Haematological and electrolyte profile of dogs suffering from chronic dermatitis

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By
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CERTIFICATE I

This is to certify that the thesis entitled " **Haematological and electrolyte profile of dogs suffering from chronic dermatitis"** submitted in partial fulfilment of the requirements for award of degree of **Master of Veterinary Science** in subject of **Veterinary Physiology** to Orissa University of Agriculture And Technology is a faithful record of bonafide and original research work carried out by Subrat Nayak, Adm. No. 02 VPY/16 under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma

It is further certified that the assistance and help received by him from various sources during course of investigation has been duly acknowledged

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This is to certify that the thesis entitled "**Haematological and electrolyte profile of dogs suffering from chronic dermatitis"** submitted by **Subrat Nayak, Adm. No. 02VPY/16** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirement for degree of **Master of Veterinary Science** in subject of **Veterinary Physiology** has been approved by the Student's Advisory Committee and the External Examiner

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Bhubaneswar (Subrat Nayak)
Dated

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ABBREVIATIONS USED

TVCC	Teaching Veterinary Clinical Complex
CAD	Chronic Atopic Dermatitis
FI	Flea Infestation
FBH	Flea Bite Hypersensitivity
CBC	Complete blood count
DLC	Differential leucocyte count
Hb %	Haemoglobin percentage
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
TEC	Total erythrocyte count
PCV	Packed cell volume
ESR	Erythrocyte sedimentation rate
TLC	Total leucocyte count
DLC	Differential leucocyte count

ABSTRACT

The present study was conducted to record the haematological and biochemical changes in chronic dermatitis in canine of all age group presented in T.V.C.C , C.V.Sc & AH , Bhubaneswar.The history , profile of dog (breed , age , sex , weight , temperature ,past deworming etc) , owner complaint and gross appearance of dogs were studied on spot . Most of dogs had intense pruritus , tough keratinised skin , dander ,bald patches , biting and chewing of paws , scratching of back etc . Some were presented with erythrema, foul odour and extensive hairloss. Blood samples were collected with prior permission of owner and attending doctor for biochemical and physiological evaluation of parameters . The physiological parameters studied were Hb %, TEC, PCV, ESR, Erythrocyte indices (MCV , MCH , MCHC), TLC ,DC , Absolute Eosinophillic Count and the biochemical parameters studied were blood Na^+ , K^+ & Cl^- concentration . It was found that the dogs were anaemic with decreased red blood cell count and lowered pack cell volume . They had significantly elevated erythrocyte sedimentation rate . Leucocytosis with eosinophilia was significant. Lymphocyte and Neutrophil concentration was found to be in normal range . There was moderate basophilia as well moderate monocytopenia . Serum Sodium and Potassium ion concentration was within normal limits but hyperkalemia was significant .

CHAPTER I

INTRODUCTION

In small animal practice , skin complications are one of most common presentation , especially in dogs . Skin is the most accessible organ and provides enough scope to the clinician for thorough investigation of underlying etiology and further evaluation . One of most common cases of skin diseases presented in T.V.C.C , O.U.A.T , Bhubaneswar is Chronic Dermatitis .

Chronic Dermatitis is prolonged irritation and inflammation of skin resulting in skin lesions . Most common presentation is long standing itching and scratching that does not get better over time.

Constant itching and scratching of skin causes frequent wound formation and healing resulting in increased collagen content of skin which is believed to be a major cause of thickened skin . The itchiness can be nonstop or intermittent even can become habitual in long run . The thickened skin is accompanied with an exaggeration of normal skin markings such as cracks, wrinkles, or scales which gives skin a leathery or bark-like appearance also known as lichenification of skin. The patch of skin that has been lichenified is called as Lichen simplex chronicus, which is also known as neurodermatitis .The skin may also look like raised patch or patches that are red or dark. The major underlying etiology behind all these are mostly the long standing cases of atopic dermatitis , contact dermatitis & parasitic infestation .

Excoriations , erythema and alopecia are amongst the important diagnostic features of dogs presented with Chronic dermatitis. The . Alopecia and erythema may be focal or generalised covering large area of skin depending on the extent and severity of skin infection. In most cases of chronic dermatitis in dogs , signs of the secondary bacterial infection in form of papules, pustules, crusts, erosions are seen. These are generally associated with broken skin , sore skin , abscession and foul smell. Symptoms of secondary yeast infection like epidermal hyperplasia, hyperpigmentation, lichenification also can be seen .

Due to constant irritation and itch , the dog remains in hyper excitable state and under constant stress . Stress is also a trigger for dermatitis exacerbations (flare-ups), leading to increased pruritus, changes in the permeability and homeostasis of the skin, and acceleration of the immune system response , hence further aggravating the condition . The

animals remains in constant state of malaise . The major setback comes in form of altered feeding pattern , in which dog becomes increasingly anorexic and this results in loss of body condition , loss of body and subcutaneous fat and the dog looks weak and unhealthy . Sleeplessness from scratching leads to fatigue, which can increase psychological distress and constant state of depression .

The skin is the outer covering of the body and is the largest organ of the integumentary system. The skin has up to seven layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs.

There are three major components of the skin. First is the hypodermis, which is subcutaneous (just beneath the skin) fat that functions as insulation and padding for the body. Next is the dermis, which provides structure and support. Last is the epidermis, which functions as a protective shield for the body.

The hypodermis is the deepest section of the skin. The hypodermis refers to the fat tissue below the dermis that insulates the body from cold temperatures and provides shock absorption. Fat cells of the hypodermis also store nutrients and energy. The hypodermis is the thickest in the buttocks, palms of the hands, and soles of the feet. As we age, the hypodermis begins to atrophy, contributing to the thinning and aging of skin.

The dermis is located between the hypodermis and the epidermis. It is a fibrous network of tissue that provides structure and resilience to the skin. While dermal thickness varies, it is on average about 2 mm thick. The major components of the dermis work together as a network. This mesh-like network is composed of structural proteins (collagen and elastin), blood and lymph vessels, and specialized cells called mast cells and fibroblasts. These are surrounded by a gel-like substance called the ground substance, composed mostly of glycosaminoglycans. The ground substance plays a critical role in the hydration and moisture levels within the skin.

The most common structural component within the dermis is the protein collagen. It forms a mesh-like framework that gives the skin strength and flexibility. The glycosaminoglycans—moisture binding molecules—enable collagen fibers to retain water and provide moisture to the epidermis. Another protein found throughout the dermis is the

coil-like protein, elastin, which gives the skin its ability to return to its original shape after stretching. In other words, elastin provides the skin with its elasticity.

Both collagen and elastin proteins are produced in specialized cells called fibroblasts, located mostly in the upper edge of the dermis bordering the epidermis.

Intertwined throughout the dermis are blood vessels, lymph vessels, nerves, and mast cells. Mast cells are specialized cells that play an important role in triggering the skin's inflammatory response to invading microorganisms, allergens, and physical injury.

The blood vessels in the dermis help in thermoregulation of the body by constricting or dilating to conserve or release heat. They also aid in immune function and provide oxygen and nutrients to the lower layers of the epidermis. These blood vessels do not extend into the epidermis. Nourishment that diffuses into the epidermis only reaches the very bottom layers. The cells in the upper layers of the epidermis are dead because they do not receive oxygen and nutrients.

The junction between the dermis and epidermis is a wave-like border that provides an increased surface area for the exchange of oxygen and nutrients between the two sections. Along this junction are projections called dermal papillae. As animal ages, dermal papillae tend to flatten, decreasing the flow of oxygen and nutrients to the epidermis.

The epidermis is the outermost layer of the skin. Categorized into five horizontal layers, the epidermis actually consists of anywhere between 50 cell layers (in thin areas) to 100 cell layers (in thick areas). The average epidermal thickness is 0.1 millimeters, which is about the thickness of one sheet of paper. The epidermis acts as a protective shield for the body and totally renews itself approximately every 28 days.

The first layer of the epidermis is the stratum basale. This is the deepest layer of the epidermis and sits directly on top of the dermis. It is a single layer of cube-shaped cells. New epidermal skin cells, called keratinocytes, are formed in this layer through cell division to replace those shed continuously from the upper layers of the epidermis. This regenerative process is called skin cell renewal. As we age, the rate of cell renewal decreases. Melanocytes, found in the stratum basale, are responsible for the production of skin pigment, or melanin. Melanocytes transfer the melanin to nearby keratinocytes that will eventually migrate to the surface of the skin. Melanin is photoprotective: it helps protect the skin against ultraviolet radiation (sun exposure).

The second layer of the epidermis is the stratum spinosum, or the prickle-cell layer. The stratum spinosum is composed of 8-10 layers of polygonal (many sided) keratinocytes. In this layer, keratinocytes are beginning to become somewhat flattened.

The third layer is called the stratum granulosum, or the granular layer. It is composed of 3-5 layers of flattened keratin—a tough, fibrous protein that gives skin its protective properties. Cells in this layer are too far from the dermis to receive nutrients through diffusion, so they begin to die.

The fourth layer in the epidermis is called the stratum lucidum, or the clear layer. This layer is present only in the palms, and soles of the feet. It is 3-5 layers of extremely flattened cells.

The fifth layer, or horny layer, is called the stratum corneum. This is the top, outermost layer of the epidermis and is 25-30 layers of flattened, dead keratinocytes. This layer is the real protective layer of the skin. Keratinocytes in the stratum corneum are continuously shed by friction and replaced by the cells formed in the deeper sections of the epidermis. In between the keratinocytes in the stratum corneum are epidermal lipids (ceramides, fatty acids, and lipids) that act as a cement (or mortar) between the skin cells (bricks). This combination of keratinocytes with interspersed epidermal lipids (brick and mortar) forms a waterproof moisture barrier that minimizes transepidermal water loss (TEWL) to keep moisture in the skin. This moisture barrier protects against invading microorganisms, chemical irritants, and allergens. If the integrity of the moisture barrier is compromised, the skin will become vulnerable to dryness, itching, redness, stinging, and other skin care concerns.

In the very outer layers of the stratum corneum, the moisture barrier has a slightly acidic pH (4.5 to 6.5). These slightly acidic layers of the moisture barrier are called the acid mantle. The acidity is due to a combination of secretions from the sebaceous and sweat glands. The acid mantle functions to inhibit the growth of harmful bacteria and fungi. The acidity also helps maintain the hardness of keratin proteins, keeping them tightly bound together. If the skin's surface is alkaline, keratin fibers loosen and soften, losing their protective properties. When the pH of the acid mantle is disrupted (becomes alkaline)—a side effect of common soaps—the skin becomes prone to infection, dehydration, roughness, irritation, and noticeable flaking.

A number of components are common to both the dermis and epidermis. These are: pores, hair, sebaceous glands, and sweat glands.

Pores are formed by a folding-in of the epidermis into the dermis. The skin cells that line the pore (keratinocytes) are continuously shed, just like the cells of the epidermis at the top of the skin. The keratinocytes being shed from the lining of the pore can mix with sebum and clog the pore. This is the precursor to acne. If oil builds up inside pores, or if tissue surrounding the pore becomes agitated, pores may appear larger.

Hair grows out of the pores and is composed of dead cells filled with keratin proteins. At the base of each hair is a bulb-like follicle that divides to produce new cells. The follicle is nourished by tiny blood vessels and glands. Hair prevents heat loss and helps protect the epidermis from minor abrasions and exposure to the sun's rays.

Sebaceous glands are usually connected to hair follicles and secrete sebum to help lubricate the follicle as it grows. Sebum also contributes to the lipids and fatty acids within the moisture barrier. Oil production within the sebaceous gland is regulated by androgen levels (hormones such as testosterone).

Sweat glands are long, coiled, hollow tubes of cells. The coiled section is where sweat is produced, and the long portion is a duct that connects the gland to the pore opening on the skin's surface. Perspiration excreted by the sweat glands helps cool the body, hydrate the skin, eliminate some toxins (i.e., salt), and maintain the acid mantle. aiding in several functions like it regulates body temperature, prevents loss of essential body fluids, and penetration of toxic substances.

Protects body from harmful effects of the sun and radiation, excretes toxic substances with sweat, provides mechanical support etc

Normal haematological parameters indicate normal skin health. Normal Haemoglobin concentration and erythrocytic indices indicates healthy blood supply to skin and absence of anaemic conditions . Normal DC, TLC indicates absence of any infection or allergy condition. PCV & ESR in normal ranges denotes absence of inflammatory disease conditions

Mineral concentration of several minerals are indicative of skin health condition. Potassium, along with sodium, helps regulate the amount of water in body’s cells. Zinc has antioxidant properties to protect against premature aging of the skin and muscles. Zinc also helps to heal skin after an injury and is highly beneficial to all parts of the immune system. Hence presence of normal levels of electrolytes like Sodium , Potassium , Chloride etc. indicates healthy body conditions .

Dehydration, the primary consequence of a sodium and potassium imbalance in the body, can impact collagen, elastin, and the moisture layer of the skin, all of which can

lead to premature ageing, wrinkling, and sagging of the skin. Dehydration can destroy the skin's natural ability to hold water in the outer layer of the skin called the stratum corneum. The stratum corneum is made up of what is called Natural Moisturising Factor (NMF), and this moisture factor binds with water to hydrate the skin. So water consumption is crucial and helps gives skin its plump, hydrated appearance and its springiness as well.

Dehydration has other ageing effects upon the skin as well, causing puffiness in the face and around the eyes , oily skin and acne, wrinkles , dry skin , loss of elasticity due to absence of moisture to fortify elastin , collagen strands - which are chiefly composed of water, begin to crack and clump together. Dehydrated skin often feels like sunburned skin—dry and irritated along with slight burning or chapped sensation. Itching is also a symptom of severely dehydrated skin.

Chronic potassium deficiency can manifest into skin problems including acne and dry skin.

Chloride plays a important role in nutrient uptake in cells, cell growth and repair, cell control, and inhibition of cellular growth, as well as motor impulses and sensory impacts . Chloride ions are the building block of hydrochloric acid, digestive juices, which is essential to our digestive system. Without proper levels of hydrochloric acid, skin cells will not have valuable nutrients to draw from, and thus become fatigued and die. Many bacterial skin conditions can be directly linked to inadequate levels of hydrochloric acid, such as acne, Candida and bacterial infections. The skin, sometimes called the third kidney, also excretes sodium chloride through sweat. Chloride is involved in the recruitment of immune cells and plays a role in the activation of these cells. The immune system, which is charged with fighting off the daily invasion of germs, also responds to debris from damaged tissue and cells. Chloride can activate a suppressed immune response and aid in clean up of waste during such inflammatory skin conditions as rosacea, eczema, psoriasis and stage two and stage three acne.

The skin plays important role in electrolyte profile of blood .Deviation in haematological parameters responsible for delay in healing of affected skin. Therefore it is important to investigate the haematological and electrolytic profile of blood in dogs suffering from chronic dermatitis. Hence current study is designed with following objectives

1) To study the haematological profile of dogs suffering from chronic dermatitis
2) To find out electrolyte status of dogs suffering from chronic dermatitis

CHAPTER II

REVIEW OF LITERATURE

Chronic dermatitis is one of most common diseases encountered in small animal practice and there has been several efforts in past to study the details of the disease and its predisposition .

2.1 Incidence

Skin diseases are considered to be largest incidence in small animal practice .

Sischo et al. (1989) investigated the regional distributions of the most commonly diagnosed skin diseases in dogs from 17 North American veterinary teaching hospitals. Between January 1983 and December 1983, 11,456 diagnoses of skin disease were made. The 10 most common diagnoses were fleabite allergic dermatitis, skin cancer, pyoderma, seborrhea, allergy, demodectic acariasis (demodicosis), sarcoptic acariasis, immune-mediated skin disease, endocrine related skin disease, and acral lick dermatitis

Murthy *et al.* (2016)studied prevalence of ectoparasites in dogs of Shimoga, Karnataka to ascertain the status of ecto-parasites infesting dogs of Shimoga region. A total of 120 dogs with the history of skin problems presented to the local hospitals and local pet clinics of Shimoga region were screened for different ecto-parasites. The ticks, fleas and lice were collected, processed and identified. The skin scrapings were also collected from the affected dogs and processed for identification of mites. Out of 120 dogs examined, 59 (49.1 %) had harboured ecto-parasites. Among 59 infested pet dogs, 22 (37.28 %) positive for Fleas, 18 (30.5 %) for ticks, 09 (15.2 %) for Lice, 07 (11.8 %) for Sarcoptic mange and 03 (5.0 %) were for Demodectic mange conditions.

Lund *et al*.(1999) tried to determine age, breed, sex, body condition score, and diet of dogs examined at private veterinary practices in the United States during 1995, and estimate prevalence of the most common disorders for these animals. 31,484 dogs examined by veterinarians in 52 private veterinary practices in the US, 8.7% of the dogs were diagnosed with atopic/allergic dermatitis, allergy or atopy.

Okudaira *et al*.(1998) had a case study on reasons behind atopic diseases prevailing in developed countries. He suggested that the development of atopic diseases depends heavily on environmental influences rather than on the genetic background. Th2 polarization due to the modern decline in bacterial and viral infections is mostly implicated.

Nesbitt *et al.(*1978) summarized the findings of 230 cases of primary allergic inhalant dermatitis , with emphasis on diagnostic techniques and treatment. Evaluation of the response to hyposensitization was given for 132 of 139 dogs so treated. a variable prevalence of atopic dermatitis has been reported. It was estimated that 30% of cases at a private dermatology referral practice had atopic dermatitis.

Epidemiology and Clinical Features of Dermatophytosis in Dogs was studied at Louisiana State University from 1981–1990 by (Diane t. Lewis , Carol s. Foil , Giselle hosgood *et al.,* 1991) . Dermatophytes were cultured from seventy of 1824 (3.8 per cent) canine samples submitted over ten years. Microsporum canis was the most common species isolated (86/131. Both male and female dogs were equally affected by dermatophytosis. There was a higher incidence in dogs less than one year of age. Mixed breed dogs (19/70), were most often affected. M. gypseum had a greater incidence of infection in summer and most often caused localized disease in dogs. In dogs, localized dermatophytosis was more common.

Thomsett *et al.* (1977) found wide variation in ring worm associated skin disorders and found it to be a less common cause in diagnostic laboratories in united kingdom

Incidence of canine dermatitis due to mycotic origin in central India was studied by (Chittawar and Rao *et al.,* 1982) . They pointed out 18.5% of cases in 12 month period had dermatitis of fungal origin. *Trichophyton mentagrophytes* was common isolate followed by *Microsporum canis , Microsporium gypseum* and *Candida albicans* . He reported dermatitis in 3 dogs due to C. albicans out of 39 positive cases.

Weiss and Weber *et al*.(1983) had cultural examination of 2,395 skin scrappings of dogs from 1970 to 1981 reporting 311 cases of dermatitis due to dermatophyses origin that accounted for about 13% of total cases presented .

60 breeds of dogs suffering from alopecia and asymptomatic were considered for study (Cutsem and Rochette et al .,1985) in Belgium. Fungus was isolated from 8.4% of dogs who were asymptomatic and 22.5 % of dogs who were having alopecia

Ahmed *et al.(*1986) examined 50 stray dogs for parasites on Zig Zag city of Egypt , amongst which 68% of dogs were found to be infested with ectoparasites . Ctenocephalides canis was commonest ectoparasite in 50% of cases followed bt Riphicephalus sanguineus elucidated from 44% of dogs mostly found in ears , thigh and external genital regions .

Nolte *et al*.(1986) had a 10 year survey including 30, 272 dogs in Germany , inferred Demodex canis in 206 cases and 15.5 % of dogs suffering from dermatitis were 3 years or above .

2.2 **Predisposing factors**

Factors affecting the prevalence of mange-mite infestations in stray dogs of Yucatán, Mexico was studied by (Rodriguez-Vivas et al.,2002). The study was carried out in 200 stray dogs. Four samples (head, thoracic-abdominal area, extremities and ear) were taken from each animal by skin scraping and examined microscopically in 10% KOH solution to detect the presence of mites and prevalence of mite species was calculated. The overall prevalence was 34%. Demodex canis (23.0%) was the most frequent mite, followed by Sarcoptes scabei var. canis (7.0%) and Otodectes cynotis (3.5%)

A Survey of ectoparasite infestations in stray dogs of Gwang-ju City, Republic of Korea by Kyu-Sung Ahn, Shin-Eui Huh, Sang-Woo Seol, Ha-Jung Kim, Kuk-Hyun Suh, SungShik Shin et al .,(2018) investigated the flea infestation among 116 outdoor dogs (57 females and 59 males) in 8 rural areas of Jeonnam Province, Republic of Korea. Thirty-three dogs (28.4%) were infested with fleas, and all dogs were infested with

Ctenocephalides canis. One dog from Hampyeong was co-infested with *Ctenocephalides felis orientis*, but no dogs were infested with *Ctenocephalides felis felis*.

2.2.1 Fungal infection

Survey of fungal isolates from canine mycotic dermatitis in Chennai (Senthil Kumar et al.,2011) was done on total of 134 clinical samples such as hair plucks and skin scrapings from dogs with cutaneous lesions of alopecia, hyperpigmentation, and scales. Amongst dermatophytes, Trichophytan spp (23%) was frequently isolated along with Microsporum sp (3%) and Epidermophytan sp (1%). Among the non dermatophytes, Aspergillus niger (28%) was frequently isolated followed by Aspergillus fumigatus (6%),Phycomycetes sp (3%), Penicillium sp 2%, Curvuleria sp (1%) and Alternaria sp (1%). Along with the molds, yeast such as Malassezia pachydermatis (30%) and Candida albicans (2%) were also isolated.

2.2.2 Canine atopic dermatitis

Common allergens of atopic dermatitis in dogs and their comparative findings based on intradermal tests (Ha-Jung Kim, Min-Hee Kang and Hee-Myung Par et al.,2010) were studied in Veterinary Medical Teaching Hospital of Konkuk University, Korea. Intradermal tests were performed on 58 dogs diagnosed with atopic dermatitis from 2004 - 2008 to compare the allergen distribution observed in the present investigation to the results from other studies conducted in Korea . There was no significant difference in gender distribution among the dogs. The most common breeds among the 58 dogs were Maltese (n = 11) and Shih-tzu (n = 11). The average age was 4.8 years. The most frequently produced a positive reaction on the intradermal tests was mold (67.3%) followed by house dust (54.5%) and house dust mites (49.1%).

The ACVD task force on canine atopic dermatitis (XII) was done to study the relationship of cutaneous infections to the pathogenesis and clinical course of canine atopic dermatitis (DeBoer et al.,2001). It was found Dogs with atopic dermatitis (AD) frequently exhibit concurrent skin infections with Staphylococcus sp. bacteria or Malassezia yeast. Staphylococci appeared to colonize atopic skin readily, and bacterial products on the skin could augment cutaneous inflammation via immediate hypersensitivity responses to the bacteria, by superantigen-mediated lymphocyte activation, or other non-specific mechanisms. Similarly, skin colonization by Malassezia yeast could contribute to clinical

signs of atopic dermatitis, yeast components could induce inflammation via non-specific mechanisms such as alteration in mediator release, or via antigen-specific hypersensitivity reactions

Inverse association between endotoxin exposure and canine atopic dermatitis was studied by (Frank A. Looringh van Beeck *et al*.,2007). In this study, indoor exposure levels of house dust mite allergens, endotoxins and fungal glucans were measured to determine their possible association withAtopic dermatitis. A case-control study including adult Labrador retrievers with (n = 28) and without (controls; n = 65) atopic dermatitis was conducted. Dust samples were collected from the living room floor and the bedding and coat of the dog and these were analyzed for house dust mite allergens Der p1 and Der f1, endotoxin and (1 → 3)-β-d-glucan levels. The endotoxin exposure level in the coats of dogs was significantly inversely associated with Atopic dermatitis (odds ratio 0.38; 95% confidence interval 0.15–0.97; $P < 0.05$). No significant difference was found in exposure levels to house dust mite allergens and fungal glucans. The results indicated that endotoxin exposure is inversely associated with CAD, suggesting a protective effect of high indoor endotoxin exposure towards the development of the condition.

Role of genetics and the environment in the pathogenesis of canine atopic dermatitis
(Petra Bizikova et al.,2015) was studied . He sited British guide dogs indicated nearly 50% of the risk of developing AD was determined by an individual's genotype.

Papular dermatitis due to *Leishmania infantum* infection in seventeen dogs was studied by (Gabriella Lombardo *et al*.,2014) . Cytological and molecular results from fine needle aspirates of papules were diagnostic in 8 out of 13 (61.5%) cases and in 14 out of 15 dogs (93.3%). Three out of the nine dogs (33%) were positive by culture from cutaneous lesions. The three isolates were identified as ITS type A, however, polymorphism was observed in the Haspb gene (PCR products of 626 bp, 962 bp and 371 bp)

Incidence of and risk factors for atopic dermatitis in a Swedish population of insured dogs was studied by (Nødtvedt A, Egenvall A, Bergvall K and Hedhammar A et al ., 2006) . The incidence of atopic dermatitis was estimated to be 1.7 cases per 1000 dog-years at risk in a population of insured Swedish dogs whose insurance claims for the period 1995 to 2002 were examined. Several factors were found to increase the risk of having a recorded

claim, including living in a city or in central or southern Sweden, being born in the autumn, and belonging to a high-risk breed. Bull terriers had the highest risk, with 21 cases per 1000 dog-years at risk, and several other breeds including boxers and West Highland white terriers also had an above average risk.

2.2.3 Demodicosis

Prevalence of Demodectic Mange in Canines of Kathmandu Valley having Skin Disorder was studied by (Denusha Shrestha et al.,2015). The prevalence of the demodicosis and its associated risk factors from 110 canines of Kathmandu valley including both sheltered and free-roaming were studied . There was significant difference ($p<0.05$) between the prevalence rate among puppy (49.0%), adult (6.9%) and senior (33.33%). Whereas, there was no significant difference ($p>0.05$) between the prevalence rate among female (22.9%) and male (36.7%). Similarly, there was no significant difference ($p>0.05$) between the prevalence rate among short hair (40.7%), medium (25.67%) and long hair (28.5%). The association between the prevalence rate among good health status (10.7%) and poor health status (55.5%) is significant ($p<0.05$). Similarly, there was significant difference ($p<0.05$) between the prevalence rate among the free-roaming (48.9%) and the owned dogs (13.1%). At last, there was no significant association ($p>0.05$) between the prevalence rate among the pure breed (27.7%), cross (25.9%) and mongrel (37.5%).

A New Species of Hair Follicle Mite from the Domestic Dog *Demodex injai* was described from the hair follicles of a domestic dog in Columbus in october 1996 by (Clifford E. Desch and Andrew Hillier et al ., 2002) . The mites occupy follicles from the orifice down to and into the sebaceous glands

Skin disease associated with the cutaneous commensal organisms *Staphylococcus intermedius, Malassezia pachydermatis* and *Demodex canis* is frequently encountered in veterinary medicine. These potentially pathogenic commensals and their ecology were discussed with particular reference to skin biology and the surface ecosystem by (Ian s. Mason Kenneth v. Mason and David h. Lloyd et al ., 1996)

Demodex injai infestation and dorsal greasy skin and hair in eight wirehaired fox terrier dogs was studied by (Laura Ordeix, Mar Bardagí, Fabia Scarampella, Lluís Ferrer and Alessandra Fondati et al 2009).

Phylogenetic relationships in three species of canine Demodex mite based on partial sequences of mitochondrial 16S rDNA was studied by (Natalia Sastre, Ivan Ravera, Sergio Villanueva, Laura Altet, Mar Bardagí, Armand Sánchez, Olga Francino and Lluís Ferrer et al .,2012) . Demodex mites were examined microscopically and classified as *Demodex folliculorum* (one sample), *D. canis* (four samples), *D. injai* (two samples) or the short-bodied species *D. cornei* (three samples). DNA was extracted, and a 338 bp fragment of the 16S rDNA was amplified and sequenced. They found out *Demodex canis* and *D. injai* were two different species, with a genetic distance of 23.3% and also found that the short-bodied Demodex mite D. cornei was a morphological variant of D. canis.

A case control study of the risk factors for canine juvenile- onset generalized demodicosis in the USA was done by (Jon D. Plant, Elizabeth M. Lund and Mingyin Yang et al .,2010) . The case control study was conducted by searching the electronic medical records of 1,189,906 dogs examined at 600 hospitals during 2006 in order to assess the risk factors associated with juvenile- onset generalized demodicosis (JOGD) in the USA. It was found that Breeds (odds ratio) found to have the greatest association with a diagnosis of juvenile- onset generalized demodicosis (JOGD) were American Staffordshire terrier (35.6), Staffordshire bull terrier (17.1) and Chinese shar- pei (7.2). Nonbreed risk factors (odds ratio) significantly associated with a diagnosis of canine juvenile- onset generalized demodicosis (JOGD) were the diagnosis of pyoderma (5.5), coccidiosis (2.7) or hookworms (1.5), short coat (1.9) and nonenrolment in a preventative care wellness plan (1.5).

2.2.4 Lice and fleas Infestation

Studies on ectoparasites of stray dogs in Ismailia City was carried out by (Asmaa Abu Zeid,,Nahla Sallam , Eman Youssef, Amal Gayar,Ahmed Abdel Aal and Hamdy Gawady et al.,2015). 50 stray dogs of different age and sex were captured from different areas of Ismailia City and examined for ectoparasites. The detected ectoparasites were six species (one tick, three fleas, one louse and one dipteran fly). All of the examined

dogs were infested with at least one species of ectoparasites. The most prevalent ectoparasite was *Ctenocephlides canis* flea (100%) followed by *Rhipicephalus sanguineus* tick (60%), chewing lice (*Heterodoxus spiniger*) (46%), *Ctenocephlides felis* flea (44%), *Hippobosca longipennis* fly (16%) and *Echidnophaga gallinacea* flea which was detected in 4% of examined dogs. Mixed ectoparasitic infestation with two or more ectoparasite was higher (84%) than single infestation (16%). The most common combination was (fleas and ticks) (28%).

Ixodid ticks, fleas and lice infesting dogs and cats in Hawassa, southern Ethiopia was studied by (Bersissa E. Kumsa and Shewit Mekonnen ert al., 2011).They investigated the prevalence, risk factors and species composition of ticks, fleas and lice infesting dogs by taking into account 200 dogs from November 2008 to April 2009. Of the dogs examined, 99.5% were infested with one or more species of ticks, fleas or lice. A total of six different species of ectoparasites were collected and identified from dogs. *Ctenocephalides felis* was the predominant species amongst the animals, with a prevalence of 82.9% on dogs. Other prevalent species on dogs included *Ctenocephalides canis* (73.8%), *Heterodoxus spiniger* (4%), nymphs of Amblyomma spp.

2.2.5 Bacterial infection

A clinico pathological investigation was carried out on skin scrapping, skin biopsy specimens, blood, and serum samples of 210 freshly registered cases of dogs with dermatological afflictions by (M. J. Sindha, B. J. Trangadia,P. D. Vihol,R. S. Parmar, and B. V. Patel et al .,2015). Out of 210 cases of dermatoses, 60 cases were of non-parasitic dermatoses, i.e., 28.57%. Of these, bacterial skin infections (pyoderma) were found to be the predominant at 80.00%, followed by other non-parasitic dermatological disorders, i.e., 11.67% and fungal skin infection, i.e., 8.33%. The dogs belonging to age group 1-3 years showed greater susceptibility to non-parasitic dermatological conditions. Breed wise incidence of pyoderma was found more in the Pomeranian breed (20.83%), whereas fungal skin affections were found to be higher in mongrel breed (60.00% and 42.86%, respectively). Male dogs showed greater involvement in bacterial, fungal, and other non-parasitic dermatoses.

The adherence of *Staphylococcus intermedius* to canine keratinocytes in normal dogs was compared to that in dogs suffering from atopic dermatitis, primary seborrhoea and bacterial pyoderma was found by (N.A.Mcewan et al .,2002).

A case control study was done by Nicla Furiani, Fabia Scarampella, Piera Anna Martino, Ilaria Panzini, Elisabetta Fabbri and Laura Ordeix et al (2011) to evaluate and compare the bacterial microflora from the conjunctival sac of dogs with atopic dermatitis and healthy dogs. Twenty- one atopic dogs without clinical and/or cytopathological signs of bacterial blepharoconjunctivitis and 21 breed- matched healthy dogs were taken .Bacteria were recovered from 12 atopic dogs and three healthy dogs. Staphylococcus pseudintermedius was the most commonly isolated species in atopic dogs (seven of 12).

P.J.Ihrke et al ,(2007) provided an overview of bacterial skin disease in the dog.

2.2.6 Scabies

Epidemiology of sarcoptic mange in free-ranging raccoon dogs (*Nyctereutes procyonoides*) and its influence on the population of masked palm civets in Yokohama, Japanin Yokohama, Japan was studied by (N.Kidoa,M.Itabashia, M.Takahashib and M.Futami et al ., 2012) .

2.3 Clinical findings

A retrospective study of localised sarcoptic mange in 10 cases of dogs was studied by (D. Pin, E. Bensignor, D.- N. Carlotti and M. C. Cadiergues et al.,2006). In each case, lesions were localised to one precise area of the skin. Pruritus was present in nine cases and absent in one. Affected areas were the feet (one case), the face and/or the pinnae (six cases), the abdominal skin (one case), the flank (one case) and the lumbar area (one case). The types of lesions were erythema, papules, lichenification, scales, crusts and alopecia. Parasites were found in all cases except one, in which anti- immunoglobulin G Sarcoptes serology was positive.

Microbiological and histopathological features of canine acral lick dermatitis was studied by (Shumaker *et al*.,2008) . Skin scrapings and dermatophyte culture were

performed. Bacteria were isolated in 30 of 31 cases. Staphylococcus intermedius was isolated in 58% of deep cultures. Twenty per cent of deep isolates were methicillin-resistant Staphylococcus species. Forty- eight per cent of cases yielded organisms defined as multidrug resistant on deep culture. Only 57% and 55% of bacteria isolated from tissue culture were sensitive to amoxicillin- clavulanic acid and cefazolin. *Microsporum gypseum* was isolated from one dog. Histopathological features included acanthosis, follicular elongation, lymphoplasmacytic dermal inflammation, folliculitis, furunculosis, perihidradenitis, hidradenitis and vertical streaking fibrosis. Cytology and superficial cultures did not correlate well with deep cultures. Surface culture predicted deep tissue isolates in eight of 22 cases.

Protein oxidative damage in the stratum corneum (Yukie Niwa et al.,2003) provided evidence for a link between environmental oxidants and the changing prevalence and nature of atopic dermatitis in Japan. An association between atopic dermatitis severity and markers of ROS- associated damage was found indicating that environmentally generated ROS may induce oxidative protein damage in the stratum corneum, leading to the disruption of barrier function and exacerbation of atopic dermatitis.

Characterization of pruritus in canine atopic dermatitis, flea bite hypersensitivity and flea infestation and its role in diagnosis was studied by (Vincent Bruet et al.,2012). Three hundred and forty- six dogs were analysed, 91 with CAD, 110 FI and 145 FBH. The period (season) of onset was not statistically different either for each dermatosis or among the three dermatoses. Some locations were highly specific for one dermatosis as follows: ventral abdomen/medial surface of thigh (chewing) and radius/carpus/tibia/tarsus (chewing) in FI; back/dorsolumbar area (chewing) and tail (chewing) in FBH; and paws (chewing/licking) and face/neck (rubbing) in CAD.

Breed- associated phenotypes in canine atopic dermatitis was studied by (Claude Favrot et al.,2010) and demonstrated the existence of substantial differences between the clinical phenotype of each breed and the whole population

Clinical and histological manifestations of canine atopic dermatitis were studied by (Petra Bizikova et al.,2015)

Wittich et al.(1941) published the first really detailed description when he reported a case of 'spontaneous allergy (atopy)' in a dog with rhinitis, conjunctivitis and urticaria. He was able to demonstrate allergic sensitisation to ragweed pollen and a response to allergen-specific immunotherapy (ASIT). Further studies suggested that pollen exposure could induce the formation of allergen-specific antibodies, and that subsequent allergen exposure could result in atopic conjunctivitis, rhinitis, asthma, pruritus and anaphylaxis, although not what we would now regard as canine atopic dermatitis.

It was first shown that dogs suffer from allergic 'eczema' in the 1930s, although these early studies were limited to food allergens (Burns et al .,1933, Schnelle et al.,1933, Pomeroy et al., 1934)

Schwartzman et al.(1965) first linked respiratory disease and pruritus with the diagnosis of 'atopy'. He proposed that atopic dogs became sensitised following inhalation of allergens. The allergen-specific IgE would then bind to mast cells, triggering the release of histamine and other mediators following subsequent allergen exposure.

2.4 Haematological and biochemical studies

Diagnostic significance of haematological changes associated with various canine dermatoses was studied by (Nair and Nauriyal et al.,2007) Blood samples were collected from 151 dogs suffering from various skin diseases, like pyoderma, fungal infection, demodicosis, scabies, arthropod reaction, flea-bite dermatitis, contact dermatitis, seborrhoea, allergy, mixed infection/infestation, otitis externa and miscellaneous dermatoses. The blood samples were examined for haemoglobin, total erythrocyte and leukocyte counts, erythrocyte sedimentation rate, haematocrit and differential leukocyte count The average value of each parameter of haemogram from the cases of dermatitis was compared with the corresponding mean of the normal control group of 13 healthy dogs. The results of the study revealed significantly lower average haemoglobin concentration in dermatitis cases especially pyoderma, demodicosis, arthropod reaction, contact dermatitis and mixed condition. Significantly lower average total erythrocyte count was observed in general and individually in pyoderma, demodicosis, arthropod reaction, flea-bite dermatitis, allergic dermatitis, seborrhoea, contact dermatitis and mixed conditions. Significant

decrease in PCV was observed for cases of arthropod reaction and contact dermatitis. In conditions like pyoderma, contact dermatitis and mixed infection/infestation, statistically significant leukocytosis was observed. Differential leukocyte count revealed neutrophilia in cases of pyoderma and contact dermatitis; lymphocytosis in contact dermatitis; lymphopenia in mixed conditions and eosinophilia in scabies, demodicosis, arthropod reaction, contact dermatitis, mixed condition and miscellaneous disorders.

Variations in standard blood count and biochemical parameters in dogs suffering from atopic dermatitis was studied by (Ozana Maria dulman, Alina anton and Gheorghe solca et al., 2015). From 15 dogs examined ,6 dogs showed raised levels for Ca and in 4 of these were correlated with low protein level.Regarding CBC, in 7 out of 15 cases ,there was an obvious response to the inflammatory process that takes place in the dermis. Lymphopenia and eosinophilia were the main findings associated with neutrophilia and the left deviation of the Arneth index (more than 80% neutrophils with 1-3lobes).This indicates a strong corticoid reaction with systemic stress and the start of an infection (increased WBC), most likely a superficial pyoderma.

Symptomatology and haemato-biochemical changes in dogs suffering recurrent Pyoderma was studied by (Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,2016). No significant difference in the decrease of PCV was recorded. However, a highly significant decrease in the values of haemoglobin and TEC was recorded. A highly significant increase in total leucocyte count, absolute neutrophil count and absolute eosinophil count were recorded.

Clinico-pathological Studies on Atopic Dermatitis in Dogs done by (Rasmeek Kaur Brar, P.S. Dhaliwal, Ashwani Kumar, Sushma Chhabra and S.K. Uppal et al 2017) . They found the haemoglobin concentration did not changed significantly in affected dogs as compared to healthy dogs. Leukocytosis was observed in atopic dogs when compared to healthy ones. Neutrophilia was also observed in atopic dermatitis affected dogs.

Case report on dermatitis due to mixed demodex and sarcoptes mites in dogs (Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al 2015) showed haematological abnormalities included reduced total erythrocyte count, haemoglobin concentration,leukocytosis,neutrophilia,and eosinophilia.

Haematobiochemical studies in dogs affected with bacterial dermatitis was studied by (Shyma and Vijayakumar et al.,2011). They found infected group of dogs had significantly lower mean values of haemoglobin content, volume of packed cells, total erythrocyte count, total leucocyte count. Differential leucocyte count in infected animals indicated neutrophila (69 per cent) and reduced levels of eosinophils and monocytes (1.75 per cent and 0.42 per cent respectively). The values of albumin were significantly lower than the mean values of control animals and that of total protein and globulin was higher than those of control animals. Hypercholesterolemia and hypoglycaemia was also seen .

Haemato-biochemical alterations in canine dermatitis was studied by (Sindha et al .,2015). The average values of Hb, PCV, and TEC were significantly lower in bacterial , fungal and other non-parasitic dermatological conditions while TLC values were found significantly higher in all cases of non-parasitic dermatoses as compared with control. The parameters such as serum globulin, A/G ratio in bacterial, fungal, and other non-parasitic dermatological conditions showed a significant difference whereas other parameters were non-significant.

Haemato-biochemical findings and thyroxin levels in canine demodicosis was studied by (Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al 2014). They found dogs affected with generalized demodicosis revealed significant reduction in total erythrocyte count and haemoglobin levels. All the dogs had normal packed cell volume. Affected dogs also showed leukocytosis accompanied by neutrophilia, eosinophilia and lymphopenia. Dogs had normal serum protein levels, normal A/G ratio, reduced serum albumin levels, increased serum globulin and cholesterol levels. Dogs with demodicosis did not show any significant difference in total T4 and free T4 levels when compare with health dogs.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental design

The present study was undertaken in Department of Veterinary Physiology & Teaching Veterinary Clinical Complex , College of Veterinary Science and Animal Husbandry, O.U.A.T , Bhubaneswar during period from February ' 18 to June '18.

About 15 dogs suffering from chronic dermatitis presented at TVCC, C.V.Sc & AH, OUAT , Bhubaneswar during above said period were taken into consideration for research purpose .

The history , profile of dog (breed , age , sex , weight , temperature ,past deworming etc) , owner complaint and gross appearance of dogs were studied on spot .

Most of dogs had intense pruritus , tough keratinised skin , dander ,bald patches , biting and chewing of paws , scratching of back etc . Some were presented with erythrema, foul odour and extensive hairloss.

The blood samples were collected with prior advice of veterinary doctor on duty were taken for study .The blood samples were examined for electrolytes and haematological status of diseased dogs .

The parameters studied were Hb %, TEC, PCV, ESR, Erythrocyte indices (MCV , MCH , MCHC), TLC ,DC , Na^+ , K^+ & Cl^- concentration.

The data will be analysed to establish electrolyte and haematological alternation if any in dogs suffering from chronic dermatitis

Fig.No. 1 **German sepherd dog presented in clinic with chronic dermatitis resulting from sarcoptic mange**

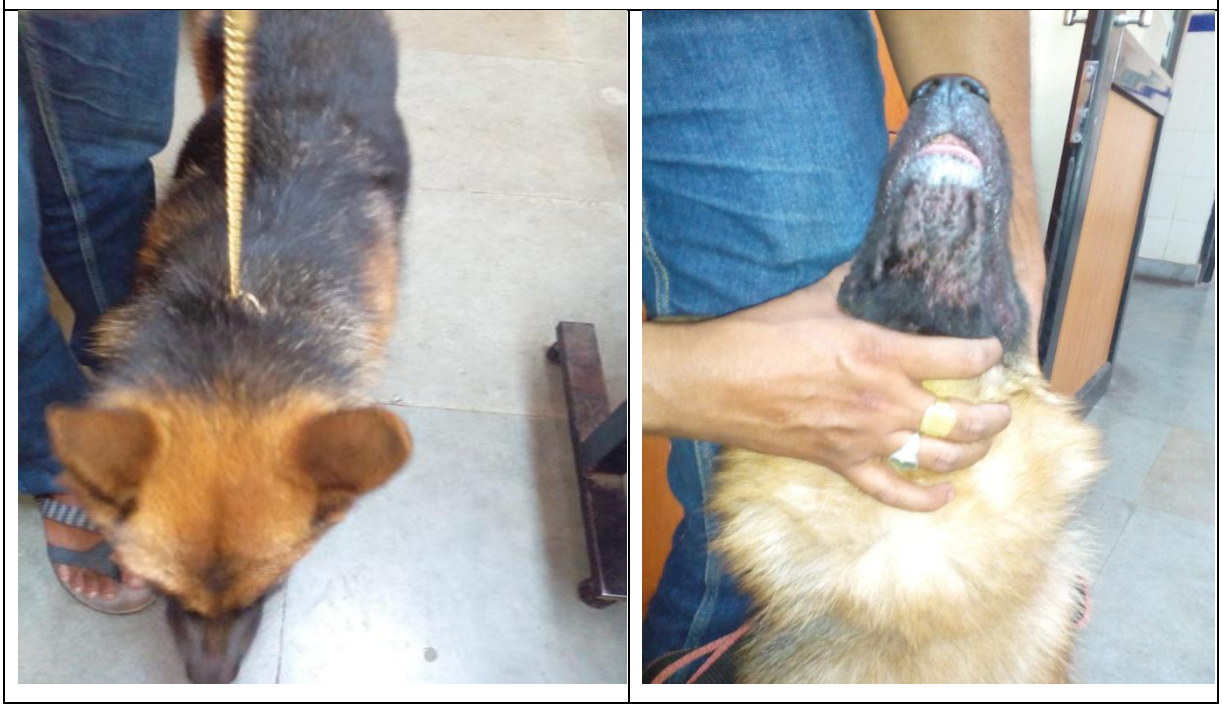
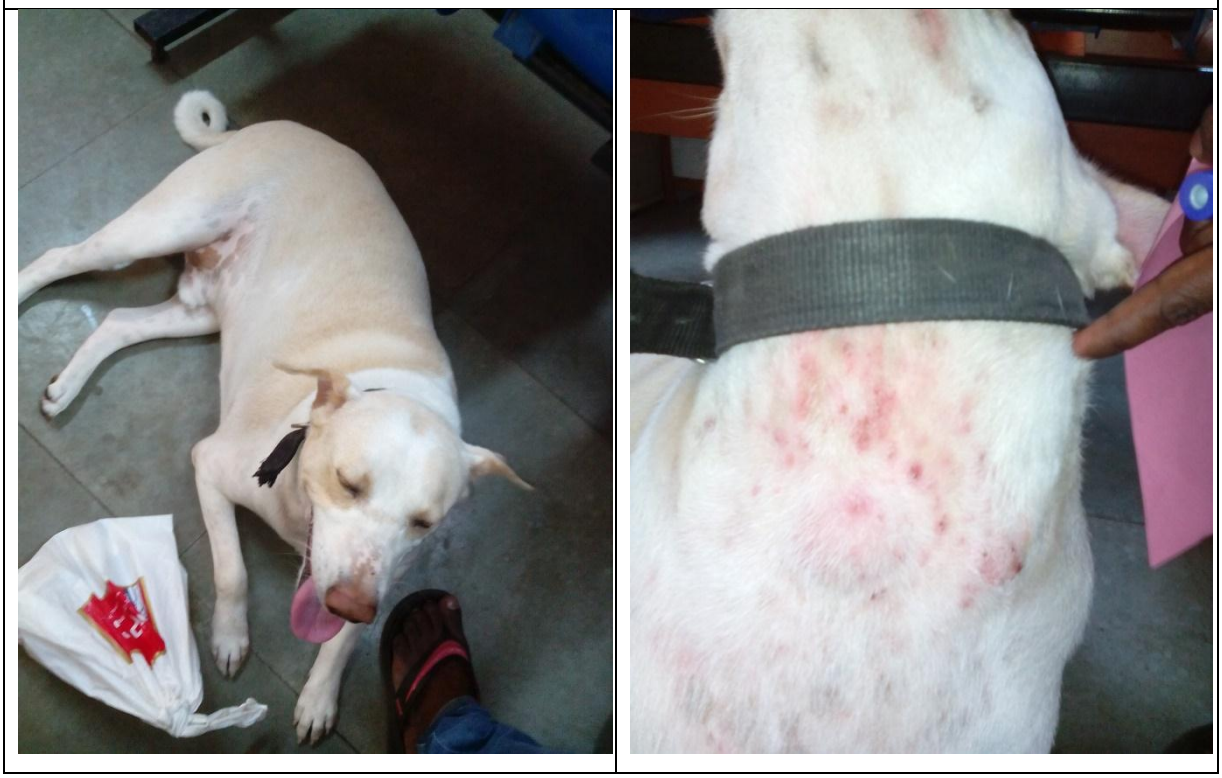


Fig.No.2 **Labrador presented in clinic with chronic dermatitis resulting from atopic dermatitis**



3.2 Blood collection

5 mL of blood was collected from dogs suffering from chronic dermatitis Cephalic and via 5 mL Dispovan single use syringe having 21G scalpvein needle .2mL of blood drawn was transferred to EDTA vial and 3 mL to clot vial . These vials were then transferred to laboratory for physiological and biochemical estimation by maintaining suitable cold chain

3.3 Haemoglobin percentage

(Hb) is the main component of the erythrocyte which is a conjugated protein and serves to transport CO_2 and O_2 in the body.

Haemoglobin concentration was studied by Sahli's Hemoglobinometer

Principle

A measured volume of blood was converted to acid hematin with dilute hydrochloric acid and the hematin solution is diluted drop-wise until it matches with brown glass standards of the hemoglobinometer and the reading is noted.

Materials/Chemicals/Apparatus required

Sahli's hemoglobinometer, 0.1N HCI, distilled water and blood sample.

Procedure

- The graduated measuring tube was filled with N/10 HCl
- Then exactly 20µl (0.02ml) of blood sample was added to the hemoglobinometer tube with the help of hemoglobin pipette.
- It was mixed properly with the help of a glass stirrer.
- The tube was allowed for to stand for 5–7 minutes for formation of acid hematin.
- Now the hematin solution was diluted with distilled water till the color matches with glass standards
- The lower meniscus was read while taking the readings.
- This gave Hb concentration as gm per 100 ml of blood or gm% or gm per dl of blood

Advantages of Sahli's method

i) Low cost

ii) Being portable can be taken in field condition.

iii) Give immediate results.

Precautions

1. Exactly 0.02 ml (20µl) of blood was taken and excess of blood adhering to outside of pipette was wiped away.
2. HCI should be exactly 0.1N.
3. There should be proper mixing of the acid and blood for complete formation of acid hematin.
4. Properly anticoagulant mixed blood should be taken.
5. There should not be any air bubble in the pipette.
6. Read lower meniscus while taking the reading in the hemoglobinometer tube

3.4 Determination of erythrocyte sedimentation rate (ESR)

ESR is defined as the speed at which erythrocytes fall down in a vertical column of undisturbed blood sample .

Principle :- Blood cells being heavier than plasma settle down when they are allowed to stand in a vertical column without disturbance

Materials required :- Wintrobe hematocrit tube , syringe with long needle.

Procedure :- It was estimated by Wintrobe hematocrit tube method.

- Wintrobe hematocrit tube was 11 cm long tube having an uniform bore of 3 mm . It was calibrated by a double 10cm scale . On the left side calibration was from top to bottom and on right side calibration is from bottom to top.
- The Wintrobe hematocrit tube was filled upto zero mark at the top with the help of a syringe and a long needle
- This tube is kept in Wintrobe's stand in a vertical position at a constant temperature
- The initial reading was noted at zero

- Final reading was taken after half an hour or one hour
- ESR was recorded in mm per Ist hour or mm per 2nd hour in real time scale.

3.5 Determination of packed cell volume(PCV) or hematocrit value

The word 'hematocrit' is derived from two Greek words, Haima meaning blood, and Krinein meaning to separate i.e. to separate blood.

Principle :-

PCV is defined as percentage of total volume occupied by packed erythrocytes when a known volume of whole blood is centrifuged at a constant temperature in a specific time.

PCV was determined by Wintrobe hematocrit tube method (Macro Hematocrit method)

Materials Required

Wintrobe tube, syringe with long needle and centrifuge machine.

Procedure

- The blood sample was taken in the syringe with long needle
- The needle was inserted in Wintrobe's hematocrit tube so that Wintrobe tube gets completely filled to the top mark 10 without any air bubbles.
- Level of blood sample was made up to mark 10 on right side of the Wintrobe tube where calibration was from bottom to top.
- The tube was put into the centrifuge machine
- It was rotated at the speed of 3000 rpm for 30 minutes.
- After centrifugation , erythrocyte mass was seen at the bottom of the tube is called as PCV.
- A white to gray layer of leucocytes and thrombocytes (platelets) occurring immediately above the red cell mass was called as buffy coat and then there was plasma.
- Then packed red cell level found was multiplied by 10
- This gave the PCV per 100 ml of blood or PCV in percentage.

Precautions

- Wintrobe tube must be clean and dry.
- There should not be any air bubble in the tube while taking blood.
- Proper amount of anticoagulant should be used
- Centrifugation should be at the constant speed.

3.6 Total erythrocyte count (TEC)

Principle Measured quantity of blood was 200 times diluted with isotonic solution (which prevented hemolysis of erythrocytes) and was placed on hemocytometer and number of cells were counted under high power of microscope.

Haemocytometer:

- Routinely used haemocytometer was Neubauer haemocytometer, which consisted of two chambers and each chamber consisting of 3 x 3 x 0.1mm dimension
- It had a volume of 0.9 cu mm.
- The ruled area in each chamber consisted of 9 large squares each of 1 x 1 x 0.1mm dimension and a volume of 0.1 mm.
- Each of the four corners squares measured 0.25 x 0.25 x 0.1mm dimension
- This had a volume of 0.00625 mm and these corner squares were used for counting of WBCs.
- The central large square was divided into 25 smaller squares each of 0.2 x 0.2 x 0.1mm dimension
- This had a volume of 0.004 cu mm.
- Each smaller square was further subdivided into 16 smaller squares.
- This central square was used for counting of RBCs.

B) **Materials /Chemicals/ Apparatus required**

Hemocytometer, Tissue paper, Microscope

RBC diluting fluid, Blood sample

This fluid should have the following properties:-

a) It should be isotonic to blood so does not cause lysis of the RBCs.

b) It should contain a preservative so that it preserves the shape of RBC and prevents autolysis for longer period and makes it possible to count cells even after several hours of dilution.

c) It should prevent agglutination of cells.

RBC diluting fluid -- **Hayem's solution**

Sodium sulfate	2.5gm
Sodium chloride	0.5gm.
Mercuric chloride	0.25 gm.
Distilled water to make	100 ml.

This RBC diluting solutions should be used within three months of preparation.

Procedure

- RBC diluting pipette was filled with blood to the mark of 0.5
- RBC diluting fluid was added up to the mark of 101
- It was mixed for two minutes
- The cover slip was placed over the hemocytometer and fixed.
- The fluid was discarded from the stem of the pipette.
- The tip of the pipette was applied at the junction of hemocytometer chamber and cover slip such that diluted blood flows under the cover slip by capillary action.
- To facilitate the uniform flow, The pipette was rotated slowly by finger.
- The counting chamber were filled without any air bubble and there was no overflow.
- It was allowed five minutes for setting of cells.
- The cells were counted in five small squares (four corners and one central square) of the central large square under high power of microscope.
- The cells were counted on lines which touched left and lower margin
- The cells which touched right and upper margin of the squares were excluded.
- The cells were added from all the five squares
- The TEC was calculated as following:

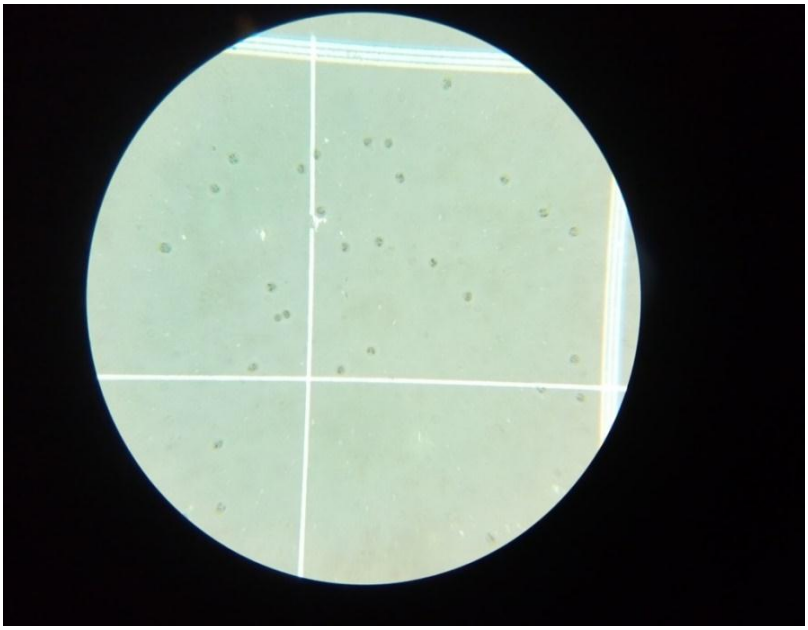


Fig.No. 3 Leucocytes seen in Neubauer's slide under 100X

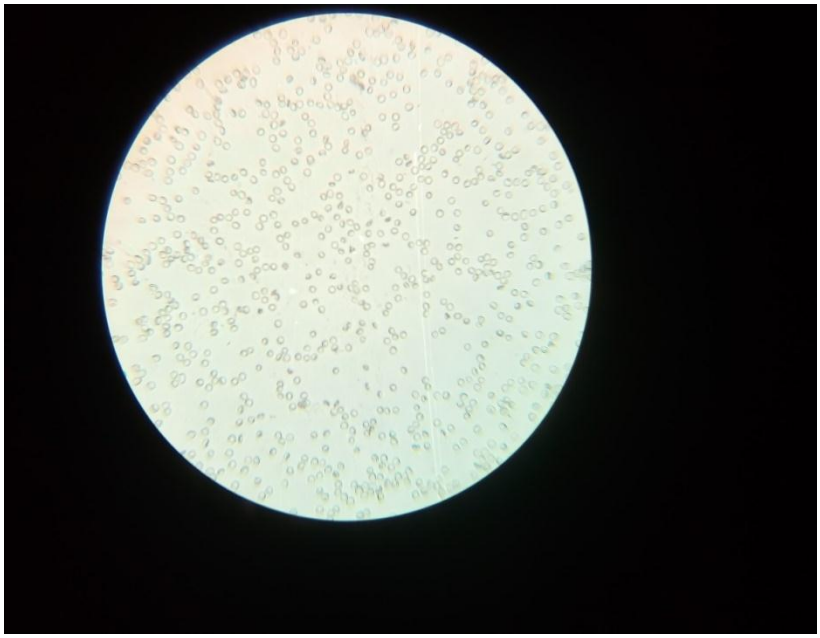


Fig.No.4 Erythrocytes seen in Neubaur's slide under 100X

Calculations

The number of cells in five medium squares or 80(16x5) small squares = 550

Depth is. 0.1mm

Length of large square is 1.0mm

Width of large square is 1.0 mm

Volume of one large square is: 1x1x0.1=0.1 cubic mm

Or

Volume of 25 medium squares is 0.1 mm^3

So volume of 5 medium squares = 1/50 mm^3

1/50 mm^3 of 200 times diluted blood has 550 numbers of RBC.

1 mm^3 of whole blood is 550 x 50 x 200 = 550 x 10,000 numbers of RBCs

Dilution of blood is 200 times

Results :-

TEC was expressed as millions per cubic mm of the blood or millions per microlitre of blood

(PCV/6) = TEC in millions per cubic mm

PCV/3= Hb gm%

Precautions

- Hemocytometer should be clean and dry.
- Exact quantity of blood was taken (upto the mark 0.5) and dilution was done carefully
- Excess blood from the tip of the pipette.was wiped off'
- There was proper mixing of blood and diluting fluid.

- Proper filling of the counting chamber was done without any air bubble
- 2-3 drops of solution were discarded from stem of the pipette before charging the hemocytometer.

3.7 Total leucocyte count (TLC)

Principle

Blood wass diluted in a special pipette with WBC diluting fluid which hemolysed the erythrocytes but not leucocytes.

These diluted cells were placed on hemocytometer and then counted under microscope.

Materials/ Chemicals / Apparatus required

Hemocytometer, Microscope, Tissue paper, WBC diluting fluid, Blood sample

WBC diluting fluid

Turks WBC diluting fluid

Glacial acetic acid	2ml
Gention violet (1% aq.solution)	1ml
Methyl violet	1 drop
Distilled water to make	100ml

WBC diluting fluid should have the following properties:

(a) Should be hypotonic to blood i.e. it should cause lysis of RBC and WBC lose their cytoplasm and their nuclei become prominent.

(b) Should have fixative to fix WBC.

(c) Should have stain to stain WBC.

Best result was obtained when this solution was used within three months of its preparation

Procedure :-

- The blood was filled up to 0.5 mark in WBC diluting pipette
- The excess blood adhering to the outside of the pipette was wiped off
- WBC diluting fluid was drawn to the 11 mark
- The diluting fluid was mixed with blood by rotating the pipette for two minutes.
- Then first few drops were discarded from the stem of the pipette .

- The cover slip was fixed on the Neubauer's slide.
- The chamber of hemocytometer was charged
- By touching the tip of pipette at the junction of edge of Neubaur's slide and cover slip liquid was poured till the chamber was filled with the diluted blood without any air bubble.
- Then cells were allowed to settle down in the chamber for five minutes.
- Then the leucocytes were counted under low power (10X) of the microscope in the four corner large squares.
- The cells which touch the left and lower margin of the square were counted
- The cells which touchrd the right and upper margin were excluded

Calculation :-

Number of leucocytes in four large squares = 160

Volume of one large square = 1x1x0.1=0.1cmm or 0.1 mm^3

Volume of four large square = 4x0.1 = 0.4 cmm or 4/10 mm^3

Thus 0.4 cmm of diluted blood contains cells =160

1 cubic mm of diluted blood contain cells = 160 x10/4

Dilution factor is 1:20

So, 1 cubic mm of blood will contain = 160 x 10/4 x 20= 160 x 50 numbers of leucocytes per cubic mm

Precautions

- Pipette and hemocytometer should be clean and dry.
- Exact measuring of blood upto mark 0.5 in the pipette and proper dilution of sample was to be done.
- Excess blood was wiped off from the tip of the pipette.
- There was proper mixing of blood and diluting fluid.
- Proper filling of the counting chamber without any air bubble had to be done**.**
- 2-3 drops of solution were discarded from stem of the pipette while charging the Neubaur's slide

3.8 Differential leucocyte count (DLC)

DLC is the counting of different types of leucocytes which are classified as:

a. Granulocytes (Neutrophil, Eosinophil and Basophil)- which contain granules in their cytoplasm and such leucocytes are neutrophils, eosinophils and basophils.

b. Agranulocytes (Lymphocytes and Monocytes)- which do not contain granules in their cytoplasm and such leucocytes are lymphocytes and monocytes.

Principle

Different leucocytes in a thin smear of blood were stained by specific stain which helps in differentiation of different leucocytes by identification of size, shape of nucleus and presence or absence of granules in the cytoplasm and colour of granules.

Materials/Chemicals/Apparatus Required:-

Microscope, Glass slide, Staining rack, Cell counter, Filter paper

Distilled water, Alcohol , Blood sample

Giemsa's stain

Giemsa powder	3.8gm
Glycerine	200 ml
Methanol	312 ml

For working stain , dilute 1:10 with water

Procedure

Preparation of the blood smear

Blood smear is prepared for following purposes

i) To determine DLC

ii) To know size and shape of erythrocytes

iii) To know parasitic or protozoan infection if any

- For preparation of blood smear , a very small drop of blood was taken on a clean ,dry and greese free glass slide .
- Another slide was picked up with smooth edge
- This was used as spreader slide.
- The spreader was slid holding it with the thumb and the middle finger
- It was moved near the drop of blood
- As it came in contact with the drop of blood, the blood did spread along the edge of spreader slide
- The spreader slide was moved forward smoothly to get a blood smear.
- It was dried quickly.
- The angle at which spreader was held ,determined the thickness of the smear.
- Greater the angle , thicker was the smear
- 30° angle was satisfactory for preparation of blood smear for DLC.
- Anterior end of the smear was called as feather end.
- Good smear should be thin, evenly spread.

Staining of the smear by Giemsa stain

- The slide was placed on the staining rack
- then the smear was fixed with a few drops of absolute alcohol for 5 minutes
- It was dried in air.
- Then freshly diluted (1:10) Giemsa stain was put on the smear for 40 minutes
- Then the smear was washed with distilled water and dried

Counting of different leucocytes

Thestained slide was examined under oil immersion lens(100x) of the microscope and 200 different leucocytes were counted with the help of their characteristic identifications and then the percent of different leucocytes was found.

The different leucocytes were identified as per following characteristics :-

Granulocytes

A. Neutrophils

- These were quite large cells, having about 10-15µm in diameter

- Nucleus was blue stained with 3-7 lobes.
- Cytoplasm was faintly pink stained with granules which are observed after careful observation.
- Increased neutrophil count was seen in acute inflammatory reactions, stress, pain bacterial or viral infections.
- Decreased neutrophil count was seen in patients treated with drugs and radiations, sequestration and deficiency of vitamin B12
- Increased neutrophil count was termed neutrophilia and decreased neutrophil count was termed as neutropenia.

B. Eosinophils

- About 10-12 µ in diameter
- Nucleus had 2 lobes
- Nucleus was deep blue stained in contrast with red cytoplasmic granules which were large, round, closely packed and give shining appearance.
- Eosinophilia is seen in allergic conditions parasitic infestation, skin diseases etc.
- Eosinopenic is seen and hypoadrenocorticism.

C. Basophils

- These were rare cells in blood having a diameter of 8-10µ with diffused nucleus and few larger granules which are blackish or dark blue stained.
- Basophilia was seen during inflammatory conditions

Agranulocytes

A. Lymphocytes

- These were small cells 8-10µ in diameter.
- There was big blue stained nucleus which occupied almost all the cell
- Nucleus was round or indented.
- Granules were absent
- Lymphocytosis is seen in viral infections, autoimmune diseases, chronic infectious disease, menstruation etc.
- Lymphopenia is seen in stress and administration of corticoids.

B. Monocytes

- These were largest cells among the leucocytes having a diameter of 18-21µ
- The nucleus was comparatively small and fill only half of the cell space
- The nucleus was kidney or horse shoe shaped.
- The cytoplasm was pink and intense stained containing cytoplasmic vacuoles.
- Granules were absent.
- Monocytosis is seen in tuberculosis, sub-acute bacterial endocarditis and protozoan infections.
- Monocytopenia is seen in diseases of the reticuloendothelial system

Precautions

- Slide must be clean, dry and non-greasy.
- Spreader edge of the spreader slide must be smooth.
- Spreader slide should be held at appropriate angle.
- Staining time should be appropriate for proper staining.

3.9 Derivation of erythrocytic indices

Definition:

Erythrocytic indices were derived values that explained cell morphology (size, volume and color) of erythrocytes in absolute terms.

These indices were termed as mean corpuscular volume (MCV) ,mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) .

These indices helped in characterizing various types of anaemias occurring due to altered cell morphology.

MCV = PCV (%) x 10/TEC ($10^{6/}$µL) μ^3 or 10^{-15} liter or Total volume(%)/Total number

Cells with low MCV are microcytic, with normal MCV are normocytic and with high MCV are macrocytic RBCs.

MCH : is defined as the average quantity of hemoglobin present in one erythrocyte.

MCH is expressed in pictogram (1 pg = 10^{-12}g)

MCH = Hb conc.(g/dl) x 10/TEC(10^6/µL) pg or µµg.

MCHC: defined as concentration of hemoglobin per hundred volume of erythrocytes.

MCHC is expressed in percent (%)

MCHC = Hb conc. (g/d) x 100/PCV (%)

Estimation of serum Na+,K+,Cl- concentration by Dry chemistry Analyser Vitros 250

3.10 Estimation of Serum Potassium (Direct Potentiometry)

Principle

The VITROS K+ slide method is performed using the VITROS K+ slides and the VITROS chemistry products calibrator Kit 2 on VITROS chemistry systems.

The VITROS K+ slide is a multilayered, analytical element coated on a polyester support that uses direct potentiometry for measurement of ionic potassium.

The slide consists of two ion selective electrodes, each containing valinomnycin (an ionophore for potassium) a reference layer, and a silver and a silver chloride layer coated on a polyester support.

A drop of serum sample and a drop of VITROS reference fluid on separate halves of these slide results in migration of both fluids toward the center of the paper bridge. A stable liquid junction is formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of potassium applied to it. The potential difference poised between the two electrodes is proportional to the potassium concentration in the sample.

Test type and conditions:

Test type	Vitros System	Appx. Incubation time	Temperature	Drop Volume	
potentiometric		3 min	25° C (77 ° F)	Sample 10 µl	Reference fluid 10 µl
	250	2 min	37 ° C (98.6 ° F)		

Materials/Chemicals/Apparatus Required

VITROS 250 System

VITROS K+ slides

VITROS chemistry products calibrator Kit 2

Fig.No. 5 : Dry chemistry Analyser Vitros 250

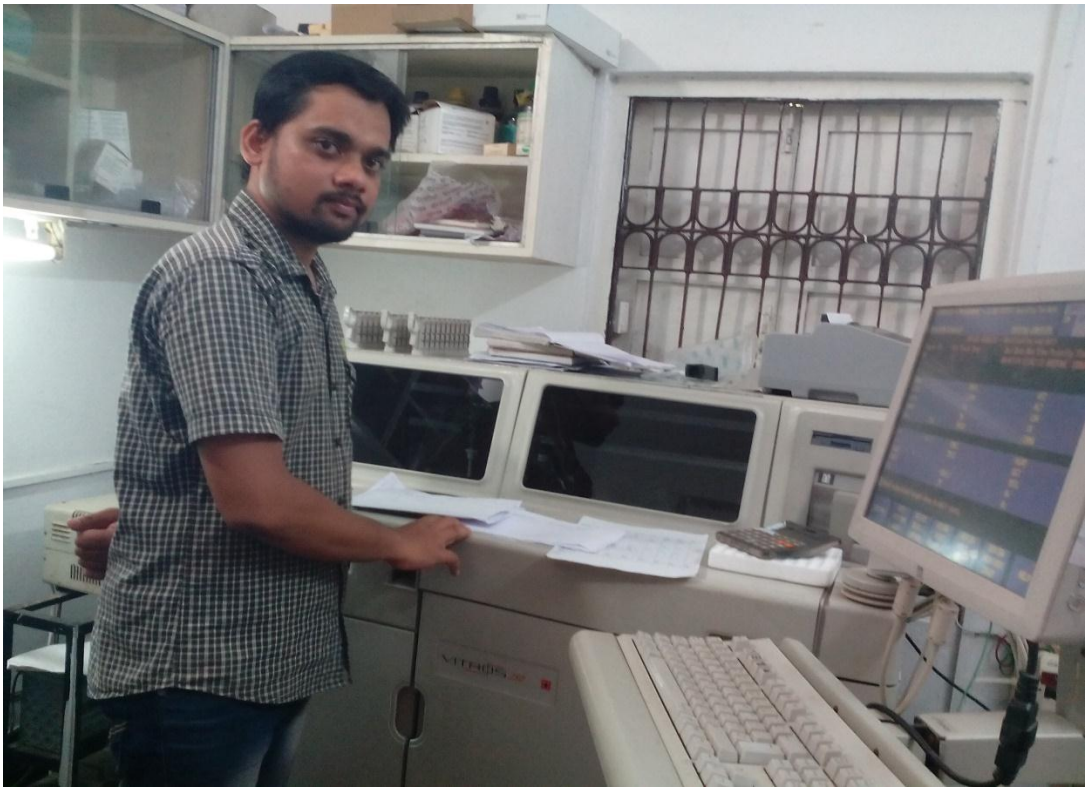


Fig.No.6 : Estimation of serum Na+,K+,Cl- concentration by Dry chemistry Analyser Vitros 250

Procedure

Specimen requirement: Serum or heparinized plasma

1) Serum sample was brought to room temperature 18°-28°C prior to analysis.
2) The serum sample was caliberated with the help of the calibrator kit provided.
3) The automated VITROS system was used to determine the K+ ion concentration.

Calculations

The VITROS chemistry system measures the potential difference in millivolts between the two electrodes of a potentiometric slide-one in contact with the sample to be analyzed and the other in contact with the electrolyte reference fluid. A linear relationship exists between the measured potential difference observed on the slide and the logarithm of K+ concentration. Once the calibration has been established for each slide lot, unknown K+ concentrations for a given sample can be determined using the software-resident math model and the measured potential difference.

3.11 Estimation of Serum Sodium (Direct Potentiometry)

Principle

The VITROS Na+ slide method is performed using the VITROS Na+ slides and the VITROS chemistry products calibrator Kit 2 on VITROS chemistry systems. The VITROS Na+ slide is a multilayered, analytical element coated on a polyester support that uses direct potentiometry for measurement of ionic potassium. The slide consists of two ionselective electrodes, each containing methyl monensin (an ionophore for sodium) a reference layer, and a silver and a silver chloride layer coated on a polyester support. A drop of patient sample and a drop of VITROS reference fluid on separate halves of the slide results in migration of both fluids toward the center of the paper bridge. A stable liquid junction is formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of sodium applied to it. The potential difference poised between the two electrodes is proportional to the potassium concentration in the sample.

Test type and conditions:

Test type	Vitros System	Appx. Incubation time	Temperature	Drop Volume	
potentiometric		3 min	25° C (77 ° F)	Sample 10 µl	Reference fluid 10 µl
	250	2 min	37 ° C (98.6 ° F)		

Materials/Chemicals/Apparatus Required

VITROS 250 System

VITROS Na+ slides

VITROS chemistry products calibrator Kit 2

Procedure

Specimen requirement: Serum

1) Serum samples were brought to room temperature 18°-28°C prior to analysis.

2) The serum samples were caliberated with the help of the calibrator kit provided.

3) Automated VITROS system was used to determine the Na+ ion concentration.

Calculations

The VITROS chemistry system measures the potential difference in millivolts between the two electrodes of a potentiometric slide-one in contact with the sample to be analyzed and the other in contact with the electrolyte reference fluid. A linear relationship exists between the measured potential difference observed on the slide and the logarithm of Na+ concentration. Once the calibration has been established for each slide lot, unknown Na+ concentrations for a given sample can be determined using the software-resident math model and the measured potential difference.

3.12 Estimation of Serum Chloride (Direct Potentiometry)

Principle

The VITROS Cl- Slide assay is performed using the VITROS Cl- Slides and the VITROS Chemistry Products Calibrator Kit 2 on VITROS Chemistry Systems. The VITROS Cl-Slide

is a multilayered, analytical element coated on a polyester support that uses direct potentiometry for measurement of chloride ions.

The slide consists of two ion-selective electrodes, each containing a protective layer, a silver

layer and a silver chloride layer coated on a polyester support.

. A drop of serum sample and a drop of VITROS Electrode Reference Fluid on separate halves of the slide results in migration of both fluids toward the center of the paper bridge.

A stable liquid junction is formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of chloride ions applied to it. The potential difference poised between the two electrodes is proportional to the chloride concentration in the sample.

Test type and conditions:

<table>
<tr><td>Test type</td><td>Vitros System</td><td>Appx.
Incubation time</td><td>Temperature</td><td colspan="2">Drop Volume</td></tr>
<tr><td rowspan="2">potentiometric</td><td></td><td>3 min</td><td>25° C (77 ° F)</td><td rowspan="2">Sample
10 µl</td><td rowspan="2">Reference
fluid
10 µl</td></tr>
<tr><td>250</td><td>2 min</td><td>37 ° C
(98.6 ° F)</td></tr>
</table>

Materials/Chemicals/Apparatus Required

VITROS 250 System

VITROS Na+ slides

VITROS chemistry products calibrator Kit 2

Procedure

Specimen requirement: Serum

1) Serum samples were brought to room temperature 18°-28°C prior to analysis.

2) The serum samples were caliberated with the help of the calibrator kit provided.

3) Automated VITROS system was used to determine the Cl^- ion concentration.

Calculations

The VITROS chemistry system measures the potential difference in millivolts between the two electrodes of a potentiometric slide-one in contact with the sample to be analyzed and the other in contact with the electrolyte reference fluid. A linear relationship exists between the measured potential difference observed on the slide and the logarithm of Cl^- concentration. Once the calibration has been established for each slide lot, unknown Cl^- concentrations for a given sample can be determined using the software-resident math model and the measured potential difference.

CHAPTER IV

RESULTS AND DISCUSSION

Different parameters were studied and following results were obtained

4.1 Haematological parameters

Different Haematological parameters were studied and results were analysed

4.1.1 Haemoglobin concentration

Sig $P < 0.01$

*Means with different superscripts differ significantly ($P < 0.01$)

The average haemoglobin content of dogs suffering from chronic dermatitis was found to be 11.47 g percent which is significantly higher ($P < 0.01$) than the healthy dogs whose average Haemoglobin content was found to be 12.98 g percent.

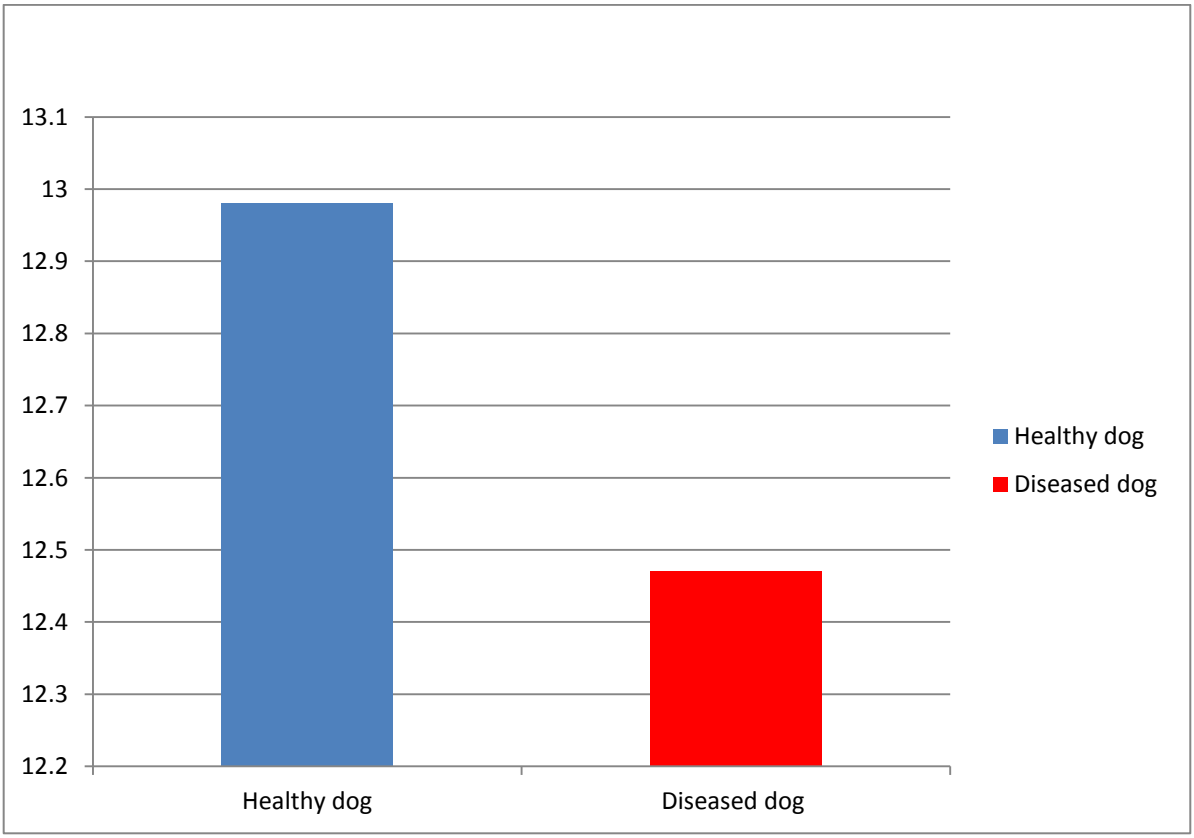
The find is in agreement with the find of Nair and Nauriyal et al.,(2007); Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,(2016); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Shyma and Vijayakumar et al.,(2011); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

The dogs suffering from chronic dermatitis were found to have low haemoglobin content and hence were said to be anaemic . This may be due to chronic ectoparasite infestation and alternation in skin resulting in continuous blood loss. Thus they need to have haematinic supplementation . If treatment is not started at behest , the condition of patients may further degrade to alaramig low levels of haemoglobin that may be life threatning.

TABLE-1 : Mean ± S.E of Haemoglobin content (g%) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	12.98 [a]	11.47 [b]
Standard deviation	0.682	1.027
Standard Error	0.215	0.325
Range	12.6 – 14.8	10.7 - 14

Fig No. 7 Bar diagram showing comparison of Hb content between healthy and diseased dogs



4.1.2 TEC concentration (millions/ µL) of blood

Sig P < 0.01

* Means with different superscripts differ significantly (P < 0.01)

The average Total Erythrocyte Concentration (TEC) of Chronic Dermatitis affected dog was found to be 4.9 million / µL of blood that was significantly lower (P < 0.01) than average Total erythrocyte concentration of normal or healthy dogs that was found to be 5.19 million /µL of blood .

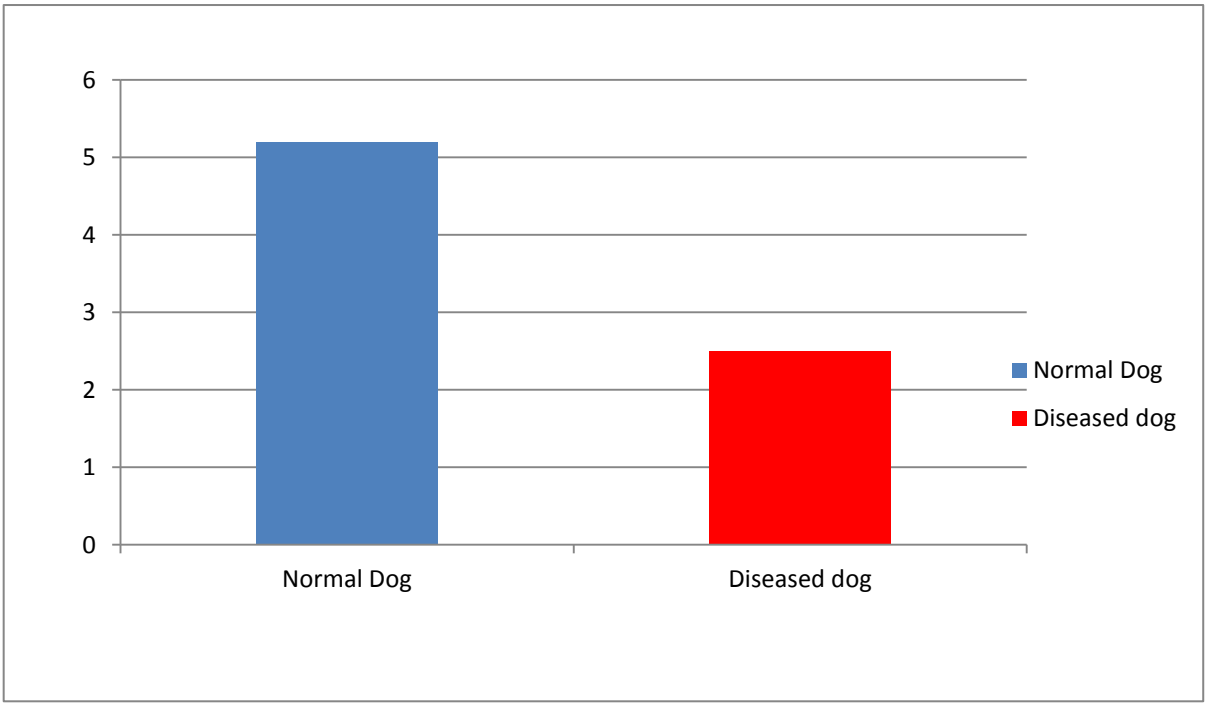
The find is in agreement with the find of of Nair and Nauriyal et al.,(2007); Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,(2016); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Shyma and Vijayakumar et al.,(2011); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

The dogs suffering from chronic dermatitis were found to have significantly low total erythrocyte concentration as compared to healthy dogs signifying erythrocytopenia. This may be due to chronic ectoparasitic infestation and breach in normal structural attributes of skin resulting in blood loss and anaemia . The chronic dermatitis affected animals must be provided with Iron suppliments and Haematinics to help body produce more red blood cells. The dogs must be treated as early as possible to prevent further degradation in condition and prevent lowering of total erythrocyte content to alaramingly serious levels.

TABLE – 2 : Mean ± S.E of TEC (millions/ µL) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	5.19[a]	4.9[b]
Standard deviation	0.182	0.1138
Standard Error (S.E)	0.057	0.036
Range	4.5 – 5.65	4.2 – 5.3

Fig. No. 8 Bar diagram showing comparison of TEC content (millions/mL) between healthy and diseased dogs



4.1.3 PCV (%) of blood

Sig P < 0.05

*Means with different subscripts differ moderately (P< 0.05)

The average Packed cell volume percentage of chronic dermatitis affected dogs was found to be 50.1% that was moderately lower (P < 0.05) than the packed cell volume of healthy dogs that was found to be 54.85%

The find is in agreement with the find of of Nair and Nauriyal et al.,(2007); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Shyma and Vijayakumar et al.,(2011); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

The dogs suffering from chronic dermatitis were found to have slightly low packed cell volume percentage than that of healthy dogs . This can be due to lower Total Erythrocyte Count .

4.1.4 ESR (mm/ 1st hr) of blood

Sig p < 0.1

* Means with different superscripts differ significantly (P < 0.01)

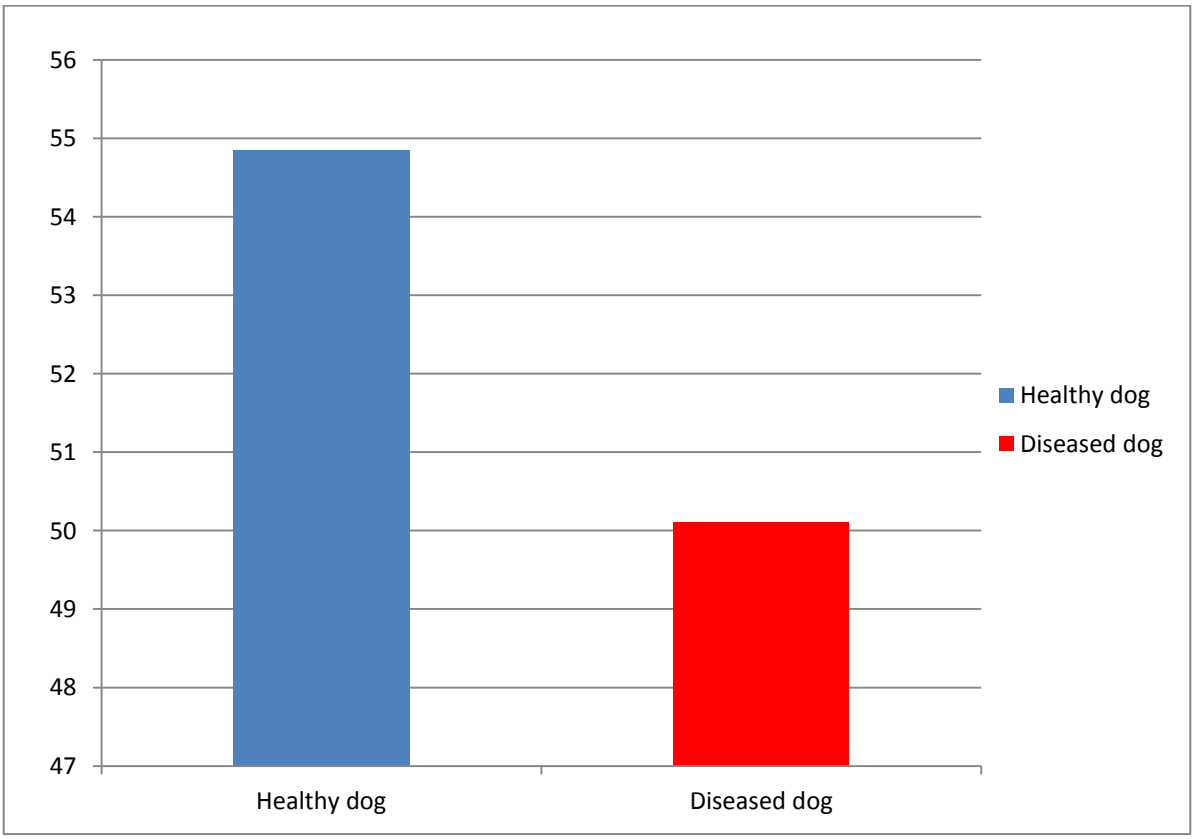
The average ESR percentage of dogs suffering from chronic dermatitis was found to be 5.3 mm/hr that was significantly higher (P < 0.01) than the average erythrocyte sedimentation rate of normal dogs that was 5.19 mm/hr .

The find is in agreement with the find of of Nair and Nauriyal et al.,(2007); Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,(2016); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Shyma and

TABLE – 3 : Mean ± S.E of PCV (%) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	54.85[a]	50.1[b]
Standard deviation	6.3	6.8
Standard Error	1.99	2.15
Range	48 - 59	41- 54

Fig. No. 9 Bar diagram showing comparison of PCV (%) content between healthy and diseased dogs



Vijayakumar et al.,(2011); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

The dogs suffering from chronic dermatitis were found to have higher ESR rate than that of normal dogs . This may be attributed to anaemic condition . Lack of normal count of red blood cells decreases the viscousity of blood significantly resulting in faster sedimentation or settling of blood cells . It can also be conferred that there is presence of infection in blood too.

4.1.5 TLC (thousand/ µL) count in blood

Sig $P < 0.01$

* Means with different subscripts differ significantly ($P< 0.01$)

The average Total leucocyte count of chronic dermatitis affected dogs was found to be 11.46 thousand / µL of blood that was significantly higher ($P < 0.01$) than total leucocyte count of normal dogs that was found to be 7.095 thousand/ µL of blood

The find is in agreement with the find of of Nair and Nauriyal et al.,(2007); Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,(2016); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Shyma and Vijayakumar et al.,(2011); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

The dogs suffering from chronic dermatitis were found to be suffering from lymphocytosis . This may indicate substantial immune response to the infection in the body or cases of allergy dermatitis in case of dogs suffering from Atopic dermatitis . Treatment should include antibiotics as well anti histamines . NSAID can also be tried in these cases .

TABLE – 4 : Mean ± S.E of ESR (mm/ 1st hr) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	5.19[a]	5 .3[b]
Standard deviation	0.182	0.1138
Standard Error	0.057	0.036
Range	3.9 - 5	4 – 6.2

Fig. No. 10 Bar diagram showing comparison of ESR (mm/ 1st hr) content between healthy and diseased dogs

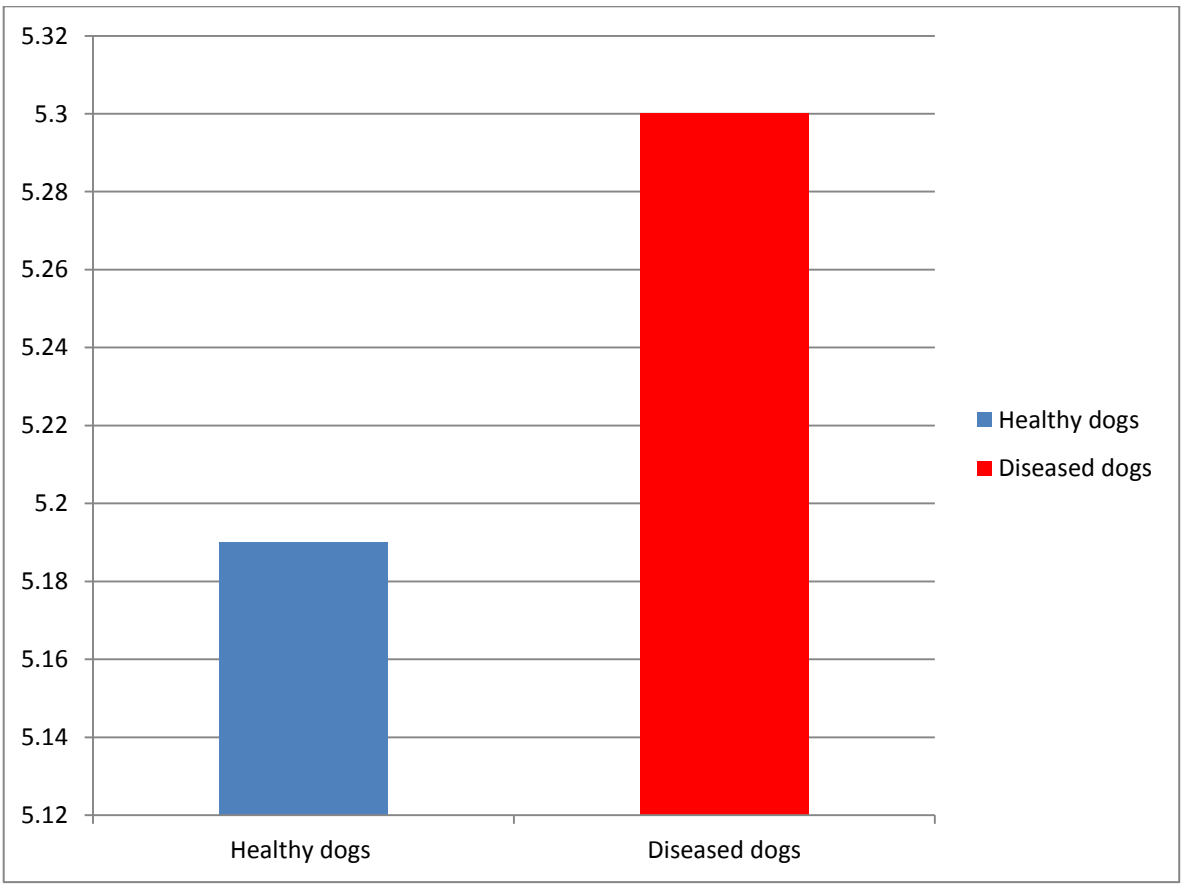


TABLE – 5 : Mean ± S.E of TLC (thousand/ µL) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	7.095[a]	11.46[b]
Standard Deviation	0 .760	2.06
Standard Error	0.24	0.65
Range	6.2 - 8.3	8.2 - 15

Fig. No. 11 Bar diagram showing comparison of TLC (thousand/ mL) content between healthy and diseased dogs

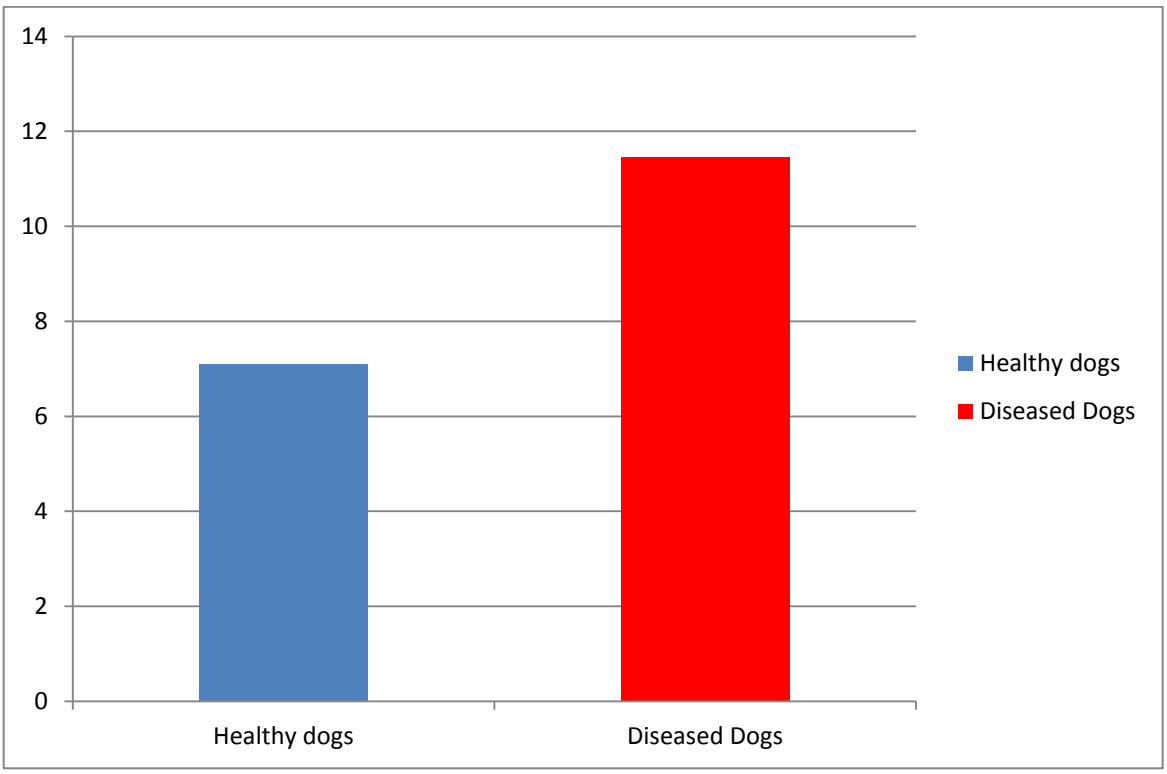
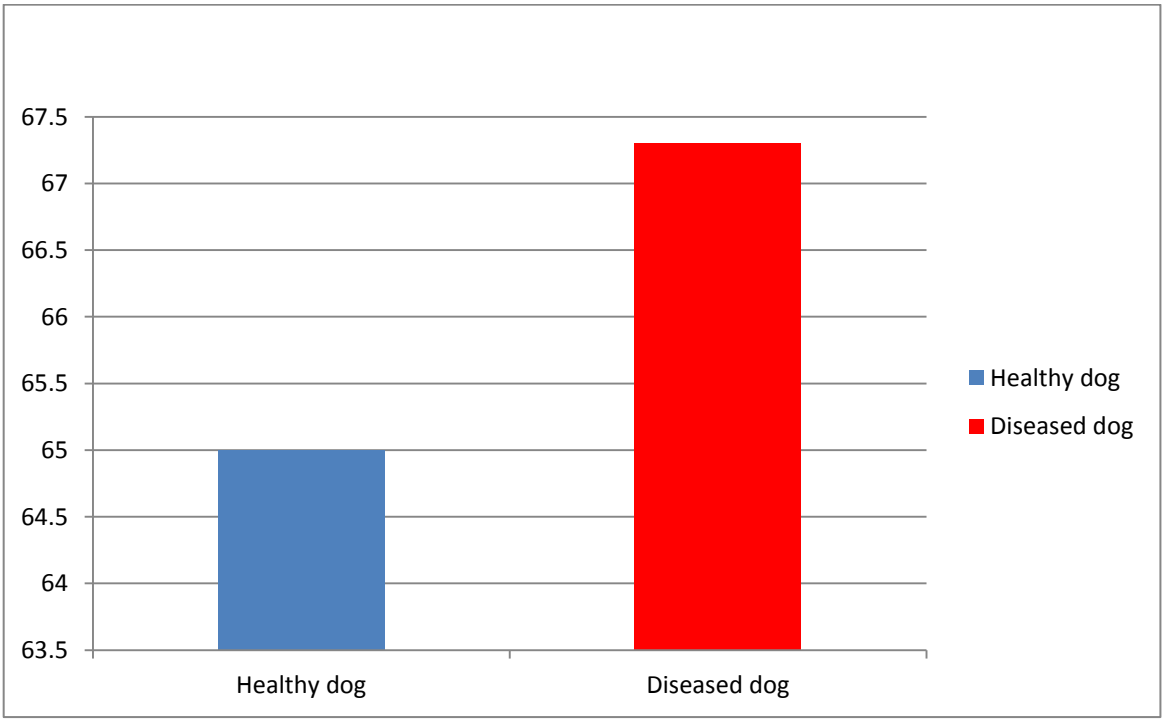


TABLE – 6 : Mean ± S.E of Blood DC (%) in dogs suffering from chronic dermatitis

Statistical attributes / LEUCOCYTES	NEUTROPHIL		EOSINOPHIL		BASOPHIL		LYMPHOCYTE		MONOCYTE	
	C	D	C	D	C	D	C	D	C	D
Mean	65^a	67.3^b	6^a	7.5^b	0.5^a	0.7^b	21^a	21.6^b	6.5^a	3.1^b
Standard deviation	3.6	4.3	1.8	2.3	0.08	0.24	12.4	18.48	4.2	6.8
Standard Error	1.14	1.36	0.57	o.73	0.025	0.075	3.92	5.85	1.33	2.15
Range	62 - 68	63 - 76	5.8- 6.2	6.3 – 8.4	0 - 0.7	0.2 – 1.6	19 - 26	18 - 26	4.8 – 8.1	2.3 – 4.9

C = CONTROLLED/HEALTHY D = DISEASED

Fig. No. 12 Bar diagram showing comparison of Neutrophile percentage between healthy and diseased dogs



4.1.6 Differential Leucocyte Count (%) in blood

4.1.6.1 Neutrophil concentration in blood

* Means with different superscripts for Neutrophil percentage do not differ significantly (P > 0.05)

The average Neutrophil concentration of chronic dermatitis affected dogs was found to be 67.3 % which was not significant with respect to the average neutrophil concentration of healthy dogs that was found to be 65%

The neutrophile concentration in chronic dermatitis dogs were found to be in normal limits and was not significantly different than that of normal healthy dogs

4.1.6.2 Eosinophile concentration in blood

Sig P > 0.01

* Means with different superscripts for Eosinophile concentration in blood differ significantly

The average eosinophile concentration in blood of dogs suffering from chronic dermatitis was found to be 7.5% that was significantly higher (P < 0.01) than the average eosinophile concentration in blood of healthy dogs that was found to be 6%

The find was found to be in accordance with of Nair and Nauriyal et al.,(2007); Ozana Maria dulman, Alina anton and Gheorghe solca et al.,(2015); Sudhakara reddy, Nalini kumari, Vaikunta rao, Rayulu and Sivajothi et al.,(2016); Sudhakara Reddy,Nalini Kumari,Sivajothi and Venkatasiva kumar et al.,(2015); Sindha et al .,(2015); Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014)

Fig. No. 13 : Bar diagram showing comparison of Eosinophile percentage between healthy and diseased dogs

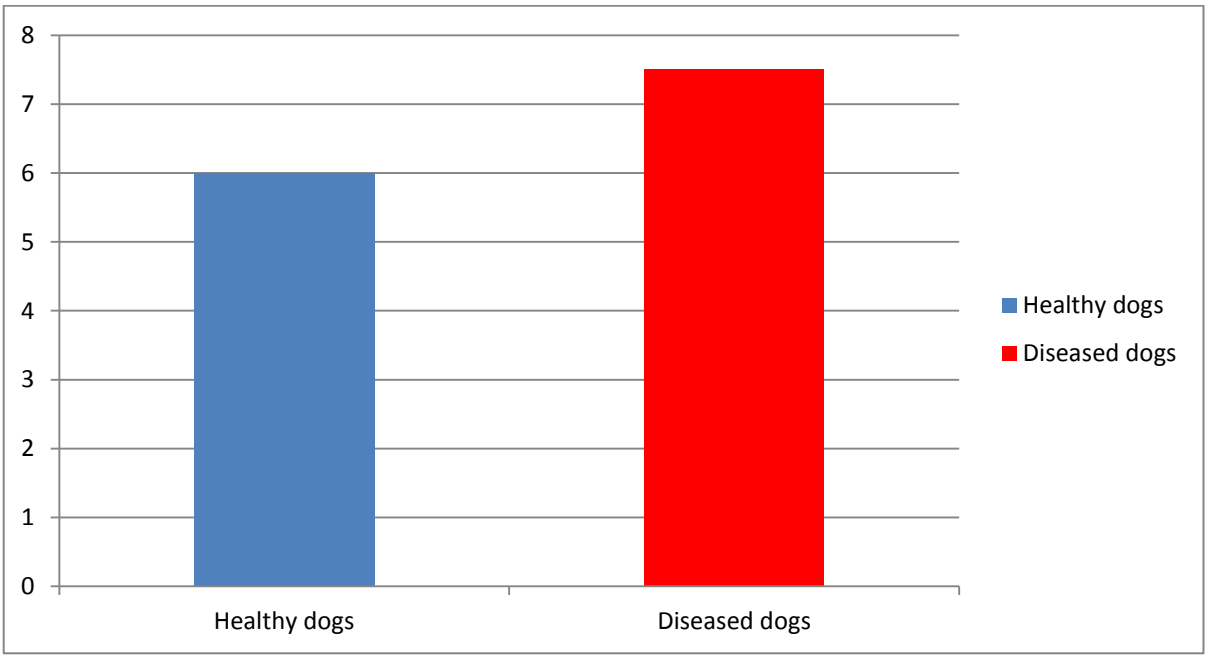
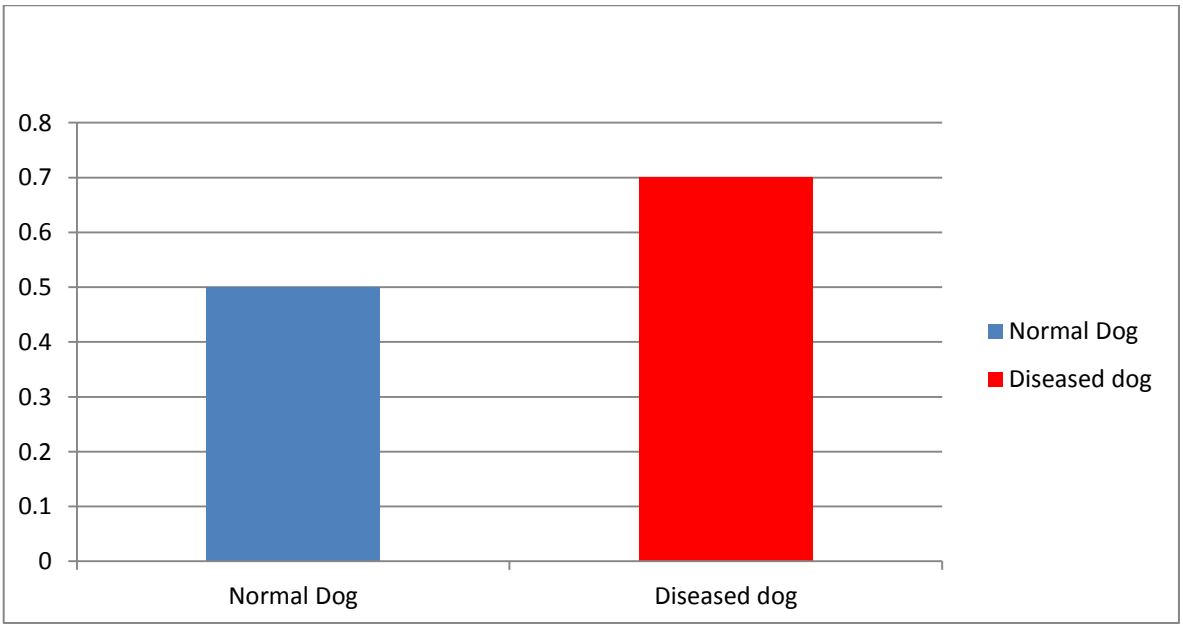


Fig. No. 14 : Bar diagram showing comparison of Basophile percentage between healthy and diseased dogs



The dogs suffering from chronic dermatitis were found to have significant eosinophilia . This may be attributed to allergy conditions and infectious aeitiology.

4.1.6.3 Basophile concentration in blood

SigP <0.05

* Means with different superscripts for Basophil percentage in blood differ with moderate significance.

The average Basophile percentage in blood of chronic dermatitis affected dogs was found to be 0.7 % which was moderaterly significant (P < 0.05 %) as compared to the average basophile percentage of healthy dogs where the average basophile percentage in blood was found to be 0.5% .

Hence the dogs suffering from chronic dermatitis were found to have slight basophilia .

4.1.6.4 Lymphocyte concentration in blood

* Means with different subscripts for lymphocyte percentage do not differ significantly (P > 0.05)

The average Lymphocyte concentration in blood of dogs suffering from chronic dermatitis was found to be 21.6 % that was not significantly different to that of average lymphocyte concentration of healthy dogs that was found to be 21%

The find was found in corrandance with Bhavanam Sudhakara Reddy, Karumuri Nalini Kumari and S. Sivajothi et al (2014) and Ozana Maria dulman, Alina anton and Gheorghe solca et al., (2015)

The blood lymphocyte percentage of chronic dermatitis affected dogs was found to be in normal range and was not significantly different than that of healthy dogs

Fig. No. 15 : Bar diagram showing comparison of Lymphocyte percentage between healthy and diseased dogs

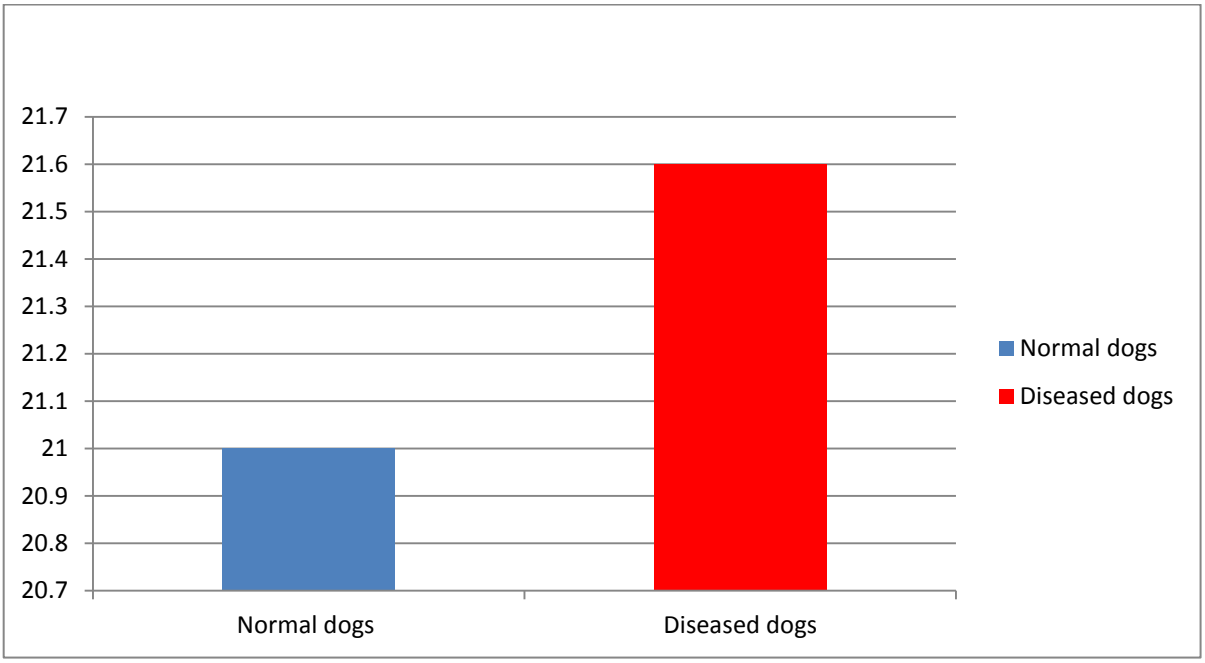
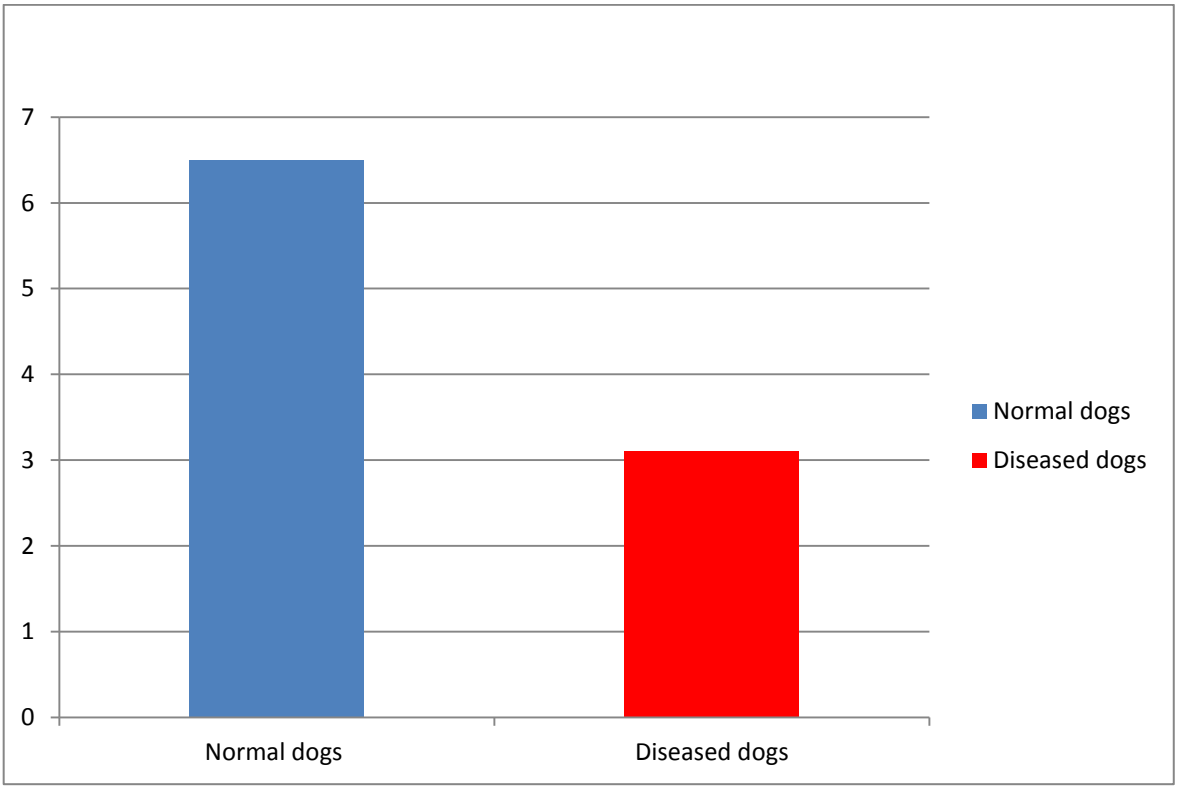


Fig. No. 16 : Bar diagram showing comparison of Monocyte percentage between healthy and diseased dogs



4.1.6.4 Monocyte concentration in blood

SigP < 0.05

* Means with different superscripts for monocyte percentage difers moderately (P < 0.05)

The average Monocyte percentage in blood of Dermatitis affected dogs were found to be 3.1 % which was moderately significant (P < 0.05) as compared to the average monocyte concentration of normal dogs that was found to be 6.5%

The find was found in accordance with the find of Shyma and Vijayakumar et al.,(2011)

Hence it was found the monocyte percentage in blood of dogs suffering from chronic dermatitis was moderately lower than that of normal dogs

4.2 Biochemical parameters

The following biochemical parameters were studied and analysed

4.2.1 Serum K+ (mmol/ L) concentration in blood

Sig P < 0.1

* Means with different superscripts differ significantly (P< 0.01)

The average serum concentration of Potassium ions in blood of chronic dermatitis affected dogs was found to be 5.34 mmol/ L of blood which was significantly higher (p < 0.01) than that of plasma potassium ion concentration that was found to be 4.85 mmol/L

Table 7 : Mean ± S.E of serum K+ concentration(mmol/L) in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	4.85	5.34
Standard Deviation	0.31	0.42
Standard Error	0.098	0.13
Range	3.9 - 5.6	4.5 – 6.5

Fig. No. 17 : Bar diagram showing comparison of serum potassium concentration (mmol/L) between healthy and diseased dogs

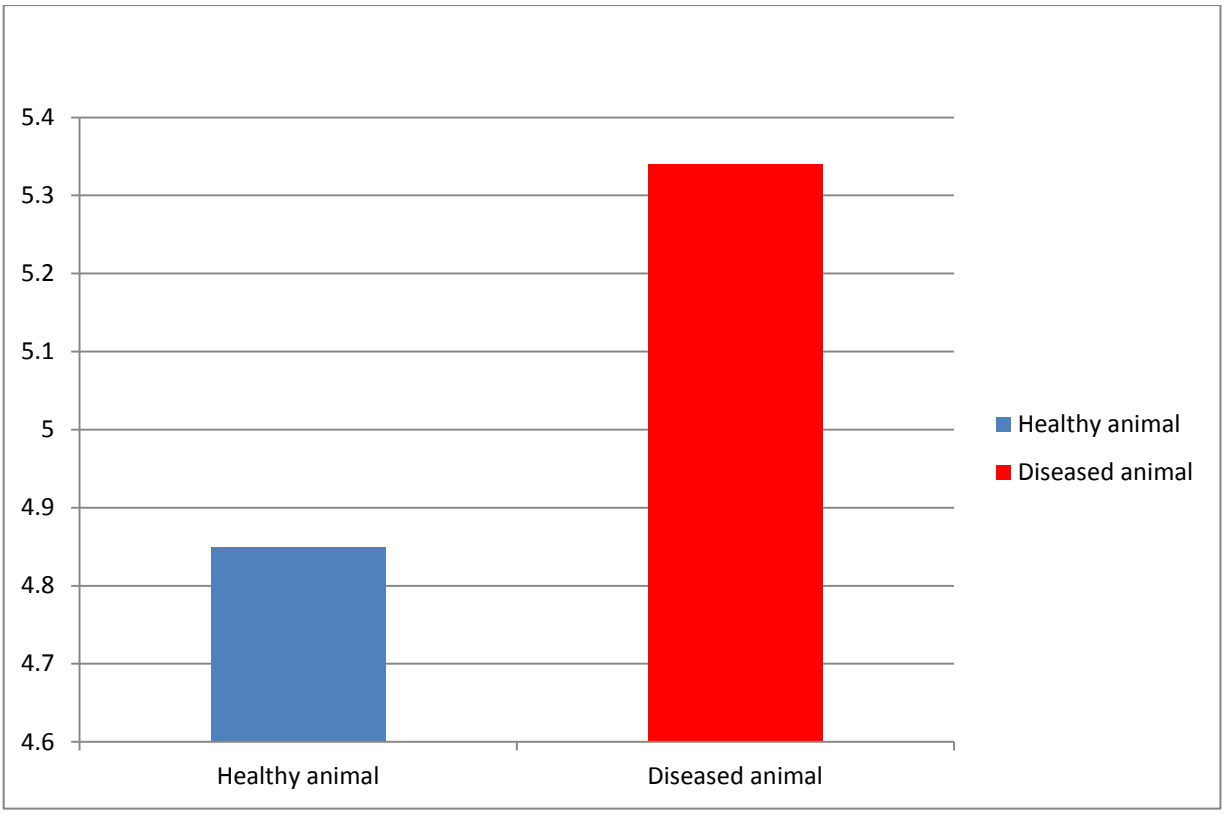
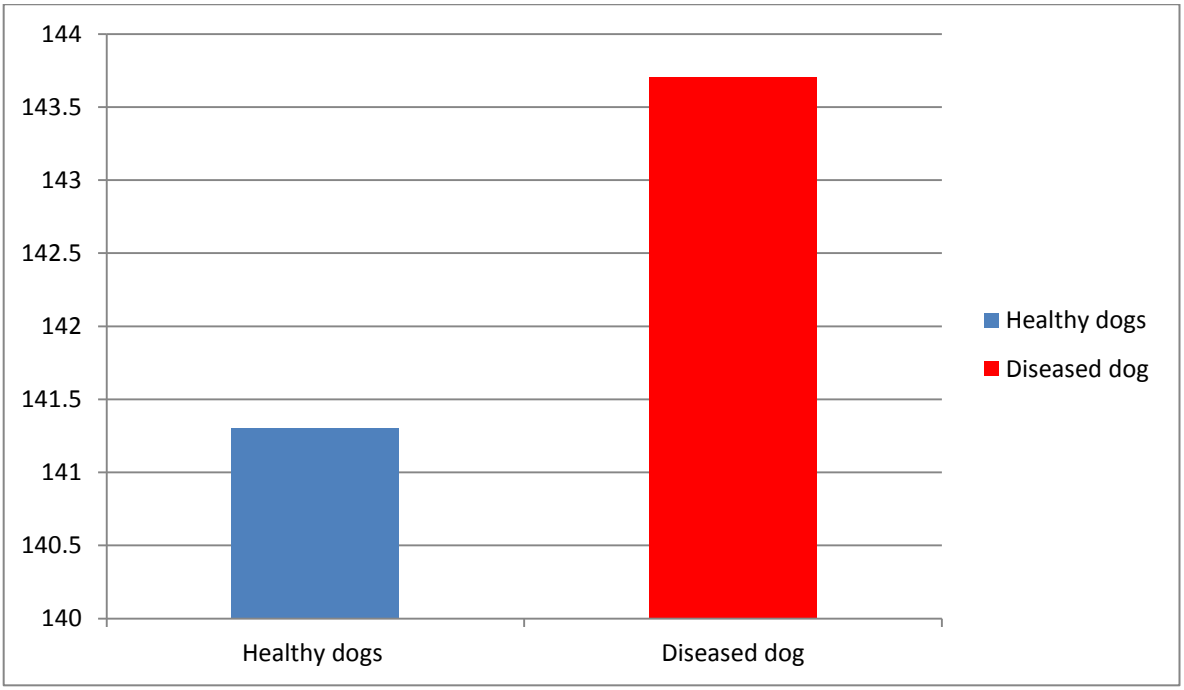


TABLE – 8 : Mean ± S.E of serum Na^+ (mmol/ L) concentration in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	141.3[a]	143.7[b]
Standard deviation	8.9	8.9
Standard Error	2.81	2.81
Range	139 – 147	139 - 148

Fig. No. 18 : Bar diagram showing comparison of serum Na+ (mmol/ L) content between healthy and diseased dogs



4.2.2 Serum Na+ concentration (mmol/L) in blood

* Means with different superscripts do not have significant difference (P > 0.05)

The average serum sodium concentration of dogs affected with chronic dermatitis was found to be 143.7 mmol/L that was not significantly different from the average average serum sodium concentration of healthy dogs

Hence serum sodium concentration of dogs affected with chronic dermatitis was found to be within normal limits .

4.2.3 Serum Cl- concentration (mmol/L) in blood

* Means with different superscripts do not differ significantly (P > 0.05)

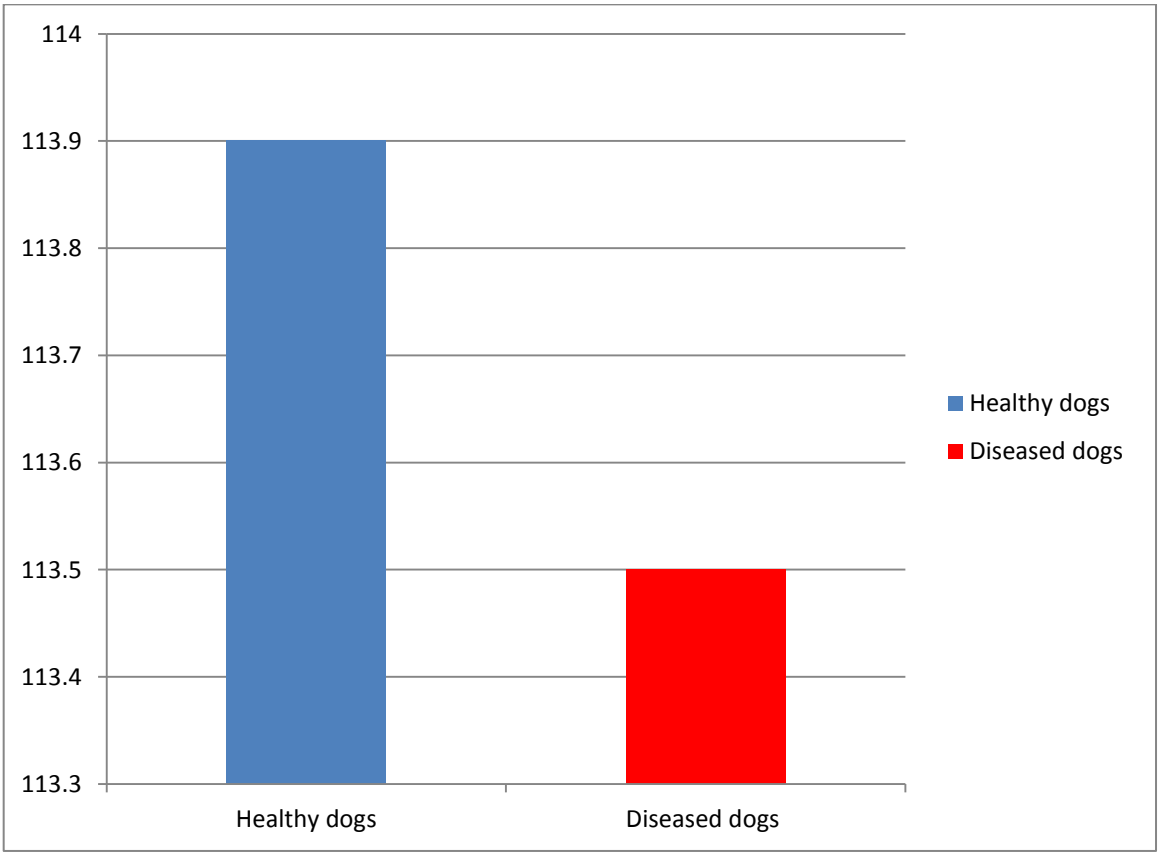
The average serum chloride concentration of dogs affected with chronic dermatitis was found to be 113.5 mmol/L that was not significantly different from the average average serum chloride concentration of healthy dogs that was found to be 113.9 mmol/L

Hence serum chloride concentration of dogs affected with chronic dermatitis was found to be within normal limits .

TABLE – 9 : Mean ± S.E of serum Cl- (mmol/L) concentration in dogs suffering from chronic dermatitis

STATISTICAL ATTRIBUTES	HEALTHY DOG (n = 10)	CHRONIC DERMATITIC DOG (n = 10)
Mean	113.9	113.5
Standard deviation	4.3	5.2
Standard Error (S.E)	1.36	1.64
Range	113.9 ± 1.36	113.5 ± 1.64

Fig. No. 19 : Bar diagram showing comparison of serum Cl- (mmol/ L) content between healthy and diseased dogs



CHAPTER – V

SUMMARY

The present study was conducted to study the haematological profile (Hb %, TEC, PCV, ESR, Erythrocyte indices (MCV , MCH , MCHC), TLC ,DC and electrolyte status (Na^+, K^+ , Cl^- concentration) dogs suffering from chronic dermatitis.

Following conclusions can be drawn for the dogs suffering from chronic dermatitis from the present research

- Haemoglobin percentage was significantly lower
- Total erythrocyte count was lower than normal
- PCV was lower than normal
- ESR was significantly higher than normal
- Total leucocyte count was significantly higher
- Blood DC showed significantly higher percentage of Eosinophil , moderately high level of Basophil, moderately lower level of monocyte ,and non significant variation in lymphocyte and neutrophil
- Serum concentration of Sodium and Chloride ions were not significantly different and were in normal range
- Serum concentration of potassium was increased significantly .

CHAPTER – VI

CONCLUSION

The dogs suffering from chronic dermatitis were found to be anaemic . They had lower concentration of red blood cells in blood . They were also found to be suffering from leucocytosis .Differential leucocyte count showed significant eosinophilia , moderate basophilia and moderate monocytopenia . The lymphocyte and Neutrophil concentration were found to be within normal limits . The dogs were also found to be hyperkalemic.

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