuru County, First County Integrated Development Plan 2013 – 2017). The approximate total number of domestic animals is about 1.7 million (KNBS, 2013). These comprise of Cattle, Sheep, goats, pigs, indigenous chicken and commercial poultry. The contribution of livestock sector to the County economy cannot therefore be underestimated. However, with emerging diseases emanating from fluoride toxicity, there is grave concern and cause to worry for both farmers and the government on the future of livestock industry in the region. It is feared that the recent climatic changes could even aggravate fluoride toxicity and the associated diseases in the area and adversely impact on livestock production. Apparently, no studies have specifically reported prevalence of fluorosis in livestock from these areas which further impede any directed intervention from any quarter.

Residents of the Kenyan Rift Valley are increasingly being troubled with fluoride toxicity arising from high levels of fluoride in drinking water and food sources. Their livestock are equally exposed to this high fluoride levels. These are likely to pose grave health debate and major drawbacks to livestock development initiatives.

High fluoride levels tend to impact negatively on productivity and reproduction of farm animals which is likely to affect food and nutrition security and livelihood of communities residing in Nakuru County. And hence the need to do a detailed study of fluoride levels in the feeds, farm water and milk, and its effects of farm animals.

Public health professionals need a good understanding of the fluoride situation in the study area in relation to livestock and to create awareness for the communities residing in the study area about the greater threats involve in consuming livestock and related products from these fluoridated areas.

1.4 Objectives of the study

1.4.1 Broad objective

To evaluate the occurrence of fluoride in feeds, water and milk and prevalence of dental fluorosis in ruminants in Nakuru County.

1.4.2 Specific objectives

The specific objectives were:

i. To evaluate the prevalence of the dental fluorosis among ruminant farm animals in Nakuru County.
ii. To determine the fluoride concentration in the livestock feeds (Boma Rhodes hay, indigenous grass, maize silage, Napier, Desmodium, Lucern, Water) and faeces in Nakuru County.
iii. To determine the fluoride concentration in hooves and milk.

1.5 Hypothesis

i) Ho: There are no domestic ruminant livestock suffering from dental fluorosis in Nakuru County.

ii) Ho: There is low high fluoride concentration in water, feeds and faeces of Cattle and Sheep.

iii) Ho: There is low high fluoride concentration in hooves and milk.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

Fluorine is a common element that does not occur in the elemental state in nature because of its high reactivity (Haritash *et al.,* 2018). It accounts for about 0.3 g/kg of the Earth's crust and exists in the form of fluoride compounds in a number of minerals (Weinstein and Davison, 2003). Fluoride occurs naturally in soils, geological rock types and waters, where natural sources are released by rock weathering processes, and it is elevated in areas of volcanic eruptions (Flueck, 2016). However, most inorganic fluoride compounds pollute the environment as a result of human activities such as during aluminum manufacturing; production and use of phosphate fertilizers (Choubisa, 2017), manufacture of glass, cement, bricks and tiles. Hydrogen fluoride (HF) alkylations in petroleum refining and in ceramic industry (Cronin et al., 2000; Ghosh et al., 2013) are also contributors to the environmental pollution. Most of the fluoride occurs in high concentrations in drinking water, which currently remains a serious problem.

In Nakuru County for example, the available water sources contain fluoride of concentration ranging from 1.0 to as high as 30 mg/L (Wambu and Muthakia, 2011). Therefore the vegetation present in these areas is likely to have high fluoride concentration. While water points get the contaminant through mineral rock solubilization, plants acquire it from soils (Cronin *et al.,* 2000; Brindha and Elango, 2011)) as well as dust fluorides blown from industrial waste (Panchal and Sheikh, 2017). Animals kept in these areas are undoubtedly candidates for fluoride poisoning (Parlikar *et al.*, 2013).

Once in the environment at sufficiently high concentration, animals acquire these compounds through food and water during grazing and water consumption (McLaughlin *et al.*, 2001; Weinstein and Davison, 2003). The amount of fluoride absorbed by the grazing animal is influenced by the solubility of the ingested fluorides, the pH in the digestive system and the presence of substances in the diet that can complex fluoride (Buzalaf *et al.*, 2015; Ranjan and Ranjan, 2015a). Since fluoride is a mineralized tissue seeker, approximately 99% of the fluoride that is retained in the body is found in bones and the dental hard tissues (de Menezes *et al.*, 2003) and are largely incorporated in actively mineralizing tissues such as bones and teeth inform of calcium hydro-sulphate crystals. Persistent exposure to high fluoride levels causes health complications in domestic animals in the form of chronic fluoride toxicity (Livesey and Payne, 2011; Flueck and Smith-Flueck, 2013).

Fluorine is a double edged element. Adequate fluoride consumption is a vital component required for proper teeth enamel formation. Additionally, due to its bone mineralization activity, fluoride has been utilized in therapeutic treatment of bone and joints (Komsa *et al.*, 2016). On the other hand, fluoride over exposure disorients normal growth and development of teeth and skeletal structure resulting in dental and skeletal fluorosis (Erdal and Buchanan, 2005). Livestock attacked with this disease suffer permanent life disorder in the affected tissues (Choubisa *et al*., 2012). While acceptable fluoride concentration levels is 1.5 mg/L in water (WHO, 2006), other studies point to the fact that progressive intake overtime can as well lead to dental mottling and staining (Sharma *et al.*, 2013). Apart from natural drinking water, other secondary sources of fluoride may include agricultural crops, fruits and animal products that can either obtain fluoride from soil absorption and ingestion of fluoride contaminated feeds respectively (Viswanathan *et al.*, 2010). Other important sources

may also include fruit drinks, tea and other beverages made from fluoride enriched water (Malinowska *et al.,* 2008). Studies conducted in African countries such as Nigeria and Tanzania (Helderman *et al.,* 1997) found out large number of citizens suffering from dental fluorosis associated with consumption of contaminated drinking water and tea (Awadia *et al.,* 2000).

Incidences of dental fluorosis have been reported in flocks and herds grazing in many parts of the world since early times (Shortt *et al.,* 1937). Dental fluorosis in livestock may build up through numerous channels. Such pathways may include intake of mineral supplements containing fluoride overtime. Such minerals include rock phosphate, and phosphatic limestone, which contain fluoride in proportion to the amount of phosphorus present. The fluoride content of some defluorinated rock phosphates commonly used in mineral supplements sometimes may constitute a considerable portion of the total fluorine ingested (Ranjan and Ranjan, 2015b). Fluorosis has been reported in grazing animals feeding from mineral supplements containing excessive amounts of fluoride; from drinking fluoride-contaminated water (Schmidt and Rand, 1952) and in animals grazing on phosphatic limestone soils, especially where the phosphatic rock appears near surface levels (Phillips, 1952). The problem affect many countries in Asia (Maiti *et al.,* 2003), Africa (Kloos and Haimanot, 1999), South America (Flueck and Smith-Flueck, 2013), Europe (Oruc, 2008) and Oceania (Death *et al.,* 2015). In affected Cattle, Sheep and goats, chronic fluorosis can be diagonized through intermittent lameness, stiffness and lesions of the bones and teeth (Choubisa, 2015). Animals normally ingest small amounts of various fluorides in their diets with no harmful effects, but excessive intake can be damaging. Several studies have placed domestic animal such as Cattles, Sheep and goats (Ulemale *et al.,* 2010) as highly sensitive to effects of fluoride toxicity.

Physiologically, fluoride is essentially desired for proper enzymatic body activities, animal growth and healthy bone development (Samal *et al.,* 2016), but excessive concentrations intake is hazardous. Once ingested, fluoride diffuses across the cell membranes and gets deposited in various body parts including the kidney, liver, skeletal and cardiac muscles (Cinar *et al.,* 2005). The excessive intake of fluoride causes injuries to these vital body organs (Hong *et al.,* 2016). Furthermore, toxic fluoride is associated with functional disruption of the thyroid glands (Dhurvey *et al.,* 2017), the brain, blood sugar regulations and animal fertility (, Basha *et al.,* 2011; Pereira *et al.,* 2011; Panda *et al.,* 2015).

Fluoride tolerance differs from one animal species to the other depending on such factors as: age, species, weight, concentration levels in feeds and exposure frequency. This means that setting tolerance limit in livestock is a major challenge. Previous study placed the bio-safe levels of fluoride in *Bos taurus*, *Ovis aries*, *Capra hircus*, *Equus caballus* and *Camellus dromedarius* to be up to 1 ppm fluoride concentration in drinking water (Choubisa, 2012). Therefore, livestock consuming water of high fluoride concentration above 1ppm could develop osteo-dental fluorosis overtime (Pruss-Ustun and WHO, 2008).

2.2 Dental fluorosis

The chemical characteristics of fluorine enable it to have a high affinity for calcium in the calcified tissues (Ganta *et al,* 2015). This is why fluoride is mostly prevalent in bones and teeth. It is believed that fluoride absorption in the teeth and the skeletal structure insulates the body from toxic fluoride circulation (Neuhold and Sigler, 1960). The effects of fluoride overexposure during dental formation are appalling and detrimental. The side effects arise overtime due to cumulative and duration exposure from several sources. The degeneration of teeth begins by becoming chalky and

opaque because of subsurface hypo-mineralization. The lose enamel then develop pits and grooves on the tooth surface. Eventually, dental fluorosis develops as a result of persistence, fluoride-induced circumstance, where the development of enamel is disrupted and hypo-mineralized (Grynpas, 1990). Therefore, an indication that excess fluoride ingestion results in dental fluorosis is evidently documented (Ulemale *et al*., 2010). Nonetheless, other studies reported that substantial amounts of fluoride consumption need to take place at formative periods of tooth to cause significant flouride trouble to ameloblasts activity (Susheela, 2001) during the secretion or period of early maturation of enamel in the domestic animals (Yan, Q. *et al.,* 2007). However, this information is derived from human dental fluorosis studies. Additional evidence could probably be required in livestock studies.

Global attention in fluorosis has been stimulated by the recognition that certain functional disabilities suffered by livestock and human are due to ingestion of excessive amounts of fluoride (Roy *et al.,* 2018). Fluorosis is endemic in at least 25 countries around the world, and is most prevalent in India, China, and parts of Africa. It is not known how many people are currently afflicted with the disease, but conservative estimates are in the tens of millions of people (WHO, 2004). Occurrences of chronic fluoride intoxication have been described in flocks and herds grazing in many parts of the world (Ulemale *et al.,* 2010). For instance, animals grazing in the vicinity of processing operations such as superphosphate plants, aluminum plants, brick kilns and steel production centers were diagonized with symptoms of fluoride toxicity (Choubisa, 1999). Fluorosis is highly significant since it often diminishes the mobility of animals at a very early age by producing varying changes in the bones such as exostosis, osteosclerosis, osteoporosis, osteophytosis etc (Choubisa, 2007). Besides these osteal abnormalities, nonskeletal changes or fluorosis

due to over exposure of fluoride have also been observed in the form of gastrointestinal disturbances, neurological disorders, reproductive dysfunctions, apoptosis, excitotoxicity, genotoxicosis, and teratogenic effects in domestic animals (Choubisa, 2012).

Currently, there is no clear information on the levels of fluoride toxicity that could cause dental fluorosis in each animal (Sohn *et al.,* 2009). Fluoride susceptibility vary from one animal to the other depending on species, feeds sources, drinking water sources, chemical form of the ingested fluoride and age (Modasiya *et al*., 2014; Acharya, 2005). The severity of dental fluorosis can further be linked to the exposure duration, and the environmental fluoride concentration (Choubisa *et al*., 2012). In addition, physical and anatomical structure of animals also affects the levels of fluorosis. For instance, fluoride solubility in the gut varies between small and large ruminants. Large ruminants have an elaborate and larger digestive system compared to small ruminants hence a higher solubility advantage (Choubisa, 2017).

Even though all the teeth are exposed to fluoride toxicity, their sensitivity to fluoride over-exposure is tooth specific and differs among the teeth. According to (Franzman *et al.,* 2006), incisors are more prone to fluoride corrosion than the molar teeth in the first three years of life. However, progressively over 6 to 8 years, the molar teeth are greatly susceptible (Levy *et al.,* 2002). The extent of dental mottling, staining and disfigurement due to fluoride attack is a factor of stage of teeth development and cumulative exposure period to fluoride (Ranjan and Ranjan, 2015b).

Fluoride attack on teeth enamel progresses in different forms. These forms have been assigned to the different grading scales that are used to classify them according to the severity of fluoride poisoning. The Dean’s Fluorosis Index (Dean, 1942) is considered as the gold standard. This index has been used predominantly for human dentition

(Jackson and Robert, 1995). Dentists examine all teeth and score the staining and discolouration according to the numbered scale as described by Dean (1942). Apart from humans, the Dean's Fluorosis Index can also be used to determine the extent of dental fluorosis in affected animals. Although the index does not consider the number of teeth affected, it is quantifiable and simple to use. Other classification index was developed by Thylstrup and Fejerskov index (TFI) which had bigger score scales ranges from 0.0 to 9.0 (Thylstrup and Fejerskov, 1978).

2.3. Fluoride in Plants

While consumption of fluoride enriched water is ranked as the main route through which both livestock and humans acquire fluorosis world wide (Roy *et al.,* 2018), exposure of fluoride to vegetation can also affect plants to varying degrees depending on many factors such as plant species, stage of growth and environmental influences (Davison and Blakemore, 1976). Furthermore, this accumulation of fluoride in plants can affect browsing and grazing livestock, causing fluorosis in animals consuming them (Choubisa *et al.,* 2012).

Increasingly more livestock are being over-exposed to fluoride through ingestion of contaminated forage plants resulting from deposition of particulate and effluent of fluoride on the plants' leave surfaces. Rain drops and overhead irrigation agitate fluoride contaminated soil upwards causing splash erosion that eventually settle on forage plants leaves and grass. Thus, thousands of livestock across the globe in fluoride endemic areas are constantly at risk of fluoride toxicity due to exposure to fluoride contaminated feeds. Additionally, habitats prone to volcanic eruptions, grazing and browsing animals are equally endangered with similar threats of fluoride deposits covering grass and plant leaves probably altering grazing and foraging behaviour.

Plants are also known to absorb fluoride from soil which is known to provide a large proportion of dietary fluoride (Baunthiyal and Ranghar, 2015). Accumulation of fluoride occurs most in plant roots and least in their fruits (Singh *et al.*, 1995) implying that browsing animals are less exposed to fluoride in their diets than grazers. This would suggest that goats, known for their browsing, could have shown fewer signs of dental fluorosis had they been left to only to browse (Choubisa, 2015). Livestock fed on these contaminated forage and grass could develop myriad of health challenges such as poor body conditions score, low reproductive rates and general anatomical deformations. Studies have documented that both male and female (Dhurvey *et al.,* 2017) animals could face sterility and other reproductive abnormalities derived from with fluoride toxicity (Choubisa, 2012). For example, in male animals, biochemical reactions in the sperm cell associated with fluoride reduce sperm count by interrupting the spermatogenesis (Yin *et al.,* 2015). Moreover, other studies have indicated a substantial reduction of life expectancy in animals inflicted with the fluoride poisoning (Choubisa, 2015). Forage plants leaves engulfed with these fluoride contaminated volcanic soils and water could also explain the increase in tooth wear among the domesticated animals. Furthermore, developing teeth affected with the excessive fluoride give rise to permanent teeth with deformed physical qualities such as toughness and coluoration leading to accelerated erosion of teeth enamel (Ulemale *et al.,* 2010).

In view of the above potential risks, the fate of fluoride in livestock and livestock products has continued to attract the world attention (Samal *et al.,* 2016). The most recent evidence reveals that livestock domesticated along the East African Rift Valley topography could be at greater threat of severe forms of fluoride toxicity (Wambu and Muthakia 2011; Wambu *et al.,* 2014). Immature animals with rapidly developing

skeleton tissues are critically predisposed to adverse fluoride atrocities (Gupta *et al.,* 2015). In as much as fluoride over exposure upscale the risk and susceptibility to dental fluorosis (Ranjan and Ranjan, 2015b), by and large, other feed nutritional components factors play a critical role to the overall effects of fluoride on teeth and skeletal tissues. Studies done by Choubisa, (2015) with Cattle, goats and buffaloes found out that both Cattle and buffaloes were more vulnerable to dental fluorosis than goats (Panchal and Sheikh, 2017). This could be explained by dietary components of goat forage feeds which are usually high in Calcium and vitamin C that are known to neutralize fluoride toxicity. Also bulk feeders like buffaloes graze too close to the ground ingesting soil rich in fluoride while goats nibble or browse on feeds raised above the ground away from soil fluoride. On the other hand, vulnerable livestock are faced with reduced animal performance with subsequent reduction in livestock productivity. Besides, affected livestock are at risk of poor quality feed availability which is the primary source of nutrient requirement for normal body functioning. Therefore it is incumbent upon farmers and other key stakeholders to be proactive and devise alternative ways to control livestock over exposure to fluoride

2.4. Fluoride in livestock products and wastes

After ingestion, the fluoride rapidly penetrates cell membranes and enters into the blood circulatory system through which some reacts with calcium and phosphorus to form calcium fluoride and phosphatic fluoride respectively (Cinar and Selcuk, 2005). Some is stored in skeletal tissues, cardiac tissues, liver, kidney, skin, adrenal glands, central nervous system, erythrocytes and teeth (Cronin *et al.,* 2010; Ulemale *et al.,* 2010). However, according to (Inkielewicz *et al.,* 2003), only 10% of fluoride is absorbed within the soft tissues from plasma. The rest is absorbed within the body skeletal tissues (Rango *et al.,* 2014). The major pathway of fluoride excretion from

the body is through the kidney. Up to 70% of the fluoride ingested is removed from the body system through urine, sweat, saliva, eggs milk and faeces (Ranjan and Ranjan, 2015b), the remainder is absorbed and retained. There is evidence that consumable animal tissues and products accumulate fluoride in significant proportions and can be detrimental to the body's normal functioning. The ion selective electrode (ISE) method has been used for several laboratory analyses for assessing and determining fluoride levels in livestock tissues, products and wastes. As the name suggest, ISE gives the selective analytic concentration measurement. It is also simple to perform and has high precision and sensitivity. The instrument indicates the electrical potential difference between itself and a reference electrode. The output potential reading is proportional to the selected level of ion concentration in the solution in a specific volume. There is also a possibility of other analysis utilizing the same sample as it is non destructive to the sample once used. During the analysis activity, any adjustment is also possible with ISE to make the concentration have same ionic strength and pH through addition of constant concentration of total ionic strength adjustment buffer (TISAB) to the solution. The TISAB act by freeing fluoride ions thus ensuring constant pH range of between 5 and 7, a level where fluoride is the predominant fluorine-containing species.

Like crops, evidence of bioaccumulation of fluoride in animal by-products provides further sources of fluoride intake to livestock (Pińskwar *et al.*, 2003). This is facilitated through consumption of feeds manufactured from raw materials of animal origin such as bone meal, feather meal, egg shell, fish meal and blood meal. Based on Nakuru County Statistical abstract by (KNBS, 2015), both large and small ruminants are primarily kept for milk and meat production while poultry is reared for both eggs and meat. Therefore the unabated consumption of fluoride-contaminated animal

products could inevitably increase the risk of human exposure to fluoride toxicity (Choubisa, 2013). Physical symptoms in animals affected by fluorosis include lameness, wasting of muscle mass (Choubisa, 2012); kidney and liver damage (Raghavendra *et al.,* 2016). Livestock enterprises would therefore suffer tremendously due to reduced reproductivity and production as a result of fluorosis influence.

2.5 Exposure to fluoride toxicity

Over exposure to fluoride through ingestion of contaminated feeds and drinking water cause fluorosis; a developmental disturbance of enamel, which occurs during teeth formation (Kanduti *et al.,* 2016). However, drinking water has been regarded as the chief source of fluoride over-exposure globally to both livestock and human (Roy *et al.,* 2018). Many countries around the world have been alarmed by fluorosis challenge affecting their population in large numbers. For example, high fluoride contamination in groundwater have been reported in the humid tropical areas of China (Guo *et al*., 2007; Wu *et al*., 2015), India (Hussain and Hussain, 2012) and in Africa (MacDonald *et al*., 2012; Rango *et al*., 2014; Kut *et al*., 2016). India for example represents one of the countries that are worst affected by fluorosis. The data reported by Saxena and Sewak, (2015) estimated approximately 66.62 million people are at greater risk of contracting fluorosis from contaminated water. In Indian's districts, majority are worst hit by fluorosis which are 50% to 100% of the population. High fluoride content in agricultural produce has been documented in various parts of India.

Plant and animal products that are commonly associated with high fluoride levels include fresh vegetables, pulses, cereals, liver and milk (Choubisa, 2012; Saxena and Sewak, 2015). Generally most plant species have in-built fluoride toxicity resistance mechanisms. However, some are much more sensitive to hydrogen fluoride which is known to be highly toxic to most plant species. In fact more sensitive plants start to

show fluoride damaging signs when exposed to a concentration level less than 1 ppb within a period of three days (Weinstein and Davison, 2003). In a study to evaluate fluoride consumption in endemic villages of India and its remedial measures, fluoride concentration in vegetables, pulses and cereals were found to have high fluoride levels ranging from 1.79 -7.33 mg/kg 2.34 -6.2 mg/kg and 1.7-14.03 mg/kg respectively in areas endemic to high fluoride levels in water ranging from 1.5 to 13.85 mg/L. The fluoride concentration in cow's milk, goat milk and buffaloes milk, was found to range from 0.41 – 6.87 mg/L (Saxena and Sewak 2015).

Sophiscated technologies have been developed by industrialized countries e.g. United States of America aimed at curbing the fluoride anthropogenic origin sources such as industrial emission (Weinstein and Davison, 2003; Komsa *et al.,* 2016) but due to high toxicity, there is threat of environmental pollution resulting from industrial discharges and gaseous effluents channeled to water bodies and air (Vengosh *et al.,* 2014). A report from Environmental Protection Agency (Parry, 1998) gave an indication of small number of citizen (1.4Million) suffering from geological fluoride contamination of water averaging between the range of 2.0 – 3.9 mg/L in 1992. Relatively large numbers of people (approximately 162 million) were reported to suffer from fluorosis traced from human activities that contaminated water to a concentration of 0.7 – 1.2 mg/L (Parry, 1998). Ethiopia recorded high levels of fluoride in traditional spices which ranged from 2.14 - 8.57 mg/kg (Nigus *et al.,* 2016). Ordinarily, tea plants (*Camellia sinensis*), are known to habour amplified amounts fluoride ions that average between 321.27±234.1 µg/g (Ashenef and Engidawork, 2013). On the other hand, in selected cereals products were found to range between 3.70 - 10.98 mg/kg while legumes recorded 1.52-11.07 mg/kg (Mustofa *et al.,* 2014). Moreover, high occurrence of fluoride in groundwater in

Kenyan Rift Valley has also been reported (Näslund and Snell, 2005; Wambu *et al*., 2014). In a research to study level of human exposure, excessive fluoride in cow milk was found to range between (0.02 - 0.34 μg/g) and vegetables (7.9–59.3 μg/g) in Elementaita regions of Nakuru County (Kahama *et al.,* (1997). For these reasons, it is hypothesized that there is likelihood of high fluoride concentration in livestock and livestock products in domesticated animals in Nakuru County and hence the need to document the information for future referenced interventions.

2.6 Fluoride occurrence in animal feeds

The variations in prevalence and severity of fluorosis effects in animal living in the same fluoride endemic villages is much more dependent upon the presence of calcium and vitamin C and D in their feeds, frequency of fluoride intake and the consistency of exposure (Miller *et al.,* 1999; Choubisa, 2015). Furthermore, prevalence and severity of fluorosis can be accelerated by; the amount of fluoride dissolved in water, environmental factors and the animal characteristics such as age, health, genetics, stress factors and the biological response of individual. (Choubisa, 2010).

Water is a vital ingredient in animal nutrition and feeding. It is common knowledge that water aids in feed ingestion and digestion, facilitate the osmo-regulation and synthesis of Cattle milk. Besides, water is an important solvent and natural vehicle for most of the naturally occurring metals both on surface and underground. Solubilization of fluoride in groundwater remains formidable threat to life and the most urgent challenge world over (Samal *et al.,* 2016). The concentration of naturally occurring fluoride is predetermined principally by the geological status occasioned by volcanic activities of an area. The majority (over 70%) of the water samples that have been tested for fluoride along the African Rift Valley exceed the recommended level of 1.5mg/L (WHO, 2006) for human consumption (Olaka *et al.,* 2016). Fluoride

bearing rocks undergo disintegration as a result of exposure to weathering processes. For instance, moving water flows against these rocks, solubilizes compacted fluoride ions which is then released and accumulates in the groundwater (Edmunds and Smedley, 2013).

Further, both livestock feed and human food are also examples of how fluoride is acquired through nutrition (Choubisa, 2015; Ranjan and Ranjan, 2015b). Factors governing the fluoride levels in the food/feed are; the soil where the crops were grown, processing point and the source of water utilized used for preparation and plant growth. Countries in humid tropics such as Europe experience generally less fluoride concentration which ranges 0.02 to 0.29 mg/kg, but food stuff such as fluoridated table salt, fish, and bottled natural mineral water may contain high fluoride concentration (Indermitte *et al*., 2009).

Under ideal conditions, fluoride in soil range between 10 mg/kg and 1500 mg/kg and most species in plants, the range is generally between 1–10 mg/g dry weight in most plant species (Baunthiyal and Ranghar, 2015). While all plants absorb fluoride contaminated water from soil through roots by passive diffusion, the rate of fluoride movement differ with each plant (Šucman and Bednář, 2012). Factors that influence fluoride uptake from soil include plant type, plant height and prevailing climatic conditions. Plants near the ground are prone to fluoride contaminated soil splash from rain and over head irrigation drops that settle on leaf surfaces. During the dry season, fluoride concentration tends to be high in plants compared to wet seasons (Kahama *et al.,* 1997). Plants are not known to harbour much fluoride to toxic levels with exception of tea plants which are shorter and can accumulate high levels of fluoride. Fortunately, the tea plant potentials as a livestock forage feed has not been documented. However, plant species like *Acacia georginae* and *Dichapetalum*

*cymosum (*Gifbaar) have the capacity to incorporate fluoride from soil and convert it to a very toxic fluoroacetate substance which is extremely poisonous to livestock (Shupe *et al.*, 1984).

Air blown fluorides (gaseous fluoride) from industrial pollution settle on plants leaves and penetrate the leaves through stomata pores (Miller *et al.*, 1999). This cumulatively interferes with photosynthesis and leaf malformation (Baunthiyal and Ranghar, 2015). Cumulative exposure to fluoride concentration in excess of 0.2 µg/m3 cause plant damage (WHO, 1984). Human agricultural activities such as inorganic fertilizer application to the soil and factory effluents are some of the sources of fluoride to plants. The most common sources of fluoride to animals are shown in the Figure 2.1 below.

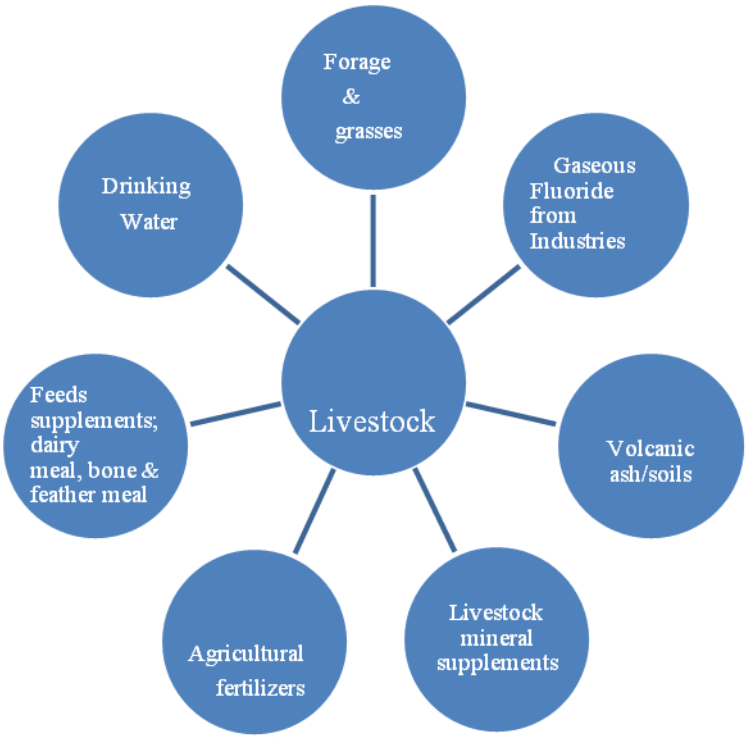

Figure. 2.1: Sources of fluoride toxicity in livestock

Grazing and browsing livestock ingest fluoride through contaminated plants though at much lower rate than those licking the contaminant directly from soil. Farmers who

practice pasture management to ensure soil cover can greatly mitigate fluoride toxicity in livestock (Loganathan *et al.,* 2008). Livestock can also avoid fluoride exposure by restricted grazing and browsing close to industrial and processing plants. Cereal products normally have less fluoride concentration. Nevertheless, studies have indicated that sorghum (*Sorghum bicolor*) planted in fluoride endemic areas tend to have high molybdenum concentration and are more vulnerable to hydrogen gas (Mac-Lean *et al.,* 1984). A research study by Lakshmi and Lakshmaiah, (1999) in rats proved that the mineral element has capacity to slow down fluoride removal through urine thereby encourages fluoride retention in mammals (Stookey and Muhler, 1962). Therefore it is imperative to take caution when formulating diet based on sorghum as a raw material.

2.7 Effects of fluorides in livestock

Several studies have concluded that overexposure to fluoride leads to myriad of detrimental effects to livestock productivity (Samal *et al.,* 2016; Roy *et al.,* 2018). These include incomplete enamel formation, teeth mottling, excessive wear of teeth (Ulemale *et al.,* 2010; Kanduti *et al.,* 2016,) and skeletal deformities. Other research studies have reported impaired oocyte maturation in animals overexposed to fluoride contamination which impair animal reproductivity (Zhou *et al.,* 2012). However, the prevalence rate, the acquisition mechanism and the major disposal channel from the livestock body remains to be undocumented in Nakuru County, Kenya.

Normal Cattle have blood levels of up to 0.2 mg fluoride per deciliter of blood and 2-6 ppm in urine. However, at 8 – 12 ppm fluoride concentration, the general animal physiological function will be curtailed (Ulemale *et al.,* 2010). Fluoride levels exceeding 2 ppm in water is toxic to animals. At 5 ppm, it produces mild teeth

lesions; at 10 ppm it causes excessive wear and tear of teeth; and if it is present at the rate of 30 ppm in water, it may produce more systemic effects (Ulemale *et al.*, 2010).

Calcium and phosphorus is present in the body soft tissues (plasma) and hard tissues (bones and teeth). Calcium and phosphorus present in skeletal as calcium hydroxyl-apatite $Ca_3 (PO_4)_2$. $2Ca (OH)_2$ crystals. When a lot of F^- is ingested in feed and water F^- displaces OH^- as they have almost similar ionic radii.

$Ca_3 (PO_4)_2 . 2Ca(OH)_2 + F^-_{aq} \Longleftrightarrow Ca_3 (PO_4)_2 . Ca(F)_2$ White colour due to OH and Brownish Colour due to presence of F2 in the calcium hydroxyapatite characteristics in bone and teeth respectively. Livestock tissues with high calcium contents such as bone and teeth start to attract and accumulate more fluoride (Pradhan *et al*., 2016) resulting in delayed mineralization of bones and teeth. The negative effects are more pronounced in actively growing and young animals (Mapham and Vorster, 2012). Furthermore, fluoride permeates within the body tissues causing irreversible damages to the liver, kidney and brain organs (Choubisa, 2012).

The maximum safe level in ruminants is 1 mg/kg body weight (Ulemale *et al.*, 2010). In feeds concentration, the maximum tolerance level ranges from 20–50 mg/kg dry weight in most species (Blakley and Barry, 2016). Poultry can tolerate as much as 200 mg/kg (Blakley and Barry, 2016). These tolerance levels vary depending on age, length of exposure and nutritional status (Panchal and Sheikh, 2017). Animals affected by its toxicity normally possess diffused and thickened bones and calcified ligaments resulting in stiffness and lameness (Pradhan *et al.*, 2016). It is well known that skeletal fluorosis is highly painful and causes enormous economic loss to the livestock keepers. Undesirable effects such as restricted animal movement, reduced life-span and sometimes premature deaths are losses incurred that are precipitated by fluoride over-exposure.

Limited information is available on the effects of fluoride in thyroid gland. However studies conducted by Zhan *et al.,* (2006) on young pigs, found out that excessive fluoride in livestock feeds led to abnormal thyroid hormone levels depressed growth hypothyroxinemia. This was further confirmed by Wang *et al*., (2009) in their studies on rats that resulted in damages to the structure of the thyroid gland and an alteration of thyroid hormone levels in serum. On the other hand, (Lohakare and Pattanaik, 2013) indicated that fluoride thyroid damage may develop in instances where animals are severely over-exposed to fluoride. The study suggests that change in levels of thyroid hormones might be due to inhibition of iodine absorption through fluoride interaction (Dolottseva, 2013).

One of the many effects of over exposure to fluoride is dental malfunction. Any problem that affects the teeth interferes with the whole feeding and mastication process. The side effects are generally noted in terms of reduced milk and wool production, staggered growth and health instability. These have obvious net effects on enterprise profitability. Studies by Ulemale *et al.,* (2010) concluded that milk production is greatly reduced when lactating dairy cows are exposed to 150 to 200 ppm fluoride concentration levels. This can be associated with binding effects of fluoride to calcium and phosphorus elements that are essential in milk synthesis.

Wool production in Sheep is also impaired with high fluoride levels. Fluoride have a biochemical effects that ultimately interferes with wool qaulity. The product at the end becomes shorter finer and less crimped (Flueck, 2016).

With the aforementioned serious negative effects of fluoride on livestock and humans, the research evidence proved that there was widespread presence of fluorides along the East Africa Rift Valley. Livestock domesticated in Nakuru County were diagonized with fluorosis at varying magnitudes. Significant levels were also found to

be present in livestock feeds, hooves and milk. The present study therefore presented vital information that would guide intervention strategies in fluoride endemic areas with an aim to mitigate the undesirable effects in livestock production.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

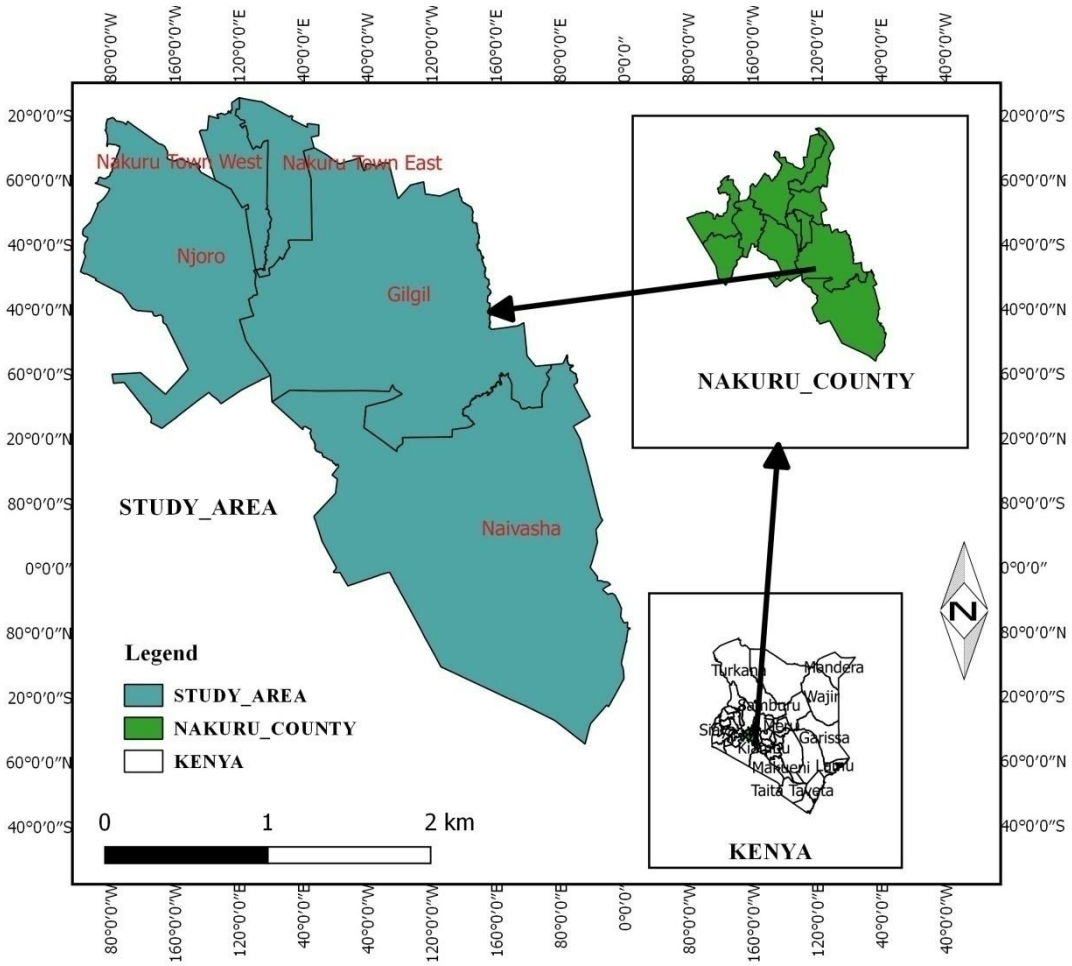

Figure 3.1: Map of Nakuru County

3.1.1 Geography

The study was carried out in Nakuru County in the Central Rift Valley region of Kenya. The county covers an area of 7,495.07 $km^{2.}$ The focal areas lie between latitudes 0^{o} 13' N and 01^{o} 10' S and between longitudes 36^{o} 30' E and longitude 35^{o} 30' W (Jaetzold *et al.*, 2009). Greater parts of the County of Nakuru are flat and are found on the floor of the Rift-Valley whereas the gently sloping areas with highlands are located to the North West around Molo bordering Kericho and Bomet Counties.

The County sits astride the Rift-Valley and most lakes such as Lake Nakuru and Lake Elementaita are found on the flatter areas.

3.1.2 The Climate

Physical features and the altitude greatly contribute to the Nakuru County climatic conditions (Jaetzold *et al*., 2009). Areas such as Rongai and parts of Subukia known for their high altitude (1980 - 2700 m) generally receive minimal rainfall of approximately 1000 mm. Greater parts of Nakuru County receive rainfall of up to 1500mm per year and lie between altitudes 900 – 1800 m above the sea level. The remaining places (Naivasha and Solai) receive between 500 to 1000 mm rainfall. The County is generally warm with minimal monthly variation in temperatures between 9° and 26°C throughout the year depending on location and altitude. It experiences two rainy seasons of March to May long rains and September to December short rains (Jaetzold *et al.,* 2009).

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3.1.3 Human population

The human population of Nakuru County stood at 1,867,461 in year 2014, comprising of 937,131 males and 930,330 Females (KNPHC, 2009). It is projected that the population will increase to 2,046,395 by year 2017 comprising of 1,026,924 males and 1,019,471 females as shown in Table 3.1. This population is likely to be affected by fluorosis through consumption of fluoride enriched-food stuffs and water. Moreover, it implies that the county government will have to invest more in public health to match the needs of the projected 'unhealthy' population.

Table 3.1: Nakuru County Human Population Projections

	Year			
Gender	2009	2014	2015	2017
Male	804,582	937,131	966,154	1,026924
Female	798,743	930,330	959,142	1,019471
Total	1,603,325	1,867,461	1,925,296	2,046,395

Source: Nakuru County, First County Integrated Development Plan, 2013-2017

3.1.4 Livestock Population

The County has a total of about 3 million domestic animals (KNBS, 2015) as shown in the Table 3.2 below. These numbers are likely to reduce in the near future if precautionary measures are not put in place to address the fluoride toxicity in the area.

Table 3.2: Nakuru County Livestock Statistics

Livestock species	Livestock population
Dairy	286,050
Beef Cattle	160,514
Goats	261,543
Sheep	436,819
Layers	295,978
Broilers	85,007
Indigenous birds	1,183,108
Turkeys	22,329
Ducks	26,208
Geese	10,375
Quails	5,120
Rabbits	88,682
Pigs	18,866
Donkeys	82,703
KTBH	12,067
Log Bee Hive	24,878
Total	3,000,247

Source: Department of Agriculture, Livestock and Fisheries, County Govt Nakuru 2014

3.2 Data Collection and Preparation

3.2.1 Site selection

The study site was divided into 4 sub counties that included; Gilgil, Njoro, Naivasha and Nakuru plus Egerton University demonstration farm within Nakuru County. These regions were chosen to cover a geological transect across the Rift valley from eastern side (Naivasha) to central parts (Gilgil and Nakuru) and western side (Njoro and Egerton). The reasons for the choice of these areas were; 1. The livestock data from each region indicate a substantial number of ruminant population that provided good number of livestock for the sampling (KNBS, 2015); 2.These regions cover Lake Naivasha, Elementeita and Nakuru that are well known for their high soil fluoride content ranging from 2.4 to 2800 ppm (Tekle-Haimanot *et al.*, 2006; Gikunju, 1990); 3. Fluoride concentration in most water sources from these areas range from 1.0 to 30mg/L (Wambu and Muthaika, 2011). 4. These areas lie on the Kenyan Rift Valley where geological fluorides are endemic. Predisposing factors e.g. soils and most waters sources are principally contaminated with both geological and anthropogenic fluorides sources due to volcanic eruptions associated with these areas and heightened human activities. Consequently, life forms things supported with the above natural resources are liable to fluoride toxicity (Baunthiyal and Ranghar, 2015).

3.2.2 Farm selection

Three livestock farms were purposively selected in each region based on their location and herd size. The farmers with at least 30 ruminants and above were considered. The visit was done prior to actual study in order to obtain the required permits. The selected farmers provided an oral informed consent and agreed to participate in the research and handling of their Cattle and Sheep. Other criteria for selection included; utilization of either exotic or indigenous pastures or fodder for feeding, and

permission to allow collection of samples of livestock and feeds in the farm for analysis. Below is the criteria that was used in identifying and recruitment of farmers for the study:

- The farmers were picked in a transect across the Rift Valley. This was important geologically.
- The farms were located within the four blocks in Naivasha, Nakuru, Gilgil and Njoro areas.
- Three farms with large herd sizes were picked from each block
- The farmer utilized established grass pastures, forage or indigenous pastures as livestock feed.
- The farmers were literate, with minimum education level of primary school.

3.2.3 Animal numbers

Ruminant numbers were determined based on two reference studies, (Choubisa, 1999; Choubisa, 2015). The study both used approximately 100 ruminants in each selected location. All ruminants (or as many as time would allow) were observed at each farm. A total number of 242 Cattle and 307 Sheep were examined and sampled. Apart from their availability, these two ungulates have been largely utilized in bio-indicative studies in bio-monitoring of environmental fluoride pollution (Kosma *et al.,* 2016; Choubisa, 2015) and to measure indirectly the impact of fluoride in humans.

3.2.4 Equipment and instruments

The following equipment and materials were used during the study; the questionnaire, gumboots, dust coats, disposable gloves, weighing band, pictorial dental grading scale, 100 ml plastic bottle, 500 ml plastic bottle, zip lock polythene bags, hoof trimmers, weighing scale, scissors, ropes and permanent markers to be utilized during

the exercise. Fluoride ion selective electrode (Model Cl-6728, Pasco scientific, and Roseville, USA) was used for the determination of fluoride concentration in water, feeds and animal tissues.

3.2.5 Dental grading and sample collection

A cross-sectional survey involving on–farm epidemiological clinical dental examination of the Cattle and Sheep was conducted to obtain estimates of the prevalence of dental fluorosis in the study area. Farm-to-farm surveys were made in the mornings and evenings to minimize disturbances for grazing hours and daily farm routine. Farm to farm movement followed North-West to South-East transect of the Rift Valley in Nakuru County which incorporated regions with variable fluoride levels. Animals were selected and picked randomly from the herd of Cattle and flock of Sheep for the clinical examinations and sample collections. Strict adherence to animal welfare and ethics of University of Eldoret for animal handling was followed. The exercise involved the staff from the Regional Veterinary Investigation Laboratory in Nakuru led by a qualified veterinarian.

For evidence of dental fluorosis, visual clinical examination of anterior teeth of livestock was done for mottling or staining using sunlight. Each animal examined was held in an upright position and then the teeth were observed for signs of dental fluorosis. Grading was done according to Dean Index of Classification (Dean, 1942). In this method, the defects were classified as normal (grade 0.0), questionable (grade 0.5), very mild (grade 1.0), mild (grade 2.0), moderate (grade 3.0) and severe (grade 4.0) scores as depicted in Plates 3.1 to 3.5 below.

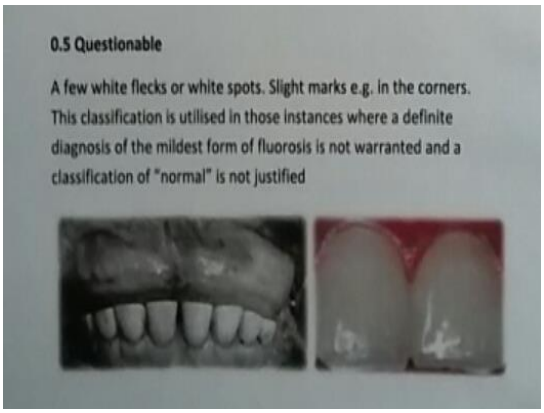

Plate 3.1: Questionable = 0.5 (few white teeth corners)

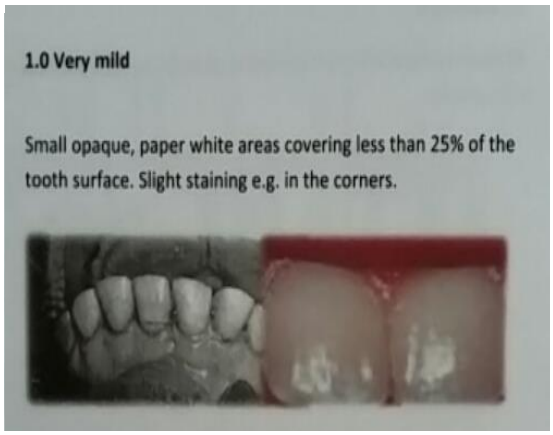

Plate 3.2: Very mild = 1.0 slight staining

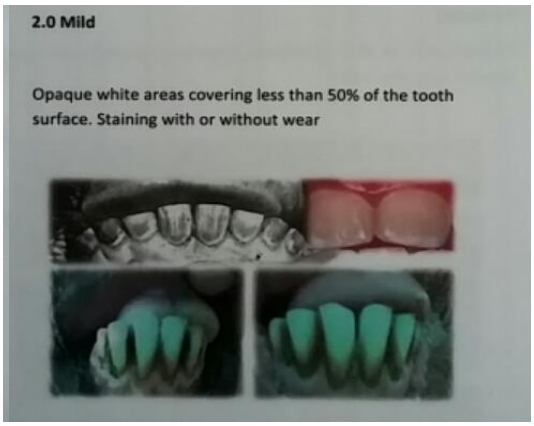

Plate 3.3: Mild = 2.0 (50% teeth staining with or without wear)

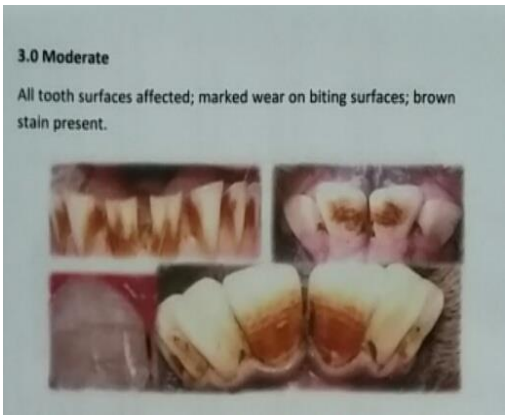

Plate 3.4: Moderate = 3.0 (All teeth surface affected, marked wear at biting surface)

Plate 3.5: Severe = 4.0 (Excessive wear, all tooth surface brown stained, discrete or confluent pitting)

During the epidemiological process, hoof samples were also obtained from all animals inspected. The veterinary officer restrained the animal. Then the hoof knife was used to trim the hooves as the trimmed hoof's samples were collected. Then the hooves were sprayed with antibiotic spray to prevent disease occurrence. Fluoride is found in higher concentration mostly in hard tissues such as teeth and skeleton formation therefore hoof concentration was used an indicative of fluoride concentration in animals' bones and the geochemical status of the surrounding ecosystem (Komsa *et al.,* 2016). For comparison of other contributing factors to dental fluorosis, information on age, weight, breed, sex, drinking water sources, site feeds and feeding systems of the livestock were simultaneously collected and recorded in the questionnaire. Fluoride concentration in drinking water correlates with that in urine and therefore concentration in urine is a bio-indicative of fluoride in water (Komsa *et al.,* 2016)

3.2.6 Animal feeds collection

The "Z" pattern random movement, procedure was used to samples of both the indigenous and established grass pastures, and forage materials from the field. The forages were cut at 5 cm from the ground to allow for re–growth. These samples were collected in triplicate and put in a well labeled zip lock polythene bags. Samples of silage and hay present in the farm were also picked and labeled.

Forage and grass samples were then air dried to remove the moisture in the shade. For complete drying, the samples were put in the oven set at 80^{o}C over night. These feeds were ground into fine powder by an electronic grinder. Then samples were milled into fine particle size to pass a 40 mm-mesh sieve and stored in a plastic bottles for later analyses.

3.2.7 Water collection

All the water type sources found in the farm were collected in triplicate in a 500 ml plastic bottle. These samples were acidified using 1.0 molar Nitric acid to prevent further chemical reaction. The bottles were then transported to laboratory for storage and analysis.

3.2.8 Faecal collection

Two methods were used. One was to collect a sample immediately it had been naturally deposited by the animal and the second was the rectal faecal sample collection which followed the procedure below:

1. Clean disposable gloves were worn on the hands and water-based lubricant applied to index and middle fingers.

2. Index and middle fingers were inserted into the rectum of the animal, one finger at a time without going deep inside. The fingers were spread to allow air into the rectum. The air duplicates fullness in the rectum and a wave of muscular movement often moved the faeces out into the hand.

3. At least 4 g of faecal matter were collected. A good sized adult pellet is about 1 g.

4. The samples were put in well labeled zipped polythene bags.

5. The sample were then transported and stored in the refrigerator at 4^0C.

The faecal samples were digested with concentrated sulphuric acid and hexamethyldisiloxane (HMSO) added so as to diffuse and trap the fluoride.

3.2.9 Milk collection

Fresh raw milk samples were obtained from randomly selected milking cows in all the regions. The milk samples were collected from a milk bucket of individual cow at milking time. Then put in a 100 ml plastic bottles and stored in a refrigerator at 4^0C tightly closed.

3.2.10 Hoof collection

Hoof clippers were used to cut hooves of small ruminants and a hoof knife was used for large ruminants. Animals were restrained by use of ropes and approximately 400 g of hoof was collected and placed into an air-tight bag and stored at $4^{o}C$. Before analysis, hooves were soaked overnight and washed thoroughly for several times to remove soil and other dirt with distilled water. The hoof samples were then dried in the oven at 80°C for complete drying. The hooves were ground into fine powder by an electronic grinder and stored in dry plastic bottle.

3.3 Sample analysis

3.3 .1 The ion selective electrode (ISE)

An ion-selective electrode (ISE) is the most commonly used method of determining fluoride concentration in a sample. This technique is simple to perform and has high precision and sensitivity in fluoride determination. ISE utilizes potentiometric analytical method, that allows only the fluoride ions (ions of interest) to pass through its membrane. The rest are blocked from passage (Bard and Faulkner, 2001). The potential difference across the membrane is generated by fluoride ions activated in the solution. This electrode potential is measured by ISE amplifier and a computer interface. The more concentrated fluoride ions are in the solution, the higher the readings. The TISAB (Total Ionic Strength Adjustment Buffer) is buffer that is added to the solution to create uniform background in ionic strength in terms of solution pH.

3.3.2 Preparation of standard sodium fluoride stock solution

Exactly 221 mg of dry Sodium fluoride (NaF) was dissolved in 250 ml of distilled water in a clean and dry volumetric flask and made up to one litre. This stock solution was stored in a polyethylene bottle. The 1.0 ml of stock solution was equivalent to 0.1 mg F

3.3.3. Preparation of calibration standard curve

Five calibration standards were prepared to cover a range of 0.1 mg/L F- to 20 mg/L F- by pipetting 0.221 g of dry sodium fluoride of the stock solution into each of 250 ml clean and dry volumetric flasks. Exactly 50 ml of total ionic adjustment buffer was added to each flask and then diluted to one litre with distilled water. The standards were then stored in properly secured polyethylene containers. The fluoride activity in the standard solution was measured and recorded in millivolts (mV) using a fluoride ion selective electrode and a calibration curve prepared by plotting the relative millivolts on the y-axis against the logarithm of the concentration of the standards on the x-axis.

3.3.4. Animal forage feeds and faecal analysis

Milled feeds and faecal samples were weighed separately to a measurement of 1.25 g and then transferred into two separate test-tubes and placed in a rack. Then 10 mL of 6 M sodium hydroxide was measured in a measuring cylinder and added to each sample in the test tubes. The mixtures were then heated in a water bath for half an hour till the feeds and faeces were completely dissolved in the test tubes. The solutions were then cooled to room temperature and each neutralized with 8M sulphuric acid. The solutions were then transferred into two separate 50 mL volumetric flasks. Distilled water was added to top up to 50 mL. Exactly 10mL from each solution was mixed with an equal volume of total ionic strength adjustment buffer (TISAB) solution into two separate a 100 mL beakers. The two samples were homogenized using a magnetic stirrer nonstop in order to magnify fluoride ions activity in the solution. The measurements were taken and recorded in milli-volts using the Ion selective Electrode of Jenway® model.

To determine fluoride concentration in the two solutions, calibration graphs were constructed from five fluoride standards in the range 0.1 to 20 mg/L was used as shown in Figures 3.4a to 3.4b.

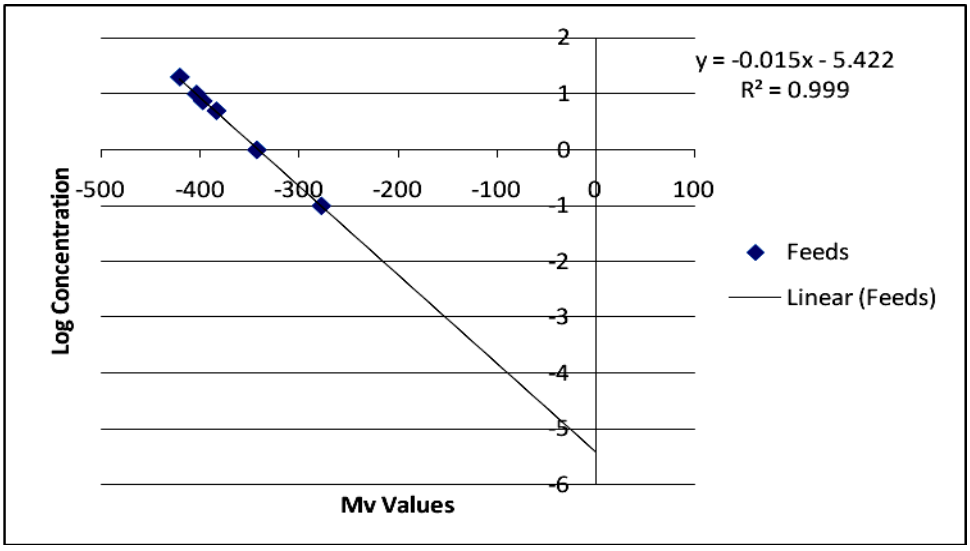

Figure 3.4a: Calibration curve for determination of fluoride concentration in feeds

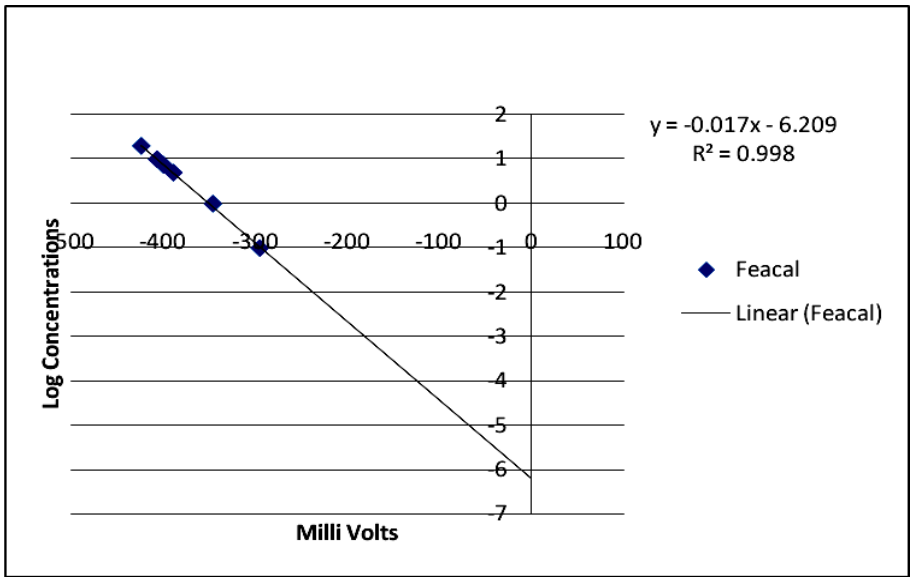

Figure 3.4b: Calibration curve for determination of fluoride concentration in faecal

The formula below was used to determine the concentration of fluoride in solid feeds and faecal samples.

$$C_s = C_l \times \frac{V}{m}$$

Where, C_s is fluoride concentration (mg/kg) in solid feeds and faecal samples, C_l is

extracted fluoride concentration (mg/L) in solution, *V* is the volume (L) of digested sample solution (50 mL, in this case), *m* is mass (kg) of feeds and faecal samples used (1.25 g, in this case).

3.3.5 Water analysis

Approximately 10 mL of water sample was mixed with an equal volume of total ionic strength adjustment buffer (TISAB) solution into a 100-mL beaker. The activity of fluoride ions in the solution were measured using Jenway® fluoride Ion Specific Electrode (ISE). During the measurements, a magnetic stirrer was used to homogenize the solution by steady continuous agitation throughout the fluoride measurement. The values were recorded in milli-volts (MV). Fluoride concentration in the solution was then determined based on a calibration curve (fig 3.5a) using five fluoride standards in the range of 0.1 to 10 ppm

3.3.6 Milk analysis

Approximately 10 mL of milk sample was poured into a 100 mL beaker and equal volume of total ionic strength adjustment buffer (TISAB) solution was added. A magnetic stirrer was used to homogenize the solution by steady continuous and rapid agitation all through to disperse fat droplets. A Jenway® fluoride Ion Specific Electrode (ISE) was immersed into the mixture to measure and record the activity of fluoride ions in the solution. The values were recorded in milli-volts (MV). The fat residues were removed from electrode after each measurement. Readings were then taken after allowing 1-2 minutes for equilibration. The fluoride concentration in the solution was then evaluated by comparing the observed readings with calibration graphs below prepared in standard solution using five fluoride standards in the range of 0.1 to 10 ppm as shown in Figures 3.5a and 3.5b

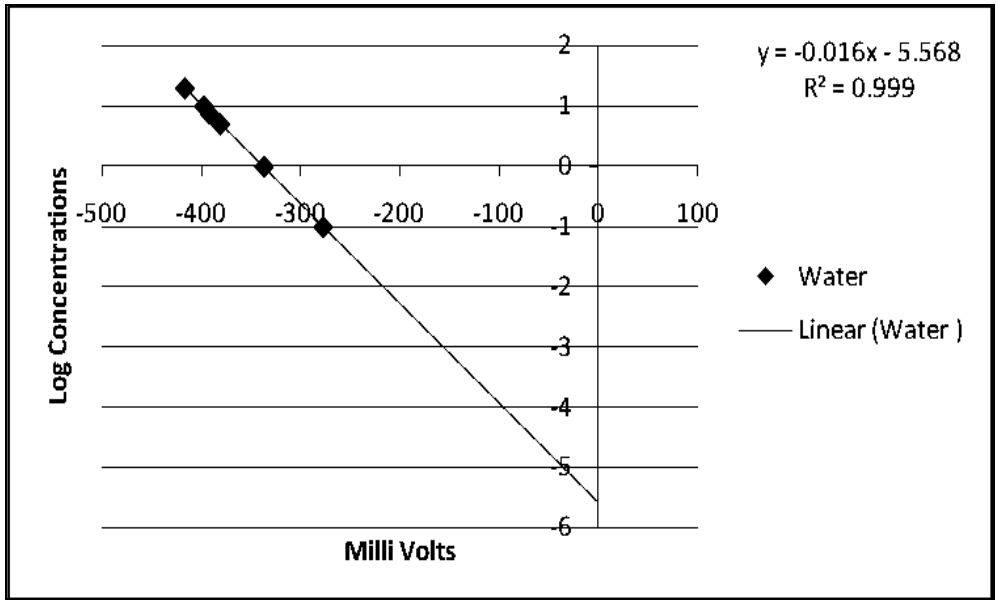

Figure 3.5a: Calibration curve for determination of fluoride concentration in water

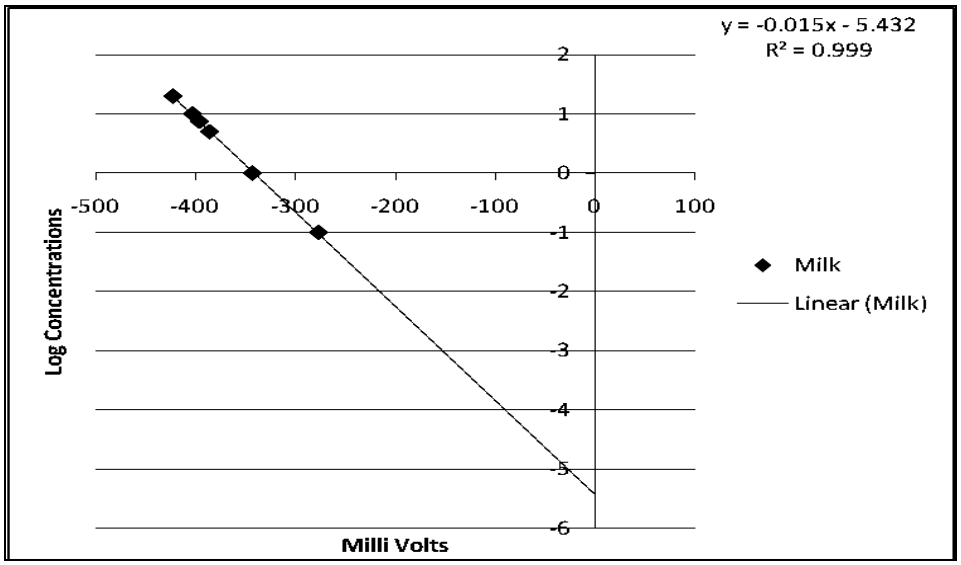

Figure 3.5b: Calibration curve for determination of fluoride concentration in milk

3.3.7 Hoof analysis

Milled hooves samples of 1.25 g were w placed in a test-tube. Before 10 mL of 6 M sodium hydroxide was added. The mixture was heated in a water bath for 30 minutes until complete dissolution was attained. The solution was cooled to room temperature and neutralized with 8M sulphuric acid. The solution was then transferred into a 50 mL volumetric flask. Distilled water was then added to top up to a 50 mL.

Approximately 10mL from each solution was mixed with an equal volume of total ionic strength adjustment buffer (TISAB) solution into a 100-mL beaker. The measurements of fluoride ions activity in the solution were recorded in milli-volts using the fluoride Ion Specific Electrode (ISE) of Jenway® model immersed into the solution. Continuous homogenization of the solution took place throughout using magnetic stirrer as the measurements were being taken.

To determine fluoride concentration in the two solutions, a calibration curve constructed from five fluoride standards in the range 0.1 to 20 mg/L was used as shown in Figure 3.6

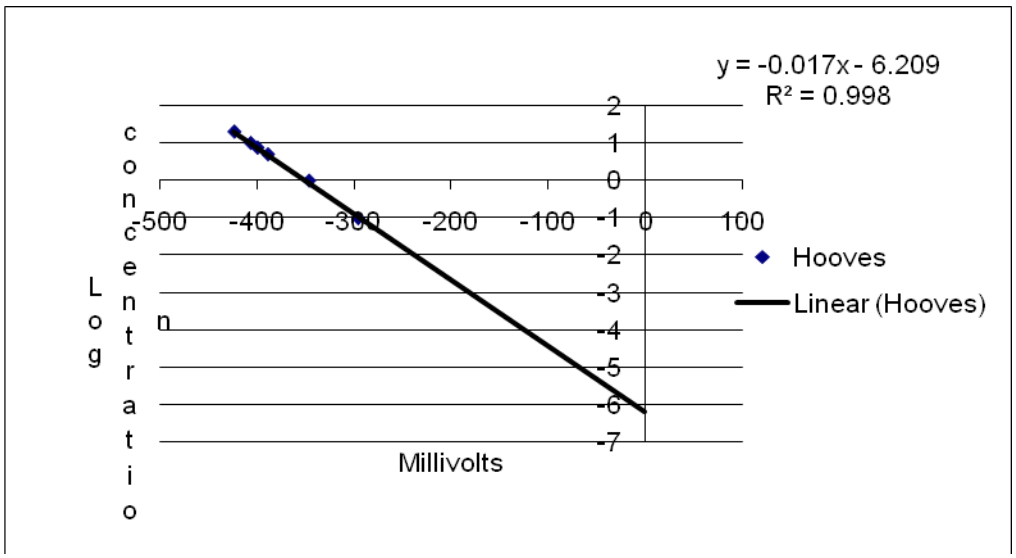

Figure 3.6: Calibration curve for determination of fluoride concentration in hooves

3.4 Statistical data analysis

The IBM SPSS version 23 of the year 2015 was used to carry out the data analysis. The dental flourosis scores were recorded as total number of animals selected and graded. The differences among regions were determined using descriptive statistics and Chi-square (X^2) test. Differences in dental flourosis based on site, breed, species and age were also analyzed using descriptive statistics. The site, breed, species and age were the independent variables while dental flourosis grading scale were the

dependent variable. Fluoride concentration in water, forage feeds, hooves and milk was analyzed by one way analysis of variance (ANOVA). The differences between the treatment means of regions were compared using Duncan's Multiple Range test at $p \leq 0.05$.

3.4.1. The statistical model

This was a block design fitted into the following equation:

Model $Yjk = \mu + bi + ejik$

Where: Yjik = Fluoride parameters tested by (age, weight, degree of mottling, breed)

μ = the underlying mean

bi = the blocking effect

ejik = error

CHAPTER FOUR

RESULTS

4.1 Level of dental fluorosis in Cattle

Fluorosis was found to be expressed in grades indices as shown here below

4.1.1 Grade score distribution

The results for the grade score distribution of the levels of fluorosis from the five sampling sites is shown in Table 4.1. Skewed distribution where majority of the scores were within the questionable (grade 0.5) to the mild (grade 2.0) scores. This trend was noticed in Egerton, Gilgil, Naivasha and Njoro regions. In Nakuru, however, the data was skewed towards the high values of grade scores (i.e. 3.0 and 4.0). On the whole, only 9.9% of the total animals scored higher grade of 3.0 and 4.0 scores compared to the majority (90.1%) of the livestock.

Table 4.1: Number of ruminants per region for each grading score

	Level of Fluorosis					
Grading Score	0.5(questionable)	1.0(very mild)	2.0 (mild)	3.0(moderate)	4.0(severe)	Total
Site						
Egerton	24	49	110	13	1	197
Gilgil	10	53	39	-	-	103
Naivasha	9	25	53	16	2	105
Nakuru	4	-	34	11	4	77
Njoro	31	18	11	6	1	67
Total	78 (14.1%)	170 (31.0%)	247 (45.0%)	46 (8.4%)	8 (1.5%)	549 100%

The majority of the animals scored between grades 0.5 up to 2.0. The most prevalent grade score among the animals was grade 2.0 which represent the mild cases. This grade appeared in all the regions apart from Gilgil which had high number of very mild cases (grade score 1) greater than the mild ones (grade 2.0).

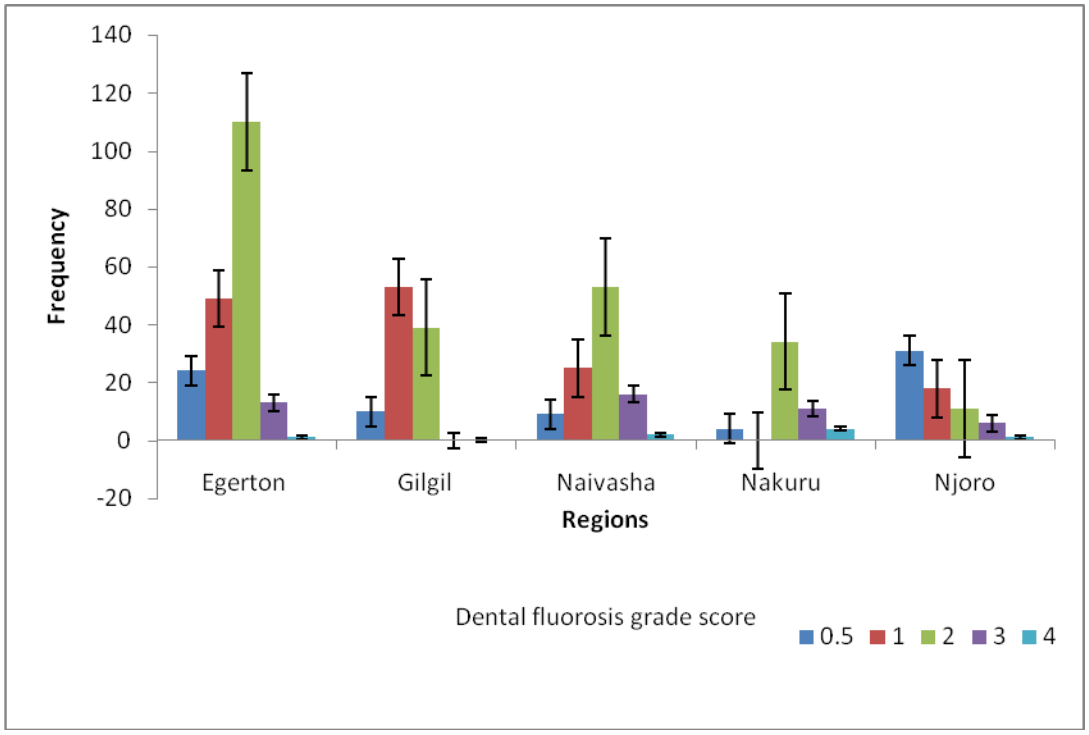

Figure 4.1: Frequency of dental fluorosis for each grading score

4.1.2 Levels of dental fluorosis in Cattle

The results for the grade score distribution of the dental fluorosis in Cattle from the five sampling sites are shown in Table 4.2. From the five regions in Table 4.2 below, the dental fluorosis score of very mild grade (36.78%) and 2.0 (29.75%) were the most prevalent followed by the questionable grade 23.55% of the Cattle population studied. Overall, 9.5% (7.85% + 1.65%) of the total Cattle sampled were troubled by moderate (3.0) to severe (4.0) grade scores. The score of 0.5 and 3.0 were frequently observed in Egerton and Nakuru respectively. Nakuru was the only location which had grade score of 4.0 with others recording nil scores.

Table 4.2: Dental fluorosis score in Cattle per region

Sites	Grading Score					Total
	0.5	1.0	2.0	3.0	4.0	
Egerton	23	24	33	0	0	80
Gilgil	6	24	6	0	0	36
Naivasha	9	14	8	3	0	34
Nakuru	0	19	21	10	4	54
Njoro	19	9	4	6	0	38
Total	57 (23.55%)	89	72	19	4	242
% total		(36.78%)	(29.75%)	(7.85%)	(1.65%)	

Ke,y: 0.5 (questionable). 1.0 (Very mild) 2.0 (Mild), 3.0 (Moderate), 4.0 Severe

4.1.3 Chi Square Analysis

The frequency of dental fluorosis in Cattle at the sampling locations as provided in Figure 4.2. However, there was a signficant differences in the occurence of dental fluorosis in Cattle among the study locations ($\chi^2 = 82.442$, df = 16, $P \leq 0.001$). Lowest levels of dental fluorosis occured at Egerton where 23 tested for grade scale 0.5 dental fluorosis, 24 were positive for scale 1.0 and 33 Cattle tested positive for grade scale 2.0 dental flourosis. At Gilgil, 24 Cattle tested positive for scale 1.0 followed by level 0.5 fluorosis (n = 6) and lowest being scale 2.0 (n = 6). In Nakuru, scale 2.0 dental fluorosis occured in large number of Cattle (n = 21), followed by level 1 (n = 19), while grade scale 3.0 occured in 10 Cattle with another 4 Cattle being affected by scale 4.0 dental fluorosis. In Njoro upto 19 Cattle had grade scale 0.5 fluorisis, followed by scale 1.0 (n = 9) , then level 3 (n = 6) and least in scale 2.0 (n = 4).

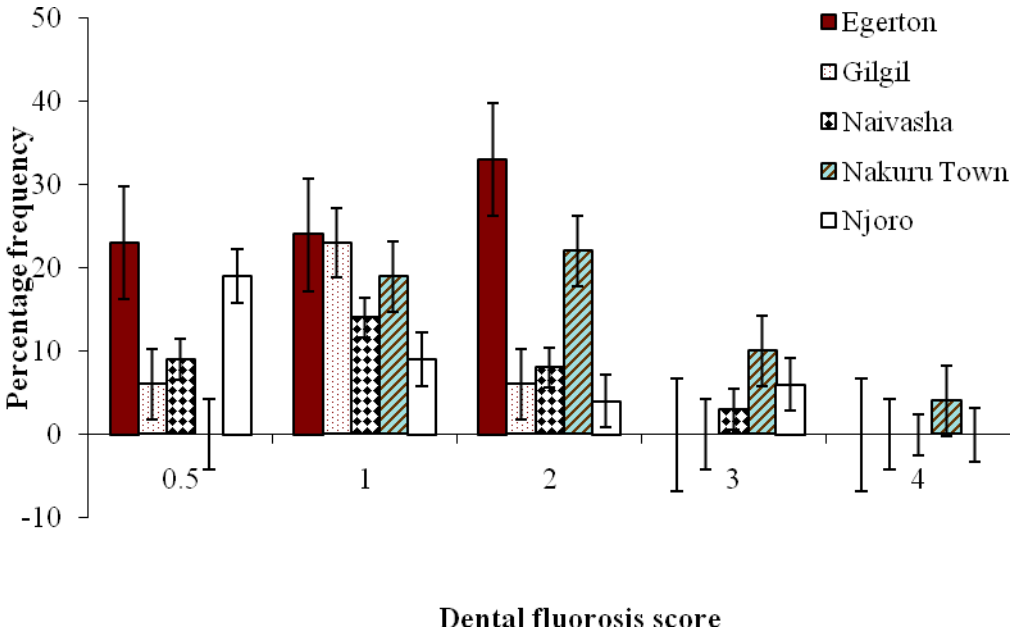

Figure 4.2: Overall frequency of dental fluorosis in Cattle

4.1.4 Dental fluorosis score among Cattle breeds

The results for the Cattle breed grade score distribution of the levels of fluorosis from the five sampling sites are shown in Table 4.3.

Table 4.3: Dental fluorosis score in Cattle breeds

	Grading Score					
	0.5	1.0	2.0	3.0	4.0	Total
Breed						
Friesian	43	71	53	18	4	189
Ayrshire	14	17	19	1	2	53
Total	57	89	72	19	6	242
% total	23.55	36.78	29.75	7.85	2.48	

Ke,y: 0.5 (questionable). 1.0 (Very mild) 2.0 (Mild), 3.0 (Moderate), 4.0 Severe

Friesian and Ayrshire Cattle which presented the majority of Cattle breeds sampled exhibited lower levels of dental mottling. The common feature noted in the two breeds was distribution of dental fluorosis with majority of animals found to have been affected by grade 1.0, 2.0 and 3.0 dental fluorosis scores. The results revealed that about 10% of Cattle experienced both moderate to severe dental fluorosis.

4.1.5 Chi – Square Analysis for breeds

The frequency of dental fluorosis in breed of Cattle at the study location as provided in Figure 4.3.There was a significant ($P \leq 0.0071$) breed differences in the occurence of dental fluorosis in Cattle among in the study locations ($\chi^2 = 11.1123$, df = 4, P = 0.0071). Friesian had higher occurence of dental flourosis than Ayrshire across all the grade scores. Upto 43, 71 and 53 Friesians had high dental flourosis compared to 14, 17, 19 for Ayrshire in the grading scale 0.5 to 3.0. However, low levels of dental flourosis of less than 6 Cattle occured at grading scale 3 and 4.

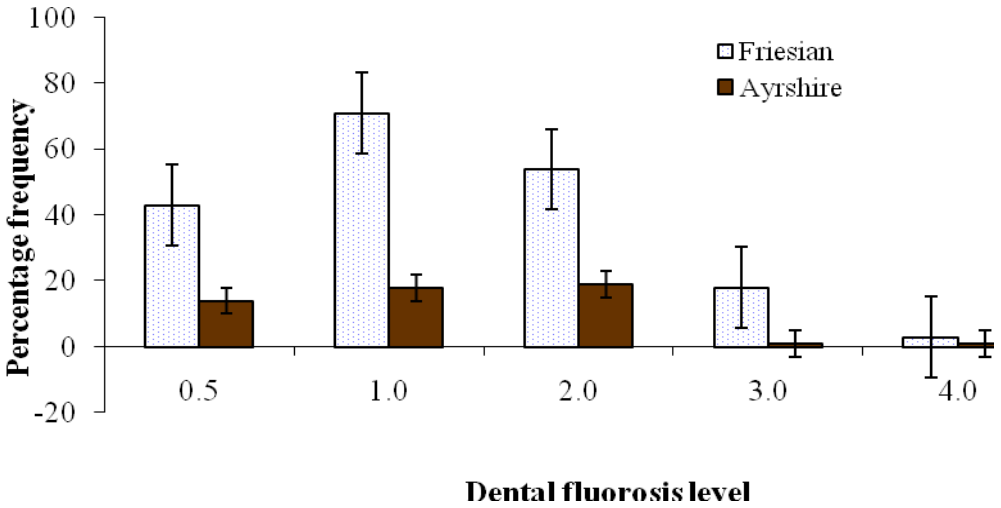

Figure 4.3: Frequency of dental fluorosis in Cattle breeds

4.1.6 Age score comparison in Cattle

The results for the age score distribution of the levels of fluorosis from the five sampling sites are shown in Table 4.4.

Table 4.4 Dental fluorosis score according to Cattle age

Grading Score	Age					Total	%total
	<0.9	1 – 1.9	2 – 2.9	3 – 3.9	>3.9		
0.5	19	17	6	6	9	57	23.55
1.0	23	12	7	4	43	89	36.78
2.0	20	10	2	4	37	73	30.17
3.0	1	6	-	2	10	19	7.85
4.0	-	-	-	2	2	4	1.65
Total	63	45	15	18	101		

Five age cohorts, < 0.9 years, 1-1.9 years, 2-2.9 years, 3-3.9 years and > 3.9 years old Cattle were considered. The elder Cattle (> 3.9 years age) were generally most affected by fluorosis. In the general comparison of the graades scores, very mild (score 1.0) and mild (score 2.0) had the highest percentages of 36.78% and 30.17% respectively. This showed that massive number of Cattle were affected within these grades category. There was a sharp drop in the proportion of Cattle exhibiting fluorosis in this range of grades 3.0 and 4.0 between the age cohort 3 - 3.9 and greater than 3.9 years. For the Cattle found with less than 2.9 year olds, the severe grade (4.0) scored nill compared to older Cattle found between geater than 3.9 years and above age cohorts which had a total of four Cattle.

4.1.7 Chi - Square Analysis for cattle age

The frequency of dental fluorosis with respect to age of the Cattle at the study location is provided in Figure 4.4. There was a significant age differences in the occurence of dental fluorosis among the study locations (χ^2 = 43.143, df = 16, P $\leq$ 0.001). Majority of the Cattle aged less than 0.9 years had dental fluorosis levels ranging from 0.5, 1 and 2. Smaller number of Cattle aged between 1 to 1.9 years scored fluorosis grade scales of 0.5, 1.0, 2.0 and 3.0. Meanwhile large majority of Cattle aged over 3.9 years had dental flourosis scale 1.0, with some registering scale 3 fluorosis and even managing to record scale 4 of dental flourosis.

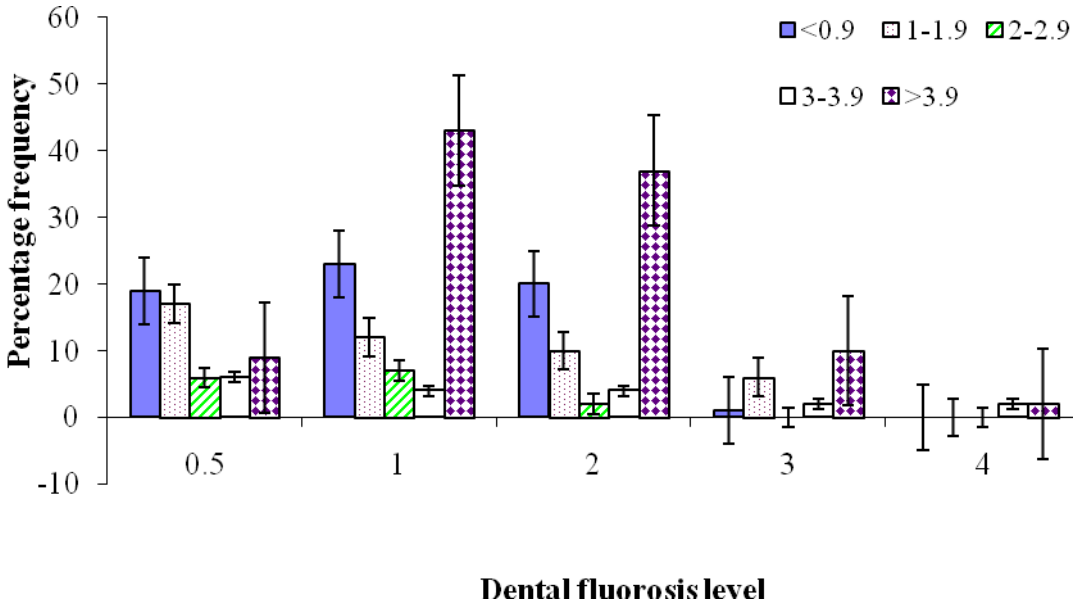

Figure 4.4: Frequency of dental fluorosis according to Cattle age

4.2. Levels of dental fluorosis in Sheep

4.2.1 Grade score comparison per region

The results for the grade score distribution of the levels of Sheep dental fluorosis from the five sampling sites are shown in Table 4.5

Table 4.5: Dental fluorosis score in Sheep per region

Sites	Grading Score prevalence					
	0.5 (Questionable)	1.0 (Very Mild)	2.0 (Mild)	3.0 (Moderate)	4.0 (Severe)	
Egerton	1	25	77	13	1	
Gilgil	4	31	33	-	-	
Naivasha	-	11	45	13	2	
Nakuru	4	5	12	1	-	
Njoro	12	9	7	-	1	
Total	21	81	174	27	4	
% total	(6.84%)	(26.38%)	(56.68%)	(8.79%)	(1.30%)	

There was variation in Sheep response to fluoride toxicity between the regions. More Sheep reported mild scores (56.7%) followed by very mild scores (26.4%). The Sheep were generally less affected with moderate (8.79%) and severe (1.30%) scores,

4.2.2 Chi – Square Analysis in Sheep

The frequency of occurence of dental fluorosis in Sheep at the study location is shown in Figure 4.5. There was a significant differences in the occurence of dental fluorosis among the study locations (χ^2 = 109.099, df = 16, P = 0.001). At Egerton, most Sheep had scale 2.0 dental fluorosis (n = 77), followed by scale 1.0 (n = 25). Scale 3.0 scored (n = 13) while both scale 0.5 and 4.0 scored (n = 1) each. At Gilgil, there was almost similar occurence of level 2.0 and level 1.0 of dental fluorosis (n = 33 and n = 31 respectively). Scale 0.5 scored (n = 4). Njoro region reported a decreasing occurence score of level 0.5, 1.0 and 2.0. Scale 3.0 and 4.0 scored n = 0 and n = 1 respectively. Very few Sheep at Egerton (n = 1), Naivasha (n = 2) and Njoro (n = 1) tested for scale 4 dental fluorosis.

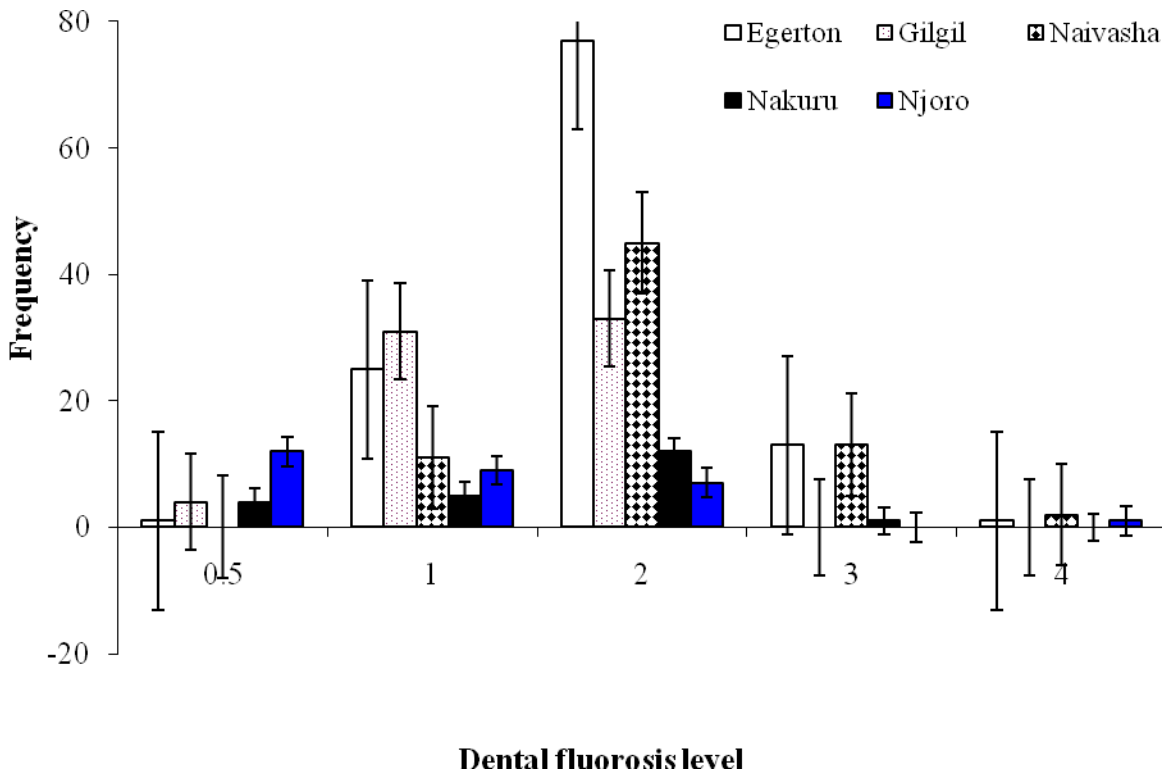

Figure 4.5: Overall frequency of dental fluorosis in Sheep

4.2.3 Comparison dental fluorosis among Sheep breed

The results for the Sheep breed grade score distribution of the levels of fluorosis from

the five sampling sites are shown in Table 4.6.

Table 4.6: Dental fluorosis score in Sheep breeds

	Grading Score				
	0.5	1.0	2.0	3.0	4.0
Breed					
Corriedale	1	25	77	13	1
Dorper	13	29	67	13	3
Maasai	4	6	9	1	-
Cross	3	21	21	-	-
Total	21	81	174	27	4
%toal	6.8%)	26.4%	56.7%)	8.8%	1.3%

All the Sheep breeds showed a skewed distribution towards the lower grade scores. On the whole, 89.9% Sheep scored between grades 0.5, 1.0 and 2.0 scales compared to higher grades scale (moderate and severe) levels that had 10.1%. From the studies, both Corriedale and doper breeds are more affected with dental fluorosis than the Maasai and Cross breeds Sheep.

4.2.4 Chi – Square Analysis in Sheep breed

The frequency of dental fluorosis in Sheep breed at the study location is provided in Figure 4.6. There was a significant breed differences in the occurence of dental fluorosis in Sheep among the study locations (χ^2 = 32.9764, df = 4, P = 0.0071). Dorper and Corriedale were the only breeds that had upto grade 4.0 fluorosis score level. Both the Dorper and Corriedale breeds each had upto 13 Sheep in grade scale 3.0 dental fluorosis. Vast majority of Sheep belonging to Dorper and Corriedale breeds had dental fluorosis graade scale 2.0. Upto 29, 25 and 21 Dorper, Corriedale and Cross breeds had grade scale 1.0 of dental fluorosis. Meanwhile, occurence of grade scale 4.0 dental fluorosis was very low among the Sheep breeds studied.

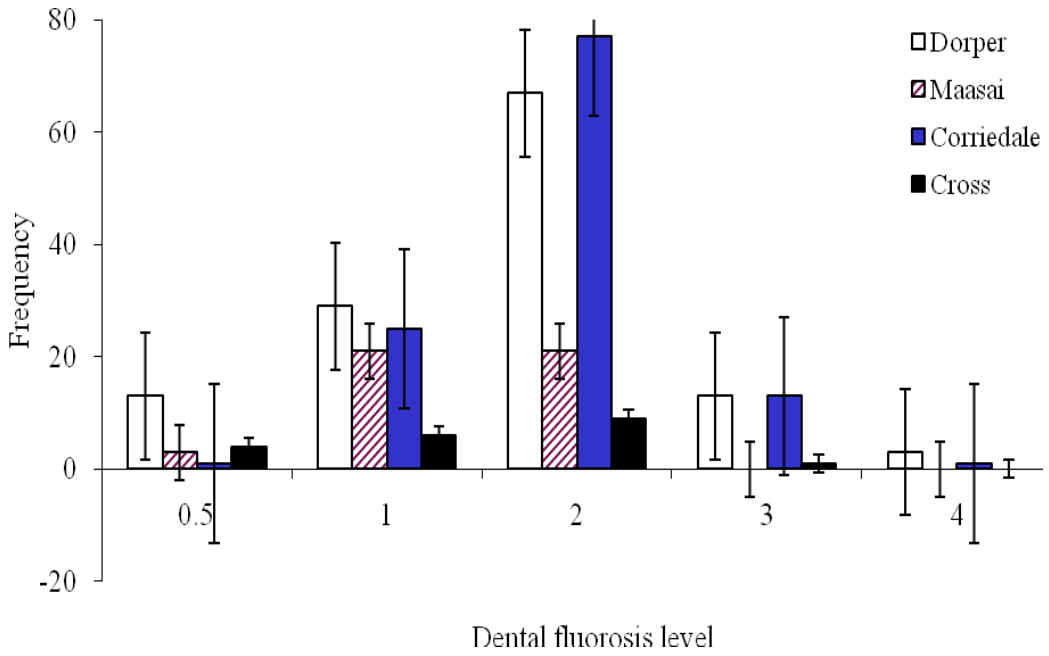

Figure 4.6: Frequency of dental fluorosis in Sheep breeds

4.2.5 Age score comparison in Sheep

The results for the age score distribution of the levels of fluorosis from the five sampling sites are as shown in Table 4.7.

Table 4.7: Dental fluorosis score according to Sheep age

Grading Score	Numbers per Age in years (yrs)					Total	% score
	<0.9	1 – 1.9	2 – 2.9	3 – 3.9	>3.9		
0.5	8	1	2	4	6	21	6.84
1.0	20	17	21	13	10	81	26.38
2.0	54	24	30	33	33	174	56.68
3.0	11	1	1	4	10	27	8.79
4.0	-	2	-	1	1	4	1.30
Total	93	45	54	55	60	307	100

Similar to Cattle, the Sheep were also grouped in five age categories of, < 0.9 years, 1 to 1.9 years, 2 to 2.9 years, 3 to3.9 years and >3.9 years old. The comparisons showed that greater proportion of Sheep were affected by dental fluorosis within dean's scores of 1.0 (26.38%) and 2.0 (56.68%) compared to corresponding values for grades 0.5, 3.0 and 4.0. There was notable trend across all the age cohorts with an increasing

number of Sheep from grade 0.5 to 3.0 scores. However, most Sheep had not reached the severe grade 4.0 but the trend is an indicative that soon they will experience severity. Therefore immediate mitigation measures need to be instituted.

4.2.6 Chi – Square Analysis for Age in Sheep

The frequency of dental fluorosis in with respect to age of the Sheep at the study locations is provided in Figure 4.7. There was a significant age differences in the occurence of dental fluorosis among Sheep at the study locations (χ^2 = 28.233, df = 12, P = 0.001). Majority of the Sheep aged less than 0.9 years scored dental fluorosis grade scale 2.0 followed by scale 1.0. The age cohort between 3-3.9 years and those aged over 3.9 years had similar number of Sheep at grade scale 2.0.

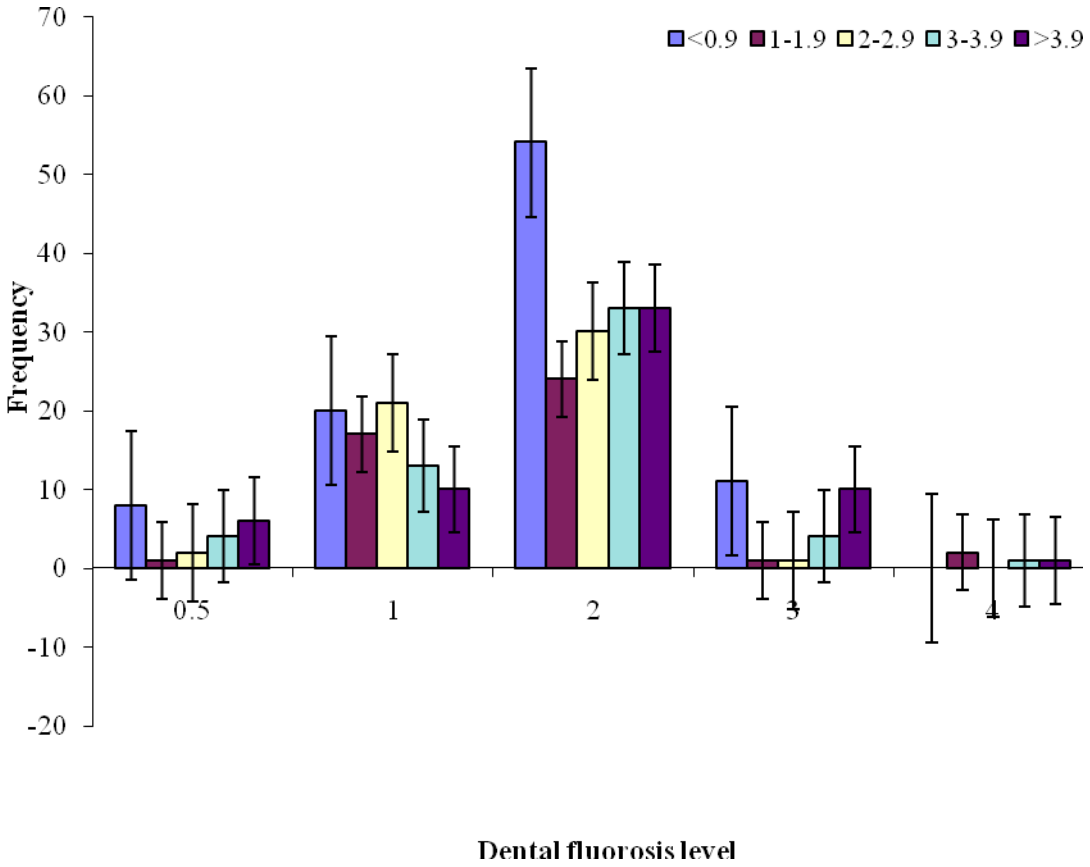

Figure 4.7: Frequency of dental fluorosis in age of Sheep

4.3 Comparing Cattle and Sheep with dental fluorosis prevalence:

Differential response to fluoride toxicity between different livestock species was then evaluated by comparing the grade score levels obtained for Cattle and Sheep in the study area. The results presented in Table 4.8, showed that both animals displayed a skewed distribution curve from median score 2.0 towards the lower scores (grade 1.0 and 0.5). A total of 219 Cattle (90.5%) out of 242 sampled for the entire study area scored questionable to mild grades of dental staining while in Sheep, 276 (89.9%) out of 307 had exhibited the same characteristics. The percentages in both species were close hence revealed a similar fluoride prevalence trends. Furthermore, only 9.5% of Cattle and 10.1% Sheep showed a prevalence of moderate to severe dental mottling.

Table 4.8: Comparing (a) Cattle and (b) Sheep to dental fluorosis per region

	Cattle						Sheep					
Grading Score	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**Total**	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**Total**
Site												
Egerton	**23(28.6%)**	**24 (30%)**	**33 (41.3%)**	**0 (0.0%)**	**0 (0.0%)**	**80**	**1 (0.9%)**	**25 (21.4%)**	**77 (65.8%)**	**13 (11.1%)**	**1 (0.9%)**	**117**
Gilgil	**6 (17.1%)**	**23 (65.7%)**	**6 (17.1%)**	**0 (0.0%)**	**0 (0.0%)**	**35**	**4 (5.9%)**	**31(45.6%)**	**33(48.5%)**	**0 (0.0%)**	**0 (0.0%)**	**68**
Naivasha	**9 (26.5%)**	**14 (41.2%)**	**8 (23.5%)**	**3 (8.8%)**	**0 (0.0%)**	**34**	**0 (0.0%)**	**11(15.5%)**	**45(63.4%)**	**13 (18.3%)**	**2 (2.8%)**	**71**
Nakuru	**0 (0.0%)**	**19 (34.5%)**	**22 (40%)**	**10 (18.2%)**	**4 (7.3%)**	**55**	**4 (18.2%)**	**5 (22.7%)**	**12(54.5%)**	**1 (4.5%)**	**0 (0.0%)**	**22**
Njoro	**19 (50%)**	**9 (23.7%)**	**4 (10.5%)**	**6 (15.8%)**	**0 (0.0%)**	**38**	**12 (41.4%)**	**9 (31.0%)**	**7 (24.1%)**	**0 (0.0%)**	**1 (3.5%)**	**29**
Total	**57 (23.6%)**	**89 (36.8%)**	**73 (30.2%)**	**19 (7.9%)**	**4 (0.2%)**	**242**	**21(6.8%)**	**81(26.4%)**	**174 (56.7%)**	**27 (8.8%)**	**4 (1.3%)**	**307**

The Plates 4.1 to 4.6 below were some of the photographs taken during the epidemiological studies of clinical examination of dental fluorosis in Nakuru County.

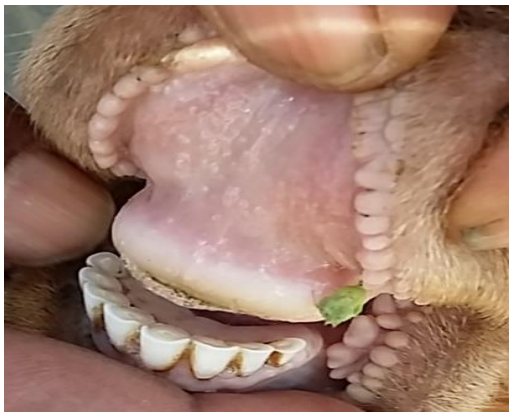

Plate 4.1: Questionable fluorosed teeth

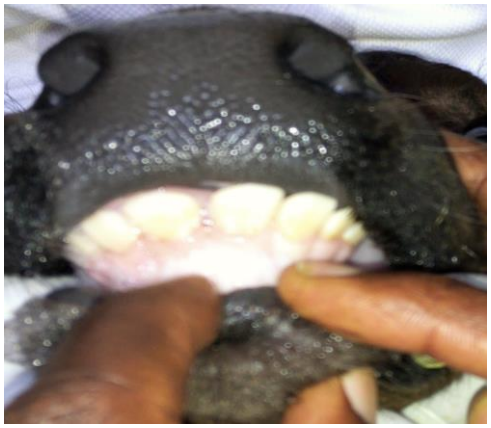

Plate 4.2: Very mild fluorosed teeth

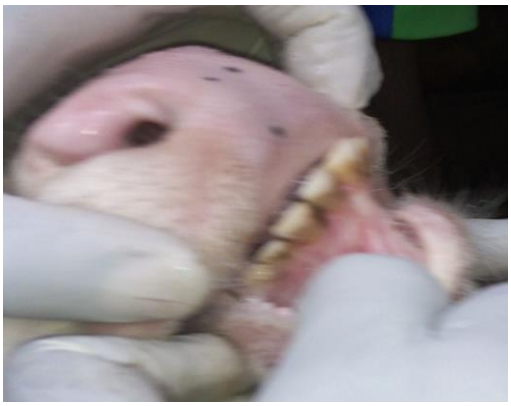

Plate 4.3: Mild fluorosed teeth

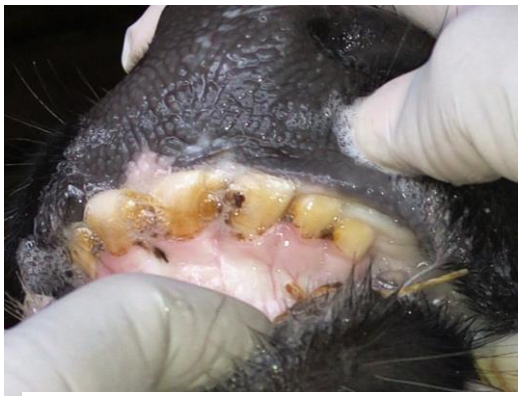

Plate 4.4: moderately fluorosed teeth

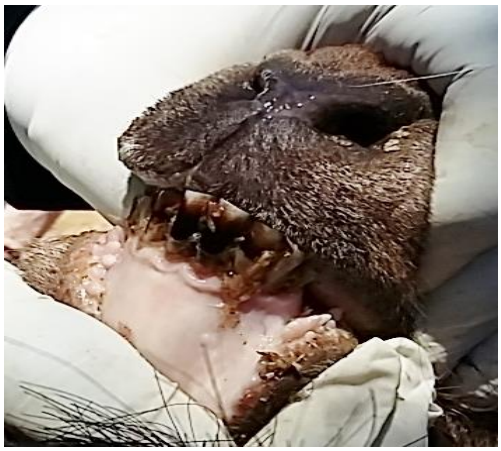

Plate 4.5: severely fluorosed teeth

Plate 4.6: Worn out teeth surface

4.4 Level of fluorides in water

The concentration of fluoride in groundwater at the sampling locations are provided in Table 4.9. There were significant ($P \leq 0.05$) spatial variation in the levels of fluorides in water (F = 52.89, df = 4 P 0.001). Highest concentration of flouride in groundwater occured in Naivasha followed by Egerton and Nakuru. Gilgil and Njoro were similar and low in concentration.

Table 4.9: Fluoride concentration in drinking water at the study areas

Sampling sites	Concentration (mg/L) ± SEM (0.433)
Naivasha	5.25 [c]
Egerton	2.75 [b]
Nakuru	2.27 [d]
Gilgil	0.36 [a]
Njoro	0.25 [a]
Overall mean	2.17mg/l

Means in the same column with the different letters superscripts are significantly different ($P \leq 0.05$) with Duncan Multiple Range

4.5 Level of fluorides in Water Sources

The concentration of fluoride in groundwater at the sampling locations are provided in Table 4.10. There were significant ($P \leq 0.05$) spatial variation in the levels of fluorides in water sources (F = 21.35, df = 2 P 0.001). Highest concentration of flouride in groundwater occured in borehole while tap water and rain water were similar and with low in fluoride concentration

Table 4.10: Fluoride concentration in different water sources at the study areas

Sampling sites	Region	Concentration(mg/L) ± SEM
Borehole	Naivasha, Nakuru,Egerton	$3.62^{b} \pm 0.409$
Rain water	Gilgil	$0.25^{a} \pm 0.006$
Tap Water	Njoro Gilgil	$0.43^{a} \pm 0.152$
Overall mean		1.43 mg/l

Means in the same column with the different letters as superscripts are significantly different ($P \leq 0.05$) with Duncan Multiple Range.

4.6 Level of fluorides in Animal Feeds

The concentration of fluoride in assorted animal feeds at the sampling locations are provided in Table 4.11. There were not significant differences in the levels of fluorides in different feeds (F = 1.928, df = 4, P = 0.111). Highest concentration of flouride in feeds occured in Gilgil followed by Njoro. Egerton, Naivasha and Nakuru were not sigificantly different in concentration of fluoride.

Table 4.11: Fluoride concentration in assorted animal feeds at the study areas

Sampling sites	Feed type	Concentration mg/kg) ± SEM (4.29)
Egerton	Indigenous grass, sunflower, barley, dairy meal, boma rhodes hay	21.70 [b]
Gilgil	Napier, indigenous grass, boma grass hay, maize stover, lucern	26.88 [a]
Naivasha	Napier, Lucerne, indigenous grass, gravellier, napier, maize silage, kikuyu grass, themeda, lemon grass, cabbages, boma grass hay.	21.84 [c]
Nakuru	Maize stover, indigenous grass, maize cobs, maize silage, boma rhodes hay, napier, Lucerne, wheat stalk hay	22.70 [d]
Njoro	Napier, maize silage, indigenous grass, boma grass hay, desmodium, Lucerne, maize cobs	23.12 [a]
Overall mean		23.25mg/kg

Means in the column with different superscripts are significantly different ($P \leq 0.05$) with Duncan Multiple Range.

4.7 Level of fluorides in Cow's Milk

The concentration of fluoride in milk at the sampling locations are shown in Table 4.12. There were significant ($P < 0.05$) differences in the levels of fluorides in different milk (F = 8.101, df = 4 P =.001. High concentration of flouride in milk occured in Nakuru at 0.147 while Egerton, Naivasha and Njoro and Gilgil were not significantly different.

Table 4.12: Fluoride concentration in cow milk at the study areas

Sampling sites	Concentration (mg/L) ± SEM (0.028)
Gilgil	0.079 [a]
Egerton	0.081 [a]
Naivasha	0.086 [a]
Njoro	0.107 [a]
Nakuru	0.147 [b]
Overall Mean	0.1 mg/l

Means in the same column with the different letters as superscripts are significantly different with Duncan Multiple Range ($P < 0.05$).

4.8 Level of fluorosis in hooves

The concentration of fluoride in hooves at the sampling locations are provided in 4.13. There were no significant ($P \leq 0.05$) differences in the levels of fluorides in different hooves ($F = 1.820$, $df = 4$, $P = 0.230$). Egerton, Naivasha and Njoro were not significantly different. Same to Gilgil and Nakuru.

Table 4.13: Fluoride concentration in hooves at the study areas

Sampling sites	Concentration (mg/kg) ± SEM (13.29)
Egerton	13.12[a]
Gilgil	16.06 [b]
Naivasha	11.74[a]
Nakuru	15.45[b]
Njoro	10.10[b]

Means in the column with different superscripts are significantly different with Duncan Multiple Range ($P \leq 0.05$).

4.9 Level of fluorosis in Faeces

The concentration of fluoride in faeces at the sampling locations are provided in Table 4.14. There were no significant differences Egerton, Naivasha and Njoro. Same to Gilgil and Nakuru. The ($P \leq 0.05$) in the levels of fluorides in different faeces ($F = 0.410$, $df = 4$, $P = 0.798$).

Table 4.14: Fluoride concentration in faeces at the study areas

Sampling sites	**Concentration (mg/kg) ± SEM (3.53)**
Egerton	17.78^{a}
Gilgil	14.06^{b}
Naivasha	18.58^{a}
Nakuru	15.72^{c}
Njoro	18.38^{a}
Overall mean	16.9 mg/kg

Means in the column with different superscripts are significantly different with Duncan Multiple Range ($P \leq 0.05$).

CHAPTER FIVE

DISCUSSION

5.1. The prevalence of Dental Fluorosis

There were variations in dental fluorosis between regions and among Cattle and Sheep as shown in Table 4.1. These could be as result of one; the varied water and soil pH in different seasons (Ghiglieri *et al.,* 2010), two; many sources of water e.g the boreholes, pans, tap waters among others that had different fluoride concentration levels. Three, the topography and climatic conditions experienced in these regions. Indeed there were evidences of fluoride contamination on the teeth enamel, however most animals were still at less alarming stages but, there is likely progression to damaging fluoride levels of dental fluorosis if mitigation measures are not put in place.

The clinical examination of teeth and the analysis of livestock forage feed, water, milk, hooves and faeces established significant fluoride concentration. The dental fluorosis in Cattle and Sheep could then be conclusively attributed to the consumption of fluoride contaminated feeds and drinking water from the region (Choubisa, 2015). Elsewhere, the study sites analysis revealed spatial differences in fluoride contamination in livestock teeth, feeds and products. These could have been caused by varied rainfall patterns, low slopes, and altitude and drainage patterns with different soils all which define these different regions (Kahama *et al.,* 1997). The frequency of occurence of dental fluorosis in Cattle and Sheep in Nakuru County were different among the sampled locations where the Gilgil and Naivasha reported more occurence of Dean's grade score 3.0 (moderate) and 4.0 (severe) while Egerton, Njoro and Nakuru reported occurence of more cases of lower forms of dental flourosis of level 0.5 (questionable), 1.0 (very mild) and 2.0 (mild)

suggesting significant levels of dental fluorosis in Naivasha and Gilgil than in Egerton, Njoro and Nakuru. The range of dental mottling scores from very mild (1.0) to severe (4.0) which was reported in the 85.8% of the total animals sampled as shown in Table 4.1.

Mottled and defective enamel is believed to be solely an indication of fluoride over-exposure during the development of the teeth. Therefore the fluorosis prevalence in Cattle and Sheep from Nakuru, Naivasha and Egerton which are in close proximity are due to fluoride concentration in the soils and water within the area. Indeed high levels of fluorides in water has been reported in Nakuru, Egerton and Naivasha (Wambu and Muthakia, 2011) due to the occurrence of rich volcanic rocks that have high content of fluoride (Jirsa *et al.,* 2013). Most rocks and soils are known to have fluoride contents. From these reservoirs, fluoride percolates downstream through ground water (Edmunds and Smedley, 2013) which empties in the drinking water sources. Studies have reported highly fluoridated lakes and other drinking water sources in Nakuru (Olaka *et al.,* 2016, Wambu and Muthakia, 2011) that are great risk to livestock and human.

In Sheep, it was observed from Table 4.5. That at high grade scores, Naivasha recorded slightly more Sheep that were affected by moderated to severe scores compared to Egerton and Njoro. This perhaps could be explained by the variations in dental fluorosis that occurred among the same species and breed despite being reared on the same farm with identical management practices. In addition, these differences could also be as a result of different seasonal weather conditions, genetical and physiological differences between the Sheep breeds sampled (Yan, D. *et al.,* 2007). Maasai and Cross breeds of Sheep are more resistance compared to Corriedale and Doper breeds as depicted in Table

4.5. Therefore it can be concluded that the Maasai Sheep and their Crosses may have an in-built genetical characteristics that safeguard them from fluoride challenges (Ganta *et al.,* 2015). However it postulated that constant exposure over time, would land more Sheep into worse stages of dental fluorosis (Choubisa, 2015).

5.2 Comparison of Prevalence rate of Dental fluorosis in both Cattle and Sheep

There was, however, slight variation in Cattle and Sheep response to fluoride toxicity between the regions. Similar results were also reported by Choubisa (2012) in the study of fluorosis in animals. At Egerton, for example, more Cattle reported questionable scores (28.6%) compared to Sheep (0.9%). Cattle were, generally, more affected than Sheep. About 23.6% of Cattle showed a questionable score (grade 0.5) compared to just 6.8% of all Sheep across all the regions as shown Table 4.8. This could be due to amount of feeds intake and differences in the metabolic processes of these two kinds of livestock (Cooke *et al,* 1990). It was observed that at high grade scores, slightly more Sheep were affected than Cattle. In the moderate (score 3.0) to severe (score 4.0) category, for example, we found that 10.1% Sheep were affected compared to 8.1 % Cattle**.** Thus, even though both Cattle and Sheep could not show significant differences in their response to fluoride toxicity, the Cattle tended to be more extensively affected than Sheep. This is consistent with what Choubisa, (2017) found out in a review of hydrofluorosis in diverse species of domestic animals in India research. Cattle are known to be highly affected with dental fluorosis (Ulemale *et al.,* 2010). This explains why relatively high numbers of Cattle were affected despite their total number being less than that of Sheep within each dean's grade scale. These differences could be attributed to the underlying anatomical

and physiological differences in the two livestock species. Cattle have larger bone structures and extensive excretory system compared to Sheep. This means that Cattle could have more efficient skeleton assimilation of fluoride minerals (Ganta *et al.,* 2015) and more efficiently get rid of unwanted fluoride in large amounts through saliva, milk and urine (Ranjan and Ranjan, 2015b) compared to Sheep.

Fluoride accumulation within the body appears to vary, even under experimental conditions and between individuals of the same species, under the same treatment (Moren *et al.,* 2007). Differences in dental fluorosis scores between animal species have also been reported. For example Choubisa, (1999) found out that Cattle, buffalo and small ruminants varied in the extent of dental fluorosis. Others factors such as differences in housing, water supply as well as variations in susceptibility, tolerance and other biological factors could as well dictate the extent of fluoride toxicity in animals. Cattle semi zero grazed. Normally enclosed in a zero grazing units or paddocks thus limiting their access to other different sources of water and calcium enriched feedstuff. The Sheep on the other hand are left to graze freely and have instinct ability choose to feed on less contaminated re-growths from plants and drink less contaminated surface water in the field (Choubisa *et al.,* 2011). Browsing characteristic nature of Sheep allow them to feed on plant leaves, pods and sprouts that are normally high in calcium, vitamin D and C which are not abate fluoride concentration (Choubisa, 2015). This could further help to qualify why Cattle are more susceptible to fluoride over-exposure than small ruminants.

5.3 Fluoride concentration in livestock feeds and drinking water

Persistent fluoride exposure through feeding and drinking contaminated water results in fluorosis. It affects mostly the developing teeth during the mineralization process of teeth

enamel (Kanduti *et al.,* 2016). Dental fluorosis is generally characterized by the presence of various enamel defects and lesions such as mottling, and increased wear, which may affect animal health and production. The high content of calcium in teeth and bones attract more fluoride deposit in these tissues (Ganta *et al.,* 2015). In this current study, the concentration of fluoride in water showed significant spatial variation ranging from the the lowest mean concentration of 0.25 mg/L in Njoro to highest mean level of 5.25 mg/L in Niavasha. Indeed the occurence of high concentration of fluorides in Naivasha and Gilgil in excess of 1.5 mg/L has been previously reported by Wambu and Muthakia, (2011) and is suspected to be as a result of volcanic topography associated with sodium bicarbonate ground water sources found in Nakuru region. Further, land characteristics that contribute to high fluoride concentration of natural groundwater depends upon geological factors, consistency of the soil, porosity of rocks, pH and temperature of the soil, complexing action of other elements, depth of wells, leakage of shallow groundwater, and chemical and physical characteristics of water (Gikunju, 1990). When groundwater percolates through rocks containing fluoride-rich minerals, fluoride leaches out and concentration may increase far above the safe level.

The assorted animal feeds had the flouride concentration levels that ranged from 21.7 to 26.88 mg/Kg dry matter and was consistently similar at all the sampling locations. Nevertheless, chronic fluorine toxicity in domestic animals can be induced by dietary fluoride concentrations of above 20 – 50 mg/Kg dry matter in most species (Blakley and Barry, 2016) over months or years, causing damage to teeth, jaw and bones. The current study reveals that the present levels of fluorine have not reached that alarming level that can cause damage to the animals even when there is prolonged exposure.

The forage and grass species are contributing factors with regard to fluoride absorption and retention and consequent levels of fluoride passed on through the livestock food chain. Forage plants absorb fluoride from soils and accumulate mostly in the roots (Zhou *et al.,* 2012). Grazing and browsing livestock obtain high fluoride concentrations from the forage roots and leaves (Singh *et al.,* 1995) as well as a significant proportion from the soil (Cronin *et al.,* 2000). For instance, Lucerne has been found to accumulate higher fluoride levels than other grasses (Botha *et al.,* 1993). The results found out that different regions showed varying degree of dental fluorosis in livestock. These regions have varied water sources and feeds available to the livestock. The pH is likely to differ between water sources and soils at different seasons (Ghiglieri *et al.,* 2010). However, drinking water is the major source of fluoride Ingested by animals of fluoride-contaminated water is widely recognized as causing significant levels of fluorosis in animals.

In the study area the majority farmers practiced semi zero grazing cattle while Sheep were left entirely to graze freely. Analysis water (Table 4.9) and forage (Table 4.11) above, indicated high fluoride concentration in these animal feeds. Therefore, the proportion of Cattle and Sheep that were suffering from dental fluorosis could be explained by the production system and feeding practices that exposed the animals to ingestion of high fluoride levels over time. Evidence suggests that more than 50% of dietary fluoride could come from soil that is ingested with feeds when grazing (Cronin *et al,* 2000). It is important to note that fluoride that are absorbed by plants, a larger proportion of it accumulate in plant roots and least in their fruits (Singh *et al,* 1995). This implies that grazing animals are more exposed to dietary fluoride than browsing animals. This would suggest that Sheep and Cattle known for their grazing were likely to show

signs of dental fluorosis compare to other animals like goats.

5.4 Dental Fluorosis and Animal Age

Dental fluorosis has been associated with accumulation of fluoride in teeth over time as a result of ingestion of contaminated feeds and drinking water. The data suggest that there was direct relationship between age and animal's dental fluorosis. Majority of Cattle (42%) observed for dental fluorosis were aged 3.9 years and above followed by 26% of younger animals at 1 year or less. In Sheep, 30% animals observed were less than 0.9 years old and 20% were 3.9 years and above. In both species, animals that were affected most were growing and mature animals. Younger animals are more susceptible to dental fluorosis because fluoride has a high affinity to calcium enriched tissues and it is incorporated in developing teeth and bone during mineralization process (Ganta *et al.,* 2015). Furthermore, fluoride is present in the milk of animals with high fluoride ingestion (Gupta *et al.,* 2015) which is another source of fluoride for the young animals, although it is also suggested that a calcium-rich protein source such as milk can be used to reduce the effects of fluorosis (Preedy, 2015). In mature animals older than 3years of age, fluoride is accumulated through many years of constant feeding on polluted feeds (Parlikar *et al.,* 2013). Variations in dental fluorosis among the age group can also indicate seasonal effects, water bodies, forage leaves and plant fruits replacement (Choubisa, 2015). It shows that the oldest cohort of Cattle may have been exposed to more severe levels of fluoride such as those precipitated by occurrence of severe droughts during there skeletal developmental stages. In the middle aged cohort of between 2 to 3.9 years old, the percentage of affected animals decreases with increasing levels of Dean's score. This could mean that this particular group of Cattle experienced more favourable conditions in

their early growth stages when the calcified tissues were most rapidly developing and they could have build up strong resistance to flouride toxicity.

5.5 Dental fluorosis among Sheep breeds

Table 4.6 Both Crosses and Maasai Sheep seem to be resistant to dental fluorosis. As the grades progressed, the Maasai and Cross breeds reported between one and zero in the moderate to severe dean's grade scores. Differences in genetics causes bone cells to respond differently to fluoride exposure (Yan, D. et al., 2007; Bronckers et al., 2009). A study by Everett et al., (2002) using highly controlled conditions for mice, found that some mice strains were far more susceptible to dental fluorosis than others to fluoride.

5.6 Level of fluoride concentration in cow milk

Excessive fluoride depresses milk production in Cattle and Sheep (Pradhan *et al.,* 2016). Lactating animals are more importantly the culprits since they are likely to consume large amounts of contaminated drinking water and forage feeds to support their physiological status. As a consequence, more fluoride concentration will be passed across to the blood via gut membranes and end up in milk (Buzalef and Whitford, 2011). The unabated consumption of fluoride-contaminated milk and milk products inevitably increases the risks of fluoride toxicity in livestock neonates and humans. In the current study, highest concentration of flouride in milk in Table 4.12 occured in Nakuru (0.147mg/l) followed by Njoro (0.107mg/l), Naivasha (0.086 mg/l), Egerton (0.081mg/l) and Gilgil (0.079 mg/l). Approximately 1.8 million people in Nakuru County (KNPHC, 2009) consume milk directly or indirectly on regular basis and therefore milk might be one other contributor to fluoride burden among residents and young animals. With this in mind, it

informed the decision to analyze the fluoride concentration levels in milk that could likely be passed on from the cow circulatory system. A study report by Gupta *et al.,* (2015) indicated that lactating cows drinking fluoride contaminated water are likely to pass on the contaminant to milk during milk synthesis. Other possible sources of likely milk contaminant are contaminated feeds and feeds supplements. It is believed that calcium in milk act as a buffer against the side effects of fluoride to consumers through its interaction between fluoride and milk (Spak *et al.,* 1995). This probably explains why there were minimum levels of fluoride concentration in the current study. While the primary sources of fluoride that cause toxicity in livestock include contaminated water, feeds and soils, available evidence show that there is no correlation between the fluoride concentration in milk and these possible sources of fluoride (Pradhan *et al*., 2016).

5.7 Level of fluorosis in animal tissues (hoof)

High concentration of flouride in hooves occured in Egerton, Gilgil, Naivasha, Nakuru and Njoro in the descending order. The accumulation of fluoride in animal hoof tissues was not uniform across the region. This perhaps could be as a result of high fluoride levels in soil water and feed from these areas. Although bones and teeth are known to be historic biomarkers for fluoride toxicity (Mehta, 2013), fluoride levels in hooves or nails can also reflect the body's fluoride burden (Buzalaf *et al.,* 2004). Available evidence shows that there is a positive correlation between concentrations in bone and hair samples (Stolarska *et al.,* 2000). Therefore collection of hooves was more practical than those of bone for which could involve slaughtering of many animals. Different tissues accumulate fluoride at different concentrations within the same species. As such, bones accumulate more than cartilage, which in turn accumulates more than skin in Siberian Sturgeon (Shi

et al., 2009); this response of tissues to fluoride ingestion can be genetic (Mousny *et al.,* 2006). In the current study, variations in dental fluorosis and fluoride burden within tissues from the same species varied greatly on the same farm despite being fed the same diet and kept under the same conditions. Differences in genetics causes hoof cells to respond differently to fluoride exposure (Yan, D. *et al.,* 2007) and a study using highly controlled conditions for mice, found out that some mice strains were far more susceptible than others to fluoride in terms of dental fluorosis (Everett *et al.,* 2002).

5.8 Ruminant breeds and dental fluorosis

It was established that among the Cattle breeds that Ayrshire were least affected by dental flourosis than Friesian. In Sheep, Dorper and Corriedale were susecptible to higher levels of dental fluorosis than Red Maasai and Crosses. Friesian and Ayrshire which presented the majority of Cattle breeds sampled exhibited lower levels of dental mottling. The common feature noted in the two breeds was a normal distribution of dental fluorosis with majority of animals found in grade 2.0 and 3.0. In Sheep breeds, there was skewed distribution towards the lower grade scores. Differences in genetics causes bone cells to respond differently to fluoride exposure (Yan, D. *et al.,* 2007; Everett *et al.,* 2002). The indigenous Sheep tolerate high fluoride levels that exotic Sheep.

5.9 Fluoride Concentration in Faeces

The body pH and type of feed consumed affects fluoride absorption across the membrane in the digestive system and amount of fluoride excreted from the body system (Buzalef and Whitford, 2011). The pH of ruminants varies from 5.5 in the rumen (Duffield *et al.,* 2004) to 2.2 in the abomasums which is highly acidic (Constable *et al.,* 2006). Low pH values in the abomasum of ruminants could result in less fluoride being absorbed into the

body from the gastrointestinal tract. In the present study, high fluoride concentration levels were excreted through faeces in different regions. Naivasha which is known for its high fluoride concentration (Wambu and Muthakia, 2011) registered the highest faecal fluoride concentration followed by Njoro, Egerton and Nakuru. Gilgil had the lowest faecal fluoride concentration compared to other sites. When there is no stronger pH gradient in the ruminant gut wall, lower absorption of fluoride across the gut lumen will be experienced. This means that more fluoride is likely to be excreted in the faeces than absorbed into the body (Moren *et al.*, 2007).

From the current work, it was found that livestock species drunk water from variety of sources. Due to ever changing climatic conditions, differences in water bodies' pH are expected to differ between the available water reservoirs at different seasons of the year (Ghiglieri *et al*, 2010).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

1. Cattle and Sheep studied showed variations in terms of fluoride content in different tissues and products which consequently when consumed by humans, may have grave implications in human health.
2. All ruminants were affected to some degree regardless of species, breed or age. Even though there was evidence of fluoride contamination in tissues, feeds, water and milk, the results revealed that most animals were still at less alarming stages. However, over time, more livestock species are likely to progress to higher scales of dental fluorosis and by extension skeletal fluorosis if mitigation measures are not put in place.
3. Fluoride concentration in water was found to range between 0.25 mg/L to 5.25 mg/L that is above the recommended of 1.5 mg/L. To lessen fluoride concentration in drinking water, there is need to enhance de-fluoridation measures to minimum levels using low cost adsorbents.
4. It is also important to provide safe water to animals in their grazing fields. Forage plants and grass species also showed significant presence of fluoride concentration ranging from 21.7 mg/kg to 26.88 mg/kg. Therefore, it is recommended to harvest and store the forage feeds during wet seasons when fluoride levels are less toxic.

From the foregoing conclusions, further recommendations are as follows:

i. There is need for further studies on low cost mitigation strategies on fluoride toxicity that are accessible and affordable to most livestock farmers. This will promote

reduction of livestock exposure to excessive fluoride through drinking water and feeds.

ii. Cattle and Sheep husbandry practices that enhance fluorosis ought to be discontinued.

iii. Further studies are recommended to investigate the influence of environmental factors including temperature and altitude on the prevalence of fluoride toxicity in livestock and their effects production.

iv. Further studies need to be conducted to assess the influence of fluoride toxicity on growth rate of immature animals, fertility and productivity so as to provide enough and effective information to the industry stakeholder on the seriousness fluoride toxicity as a threat to livestock development.

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APPENDICES

APPENDIX I: QUESTIONNAIRE FOR DENTAL EPIDEMIOLOGY SURVEY

Fluorosis in Ruminant Livestock

Sub County……..Location……

GPS - Coordinates……..

Farmer Name……

Contact……..

Code…….

Complete a separate row for each animal on the property (expand table as required)

	ITEM CODE		Age	Breed	Weight	Dental fluorosis scale 1-5	Photographs of animal been taken, record unique identifiers for all photographs	Other observations
LARGE RUMINANTS	1	BULLS						
	2	COWS						
	3	STEERS						
	4	HEIFERS						

	5 & 6	CALVES (note gender)						
	ITEM CODE		Age	Breed	Weight	Dental fluorosis scale 1-5	Have photographs of animal been taken, record unique identifiers for all photographs	Other observations
SMALL RUMINANTS	8	SHEEP (note gender)						
	8							
	8							
	8							
	8							
	8							
	8							
	8							
	8							
	8							
	8							
	9	GOATS						

		(note gender)						
	9							
	9							
	9							
	9							
	9							
	9							
	9							

APPENDIX II: DEAN INDEX OF GRADING SCALE FOR DENTAL FLUOROSIS

Table 1. *Dean's index criteria*

Score	Criteria	Definition
0	Normal	Smooth, bright, pale creamy-white translucent surface. No white discoloration of teeth.
1	Questionable	A few white flecks or white spots mainly on the edge of the incisors and cusps.
2	Very mild	Small opaque white areas covering less than 25% of the tooth surface.
3	Mild	Opaque white areas covering less than 50% of the tooth surface.
4	Moderate	All tooth surfaces are affected; a marked deterioration of occlusal surfaces; brown stains may be present.
5	Severe	All tooth surfaces are affected; discrete or confluent holes; brown stains present.

APPENDIX III: ANALYSIS OF VARIANCE TABLES FOR DRINKING WATER, FEEDS, MILK FEACES, HOOVES AND AVAIALBLE WATER SOURCES.

ANOVA	Drinking water	Sources of water	Feeds	Feaces	Milk	Hooves
df	4	2	4	4	4	4
F	52.809	21.350	1.928	0.410	8.101	1.820
P Value	0.001	0.001	0.111	0.798	0.001	0.230
Significance	S	S	NS	NS	S	NS

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