STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

BY

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(INDIA)

2024

DECLARATION OF STUDENT

I hereby declare that the experimental Research work and interpretation of the thesis entitled "**STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH"** or part thereof has not been submitted for any other degree or diploma of any University, not the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Alone Pallavi Dipakrao has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, "**STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH"** submitted by her is the result of research work is sufficient to warrant its presentation to the examination in the subject of **Veterinary Clinical Medicine, Ethics and Jurisprudence** for the award of Master of Veterinary Science (M.V.Sc.) degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

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Appendix -C (ii)

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH"** submitted by Miss. **Alone Pallavi Dipakrao** of the Maharashtra Animal and Fishery Sciences University, Nagpur, in partial fulfillment of the requirement for the degree of Master of Veterinary Science has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Dedicated to my Beloved Family

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Date:

Place: Mumbai

(Pallavi Alone)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Sr. No.	Abbreviation	Name
1	%	Percent
2	% B/B_0	Percent binding at zero standard
3	<	Greater than
4	>	Lesser than
5	±	Plus or minus
6	µl	Microliter
5	^{0}F	Degree Fahrenheit
6	^{125}I	Iodine – 125 (Radioisotope)
7	A	Albumin
8	ALP	Alkaline phosphatase
9	ALT	Alanine Aminotransferase
10	ANOVA	Analysis of Variance
11	AST	Aspartate transaminase
12	B	Basophils
13	B/F	Bound to Free ratio
14	B/T	Bound to Total ratio
15	B_0/T	Zero binding to toal binding ratio
16	BRIT	Board of Radiation and Isotope Technology
17	BSDPHA	Bai Sakarbai Dinshaw Petit Hospital for Animals
18	BUN	Blood Urea Nitrogen
19	cmm	Cubic millimeter
20	CPM	Count per minute
21	CV	Coefficient of variation
22	DB	Direct Bilirubin
23	DIT	Diiodothyronine
24	dl	Deciliter
25	E	Eosinophils
26	EDTA K3	Tripotassium ethylenediaminetetraacetic acid
27	ELISA	Enzyme-linked Immunosorbent Assay
28	*et al.*	Et alia (and others)
29	Fig	Figure

30	fT_4	Free Thyroxine
31	G	Globulin
32	GGT	Gamma-Glutamyl Transferase
33	gm	Gram
34	H/O	History of
35	Hb	Hemoglobin
36	HCT	Hematocrit
37	I	Iodine
38	IU/L	International Units per liter
39	kg	Kilogram
40	L	Lymphocyte
41	M	Monocyte
42	MCH	Mean Corpuscular Hemoglobin
43	MCHC	Mean Corpuscular Hemoglobin Concentration
44	MCV	Mean Corpuscular Volume
45	µIU/ml	Micro international unit per ml
46	mg	Milligram
47	MIT	Mon iodothyronine
48	ml	Milliliter
49	n	Sample size
50	N	Neutrophils
51	N. A.	Not Applicable
52	ND	Nondescript
53	NDDB	National Dairy Development Board
54	ng	Nanogram
55	ng/ml	Nanogram per milliliter
56	nmol/l	Nanomole per Liter
57	ºC	Degree Celsius
58	P	Calculated probability (p-value)
59	pg	Picogram
60	pg/ml	Picogram per milliliter
61	PLT	Platelets
62	pmol/l	Picomole per Liter
63	cTSH	Canine thyroid stimulating hormone
64	Q. C.	Quality Control
65	RBC	Red Blood Cells
66	RIA	Radio Immuno Assay
67	rpm	Rotations per minute

68	S.I.	System International
69	Se	Selenium
70	SE	Standard Error
71	Sr. No.	Serial Number
72	T_3/ TT_3	Total Triiodothyronine
73	T_4/ TT_4	Total Thyroxine
74	TB	Total Bilirubin
75	TEC	Total Erythrocyte Count
76	TLC	Total Leucocyte Count
77	TP	Total Proteins
78	TRH	Thyrotropin Releasing Hormone
79	TSH	Thyroid Stimulating Hormone
80	TVCC	Teaching Veterinary Clinical Complex
81	UD	Undetected
82	VLDL	Very Low Density Lipoprotein
83	WBC	White Blood Cells
84	μg	Microgram

Introduction

1. INTRODUCTION

Animals' bodies are complex and perfect systems, controlled by the nervous system and the endocrine system. The endocrine system, slower but more robust, performs many bodily functions by synthesizing and releasing chemical messengers, making them a representation of nature's most complex systems. Endocrinology studies the internal secretions produced by endocrine glands, which release hormones in response to internal or external stimuli. These hormones are secreted directly into the bloodstream through an endocrine mechanism, while exocrine glands use a ducal system to release their secretions in areas leading to the exterior of the body. The primary endocrine glands in an animal are the pituitary gland, thyroid, parathyroid, pancreas, adrenal glands, and gonads *(*Vala *et al.*2013).

The thyroid gland in dogs is made up of two distinct lobes that are joined by an isthmus, and it is situated in the cervical ventral region, lateral and ventral to the fifth and eighth tracheal rings. Because of the actions of T_3 and T_4, the thyroid is the primary organ that controls metabolism. Under the stimulus of TRH generated by the hypothalamus, TSH, which originates in the adenohypophysis, stimulates the thyroid (Kumar *et al.* 1978).

. Any disruption of the hypothalamic-pituitary-thyroid axis can lead to hypothyroidism, which is characterized by a decrease in the thyroid's ability to produce thyroid hormones ((John Wiley & Sons, 2020). Hypothyroidism in the dog can be classified as primary, secondary, and tertiary depending upon whether the cause resides in the thyroid gland, pituitary gland, or hypothalamus, respectively. The most common form of hypothyroidism is primary hypothyroidism, caused by thyroid gland destruction. Rare cases have been documented of secondary hypothyroidism, which is brought on by a reduction in the pituitary's secretion of thyroid-stimulating hormone (TSH). hypothalamic dysfunction causes tertiary hypothyroidism in dogs. Thyroid peroxidase deficiency has been linked to congenital hypothyroidism in rat and toy fox terriers. Congenital hypothyroidism also affects giant schnauzers, most likely as a result of a TSH or thyrotropin-releasing hormone (TRH) deficit (Lathan 2012).

Spayed females and neutered males are at increased risk when compared to sexually intact animals (John Wiley & Sons, 2020). The highest incidence of hypothyroidism occurs in the age group of 5-10 years followed by 1 to 5 years and lowest in the age group of more than 10 years. In the case of breeds, the highest incidence was observed in Labradors and non-descript breeds followed by Golden retrievers and German shepherds (Gupta *et al.* 2023).

Hypothyroidism is associated with alterations in several metabolic pathways due to the central role of the thyroid hormone in intermediate metabolism. Hyperlipidemia is common in canine hypothyroidism and is reflected by increased serum cholesterol and triglyceride concentrations in 75–90% of the cases (Panciera,1994; Dixon *et al*. 1999).

Metabolic abnormalities most frequently linked to hypothyroidism included metabolic indicators, especially lethargy, obesity or weight gain, intolerance to exercise, and dermatological abnormalities such as bilateral alopecia, rat tail appearance, hyperpigmentation, pruritus, and poor coat quality (Kour *et al.* 2021). Increased incidence of pyoderma, folliculitis, generalized demodicosis, and Malassezia infections as a result of immunosuppression. Neurologic dysfunction like Peripheral neuropathy may result in exercise intolerance, ataxia, weakness, conscious proprioception deficits, and hyporeflexia. Peripheral and central vestibular disease is also reported (Lathan 2012).

To evaluate thyroid function, serum levels of thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (fT_4), and cTSH must be measured. One of the main goals of the thyroid function test diagnosis in veterinary clinical practice is the identification of hypothyroidism. Nowadays, equilibrium dialysis, radioimmunoassay, chemiluminescent, immunoassay, gamma scintigraphy, standard equilibrium dialysis, and modified equilibrium dialysis are among the diagnostic methods available.

Immunoassays target particular molecules of interest and measure their concentration in a sample by making use of the great specificity and huge diversity of antibodies. The initial immunoassay to be created was documented by Yalow and Berson in 1959.

Engvail and Perlman described a method in 1971 that involved immobilizing antigens on a microplate well, permitting the antiserum to incubate, and then employing an enzyme-linked anti-immunoglobulin antibody to measure the amount of antibody in the antiserum. This method is the enzyme-linked immunosorbent assay (ELISA). The EIA (ELISA) was developed by Van Weemen and Schuurs4 (independently of Engvail and Perlman) for the quantification of antigens rather than antibodies.

The evaluation of thyroid function and the diagnosis of hypothyroidism in dogs can be greatly improved through the use of a valid assay for the determination of canine TSH. The canine TSH test kit is a microplate-format solid phase enzyme immunometric assay (ELISA) designed for quantitative measurement, coated with a monoclonal antibody specific for canine TSH.

The use of RIA in measuring hormones for veterinary clinical use has been extensively researched in India (Dadke *et al.* 2018, Roopali *et al.* 2020, Galdhar *et al.* 2021, Jayabhaye *et al*. 2021, Galdhar *et al*. 2022; Salutgi *et al*. 2023). The Department of Veterinary Clinical Medicine, Veterinary Nuclear Medicine including the radioisotope laboratory of Mumbai Veterinary College, established a reference range for canine thyroid hormones viz. T_3, T_4, and fT_4 by Radio Immune Assay. However, due to the unavailability of canine-specific TSH RIA kit, studies on canine TSH and its association with TT_3, TT_4, and fT_4 were not studied in euthyroid and hypothyroid dogs. Considering this need and the availability of a hormonal assay laboratory at Mumbai Veterinary College, the present research work is proposed with the following objectives-

1) To establish data about canine TSH and its association with total triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine(fT_4) in healthy dogs.
2) To study alterations in canine TSH, total triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) in suspected cases of thyroid dysfunction, if any.

Review of Literature

REVIEW OF LITERATURE

The detailed review of literature related to the research work entitled **"STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH"** is described in this chapter under the following headings:

2.1 Health status of dogs
2.2 Clinical signs and symptoms
2.3 Haemato-biochemical finding in thyroid dysfunction
2.4 Radio Immuno Assay
2.5 Thyroid Gland and Thyroid hormone status (TT3, TT4, fT4 &cTSH)
2.6 Alteration in thyroid hormones and function due to drugs

2.1 Health status of dogs

Klaassen (1999) described reference ranges of biochemical parameters in canines are as follows: Alanine transaminase (12-118 U/L), Albumin (2.7-4.4 g/dl), Globulin (1.6-3.6g/dl), Alkaline Phosphatase (5-131 U/L), Aspartate transaminase (15-66U/L), Blood Urea Nitrogen (6-25 mg/dl), creatinine (0.5-1.6 mg/dl), Total Protein (5- 7.4 g/dl), Glucose (70- 138mg/dl), Cholesterol (92-324 mg/dl) and Triglyceride (29-291 mg/dl).

Kraft *et al.* (1999) described reference ranges for hematology in healthy dogs such as Red Blood Cells (5-10x 10^{12} /L), White blood cells (6-18x 10^{9} /l), Lymphocytes (15-38%), Monocyte (0-4%), Eosinophils (0-6%), Basophils(0-1%),Hb (9.02-14.98g/dl), PCV(0.30-0.44l/l), MCV (40-55fl), Mean Corpuscular Hemoglobin (12.89-16.11pg), Mean Corpuscular Hemoglobin concentration (30.61-35.44g/dl), and Platelets (180-550x 10^{9}/l).

Mortiz *et al.* (2004) established reference for hematology in healthy dogs such as Red Blood Cell (5.68-9.08 x 10^{12} /L), White blood cell (5.84-20.26 x 10^{9} /l), Lymphocytes (18.97- 41.28 %), Monocyte (3.12-6.94%), Eosinophils (1.23- 9.33%), Basophils(0.19-0.70%),Hb (13.77-20.38 g/dl), PCV(0.42-0.62 l/l), MCV (62.7-74.56 fl), Mean Corpuscular Hemoglobin (20.46-24.81), Mean Corpuscular Hemoglobin concentration (31.61-34.35 g/dl), RDW 12.00-13.15%) and Platelets (173.05-486.50x 10^{9}/l).

Sanderson (2010) described BCS as a subjective and semiquantitative method for evaluating body fat that combines visual assessment (from the top and side) and palpation (waist, ribs abdominal tuck, and dorsal spinous processes at tail base) to assess adipose tissue mass. Two numeric scales are used for assigning BCS in dogs, a 5-point scale and a 9-Point Scale. For example, a dog with a BCS of 4 could be ideal or overweight, depending on which scale is used. However, if the BCS is recorded as 4/9 or 4/5, it is easy to determine the appropriateness of the dog's body weight.

Gebin Li *et al.* (2012) supplemented five-point BCS with body fat percentage, as calculated using morphometric measurements, can increase the ability to detect overweight in small-sized dogs, by increasing the sensitivity for detecting alterations in plasma metabolite levels, especially those of lipid metabolites, such as nonesterified fatty acids, total cholesterol, and triglycerides, induced as a result of increasing adiposity in overweight dogs. Therefore, body fat percentage should be used in tandem to complement the five-point BCS system to detect and diagnose overweight in dogs.

Feldman *et al.* *(*2015) described conversion factors for thyroid hormones from the traditional unit to the S.I. unit.

Waters DJ (2015) defined healthy dogs as free from sensory problems, and health problems such as allergies, teeth and joint problems, dysplasia, epilepsy, reproductive issues, heart failure, diabetes, thyroid problems, cancer, and infections.

Badavi *et al.* (2020) investigated hematological normal references ranges of healthy dogs as followed: RBC 5.4-8.84 ×10 6 / µL, Hb 13.1-19.6 g/dl, HCT 37.79-60.22%, MCV 65-76 fl, MCH 21.2- 26.1 pg, MCHC 31-38.2 g/dl, RDWS 24.4-39.5 fl, RDWC 13.3-19.6%, reticulocyte 0.05-2%, PLT 137-504 ×103/ µL, PCT 0.21-0.63%, MPV 11.3-17.7 fl, PDWS 12.8-35 fl, PDWC 36.3-46.6, PLCC 62-173 ×10 3/ µL, PLCR 25.9-59.3%, PT 6-13 second, PTT 15-27 second, CT 3-35 minutes,CR percentages 0.05-0.37, WBC 6.15-19.04 × 103/µL, lymphocyte 0.61-5.19 × 10 3/ µL and 8-60%, neutrophil 2.42-14.47 × 10 3/µL and 28-88%, monocyte 0-1.82 × 10 3/µL and 0-16%, eosinophil 0-1.91 × 103/ µL and 0-20% and basophils was zero. Also, biochemical tests were revealed the normal range as follows' 18.7-95.3 U/L, AST 11.8-50.7 U/L, bilirubin 0.50-0.59 mg/dl; blood urea 20.40-44.80 mg/dl, creatinine 0.55-2.03 mg/dl, total protein 4.1-11.6 g/dl, albumin 1.65-4.28 g/dl and glucose 57.7-112 mg/dl.

Liyanage et al. (2022) compared the BCS assessments performed by the owners of 95 large-sized, purebred dogs without and with the guidance of a 5-point BCS chart. Only 23 of 95 dog owners accurately assessed the BCS of their dogs and the correct assessments significantly increased after being guided by a BCS chart (50/95, $p < 0.001$). The present findings suggest that BCS charts are useful for reducing owner-misperception of canine body condition in large- sized, purebred dogs.

2.2 Clinical signs and symptoms

Milne KL and Hayes HM Jr (1981) reported that neutered females had a significantly greater risk and neutered males had a slightly higher risk of developing hypothyroidism than intact bitches or dogs.

Gonzalez and Quadri (1988) studied the effects of advancing age on basal serum concentrations of thyroxine (T_3 and thyrotropin (TSH), on T~ responses to TSH, and on TSH responses to thyrotropin-releasing hormone (TRH) in female beagle dogs belonging to four age group: prepubertal (11.4 + 0.2 (SD) weeks), adult (2.1 ± 0.3 years), middle-aged (6.5 _+ 0.2 years), and old (12.4 _+ 0.3 years). They reported that serum T4 levels in the old dogs were significantly lower than those in the adult dogs. There were no significant differences in serum TSH concentrations among the four age groups.

Jaggy *et al.* (1994) described Neuromuscular signs in association with hypothyroidism are described in **29** dogs. Eleven dogs had lower motor neuron signs, **9** had peripheral vestibular deficits, **4** had megaesophagus, and **5** had laryngeal paralysis.

Dixon *et al.* (1999) reported most common clinical characteristics associated with hypothyroidism were metabolic signs (84 percent of cases), particularly lethargy (76 percent), obesity or weight gain (44 percent), and exercise intolerance (24 percent); and dermatological abnormalities (80 percent), including alopecia (56 percent), poor coat quality (30 percent) and hyperpigmentation (20 percent).

Lathan (2012) reported Common signs in hypothyroid dogs include weight gain, lethargy, and weakness; cold intolerance or heat-seeking behavior; and mental dullness. Dermatologic changes are common which include Alopecia, seborrhea, and pyoderma, bilaterally symmetric alopecia in areas of increased wear (eg, lateral thorax, tail), Slow regrowth of clipped hair, Dry and brittle hair coat, Hyperpigmentation, hyperkeratosis, myxedema, and otitis externa, Increased incidence of pyoderma, folliculitis, generalized demodicosis, and Malassezia infections as a result of immunosuppression. Neurologic dysfunction like Peripheral neuropathy may result in exercise intolerance, ataxia, weakness, conscious proprioception deficits, and hyporeflexia. Peripheral and central vestibular diseases have been reported.

Parry (2013) suggested that as in euthyroidal sick syndrome. abnormal findings on thyroid function tests are found to occur in the setting of a non-thyroidal illness. Numerous diseases can suppress the levels of circulating thyroid hormones due to hypothalamic or pituitary gland suppression, resulting in reductions in TSH secretion, thyroid hormone synthesis, and concentrations or binding ability of circulating proteins, as well as inhibition of T_3 to T_4 conversion. In euthyroid sick syndrome, the level of T_4 suppression correlates with the severity of the disease. FT4 levels may also be suppressed, although typically to a lesser degree than T_4 levels. Serum TSH levels may be normal or increased.

Srikala and Kumar (2014) reported primary hypothyroidism caused by thyroxine (T_4) deficiency and peripheral disorders in euthyroid dogs, which mimic hypothyroidism in the study undertaken on 10,172 dogs presented at the clinics with skin and coat incongruity, revealed clinical manifestations suggestive of hypothyroidism and low level of total thyroxine (T_4) indicating primary hypothyroidism. The common clinical signs were bilateral alopecia, rat-tail appearance, dry and lusterless coat, obesity, exercise intolerance, and lethargy. Most of the cases were acquired hypothyroidism, the rest were congenital. The disorders in primary hypothyroidism were related to dermatological, metabolic, ocular (neuromuscular, renal, cardiovascular, and musculoskeletal systems. Dermatological lesions in primary hypothyroidism revealed complicities of *Malassezia* *pachydermatis*, *Demodex canis*, *Staphylococci* and *Escherichia coli* bacteria. Metabolic disorder displayed exercise intolerance, lethargy, obesity, and dyspnea at rest. Ocular disorder manifested corneal lipidosis. Neuromuscular disorders constituted seizures, paraplegia, and facial paralysis. Renal disorder constituted poly urea with increased levels of blood sugar, BUN, and serum creatinine. The common cardiovascular abnormality was dilated cardiomyopathy. Musculoskeletal disorder reflected lameness due to osteoarthritis.

Jaiswal *et al.* (2018) stated that nonthyroidal illness syndrome (NTIS, Euthyroid Sick Syndrome) refers to the suppression of serum thyroid hormone concentrations that occur in euthyroid patients due to concurrent illness. Disorders that are frequently associated with NTIS in dogs include neoplasia, renal disease, hepatic disease, cardiac failure, neurologic disease, inflammatory disorders, and diabetic ketoacidosis.

Haseltine *et al.* (2019) defined Euthyroid Sick Syndrome as a syndrome that refers to a condition in which nonthyroidal illness suppresses the concentration of circulating thyroid hormones. The mechanism is complex and likely involves changes in hormone distribution and metabolism and altered binding of hormones to proteins.

Roopali *et al.* (2020) conducted a study on the prevalence of canine hypothyroidism in the Durg-Bhillai, Raipur, and Rajnandgaon districts of Chhattisgarh state. Dogs between 6-8 years of age (52.381%) were more susceptible to hypothyroidism. The prevalence of hypothyroidism was higher in male dogs (59.52%) as compared to females (40.48%). Hypothyroidism in dogs was found to be more prevalent in the Labrador breed followed by Golden Retriever, Spitz, German Shephard, Pug, Saint Bernard and Rottweiler respectively. Dogs having body weight above 40 kg were more susceptible to hypothyroidism accounting for a prevalence rate of 57.14%.

Raja ***et al.*** **(2021)** observed that males and females were equally susceptible to hypothyroidism and there was no sexual susceptibility.

Kour et al. (2021) studied on the prevalence of hypothyroidism for 2 year at small animal OPD of university, Ludhiana, Punjab. (0.174%). The most common clinical characteristics associated with hypothyroidism were metabolic signs, particularly lethargy (51.43%), obesity or weight gain (80.08%), exercise intolerance (68.57%) and dermatological abnormalities including bilateral alopecia (85.71%), rat tail appearance (71.42%), hyperpigmentation (28.57%), pruritus and poor coat quality (14.28% each).

Boretti (2022) stated that the progression of the disease is slow, and clinical signs are not expected to occur unless at least 75% of the thyroid is affected. As thyroid hormones have a wide variety of physiological effects on many organ systems, clinical signs of hormone deficiency are broad and unspecific. Often, they are attributable to a decreased metabolic rate such as lethargy, unwillingness to walk, exercise intolerance, and weight gain despite normal appetite. Other common findings are dermatological abnormalities, including seborrhea, hair thinning, and alopecia, particularly affecting the flanks and tail, skin hyperpigmentation, and superficial pyoderma. Less common signs include neuromuscular, gastrointestinal, cardiovascular, ocular, and reproductive 2 abnormalities. Most important routine clinicopathologic changes include mild to moderate non-regenerative anemia, hypercholesterolemia, and hypertriglyceridemia.

Gori ***et al.*** **(2023)** reported Approximately 45% of hypothyroid dogs had gastrointestinal signs, especially constipation and diarrhea. all hypothyroid dogs had a significant improvement in gastrointestinal signs after thyroid therapy. The improvement in gastrointestinal signs following treatment with levothyroxine supports a possible association between gastrointestinal signs and hypothyroidism. (pmc)

2.3 Haemato-biochemical finding in thyroid dysfunction

Sullivan *et al.* (1993) stated that Anemia reported in hypothyroid dogs resulted from reduced thyroxine concentration as it can directly affect erythroid stimulation and indirectly by increasing the cellular oxygen demands of erythropoietic cells.

Panciera (1994) observed hypothyroidism with alterations in several metabolic pathways due to the central role of the thyroid hormone in intermediate metabolism. Hyperlipidemia is common in canine hypothyroidism and is reflected by increased serum cholesterol and triglyceride concentrations in 75–90% of the cases.

Dixon *et al.* (1999) reported the laboratory reference limits the most common biochemical and hematological abnormalities were increased concentrations of triglycerides (88 percent), cholesterol (78 percent), glucose (49 percent), and fructosamine (43 percent), and increased activities of creatine kinase (35 percent), and decreased concentrations of inorganic phosphate (63 percent), and a low red blood cell count (40 percent). Hyperlipidemia is common in canine hypothyroidism and is reflected by increased serum cholesterol and triglyceride concentrations in 75– 90% of the cases.

Panciera (2001) reported Anemia is a frequent finding in hypothyroid which is nonregenerative and typically normocytic and normochromic. platelet number is increased and platelet size is decreased. Platelet aggregation has been reported to be normal in dogs with hypothyroidism, although details of the methodology and results were lacking.

Rochen *et al.* (2003) stated that thyroid hormones improve the ability of insulin to stimulate glucose disposal related to insulinemia. This phenomenon may be highly sensitive, because it was only apparent at low thyroid hormone levels. The ratio of the incremental increase in glucose disposal to the increase in plasma insulin was significantly improved after thyroid hormone treatment in hypothyroid patients (0.05). It was also increased in healthy volunteers, but not significantly.

Rose *et al.* (2005) described Hypothyroidism in rats decreases peripheral glucose utilization by decreasing insulin responsiveness.

Inteeworn (2008) reported in the hypothyroid group, basal glucose concentrations were mildly decreased, whereas basal insulin was increased. Insulin sensitivity was reduced in the hypothyroid group. Hypothyroidism negatively affects glucose homeostasis by inducing insulin resistance.

Gommeren et al. (2009) describe decreased GFR in hypothyroid dogs and an increase in GFR after re-establishment of euthyroidism. This study also showed that serum creatinine concentration was slightly increased or remained within the reference range in hypothyroid dogs. This observation confirms that serum creatinine concentration is an unreliable indicator of kidney function in hypothyroid dogs while serum creatinine concentration can be increased in hypothyroidism because of reduced GFR, but also may be increased because of creatinine generation from possible myopathy and rhabdomyolysis.

Kawa *et al.* (2010) reported that RBC, Hb, and HCT in patients with hyperthyroidism were significantly higher than control groups while RBC and Hb were decreased in hypothyroidism, while HCT was increased. They also showed that MCH and MCHC were lower in both groups in comparison with a control group and MCV was increased in two groups of hypothyroidism and hyperthyroidism after his studies in human patients.

Michal Mazaki-Tovi *et al.* (2010) studied gender-matched hypothyroid (n = 25) and healthy (n = 25) client-owned dogs within comparable age and body condition score (BCS) ranges. Fasted blood samples were collected from each dog and analyzed for glucose, cholesterol, triglyceride, leptin, and insulin concentrations where Leptin and insulin concentrations were significantly higher in the hypothyroid compared to normal dogs following adjustment for potential confounders and no significant difference in glucose concentration between the hypothyroid and normal groups following adjustment for BCS. This study showed that canine hypothyroidism is associated with increased serum leptin and insulin concentrations, neither of which may be attributed to obesity alone.

Pearce (2012) stated that Thyroid hormone has multiple effects on the regulation of lipid synthesis, absorption, and metabolism. Studies consistently demonstrate elevated levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B, lipoprotein(a), and possibly triglycerides in individuals with overt hypothyroidism, all of which are reversible with levothyroxine therapy.

Jaiswal *et al.* (2018) observed mild non-regenerative anemia on the complete blood count and an increased serum cholesterol concentration on a serum biochemistry panel. Low serum T_4, free T_4, and increased serum TSH concentrations in a dog with appropriate clinical signs and clinicopathological abnormalities in the diagnosis of hypothyroidism, especially if systemic illness is not present.

Kaur et al. (2021) studied on prevalence of hypothyroidism for 2 year at small animal OPD of university, Ludhiana, Punjab. (0.174%). The mean total thyroxine level in hypothyroid dogs was significantly lower, whereas the mean thyroid-stimulating hormone (TSH) level was significantly higher as compared to healthy dogs. The haemato-biochemical changes included elevated TLC, hypercholesterolemia, hyper-triglyceridemia, significantly higher ALP, hypocalcemia, and hypophosphatemia.

2.4 Radio Immuno Assay:

Jensen (1999) evaluated analytical performance using total error criteria of a commercial enzyme immunometric assay for the determination of endogenous canine thyrotropin (TSH). The results of the present study showed that the canine TSH assay had good to excellent analytical performance.

Pawar (2009) studied six clinical cases of hypothyroidism in dogs based on history, clinical examination, hematology, and biochemical parameters. Thyroid profiling, electrocardiography (E.C.G), and Gamma Scintigraphy was done to confirm subsequent findings. He recommended that investigations like thyroid profile, E.C.G., and 99mTc-pertechnate uptake percentage in comparison with salivary gland were useful in assessing the thyroid function. These tests showed a positive correlation in the confirmation of canine hypothyroid cases. Thyroid profile (CLIA) with 99^{m}Tc-pertechnate uptake percent showed a better correlation.

Galdhar and Gaikwad (2015) reported that in India, veterinary-specific RIA kits are not available. Whatever work is undertaken in this area is based on the use of human-based commercial RIA kits, which are manufactured primarily for the diagnosis of human diseases. Therefore, direct use of these kits may have or may not have justifiable clinical utility always. Hence, it is vital to validate these kits for veterinary use in physiological and pathological conditions.

Naik (2016) undertook studies on fifty-nine cats over a period of eight months. The serum concentrations of total Triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) were measured in all cats using RIA. Estimation of total Triiodothyronine (TT_3) was carried out as per standard method but estimation of total Thyroxine (TT_4) and free Thyroxine (fT_4) were carried out by modifying the standard procedures. The alterations in serum concentration of thyroid hormones were also undertaken in healthy cats concerning age, breed, sex, diet, time of collection, and usages of litter. The mean serum concentration of Triiodothyronine (tT_3 ng/ml), total Thyroxine (tT_4 ng/ml), and free Thyroxine (fT_4 pg/ml) in healthy cats was 0.45±0.08 S.E., 21.98±2.94 S.E. and 24.4±2.24 S.E respectively. The alterations in serum concentration of free Thyroxine (fT_4) between age groups 1-3 years, 3-6 years, and 6-9 years were found to be significant. No significant alterations were observed in the thyroid profile between breeds, sexes, or different diets.

Galdhar and Gaikwad (2017) stated RIA's principal and application in veterinary clinical practice and research. The author also stated general assay terminologies along with quality control parameters to be done for performance evaluation.

Galdhar and Gaikwad (2017) reported the application of radiation in veterinary practice. The author also described radiation exposure and biological effects and radiation protection and safety to counter them.

Dadke (2018) concluded by screening a of total 107 dogs for thyroid hormone levels. The commercially available BRIT-manufactured RIA kits, designed for human biological samples along with their prescribed procedure could be used for the estimation of canine TT_3 and TT_4, however, author had partially modified the procedure for estimation of canine fT_4 by adding one extra wash with wash buffer to improve percent binding of tracer and remove traces of proteins. The Mean serum concentration of Total Thyroxine (TT_4 nmol/l), Total triiodothyronine ($TT_{3,}$ nmol/l), and Free thyroxin (fT_4, pmol/l) in healthy dogs were 29.67±1.43, 1.03±0.02 and 9.07±0.52 respectively by Radio Immuno Assay. A significant difference was recorded in the thyroid profile of healthy, nonthyroidal illness, and hypothyroid dogs. This established data can be used as reference data to ascertain thyroid health in the canine population in India.

Jayabhaye (2019) studied the thyroid hormone profile of 100 pigs in India. He used commercially available BRIT-manufactured RIA kits designed for human samples with the prescribed procedure. He concluded the mean serum concentration of total triiodothyronine (TT_3, nmol/l), total thyroxine (TT_4 , nmol/l), and free thyroxin (fT_4, pmol/l) in healthy pigs were 0.81±0.04 (Range: - 0.15-2.30);41.58±1.23 (Range: 17.37-70.79) and 5.31±0.18 (Range: 2.57-10.81) respectively by Radio Immuno Assay. He also concluded that commercially available BRIT- manufactured RIA kits, designed for human use along with their prescribed procedure could be used for the estimation of swine TT_3 and TT_4, however for estimation of swine fT_4 need to carry out partial modification in the prescribed procedure.

Galdhar *et al.* (2022) studied thyroid function in dogs in comparison with that of humans in 192 hypothyroid cases of dogs from the clinical setup of constituent colleges of MAFSU (*viz*. Mumbai, Nagpur, Parbhani, Shirwal, Udgir, and Akola) were ethically enrolled to examine thyroid hormones levels (TT_3, TT_4 and FT_4). Thyroid hormones were estimated using RIA. The results of the present study recorded mean serum TT_3, TT_4, and FT_4 values in healthy dogs as 1.29±0.04 nmol/l,28.17±1.18 nmol/l and 13.03±0.68 pmol/l respectively. The reference interval (25th to 75th percentile) for TT_3, TT_4 and FT_4 was found to be 0.88-1.51 nmol/l, 15.70-35.29 nmol/l and 7.80-14.75 pmol/l respectively. The median for TT_3, TT_4, and FT_4 was found to be 1.13nmol/l, 24.54 nmol/l, and 10.00 pmol/l respectively. The baseline serum thyroid hormone concentration would be valuable for identifying thyroid dysfunction in dogs.

2.5 Thyroid Gland and Thyroid hormone status (TT_3, TT_4, fT_4 & cTSH):

Peterson *et al.* (1997) reported progressive loss of thyroid gland function in a human patient with hypothyroidism, increased T_4 concentration.

Scott-Moncrieff and Nelson (1998) reported Median baseline TSH concentration was 0.25 ng/ml (range, 0.03 to 0.44 ng/ml) in healthy dogs, 0.93 ng/ml (0.21 to 3.5 ng/ml) in hypothyroid dogs and 0.21 ng/ml (0.03 to 0.63 ng/ml) in euthyroid dogs with concurrent diseases.

Salvator *et al.* (2016) stated that Thyroxine (T_4) and 3, 5, 3′-triiodothyronine (T_3) are iodine-containing amino acids. Thyroid hormone synthesis requires iodine and is dependent upon the ingestion of adequate iodide from the diet. Iodide is actively transported from the extracellular fluid into the thyroid follicular cell by the sodium-iodine symporter (NIS), where it is rapidly oxidized by thyroid peroxidase (TPO) into a reactive intermediate. At the apical membrane, iodine is incorporated into the tyrosine residues of Tg. TPO also catalyzes the coupling of the non-biologically active iodinated tyrosine residues (monoiodotyrosine [MIT], and diiodotyrosine [DIT]) to form the biologically active iodothyronines-T4 and T3. These iodination reactions are referred to as organification.

H.S. Kooistra (2000) reported pulsatile secretion of TSH in dogs during hypothyroidism and only small fluctuations in plasma TSH concentrations during euthyroidism in the study of 7 beagle bitches where the plasma profiles of TSH analyzed by collecting blood samples every 10 min for 6 hr, both before and after induction of primary hypothyroidism. After induction of primary hypothyroidism, a 37-fold increase in mean basal plasma TSH concentration and a 34-fold increase in mean area under the curve for TSH were found.

Dixon (2001) stated that the combination of reduced T_4 and increased cTSH value is highly specific for hypothyroidism. however, approximately 20 % of hypothyroid dogs have reference range cTSH values.

Kemppainen and Behrend (2001) The use of canine TSH measurement can be summarized as follows: 1) An elevated TSH concentration together with a low T4 value or low free T_4 by dialysis indicates a high likelihood of hypothyroidism. 2) A normal TSH concentration with a normal total T4 value or normal free T_4 by dialysis is interpreted as a normal thyroid with high likelihood. 3)The canine TSH assay has a significant rate of false-positive and false-negative results. 4) Measurement of canine TSH alone is not recommended for the diagnosis of hypothyroidism in dogs.

Gulzar *et al.* (2014) studied 1728 adult dogs aged one year or greater for the prevalence of hypothyroidism from September 2010 to March 2011 in the Pet Section, Teaching Veterinary Clinical Complex (TVCC), LUVAS, Hisar. Quantitative measurement of tri-iodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH) in the serum of each blood sample of a dog was done by electrochemiluminescence immunoassay (ECLIA) using COBAS-E immunoassay analyzer. Based on low levels of T_3, T_4, and higher levels of TSH, a total of 8 dogs were diagnosed with hypothyroidism. All these eight dogs had T_3, and T_4 levels less than the normal range of 48.00–154.00 ng/dl and 1.50–3.60 µg/dl respectively and the level of TSH was greater than the normal range of 0.30–0.60 ng/ml in all these hypothyroid dogs. In Hisar and adjoining areas, the prevalence of hypothyroidism was found to be 0.4 percent.

Haseltine (2019) stated Total T4 concentration is a useful screening test for hypothyroidism. The sensitivity of this test for the diagnosis of canine hypothyroidism is reported to be 89% to 100%. If the T_4 concentration is well within reference range, the dog is likely euthyroid and further thyroid testing is not required. Free T_4 (fT_4) and thyroid-stimulating hormone (TSH) are evaluated only if the T_4 concentration is low. When a dog suspected to have hypothyroidism has a low total T_4 concentration, fT_4 and/or TSH concentrations must be evaluated to help confirm or refute the diagnosis. If the TSH concentration is high, hypothyroidism can be diagnosed. However, 13% to 38% of hypothyroid dogs have normal TSH concentrations so a normal TSH concentration does not exclude the diagnosis. Because of this limitation, it is often helpful to evaluate fT_4 and TSH simultaneously as confirmatory tests. If the fT4 is low, a diagnosis of hypothyroidism can be made. If T_4 is low and fT_4 is within reference range, hypothyroidism cannot be diagnosed, and T_3 concentrations vary widely and are not diagnostically useful.

Ryad *et al.* (2021) compared thyroid hormone profiles in hypo-and euthyroid dogs, a significant reduction in TT_4 and fT_4 was observed in hypothyroid patients compared to euthyroid patients, while TSH showed significant elevation in hypothyroid patients compared to euthyroid patients. Significant elevation of cholesterol, triglycerides, and ALP was recorded in hypothyroid patients compared to euthyroid patients.

Kaur *et al.* (2021) reported that in hypothyroid dogs, the mean total thyroxine level (0.44 ± 0.61 vs. 2.17 ± 0.26 µg/ dL) was significantly ($p \leq 0.01$) lower, and mean TSH levels (6.79 ± 0.01 vs. 0.95 ± 0.04 ng/mL) was significantly ($p \leq 0.01$) higher as compared to that of healthy dogs (T4 (µl/dl) =1.21 ± 0.65 and TSH (ng/ml) = 0.09- 1.08 ng/ml).

Gupta *et al.* (2023) studied on 25 cases of hypothyroid dogs in the Mumbai region. Based on the thyroid profile by the ECLIA method, all 25 cases were found with low T_4 and T_3, whereas TSH values were found within the normal and subnormal range.

2.6 Alteration in thyroid hormones and function due to drugs

Daminet *et al.* (1999) studied the short-term effects of prednisone and phenobarbital on serum total thyroxine (tT4), free thyroxine (ff4), and thyroid stimulating hormone (TSH) evaluated in Twenty-six beagles (euthyroid) dogs. Phenobarbital therapy in this study did not affect serum tT4, ff4, or TSH concentrations. Prednisone therapy significantly decreased serum tT4 and ff4 but did not affect serum TSH concentrations.

Daminet and Ferguson (2003) stated Glucorticoids ↓ TT_4, ↓ OR = FT_4 and ↓OR = TSH; Phenobarbital ↓ OR = TT_4, ↓ OR = FT_4 and = or ↑ TSH; Sulfonamides (30 mg/kg q12h) ↓ TT_4, ↓ FT_4 and ↑ TSH; Propranolol unchanged TT_4, FT_4 and TSH; Carprofen ↓ TT_4, ↓FT_4 and ↓ TSH; Potassium bromide unchanged TT_4, FT_4 and TSH hormones.

Lathan (2012) reported Several medications can affect thyroid hormone concentrations these drugs (except sulfonamides) rarely cause clinical hypothyroidism but can interfere with test results. Glucocorticoids decrease TT_4, fT_4, and sometimes TSH in a dose-dependent manner. Phenobarbital can cause decreased TT_4 and fT_4 and slightly increased TSH but does not cause clinical hypothyroidism. Sulfonamides block T_3 and T_4 synthesis and long-term administration can cause clinical hypothyroidism with increased TSH which is reversed by discontinuation. Potassium bromide does not appear to affect thyroid function. Aspirin decreases TT_4 and fT_4 concentrations. Carprofen, meloxicam, and deracoxib do not appear to significantly affect thyroid function.

Boretti (2022) stated Glucocorticoids, Phenobarbital, Potassium bromide, Imepitoin, Clomipramine, Sulfonamides, and Carprofen are commonly used drugs that may alter circulating hormones.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH was carried out on a total of seventy-seven (n=77) dogs at Department Veterinary Clinical Medicine, Ethics and Jurisprudence and Department of Veterinary Nuclear Medicine including Radio Isotope Laboratory, Mumbai Veterinary College, Parel, Mumbai-400012, Maharashtra Animal and Fishery Science University Nagpur.

3.1 Statutory permission to undertake the research work:

The present study was initiated after permission from the Board of Studies in Veterinary Clinical Medicine, Ethics and Jurisprudence, Resolution no: VCM/7/2023. Dated: 25/07/2023, Ethics Committee No.MVC/IEC-VCR2023/VCM-M-3

3.2 Selection of dogs

In the present study, a total of seventy-seven (n=77) dogs were selected from different breeds in Mumbai. The study was conducted over a period of 6 month i.*e.* between October 2023 to February 2024. All dogs were included with dueconsent of the owner (Appendix A) and permissions. The dogs included in the study were from TVCC (Teaching Veterinary Clinical Complex), Mumbai Veterinary College, Mumbai, and BSDPHA (Bai Sakarbai Dinshaw Petit Hospital for Animals), Parel, Mumbai. The selected dogs were grouped as apparently healthy dogs (n=67) and suspected clinical hypothyroid dogs (n=10).

3.2.1 Inclusion and exclusion criteria for the establishment of thyroid profile in healthy dogs:

Inclusion criteria:

- ✓ Dogs of either sex and age (above one year).
- ✓ Any breed including nondescript dogs included in the study.
- ✓ clinically healthy Dogs and no endocrinopathy were included in the study

Exclusion Criteria:

- ✓ Dogs presently being treated for any endocrinopathies including thyroid dysfunction.
- ✓ Pregnant and nursing bitches.
- ✓ Dogs on medicines that can potentially alter thyroid functioning and thyroid hormones estimation (viz. steroid, methimazole and L-thyroxine, perchlorate or iodine therapy, sulphonamide, etc.).

3.2.2 Selection of hypothyroid dogs:

Dogs showing characteristic clinical signs such as bilateral alopecia, rat-tail, obesity, and lethargy were included in dogs with suspected hypothyroidism group.

The summary of the selected healthy dogs and suspected clinical hypothyroid dogs are shown in Table 1 and Table 2 respectively.

3.3 Anamnesis and Clinical Examination:

After enrolment of clinical cases history was taken regarding appetite, diet, age, sex, breed, deworming, and vaccination. After history taking all the dogs were examined clinically for body and coat condition, the color of a mucous membrane, hydration status, body weight, heart rate(beats/min), respiration rate(beats/min), rectal temperature(°F), palpation of cervical and abdominal region, auscultation and systolic blood pressure (mm Hg).

Table 1 Summary details of healthy Dogs(n=67)

Sr. No.	Sample Code	Age (Yr.)	Breed	Sex	Body Weight (Kg)
1	0-1	7.5	ND	Female	28 Kg
2	0-2	3	ND	Male	20 Kg
3	0-3	7	ND	Female	21 Kg
4	0-4	4	ND	Male	35 Kg
5	0-5	4	Labrador	Female	25 Kg
6	0-6	3.5	ND	Male	22 Kg
7	0-7	5	ND	Female	28 Kg
8	0-8	4.6	ND	Male	32 Kg
9	0-9	14	ND	Male	20 Kg
10	0-10	2	ND	Male	15 Kg
11	0-11	4	ND	Male	25 Kg
12	0-12	8	ND	Female	22 Kg
13	0-13	3	Golden Retriever	Male	28 Kg
14	0-14	4	ND	Female	12 Kg
15	0-15	4	ND	Male	15 Kg
16	0-16	3	Labrador	Male	35 Kg
17	0-17	8	ND	Male	20 Kg
18	0-18	5	ND	Male	20 Kg
19	0-19	6	ND	Male	28 Kg
20	0-20	4	ND	Male	17.2 Kg
21	0-21	1	ND	Female	20 Kg
22	0-22	8	Labrador	Female	25 Kg
23	0-23	1	ND	Female	20 Kg
24	0-24	9	ND	Female	25 Kg
25	0-25	1	Golden Retriever	Female	25.5 Kg
26	0-26	5	German shepherd	Male	30 Kg
27	0-27	4	Labrador	Male	26 Kg
28	0-28	3	ND	Male	35 Kg

29	0-29	4	Labrador	Male	36.4 Kg
30	0-30	4	German shepherd	Male	30 Kg
31	0-31	6	ND	Male	30 Kg
32	0-32	4	German shepherd	Female	24.5 Kg
33	0-33	4	ND	Male	20 Kg
34	0-34	5	ND	Male	20 Kg
35	0-35	2.5	German shepherd	Male	26 Kg
36	0-36	5	Dobermann	Male	25 Kg
37	0-37	3	ND	Female	23 Kg
38	0-38	4.5	Pomeranian	Male	12 Kg
39	0-39	1	German shepherd	Male	29 Kg
40	0-40	3	Pomeranian	Male	10 Kg
41	0-41	3	Rottweiler	Female	28 Kg
42	0-42	6.5	Golden Retriever	Male	45 Kg
43	0-43	6	ND	Male	12 Kg
44	0-44	1.5	Chow chow	Male	17 Kg
45	0-45	5	ND	Male	18.3 Kg
46	0-46	4	ND	Male	20 Kg
47	0-47	6	Labrador	Male	30 Kg
48	0-48	5	ND	Male	25 Kg
49	0-49	5	ND	Male	20 Kg
50	0-50	4	ND	Female	21 Kg
51	0-51	3	ND	Female	26 Kg
52	0-52	5	ND	Female	25 Kg
53	0-53	5	Labrador	Female	30 Kg
54	0-54	1.6	Labrador	Female	20 Kg
55	0-55	6	ND	Male	15 Kg
56	0-56	5	ND	Female	19 Kg
57	0-57	9	ND	Male	20 Kg
58	0-58	5	ND	Female	25 Kg

59	0-59	3	Labrador	Male	30
60	0-60	4	Labrador	Female	28
61	0-61	13	ND	Male	23
62	0-62	4	ND	Male	25
63	0-63	6	ND	Female	23
64	0-64	3	ND	Male	29
65	0-65	1.5	ND	Male	20
66	0-66	5	ND	Female	21
67	0-67	4	Labrador	Male	30.7
Mean	------	4.64	------------------------	----------	23.98
SD	------	2.42	------------------------	----------	6.42
SE	-------	0.30	------------------------	----------	0.78
Mean ± SE	------	4.64±0.30	--------------------------	----------	23.98±0.78

Table 2 Summary details of suspected hypothyroid dogs(n=10)

Sr. No.	Sample Code	Age (Yr.)	Breed	Sex	Body Weight (Kg)
1	HT-1	3	ND	Male	28
2	HT-2	12	Labrador	Male	30
3	HT-3	2	ND	Male	20
4	HT-4	8	ND	Male	30
5	HT-5	5.6	Siberian Husky	Male	41
6	HT-6	7	Shitzu	Female	7
7	HT-7	5	Tibetan Mastiff	Female	32
8	HT-8	6	ND	Female	15.3
9	HT-9	11	ND	Female	18
10	HT-10	5	ND	Male	19.5
Mean	-----------	6.46	-------------	-------------	24.08
SD	------------	3.18	-------------	-------------	9.88
SE	------------	1.01	-------------	-------------	3.12
Mean ± SE	-----------	6.48 ± 1.01	-------------	------------	24.08 ± 3.12

3.4 Blood Collection:

About 5 ml of blood samples were collected from each dog. Immediately after collection of a blood sample, 2 ml was transferred to EDTA vials, and the remaining 3 ml was kept for harvesting of serum for estimation of sero-biochemistry and thyroid profile. Serum samples for thyroid profile estimation were stored at -20C until the time of analysis.

3.5 Hematological analysis:

Five milliliters (5ml) of blood samples were collected from each dog from a cephalic vein and/or saphenous vein. Immediately after the collection of blood, 2.0 ml of blood was collected in EDTA K3 tubes and mixed properly. Samples were analyzed immediately for a complete blood count by using an automatic hematology analyzer (Orphee Mythic 18).

The following hematological parameters were studied: -

1. Hemoglobin (Hb, gm %)
2. Packed Cell Volume (PCV, %)
3. Total Erythrocyte Count (TEC, $\times 10^6$/ µl)
4. Total Leucocyte Count (TLC, $\times 10^3$/ µl)
5. Differential Leucocyte Count
6. Neutrophils (N, %)
7. Lymphocyte (L, %)
8. Basophils (B, %)
9. Monocyte (M, %)
10. Eosinophils (E, %)
11. Platelets (PLT, $\times 10^3$/µl)
12. Mean Corpuscular volume (MCV, fl)
13. Mean Corpuscular Hemoglobin (MCH, pg)
14. Mean Corpuscular Hemoglobin Concentration (MCHC, gm/dl)

3.6 Biochemical Analysis:

Out of 5 ml collected blood sample remaining 3 ml was stored in sterile plain tubes and left for 30 minutes at room temperature. Then samples were centrifuged using a centrifuge machine (Remi instruments) at 2000 rpm for 10 minutes. The clear supernatant (serum) was aspirated using pipettes into sterile serum tubes and analyzed for biochemical parameters. After biochemical analysis, the serum sample was stored at -20ºC until the estimation of thyroid hormones. Serum biochemical parameters were analyzed by using Em 200 Transcasia which uses the principle of calorimetry. The following parameters were analyzed:

3.6.1. Liver function tests:

Total bilirubin (TB, mg/dl), Direct bilirubin (DB, mg/dl), Indirect bilirubin (mg/dl), Alkaline phosphatase (ALP, IU/L), Aspartate transaminase (AST, IU/L), Alanine transaminase (ALT, IU/L), Total proteins (TP, gm/dl), Albumin (A, gm/dl), Globulin (G, gm/dl).

3.6.2 Kidney function tests:

Blood urea nitrogen (BUN, mg/dl)
Creatinine (mg/dl)

3.6.2 Serum cholesterol (mg/dl):

Serum cholesterol (mg/dl)

3.6.3 Serum triglycerides (mg/dl):

Serum triglycerides (mg/dl)

3.6.4 Random blood glucose (mg/dl):

Random blood glucose was estimated by using an ACCU-CHECK glucometer (Roche Diabetes Care Medical Systems). A drop of blood was used for the estimation of blood glucose just after blood collection and before transferring to the EDTA K3 vial **(Plate 1).**

3.7 Thyroid profile using Radio Immuno Assay (RIA):

Thyroid hormones were estimated using RIA and the procedure was carried out at the Radio Isotope Laboratory, Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai. Commercial RIA kits for human purposes, manufactured by Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, Mumbai-94 and by Immunotech s.r.o., Radiova 1122/1, 102 00 Prague 10, Czech Republic, Beckman Coulter Company, wer e procured for thyroid profile estimation in dogs.The details of the kits procured for thyroid profiling are presented in Table 3 and RIA was undertaken as per safety guidelines recommended by Radiological Safety Officer.

Table 3 Details of RIA kits procured

Sr. No.	RIA Kit Code No.	Description of kits
1	IM1363 BECKMAN COULTER	Antibody Coated tube based kit for RIA of free thyroxin (fT_4)
2	BRIA MAG 3B	Magnetizable particle-based RIA KIT of triiodothyronine (TT_3)
3	BRIA MAG 4B	Magnetizable particle-based RIA KIT of Thyroxine (TT_4)

3.7.1 Estimation of Total Triiodothyronine (TT_3) and Thyroxine (TT_4):

Total Triiodothyronine (TT_3, ng/ml) and Total Thyroxin (TT_4) were measured as per the standard procedure outlined by the manufacturers.

Procedure:

1. Set up assay as shown in Table 4 and Table 5 respectively.
2. Place all tubes (except total count tubes) on a magnetic rack for 20 minutes.
3. Invert the rack gently to discard the supernatant.
4. Drain off the traces of supernatant by placing the inverted rack on absorbent tissue.
5. Count tubes for 1 min in the gamma counter. The Genesys Geni series, a NaI-based six-well detector by Laboratory Technologies Inc. was used.
6. Calculate results to get % B/B_0.
7. Plot standard curve of % B/B_0 against standard concentrations of (TT_3, ng/ml and TT_4, ng/ml) on semi-log graph paper.
8. Read sample value directly from the standard curve

3.7.2 Estimation of free Thyroxine (fT_4, pg/ml):

Estimation of free thyroxine was done using the standard procedure outlined by the manufacturer Table 6. Modifications in the manufacturer’s assay procedure undertaken by Dadke (2018) were followed during the procedure.

Calculations

1. Calculate the average corrected CPM (counts per minute) for each pair ofassay tubes.
2. Drawn the standard curve by plotting the corrected average CPM on the Y-axis against the standard concentration on the X-axis using a semi-log graph.

Table 4 Assay Flow Chart of Total Triiodothyronine (TT_3)

1	2	3	4	5	6	7
Tube No	**Standard/Sample (µl)**	**I^{125}- T3 (µl)**	**Anti T3 magnetic particle suspension (µl)**	Vortex and incubate the tubes for 3 hr. at room temperature on a shaker at 300rpm.	**Wash solution (µl)**	Mix and place the tubes except T1 & T2 firmly on the magnetic rack for 20 min. Decant the supernatant by inverting the rack. Measure the radioactivity
T_1, T_2	-	100	-			
Co	(50 µl Std A): 0 ng/ml	100	100		1000	
C_1	(50 µl Std B): 0.3 ng/ml					
C2	(50 µl Std C): 0.6 ng/ml					
C_3	(50 µl Std D): 1.2 ng/ml					
C4	(50 µl Std E): 2.4 ng/ml					
C5	(50 µl Std F): 4.8 ng/ml					
Control A	(50 µl Control A): 1.55 ± 0.5 ng/ml					
Control B	(50 µl Control B): 3.00 ±1.0 ng/ml					
Recovery	50 µl (25 µl of std + 25 µl of sample)					
Sample	(50 µl Sample)					

*Procedure was undertaken as per serial no. 1 to 7 for testing samples.

Table 5 Assay Flow Chart of Total Thyroxine (TT_4)

1	2	3	4	5	6	7
Tube No	**Standard/Sample (µl)**	**I^{125} - T4 (µl)**	**Anti T4 magnetic-particle suspension (µl)**	Vortex and incubate the tubes for 2hr at room temperature.	**Wash solution (µl)**	Mix and place the tubes except T_1 & T2 firmly on the magnetic rack for 20 min. Decant the supernatant by inverting the rack. Measure the radioactivity
T1, T2	-	100	-		-	
C_o	(25 µl - Std A): 0 ng/ml	100	100		1000	
C1	(25 µl - Std B): 15 ng/ml					
C_2	(25 µl - Std C): 30 ng/ml					
C_3	(25 µl - Std D): 60 ng/ml					
C4	(25 µl - Std E): 120 ng/ml					
C_5	(25 µl - Std F): 240 ng/ml					
Control A	(25 µl - Control A): 61 ± 10 ng/ml					
Control B	(25 µl - Control B): 99 ± 20 ng/ml					
Recovery	25 (12.5 µl of std + 12.5 µl of sample)					
Sample	(25 µl - Sample)					

*Procedure was undertaken as per serial no 1 to 7 for testing samples.

Table 6 Assay Flow Chart of free Thyroxin (fT_4):

1	2	3	4
Tube No.	**Standards/ Samples (µl)**	**Tracer**	**Ligand**
C0	25 µl	400 µl	100 µl
C1	25 µl		
C2	25 µl		
C3	25 µl		
C4	25 µl		
Control A	25 µl		
Recovery	25 µl (STD + SAMPLE)		
Samples	25 µl		

Steps	Calibrators and control	Serum	Tubes for total count
1. Additions To antibody-coated tubes add successively:			
calibrators or control, serum sample	25µl	25µl	25µl
Tracer	400µl	400µl	400µl
Ligand	100 µl	100 µl	100 µl
Mix (Vortex gently 1-2 sec)			
2. Incubation: Incubate for 1 hour with shaking at 18-25ºC (>400 rpm)			
3. Counting: Aspirate carefully the contents of tubes (expect two tubes for the count). Count bound CPM (B) and total CPM (T) for 1 min.			

*Procedure was undertaken as per Steps.

3.8 Quality Control Parameters Studied

In the present research work quality control parameters like estimation of control samples and recovery percentage were studied to increase confidence level.

3.9 Conversion of Thyroid Hormone units:

The units of TT_3, TT_4, and fT_4 were converted to S.I. units by multiplying thevalues obtained in traditional units using conversion factors given in Table 7.

Table 7 Conversion factors for thyroid hormones units (Schenk *et al.*, 2017)

Sr. No.	Hormone	Traditional Unit	Conversion Factor	SI Unit
1	TT_3	ng/ml	1.536	nmol/l
2	TT_4	ng/ml	1.287	nmol/l
3	FT_4	pg/ml	1.287	pmol/l
4	TSH	µIU/ml	-------	-------

3.10 Statistical analysis:

The mean and standard error for each parameter of collected data was calculated and analyzed statistically for comparison as per the methods suggested by Snedecor and Cochran (2004).

3.11 Estimation of TSH by canine TSH ELISA KIT.

Standard Concentration

Standard	S0	S1	S2	S3	S4	S5
Concentration (µlU/ml)	0	0.625	1.25	2.5	5	10

Plate 1. Glucometer

Plate 2. Canine thyroid stimulating hormone ELISA kit set up.

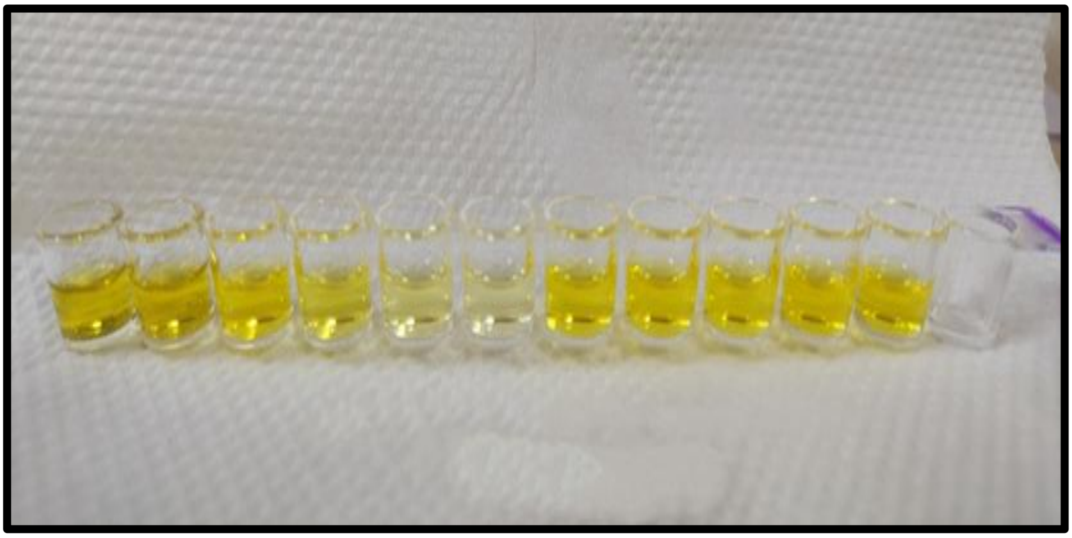

Plate 3. standard solution of ELISA plate

Plate 4. Color development on the addition of stop solution

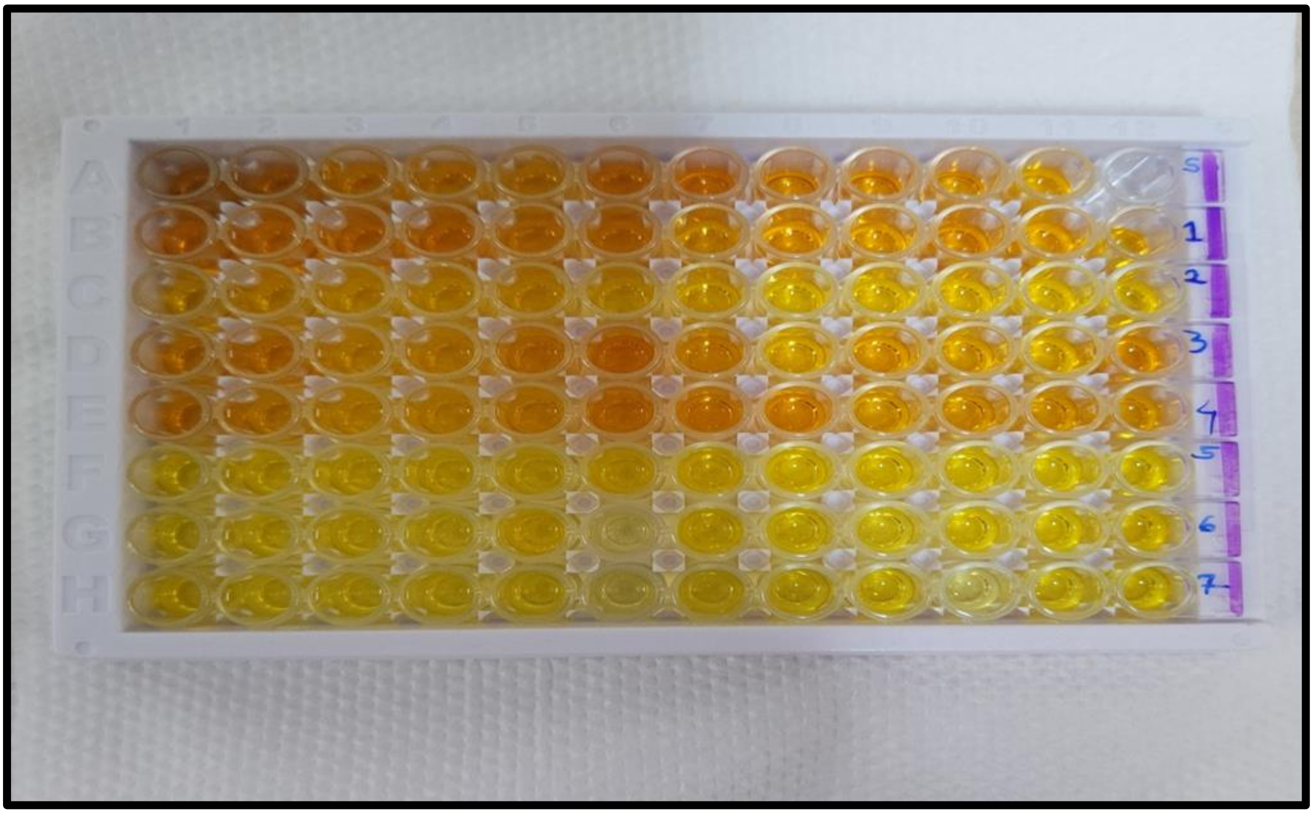

Plate 5. ELISA plate with a solution.

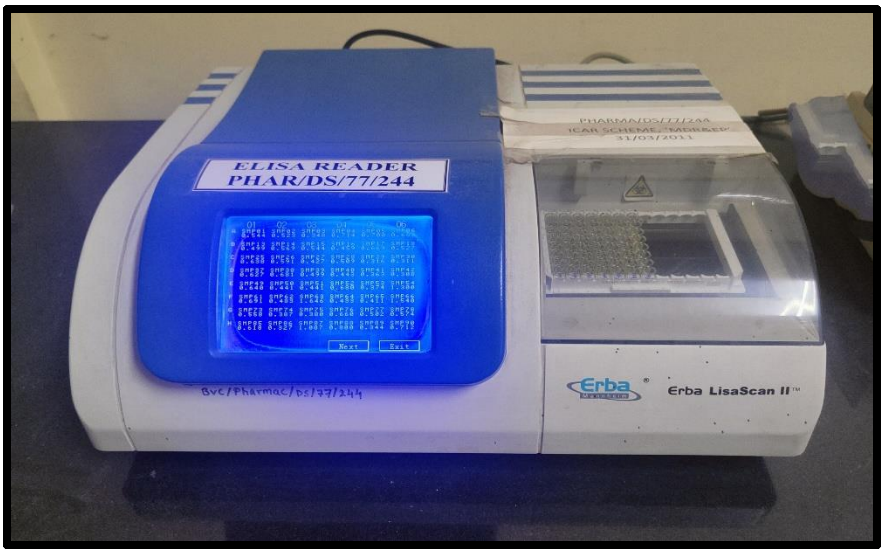

Plate 6. ELISA Reader

Assay Procedure:

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay.

1) Prepare and sample as instructed.

2) Set a blank well without any solution.

3) Add 50 µl of standard or sample per well.

4) Add 50 µl of conjugate to each well (not to blank well).

5) Mix well and incubate at 37°C for 60 min.

6) Aspirate each well and wash 3 times with wash buffer (200 µl).

7) Add 50 µl HRP –avidin to each well (not to blank well).

8) Mix well and incubate at 37°C for 30 min.

9) Aspirate each well and wash 3 times with wash buffer (200 µl).

10) Add 50 µl Substrate A and 50 µl Substrate B to each well.

11) Mix well and incubate at 37°C for 15 minutes (Protect from light).

12) Add 50 µl of stop solution to each well (plate 4 and plate 5).

13) Determine the optical density of each well within 10 min, using a microplate reader set to 450 nm.

The details of the ELISA kit procedure are presented in Table 9.

Calculation of results:

The standard curve is constructed by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and drawing a best-fit curve through the point on the graph. For each sample locate optical density on the x-axis and read off the corresponding concentration on a vertical axis.

Table 8 Assay Flow Chart for cTSH ELISA

1	2	3	4	5	6	7	8
Well no.	**Standard/ Sample(µl)**	**Conjugate**	**Washing**	**Addition of HRP-avidin**	**Washing**	**Addition of Substrate A and Substrate B**	**Addition of Stop Solution**
S0	50 µl:	Add 50µl to each well (except the blank well) Mix well and incubate at 37°C for	Aspirate and wash 3 times with wash solution(200µl).	Add 50µl HRP-avidin to each well (except the blank well). Incubate 30 min at 37°C for 30 min.	Aspirate and wash 3 times with wash solution(200µl).	Add 50µl Substrate A and Substrate B to each well Incubate 15 min at 37°C for 15 min. **(Protect from light.)**	Add 50µl Stop Solution to each well. Read at 450nm within 10 min.
S1	50 µl						
S2	50 µl						
S3	50 µl						
S4	50 µl						
S5	50 µl						
BLANK							
The procedure was undertaken as per serial no 1 to 8							

Results and Discussion

4. RESULTS AND DISCUSSION

4.1 To establish data about cTSH and its association with total Triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) in healthy dogs.

Present research work was carried out to establish data about Canine Thyroid Stimulating Hormones in dogs by using the ELISA technique and total triiodothyronine (TT_3), total thyroxine (TT_4), and free thyroxine (fT_4) in healthy dogs by radio immune assay.

The present study was carried out on a total of seventy-seven (n=77) dogs where 67 dogs were healthy and 10 dogs were hypothyroid.

4.1.1 Anamnesis and clinical findings:

Dogs' history regarding diet, vaccination, and deworming status were documented in Table 9. Clinical findings related to respiration rate (Breaths/min), heart rate (Beats/min), rectal temperature (°F), capillary refill time (Sec), body condition score, mucous membrane color, systolic blood pressure (mm Hg), and dermatological abnormalities were collected and presented in Table 10.

4.1.2 Hematological findings of healthy dogs:

Mean ± SE values of a hematological profile of healthy dogs (n=67) are presented in Table 11. In the case of healthy dogs, the Mean ± SE of Hemoglobin, Packed Cell Volume, Total Erythrocyte Count, and Total Leucocyte Count were 14.01±0.28 gm/dl, 40.52±0.72 %, 8.12±1.31 × 10^6/cmm and 15.12 ± 0.65×10^3/cmm respectively. For differential leukocyte count, the mean percentage of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were 70.72 ± 1.23 %, 24.36±1.03 %, 3.03 ± 0.41 %, 1.18 ± 0.06 %, and 0.01±0.01% respectively. For MCV, MCH, and MCHC, Mean ± SE values were 64.67±0.94 fl, 23.02±0.64 pg, and 34.10 ± 0.30 gm/dl respectively. The mean ± SE value of the platelet was 2.52 ± 0.09 /cu mm. All hematological parameters findings of reported dogs were in correlation with the reference values of Klaassen (1999).

Table 9. Anamnesis of healthy dogs (n=67).

Sr. No.	Sample code	Appetite	Diet	Vaccination Status	Deworming Status
1	0-1	Normal	Mixed	Yes	Yes
2	0-2	Normal	Mixed	Yes	Yes
3	0-3	Normal	Mixed	Yes	Yes
4	0-4	Normal	Mixed	Yes	Yes
5	0-5	Normal	Mixed	Yes	Yes
6	0-6	Normal	Mixed	Yes	Yes
7	0-7	Normal	Mixed	Yes	Yes
8	0-8	Normal	Mixed	Yes	Yes
9	0-9	Normal	Mixed	Yes	Yes
10	0-10	Normal	Mixed	Yes	Yes
11	0-11	Normal	Mixed	Yes	Yes
12	0-12	Normal	Mixed	Yes	Yes
13	0-13	Normal	Mixed	Yes	Yes
14	0-14	Normal	Mixed	Yes	Yes
15	0-15	Normal	Mixed	Yes	Yes
16	0-16	Normal	Commercial	Yes	Yes
17	0-17	Normal	Commercial	Yes	Yes
18	0-18	Normal	Commercial	Yes	Yes
19	0-19	Normal	Commercial	Yes	Yes
20	0-20	Normal	Commercial	Yes	Yes
21	0-21	Normal	Commercial	Yes	Yes
22	0-22	Normal	Commercial	Yes	Yes
23	0-23	Normal	Mixed	Yes	No
24	0-24	Normal	Mixed	Yes	Yes
25	0-25	Normal	Mixed	Yes	Yes
26	0-26	Normal	Mixed	Yes	Yes
27	0-27	Normal	Mixed	Yes	No
28	0-28	Normal	Mixed	Yes	Yes
29	0-29	Normal	Mixed	Yes	Yes
30	0-30	Normal	Mixed	Yes	Yes
31	0-31	Normal	Mixed	Yes	Yes
32	0-32	Normal	Mixed	Yes	Yes
33	0-33	Normal	Mixed	Yes	Yes
34	0-34	Normal	Mixed	Yes	Yes
35	0-35	Normal	Mixed	Yes	Yes
36	0-36	Normal	Homemade	Yes	No
37	0-37	Normal	Mixed	Yes	Yes
38	0-38	Normal	Mixed	Yes	Yes
39	0-39	Normal	Mixed	Yes	Yes
40	0-40	Normal	Commercial	Yes	Yes
41	0-41	Normal	Commercial	Yes	Yes
42	0-42	Normal	Commercial	Yes	Yes
43	0-43	Normal	Commercial	Yes	Yes

44	0-44	Normal	Commercial	Yes	No
45	0-45	Normal	Commercial	Yes	Yes
46	0-46	Normal	Commercial	Yes	Yes
47	0-47	Normal	Commercial	Yes	Yes
48	0-48	Normal	Commercial	Yes	Yes
49	0-49	Normal	Commercial	Yes	Yes
50	0-50	Normal	Commercial	Yes	Yes
51	0-51	Normal	Commercial	Yes	Yes
52	0-52	Normal	Commercial	Yes	Yes
53	0-53	Normal	Commercial	Yes	Yes
54	0-54	Normal	Commercial	Yes	Yes
55	0-55	Normal	Commercial	Yes	Yes
56	0-56	Normal	Mixed	Yes	Yes
57	0-57	Normal	Mixed	Yes	Yes
58	0-58	Normal	Mixed	Yes	Yes
59	0-59	Normal	Mixed	Yes	Yes
60	0-60	Normal	Homemade	Yes	No
61	0-61	Normal	Homemade	Yes	Yes
62	0-62	Normal	Homemade	Yes	Yes
63	0-63	Normal	Homemade	Yes	Yes
64	0-64	Normal	Homemade	Yes	Yes
65	0-65	Normal	Homemade	Yes	Yes
66	0-66	Normal	Homemade	Yes	Yes
67	0-67	Normal	Homemade	Yes	No

Table 10 Records of Clinical Examination of healthy dogs (n=67)

Sr. No.	Sample Code	Respiration Rate (Breaths/min)	Heart Rate (Beats /min)	Rectal Temperature (°F)	Capillary Refill Time (Seconds)	Mucous Membrane	B.C.S.	Blood Pressure (mmHg)
1	0-1	30	101	101	2	Pink	3	120
2	0-2	28	100	102.5	2	Pink	3	100
3	0-3	26	98	101.5	3	Pink	3	110
4	0-4	30	91	102	2	Pink	3	130
5	0-5	22	90	101	2	Pink	3	130
6	0-6	24	88	100	3	Pink	3	113
7	0-7	27	82	101	2	Pink	3	120
8	0-8	27	101	102	2	Pink	3	120
9	0-9	29	79	102	2	Pink	3	120
10	0-10	28	81	102	2	Pink	3	110
11	0-11	21	96	100	2	Pink	2	120
12	0-12	33	94	102	3	Pink	3	100
13	0-13	26	83	102	2	Pink	2	100
14	0-14	29	91	102	2	Pink	3	110
15	0-15	24	86	101	2	Pink	3	115
16	0-16	27	95	102	2	Pink	2	120
17	0-17	26	83	102	2	Pink	2	100
18	0-18	30	100	102	2	Pink	3	90

19	0-19	30	93	102	2	Pink	2	100
20	0-20	28	86	101	2	Pink	3	105
21	0-21	25	100	100	2	Pink	3	110
22	0-22	24	82	99.2	3	Pink	2	120
23	0-23	28	100	100	2	Pink	2	125
24	0-24	26	92	100.8	2	Pink	3	95
25	0-25	31	90	103	2	Pink	2	115
26	0-26	27	91	101	2	Pink	3	125
27	0-27	24	80	100	2	Pink	3	130
28	0-28	30	130	101	2	Pink	3	125
29	0-29	32	106	101	2	Pink	3	100
30	0-30	28	84	102	2	Pink	3	120
31	0-31	30	86	100	2	Pink	3	100
32	0-32	26	98	101	2	Pink	3	95
33	0-33	27	81	100	2	Pink	3	85
34	0-34	25	81	102	2	Pink	3	100
35	0-35	28	92	102	2	Pink	3	100
36	0-36	30	91	102	2	Pink	3	120
37	0-37	30	84	101	2	Pink	3	105
38	0-38	27	95	101	2	Pink	3	100
39	0-39	25	91	102	2	Pink	3	120
40	0-40	28	86	99.9	3	Pink	3	105
41	0-41	28	85	101	2	Pink	3	95
42	0-42	22	90	100	2	Pink	3	85

43	0-43	29	81	100	2	Pink	3	110
44	0-44	27	89	101	2	Pink	3	110
45	0-45	28	87	100	2	Pink	3	100
46	0-46	24	84	101.2	3	Pink	3	120
47	0-47	25	87	100	2	Pink	3	120
48	0-48	28	94	101	2	Pink	3	110
49	0-49	30	89	102	2	Pink	3	105
50	0-50	27	92	102	2	Pink	3	90
51	0-51	25	97	100	2	Pink	2	125
52	0-52	27	101	102	2	Pink	2	110
53	0-53	25	84	100.5	3	Pink	3	105
54	0-54	22	100	102	2	Pink	2	105
55	0-55	27	98	103	2	Pink	2	110
56	0-56	30	96	101	2	Pink	2	120
57	0-57	31	79	101	2	Pink	3	95
58	0-58	30	84	102	2	Pink	2	110
59	0-59	24	87	101	2	Pink	2	90
60	0-60	27	85	100	3	Pink	3	110
61	0-61	30	100	102	2	Pink	3	100
62	0-62	27	101	99.8	3	Pink	3	110
63	0-63	30	91	101	2	Pink	2	105
64	0-64	30	98	102	2	Pink	3	120
65	0-65	27	84	101	2	Pink	3	95
66	0-66	25	87	100	2	Pink	3	100

67	0-67	28	90	100	2	Pink	3	120
	Mean	27.97	91.58	101.14	2.13	---------	2.76	109.85
	SD	2.66	8.06	0.90	0.34	----------	0.43	9.53
	SE	0.32	1.06	0.11	0.04	----------	0.05	1.16
	Range	22-35	79-130	99.20-103	2-3	----------	2-3	90-130

4.1.3 Biochemical finding of healthy dogs:

Mean ± SE values of biochemical parameters of healthy dogs (n=67) are presented in Table 12. The mean concentrations of Blood Urea Nitrogen and Creatinine were 16.02 ± 0.87 mg/dl and 0.96 ±0.03 mg/dl. Mean ± SE of Total, Direct, and Indirect Bilirubin were 0.29 ± 0.01 mg/dl, 0.12 ± 0.00 mg/dl, and 0.17 ± 0.01 mg/dl respectively. Average values of ALP, AST, ALT, Total Protein, Albumin, and Globulin were 120.11 ± 7.43, 42.24 ± 2.44 IU/L, 39.91±2.14 IU/L, 7.30 ± 0.12 gm/dl, 2.87 ± 0.05gm/dl and 4.40 ± 0.13 gm/dl respectively. Mean ± SE for triglycerides, cholesterols, and glucose were 121.30 ± 5.76 mg/dl, 191.17 ± 6.85 mg/dl and 83.61±1.04 mg/dl respectively. All Biochemical parameters findings of reported dogs were in correlation with the reference values of Klaassen (1999).

4.1.4 Thyroid Profile (TT_3, TT_4, fT_4 and cTSH) of Healthy Dogs:

Thyroid profile data (TT_3, TT_4, and fT_4) of healthy dogs was established using commercially available RIA kits designed for human purposes by BRIT, Vashi, Mumbai, and by *BECKMAN COULTER* supplied by IMMUNOTECH *S.R.O. Radiova 1122/1, 10200 Prague10, Czech Republic* following the standard protocol suggested by the manufacturer as mentioned in materials and methods (Table 5, Table 6 and Table 7 for TT_3, TT_4 and fT_4 respectively). The Thyroid profile of TSH was established using a commercially available Canine Thyroid Stimulating Hormone ELISA kit designed for canine purposes by CUSABIO BIOTECH CO., LTD. (WUHAN HUAMEI BIOTECH). The complete thyroid profile of healthy dogs (n=67) is presented in Table 13.

All the Assay passed all recommended quality control parameters given by the manufacturer. The details of the quality control parameters of TT_3, TT_4, and fT_4 are summarized in Table 14, Table 15, and Table 16 respectively.

Table 11. Detail of hematological parameters of healthy dogs (n=67)

Sr. No	Parameter	Mean ± S. E	Reference Range (Klaassen 1999)
1	Hemoglobin (Hb) (gm%)	14.01 ± 0.28	12.1-20.3
2	Packed Cell Volume (PCV) (%)	40.52 ± 0.72	36-60
3	Total Erythrocyte Count (TEC) (10^6/cmm)	8.13 ± 1.31	4.8-9.3
4	Total Leucocyte Count (TLC) (10^3/cmm)	15.12 ± 0.65	4.00-15.5
5	Differential Leucocyte Count (%)		
	Neutrophils	70.72 ± 1.23	50-85
	Lymphocyte	24.36 ± 1.03	15-30
	Monocyte	1.18 ± 0.06	3-8
	Eosinophils	3.03 ± 0.41	2-10
	Basophils	0.01 ± 0.01	Rare
6	Mean Corpuscular Volume (MCV) (fl)	63.39 ± 0.51	58-79
7	Mean Corpuscular Hemoglobin (MCH) (pg)	20.26 ± 0.21	19-28
8	Mean Corpuscular Hemoglobin Concentration (MCHC) (gm/dl)	31.93 ± 0.15	30-38
9	Platelets (PLT) (/cu mm)	252000 ± 900	170000-400000

Table 12. Details of biochemical parameters of healthy dogs (n=67)

Sr. No	Parameter	Mean ± S. E	Reference Range (Klaassen 1999)
1	Blood Urea Nitrogen (BUN) (mg/dl)	16.02 ± 0.87	6-25
2	Creatinine (mg/dl)	0.96 ± 0.03	0.5-1.6
3	Total Bilirubin (mg/dl)	0.29 ± 0.01	0-0.6
4	Direct Bilirubin (mg/dl)	0.12 ± 0.00	0.1-0.3
5	Indirect Bilirubin (mg/dl)	0.17 ± 0.01	0-0.3
6	Alkaline Phosphatase (ALP) (IU/L)	120.11 ± 7.43	5-131
7	Aspartate transaminase (AST) (IU/L)	42.24 ± 2.44	15-62
8	Alanine transaminase (ALT) (IU/L)	39.92 ± 2.15	12-118
9	Total Protein (gm/dl)	7.30 ± 0.12	5.0-7.4
10	Albumin (gm/dl)	2.87 ± 0.05	2.7-4.4
11	Globulin (gm/dl)	4.40 ± 0.13	2.30-4.50 (Merck's Veterinary Manual, 2018)
12	Serum Cholesterol (mg/dl)	191.17 ± 6.85	92-324
13	Serum Triglyceride (mg/dl)	121.30 ± 5.76	29-291
14	Random Blood Glucose (mg/dl)	83.61 ± 1.04	70-138

Table 13 Thyroid profile of healthy dogs (n=67)

Sr. No.	Sample	TT_3 (nmol/l)	TT_4 (nmol/l)	fT_4 (pmol/l)	cTSH (µIU/ml)	TT_3/TT_4	TT_4/FT_4	TT_4/TT_3	fT_4/TT_4
1	0-1	0.90	35.00	19.00	1.86	0.03	1.84	38.89	0.54
2	0-2	0.72	14.00	11.50	1.95	0.05	1.22	19.44	0.82
3	0-3	0.60	17.00	6.10	1.79	0.04	2.79	28.33	0.36
4	0-4	0.64	25.00	9.40	1.97	0.03	2.66	39.06	0.38
5	0-5	0.72	24.00	13.50	1.99	0.03	1.78	33.33	0.56
6	0-6	0.64	30.50	10.80	1.96	0.02	2.82	47.66	0.35
7	0-7	0.69	18.00	6.60	1.90	0.04	2.73	26.09	0.37
8	0-8	0.63	21.00	13.00	2.07	0.03	1.62	33.33	0.62
9	0-9	0.37	29.50	12.50	2.07	0.01	2.36	79.73	0.42
10	0-10	0.72	19.00	14.00	2.03	0.04	1.36	26.39	0.74
11	0-11	0.49	14.00	13.50	1.84	0.04	1.04	28.57	0.96
12	0-12	0.57	26.00	15.20	1.96	0.02	1.71	45.61	0.58
13	0-13	0.70	20.50	2.80	1.87	0.03	7.32	29.29	0.14
14	0-14	0.60	30.00	14.00	2.03	0.02	2.14	50.00	0.47
15	0-15	0.62	37.00	10.90	2.14	0.02	3.39	59.68	0.29

16	0-16	0.70	34.00	12.70	1.9	0.02	2.68	48.57	0.37
17	0-17	0.52	36.00	9.60	1.95	0.01	3.75	69.23	0.27
18	0-18	0.49	14.00	14.50	1.97	0.04	0.97	28.57	1.04
19	0-19	0.56	19.00	16.00	2.03	0.03	1.71	33.93	0.84
20	0-20	0.70	20.50	12.00	2.08	0.03	1.56	29.29	0.59
21	0-21	0.85	21.00	13.50	2.1	0.04	2.23	24.71	0.64
22	0-22	0.70	25.00	11.20	2.08	0.03	1.47	35.71	0.45
23	0-23	0.90	17.00	11.60	2.06	0.05	2.26	18.89	0.68
24	0-24	1.23	24.00	10.60	2.04	0.05	1.79	19.51	0.44
25	0-25	0.96	25.00	14.00	2.1	0.04	1.63	26.04	0.56
26	0-26	1.11	21.00	12.90	2.12	0.05	1.84	18.92	0.61
27	0-27	1.23	19.31	10.50	1.99	0.06	1.34	15.72	0.54
28	0-28	0.92	15.44	11.50	2	0.06	1.38	16.77	0.74
29	0-29	1.11	19.31	14.00	1.94	0.06	1.10	17.47	0.73
30	0-30	0.77	15.44	14.00	2.02	0.05	1.37	20.11	0.91
31	0-31	0.89	21.88	16.00	2.04	0.04	1.34	24.58	0.73
32	0-32	1.69	16.73	12.50	1.9	0.10	2.19	9.90	0.75
33	0-33	0.95	21.88	10.00	2.17	0.04	1.68	23.03	0.46
34	0-34	1.04	19.31	11.50	2	0.05	2.57	18.49	0.60
35	0-35	1.11	29.60	11.50	2.15	0.04	1.64	26.79	0.39

36	0-36	1.04	18.02	11.00	1.93	0.06	2.70	17.25	0.61
37	0-37	1.99	28.31	10.50	2.07	0.07	1.29	14.23	0.37
38	0-38	0.94	18.02	14.00	1.99	0.05	1.93	19.25	0.78
39	0-39	0.92	19.31	10.00	2.17	0.05	1.20	20.95	0.52
40	0-40	0.41	18.02	15.00	2.03	0.02	2.48	44.38	0.83
41	0-41	0.61	19.31	7.80	1.86	0.03	0.97	31.42	0.40
42	0-42	0.55	15.44	16.00	1.97	0.04	1.06	27.93	1.04
43	0-43	0.92	15.44	14.50	2.07	0.06	1.29	16.75	0.94
44	0-44	1.04	19.31	15.00	2.18	0.05	1.24	18.48	0.78
45	0-45	0.92	16.73	13.50	2.08	0.06	1.21	18.15	0.81
46	0-46	0.86	18.01	14.90	2.11	0.05	1.93	20.95	0.83
47	0-47	1.23	19.31	10.00	2.09	0.06	1.52	15.72	0.52
48	0-48	0.92	16.73	11.00	2.09	0.06	1.51	18.15	0.66
49	0-49	1.04	18.67	12.40	2.11	0.06	1.86	17.88	0.66
50	0-50	0.86	25.10	13.50	2.14	0.03	2.16	29.18	0.54
51	0-51	1.04	25.74	11.90	2.18	0.04	1.10	24.66	0.46
52	0-52	0.92	15.44	14.00	2.17	0.06	1.85	16.76	0.91
53	0-53	1.53	25.74	13.90	1.94	0.06	2.49	16.82	0.54
54	0-54	0.93	32.18	12.90	2.14	0.03	1.49	34.60	0.40
55	0-55	0.92	28.31	19.00	1.95	0.03	1.29	30.78	0.67

56	0-56	0.92	16.73	13.00	2.18	0.06	1.05	18.15	0.78
57	0-57	0.92	14.16	13.50	2.18	0.06	1.11	15.46	0.95
58	0-58	0.14	15.96	14.40	2.01	0.01	1.29	110.82	0.90
59	0-59	0.78	19.31	15.00	2.06	0.04	1.62	24.64	0.78
60	0-60	1.22	21.88	13.50	2.02	0.06	1.03	17.93	0.62
61	0-61	0.82	18.02	17.50	2.16	0.05	1.63	21.97	0.97
62	0-62	0.82	24.45	15.00	2.19	0.03	1.10	29.82	0.61
63	0-63	0.50	16.73	15.20	1.96	0.03	0.66	33.46	0.91
64	0-64	0.56	5.14	7.80	2.06	0.11	0.88	9.18	1.52
65	0-65	1.10	14.00	16.00	2.13	0.08	0.88	12.73	1.14
66	0-66	0.90	7.20	11.00	2.02	0.13	0.65	8.00	1.53
67	0-67	0.82	11.58	8.10	1.87	0.07	1.43	14.12	0.70
Mean		0.85	20.81	12.59	2.03	0.05	1.78	28.09	0.67
SD		0.30	6.43	2.86	0.10	0.02	0.94	16.89	0.26
SE		0.04	0.79	0.35	0.01	0.00	0.11	2.06	0.03
Mean ± SE		0.85 ± 0.04	20.81 ± 0.79	12.59 ± 0.35	2.03 ± 0.01	0.05	1.78 ± 0.11	28.09 ± 2.06	0.67 ± 0.03

Table 14 Summary of Quality Control parameters of Total Triiodothyronine (TT_3, ng/ml)

Details of Data

Tube Details	Counts per minutes	Average Counts per minutes	Corrected Counts per minutes	Percent CV	Percent B/B0	Percent B/T
Total Count a	41666	41574.5	41547.5	0.31	------	100
Total Count b	41483					
Calibration 0 a	16581	16970.5	16943.5	3.25	100	40.78
Calibration 0 b	17360					
Calibration 1 a	15816	15811.5	15784.5	0.04	93.16	37.99
Calibration 1 b	15807					
Calibration 2 a	13675	13996.5	13969.5	3.25	82.44	33.22
Calibration 2 b	14318					
Calibration 3 a	11562	11434	11407	1.58	67.32	27.46
Calibration 3 b	11306					
Calibration 4 a	8568	8412.5	8385.5	2.61	49.49	20.18
Calibration 4 b	8257					
Calibration 5 a	4981	4980	4953	0.03	29.23	11.92
Calibration 5 b	4979					
Control I a	11222	11191	111164	0.39	65.90	26.87
Control I b	11160					
Control II a	9469	9301.5	9274.5	2.55	54.74	22.32
Control II b	9134					
Recovery Tube A	12481	12548.5	12521.5	0.76	73.91	30.14
Recovery Tube B	12616					

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	**40.78 %**
3	**80 % Intercept**	0.66 ng/ml
4	**50 % Intercept**	2.2 ng/ml
5	**20 % Intercept**	7.1 ng/ml
6	**QC sample -1**	**1.2 ng/ml *(0.9 ± 0.3)**
7	**QC sample -2**	**1.85 ng/ml * (1.8 ± 0.4)**
8	**Recovery**	100 %

(*Observed value)

Table15 Summary of Quality Control parameters of Total Thyroxin (TT_4, ng/ml)

Tube Details	Counts per Minutes	Average Counts per Minutes	Corrected counts per minute	Percent CV	Percent B/B0	Percent B/T
Total Count a	53359	52425.5	52392.5	2.52	---- -	100
Total Count b	51492					
Calibration 0 a	34111	34502	34469	1.60	100	66
Calibration 0 b	34893					
Calibration 1 a	23931	24635	24603.5	4.05	0.72	47
Calibration 1 b	25342					
Calibration 2 a	16672	16086	16053	5.15	0.47	31
Calibration 2 b	15500					
Calibration 3 a	12348	11581	11548	9.37	0.34	22
Calibration 3 b	10814					
Calibration 4 a	6310	6240.5	6207.5	1.57	0.18	12
Calibration 4 b	6171					
Calibration 5 a	3904	3988	3955	2.98	0.11	8
Calibration 5 b	4072					
Control I a	10980	10444	10411	7.26	0.30	20
Control I b	9908					
Control II a	8641	8533	8500	1.79	0.25	16
Control II b	8425					
Recovery Tube A	10853	9785.5	9750.5	15.46	0.29	19
Recovery Tube B	8714					

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	**65.78 %**
3	**80 % Intercept**	10 nmol /l
4	**50 % Intercept**	33 ng/ml
5	**20 % Intercept**	101 ng/ml
6	**QC sample -1**	**68 ng/ml*(60 ± 10)**
7	**QC sample -2**	**90 ng/ml *(110 ± 20)**
8	**Recovery**	96 %

(*Observed value)

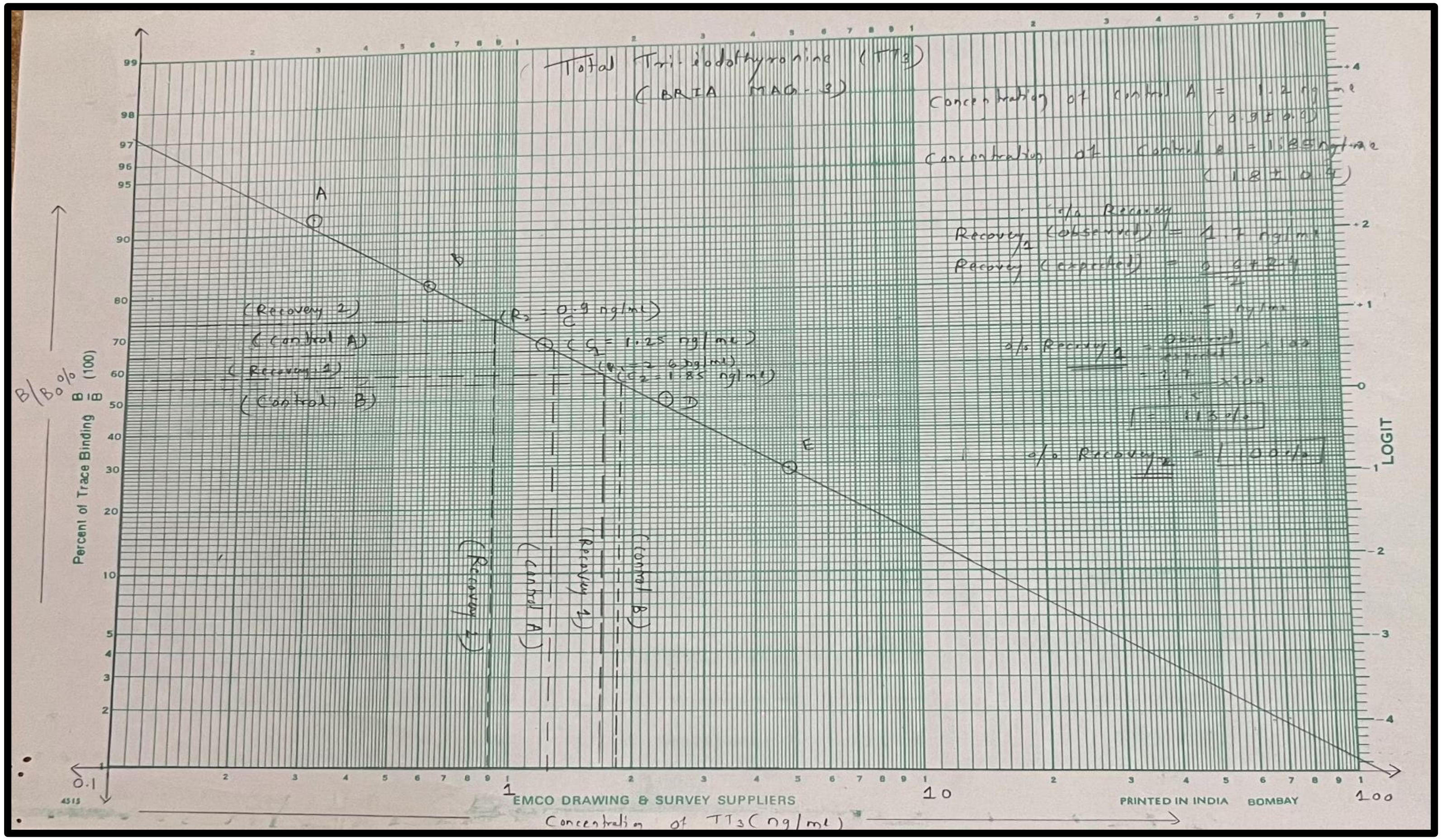

Plate 7 Graph of TT_3 (nmol/l)

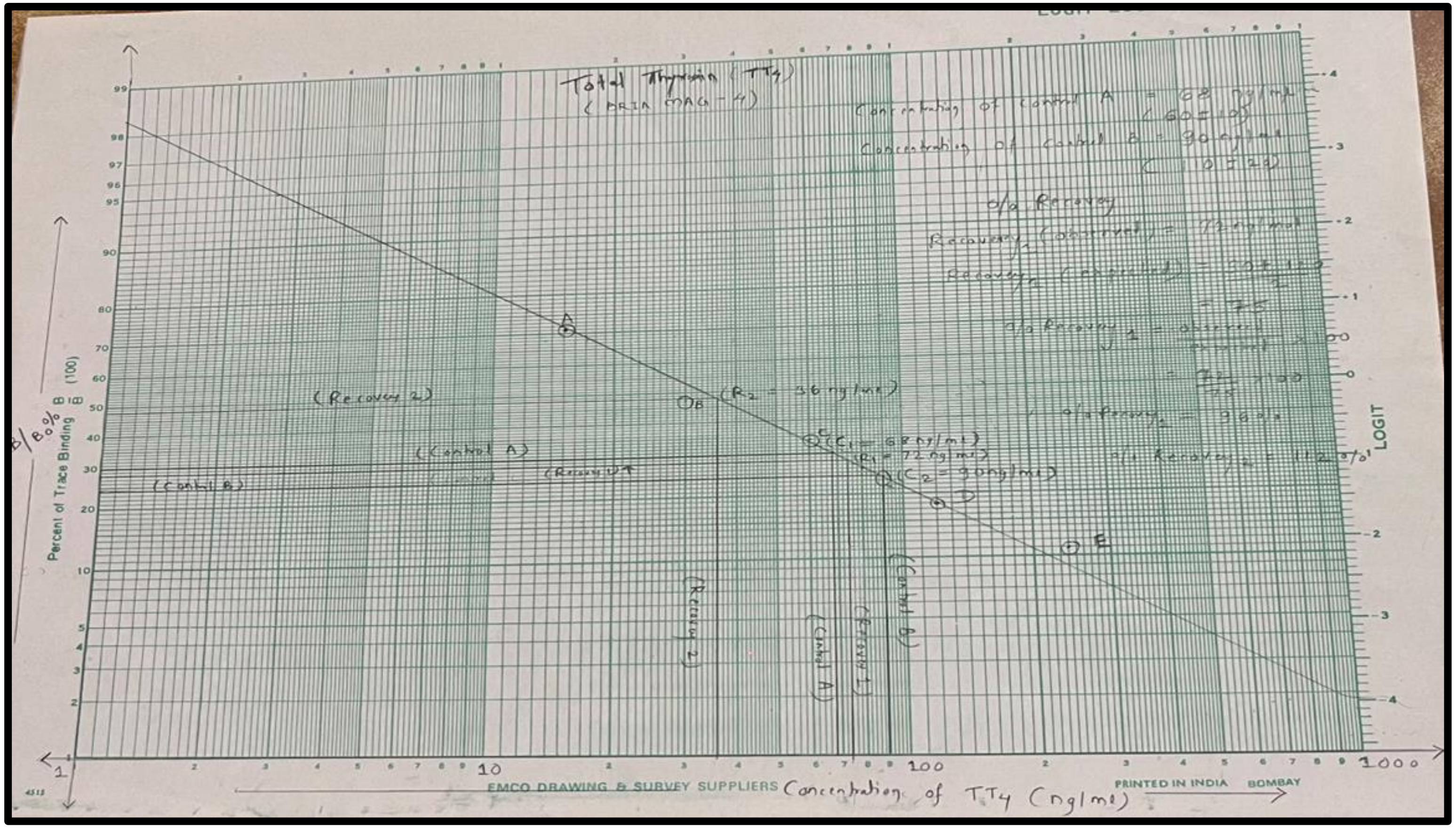

Plate 8 Graph of TT_4 (nmol/l)

Table 16 Summary of Quality Control parameters of Free Thyroxin (fT_4 pmol/l)

Details of Data

Tube Details	Counts per minutes	Average Counts per minutes	Corrected Counts per minutes	Percent CV	Percent B/B0	Percent B/T
Total Count a	61449	61344	61313	0.24	---	100
Total Count b	61239					
Calibration 0 a	31391	31283.5	31252.5	0.49	100	50.97
Calibration 0 b	33376					
Calibration 1 a	23367	23642.5	23611.5	1.65	75.55	38.51
Calibration 1 b	23918					
Calibration 2 a	13603	13792	13761	1.94	44.03	22.44
Calibration 2 b	13981					
Calibration 3 a	5748	5657	5626	2.27	18.00	9.18
Calibration 3 b	5566					
Calibration 4 a	2927	2850.5	2819.5	3.80	9.02	4.60
Calibration 4 b	2774					
Control I a	11567	11922.5	11891.5	4.22	38.05	19.39
Control I b	12278					
Recovery Tube A	6660	6679.5	6648.5	0.41	21.27	10.84
Recovery Tube B	6699					

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	50.97 %
3	**80 % Intercept**	2.1 pmol/l
4	**50 % Intercept**	9 pmol/l
5	**20 % Intercept**	23 pmol/l
6	**QC sample -1**	**12.5 pmol/l*(10.9-16.3)**
7	**% Recovery**	114 %

(* Observed value)

4.1.4.1 Estimation of control samples

Control samples present with the kits were estimated with each assay and compared with the reference range of control samples given by manufacturers to validate the accuracy and precision of assays. The summary of quality control parameters of TT_3, TT_4, and fT_4 is shown in Table 14, Table 15, and Table 16 respectively.

4.1.4.2 Recovery Percentage

Calculation of recovery percentage for each assay as one of the quality control parameters to increase the level of assurance. Recovery estimation was done by selecting half of one of the standards and half of one of the dog serum samples. The percent recovery is obtained by comparing the observed value with the expected one.

Recover %= (Observed Recovery ÷ Expected recovery) × 100

4.1.4.3 Total Triiodothyronine (TT_3, nmol/l)

The values of TT_3 were estimated from the graph shown in plate 7 which was plotted according standard protocol given by the manufacturer. Scatter plot frequency distribution and box and whisker plot of TT_3 are shown in Fig. 1, Fig. 5, and Fig.9 respectively. The box and whisker plot of TT_3 represents the middle half of data (25th and 75th percentile) 0.64-0.96 nmol/l, 'T'bar represents the range of TT_3 (0.37-1.23 nmol/l) and the median line represents by 0.86 nmol/l. The mean ± SE value of TT3 of healthy dogs was 0.85 ± 0.04 nmol/l which was in accordance with the reference range reported by Shiel *et al*. (2010), Cote (2015), Randolph *et al.* (2015) and Morre *et al.* (2017) shown in Table 17.

4.1.4.4 Total Thyroxin (TT_4, nmol/l)

The mean ± SE value of TT_4 of healthy dogs was 20.81±0.79 nmol/l. The mean concentration of TT_4 for healthy dogs in a recent study concurs with the reported data by Shiel *et al* (2010), Shadwick *et al.* (2013), Dadke *et al.* (2018), and Galdhar *et al.* (2022) which summarized in Table 18. The values of TT_4 were estimated from the graph shown in plate 8 which was plotted according to standard protocol given by the manufacturer. The scatter plot, frequency

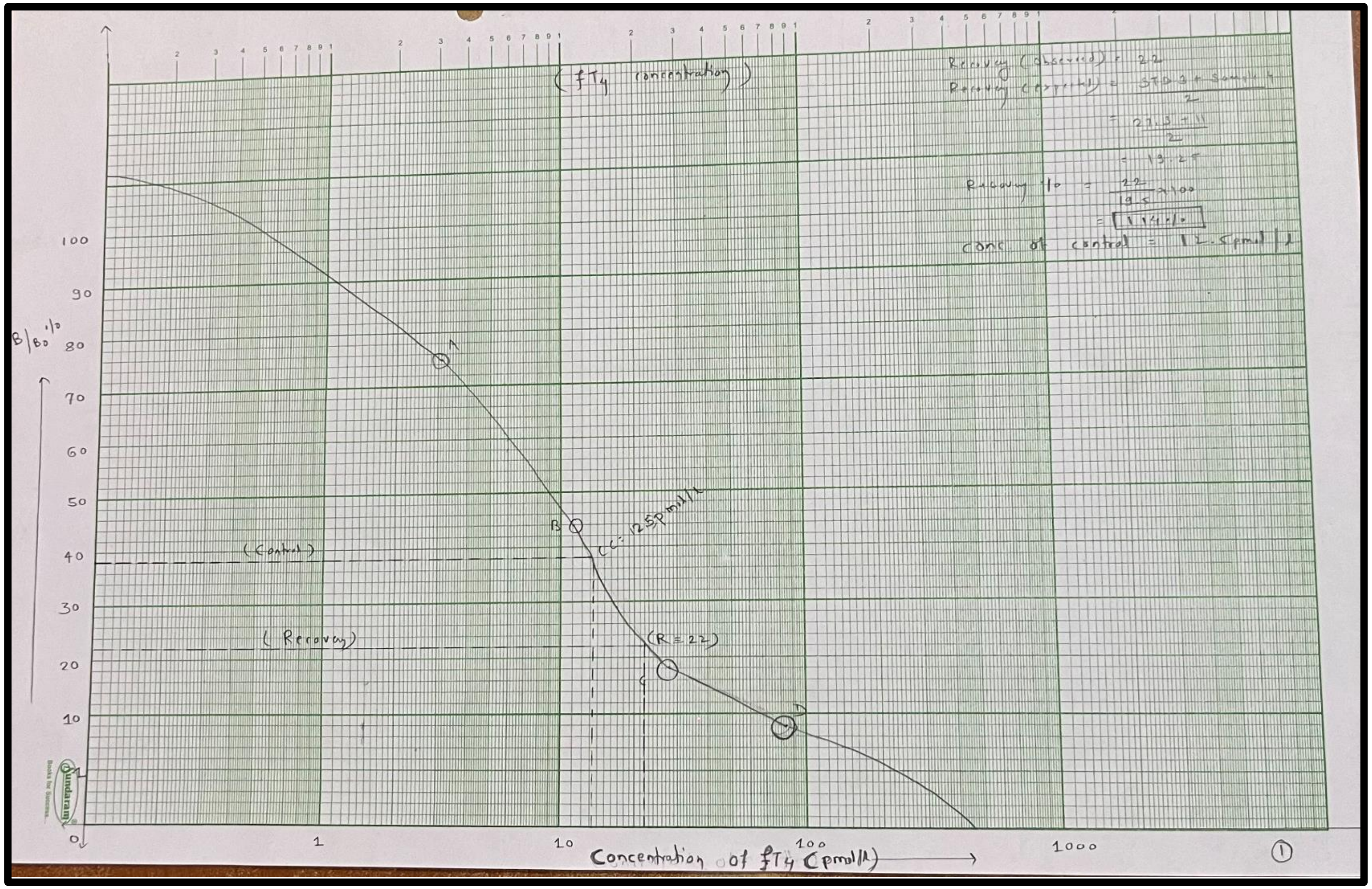

Plate 9 Graph of fT_4(pmol/l)

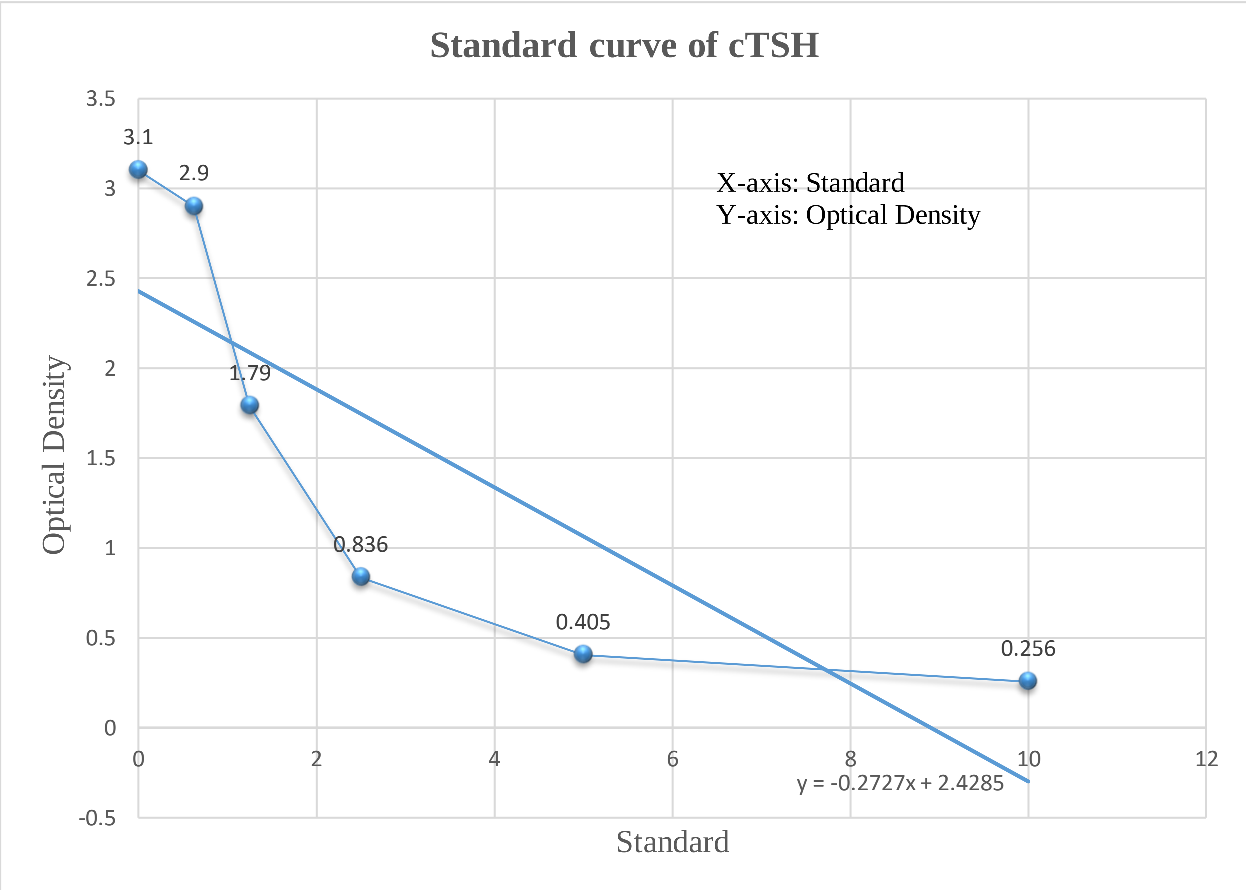

Plate 10 Standard curve of cTSH assay (cTSH ELISA)

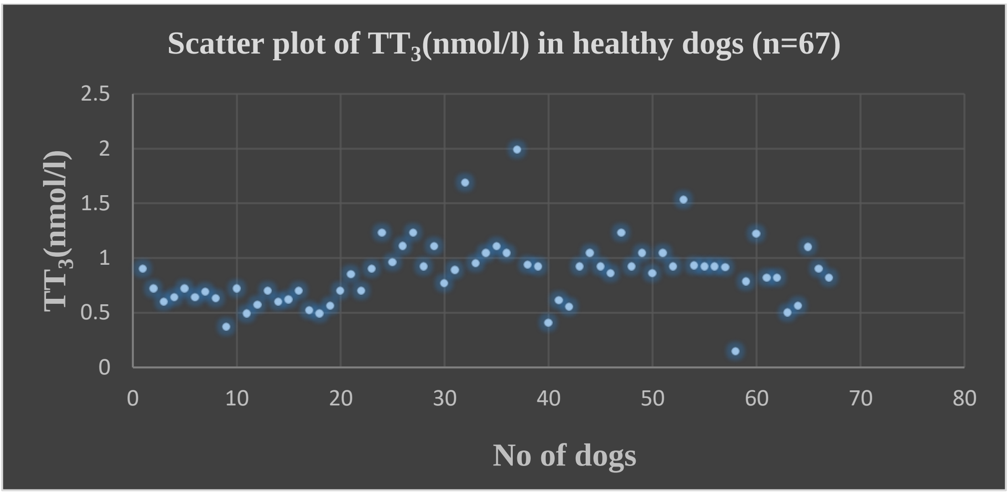

Figure 1 Scatter plot of TT_3 (nmol/l) in healthy dogs (n=67)

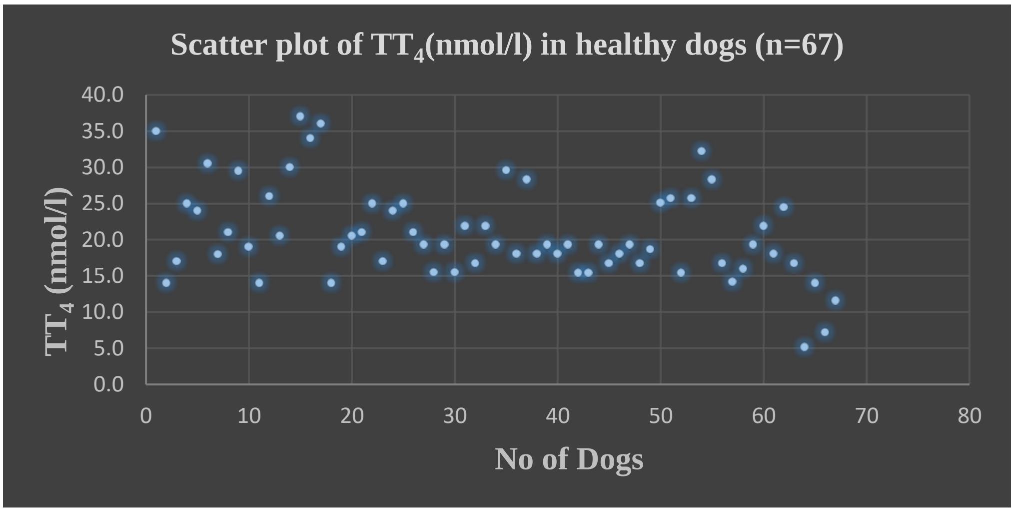

Figure 2 Scatter plot of TT_4(nmol/l) in healthy dogs (n=67)

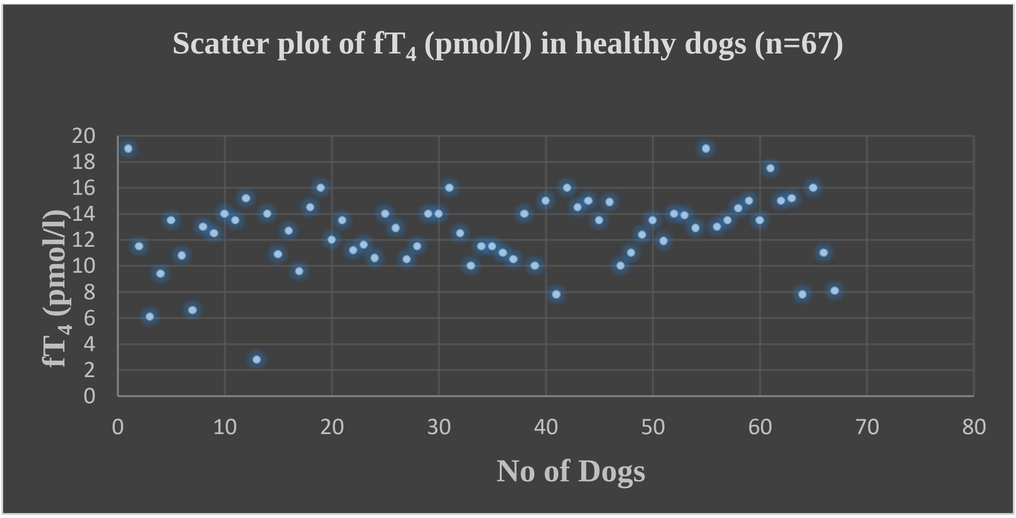

Figure 3 Scatter plot of fT_4 (pmol/l) in healthy dogs (n=67)

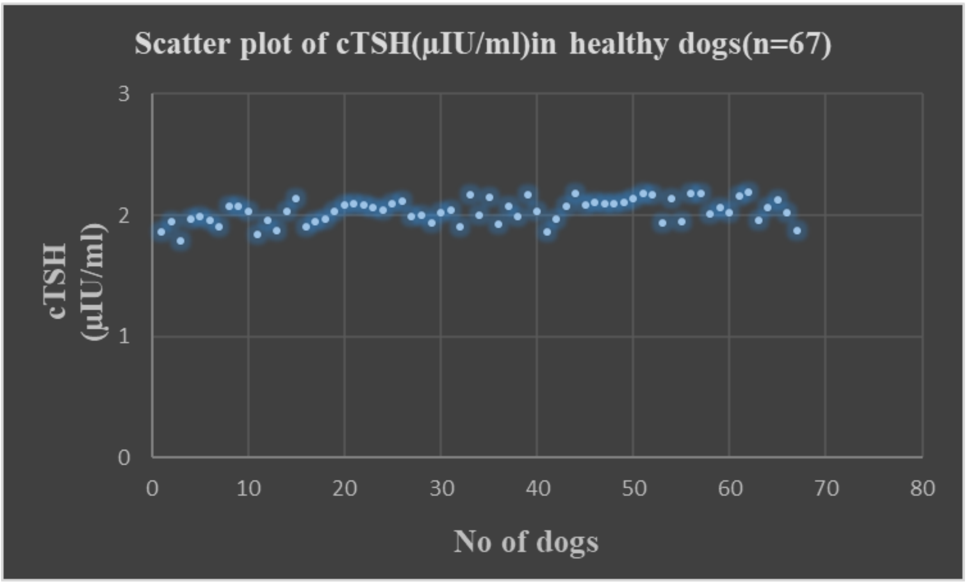

Figure 4 Scatter plot of cTSH(µIU/ml) in healthy dogs (n=67)

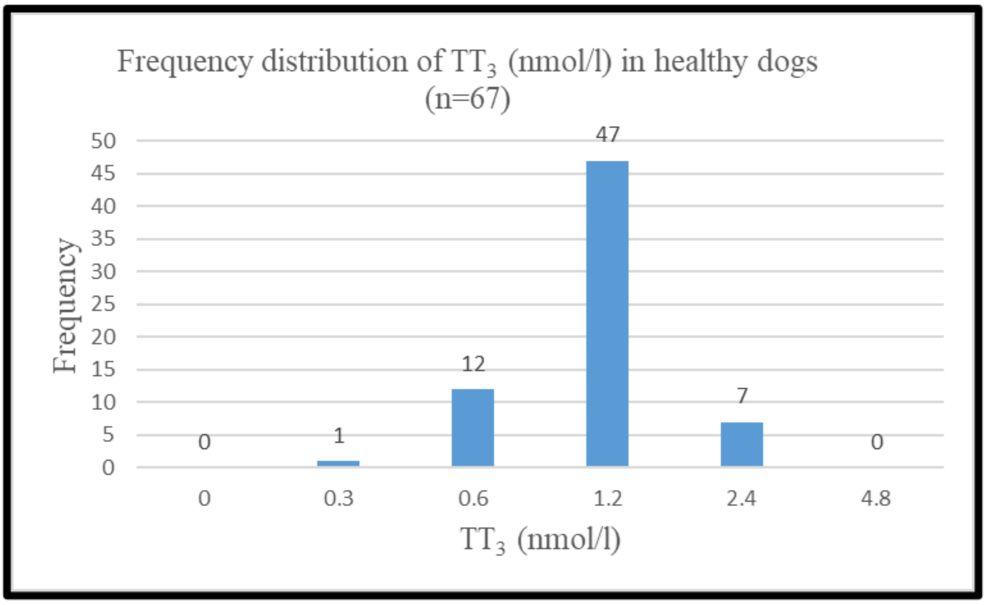

Figure 5 Frequency distribution of TT_3(nmol/l) in healthy dogs (n=67)

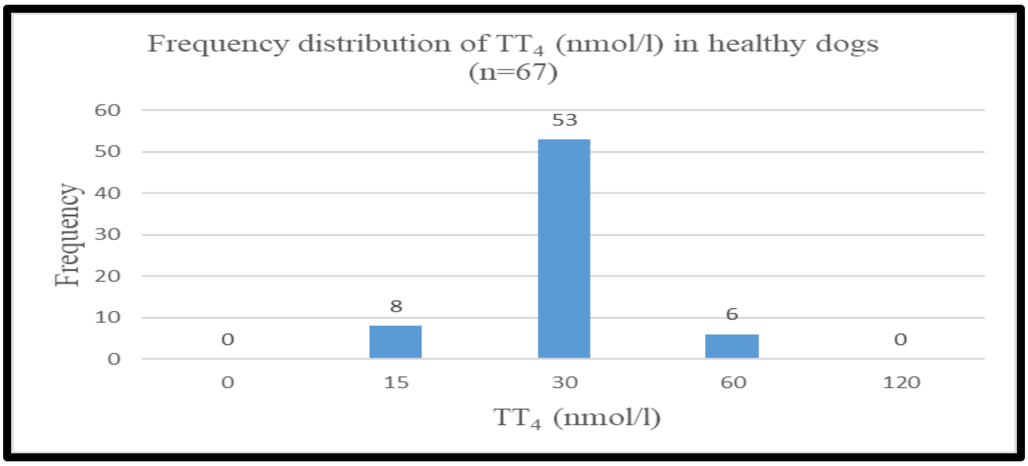

Figure 6 Frequency distribution of TT_4(nmol/l) in healthy dogs(n=67)

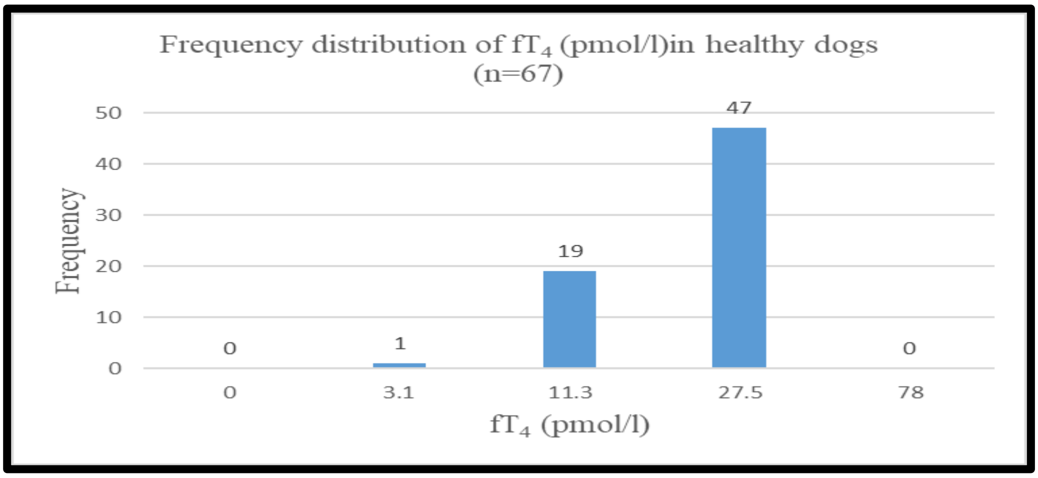

Figure 7 Frequency distribution of fT_4(pmol/l) in healthy dogs (n=67)

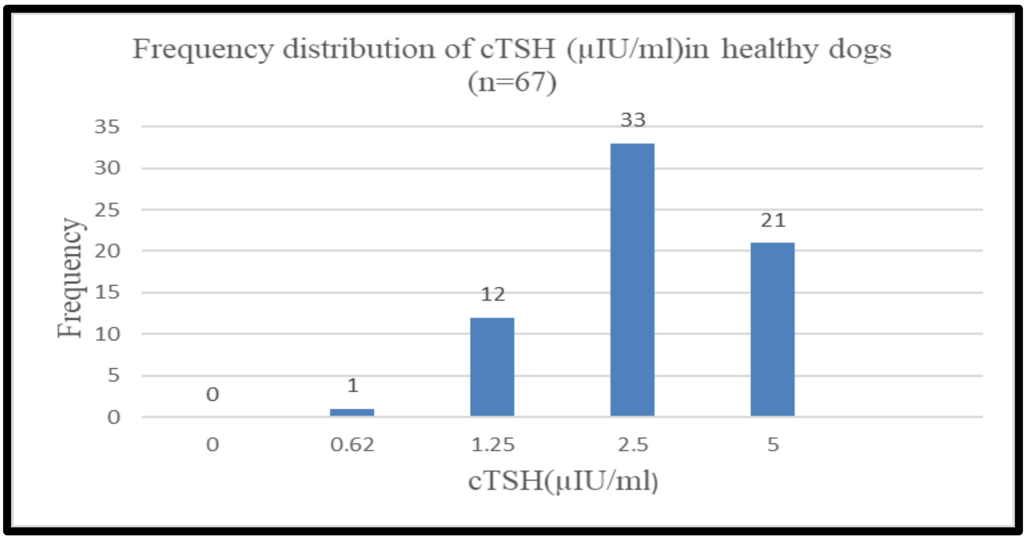

Figure 8 Frequency distribution of CTSH (µIU/ml) in healthy dogs (n=67)

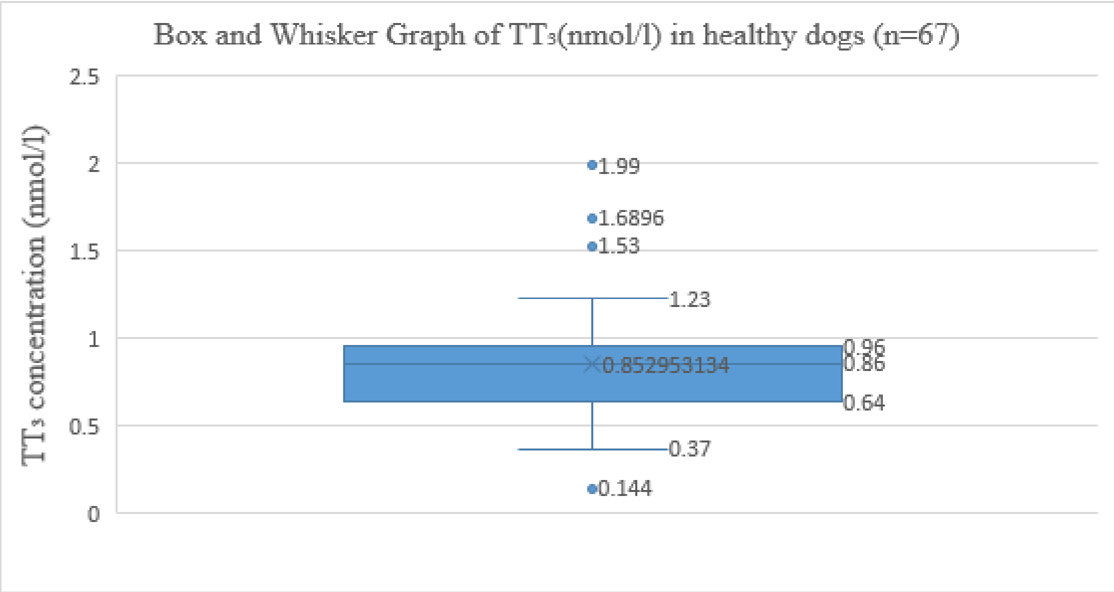

Figure 9 Box and Whisker Graph of TT_3 (nmol/l) concentration in healthy dogs (n=67)

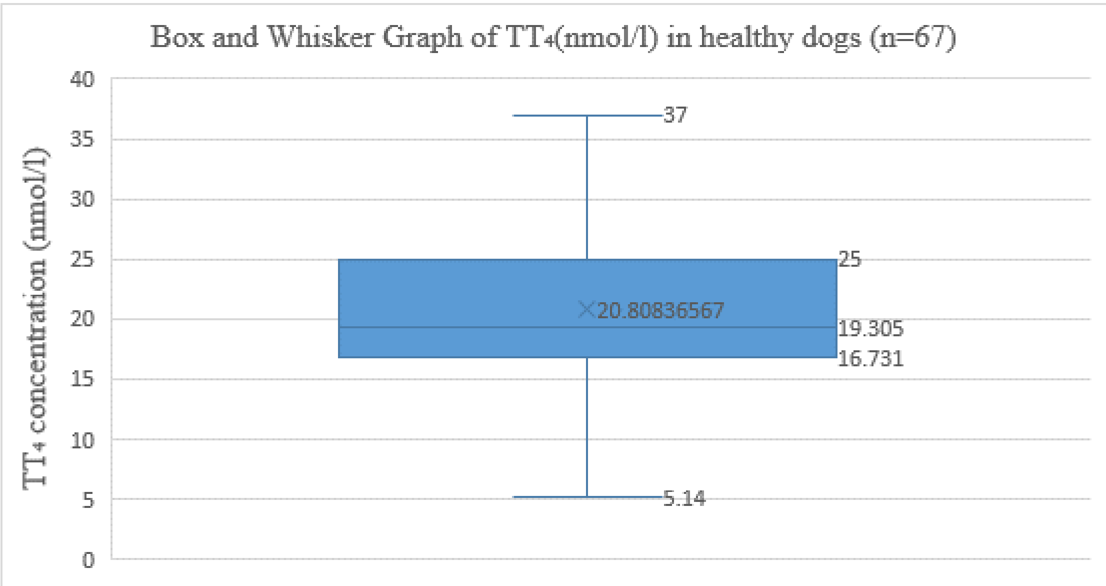

Figure 10 Box and Whisker Graph of TT_3 (nmol/l) concentration in healthy dogs (n=67)

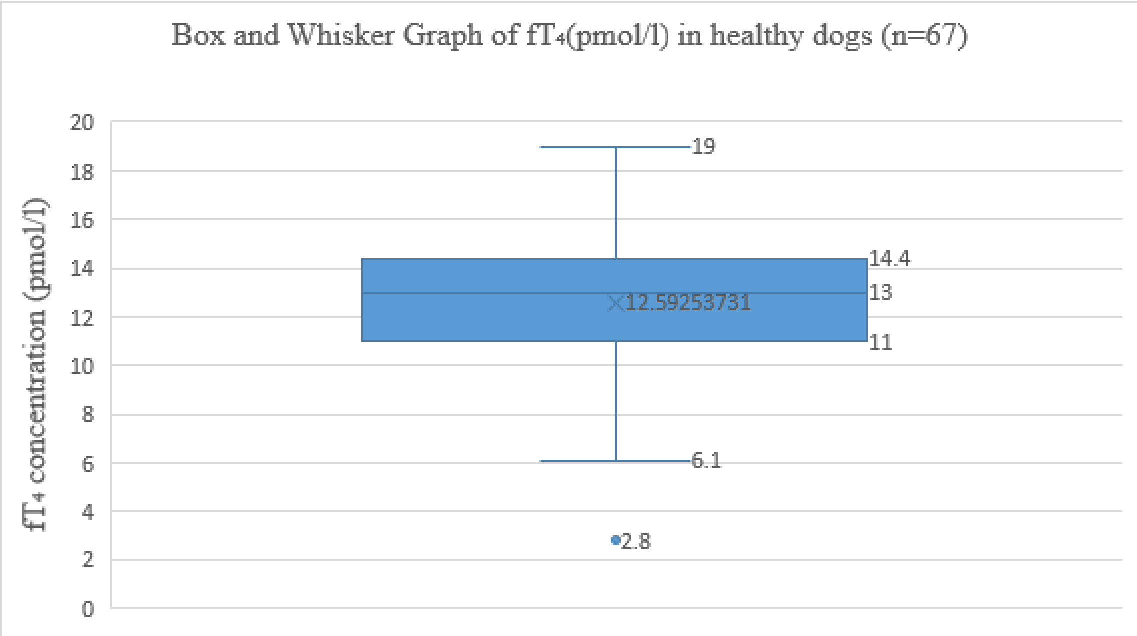

Figure 11 Box and Whisker Graph of fT_4 (pmol/l) concentration in healthy dogs (n=67)

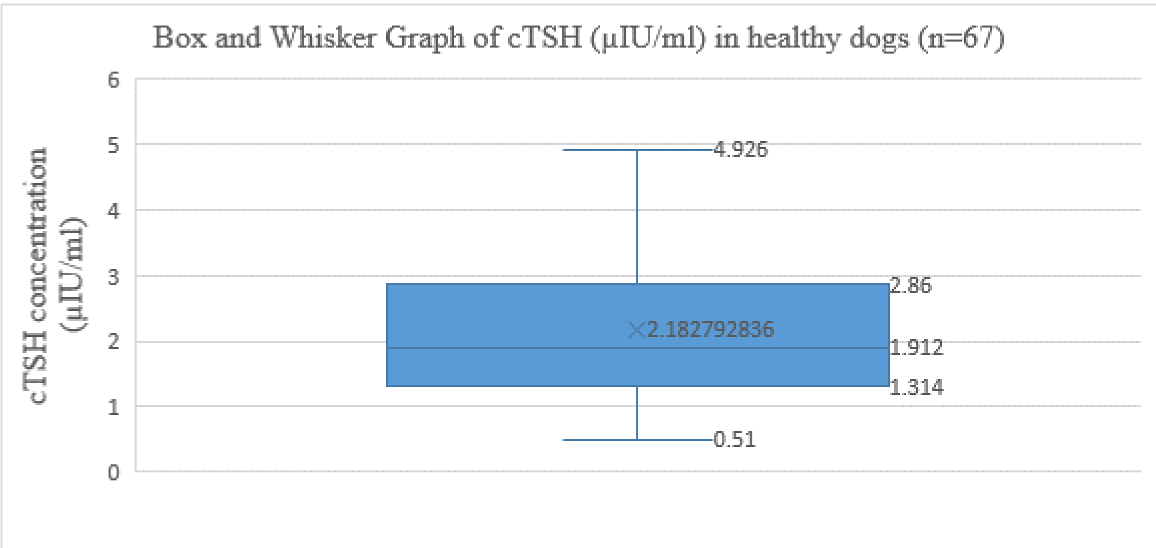

Figure 12 Box and Whisker Graph of cTSH (µIU/ml)) concentration in healthy dogs (n=67)

distribution, and box and whisker plot of TT_4 are shown in Fig. 2, Fig 6, and Fig. 10 respectively. The box and whisker plot of TT_4 represents the middle half of data (25th and 75th percentile) 16.73 – 25.00 nmol/l, 'T'bar represents the range of TT_4 (5.14-37 nmol/l), and the median line represented by a horizontal line in a box (19.30 nmol/l).

4.1.4.5 Free Thyroxine (fT_4, pmol/l)

Using the graph in Plate 9, the free thyroxine values were calculated. In the case of healthy dogs, the Mean ± SE of fT_4 was 12.59 ± 0.35 pmol/l which was in accordance with the reference range reported by Shiel *et al* (2010), Shadwick *et al* (2013), Dadke *et al* (2018) and Galdhar *et al* (2022). A summary of the same is shown in Table 19. Frequency distribution, scatter plot, and box and whisker plot of fT_4 are shown in Fig.3, Fig.7, and Fig. 11 respectively. The box and whisker plot of fT_4 represents the middle half of data (25th and 75th percentile) 11- 14.4 pmol/l, 'T'bar represents the range of T (6.1-19 pmol/l), and the median line represented by a horizontal line in a box (13 pmol/l).

4.1.4.6 Thyroid Stimulating Hormone (TSH, µIU/ml)

The values of cTSH were estimated from the graph shown in plate 10 which was plotted according to the standard protocol given by the manufacturer. The mean value of cTSH of healthy was 2.03 ± 0.01 µIU/ml which was in correlation with the reference range reported by Boretti *et al.* (2022) and Kemppain and Behrend (2001). A summary of the same is shown in Table 20. Frequency distribution, scatter plot, and box and whisker plot of cTSH shown in Fig.4, Fig.8, and Fig. 12 respectively. The box and whisker plot of cTSH represents the middle half of data (25th and 75th percentile) 1.96- 2.11 µIU/ ml, 'T'bar represents the range of T (1.79- 2.19 µIU/ml) and the median line represents by a horizontal line in a box (2.03 µIU/ml).

4.1.4.7 Establishment of reference range

The reference range (25th and 75th percentile) for TT_3, TT_4, fT_4, and cTSH of the present study was found to be 0.64-0.96 nmol/l, 16.73-25.00 nmol/l, 11-14.4 pmol/l and 1.96-2.11µIU/ ml respectively.

Table 17 TT_3 values reported by other authors

Sr. No.	Parameters	Recorded values of present study		Shiel *et al.* (2010)	Randolph *et. al* (2015)	Morre *et al.* (2017)	Dadke *et al*. (2018)	Galdhar *et al.* (2022)
1	TT_3 (nmol/ l)	Mean ± SE	0.85-0.04	0.5-2.5	0.77-1.8	0.46-1.07	1.03 ± 0.02	1.29 ± 0.04
		25th and 75th percentile	0.64-0.96					0.88-1.51

Table 18 TT_4 values reported by other authors

Sr. No.	Parameters	Recorded values of present study		Shiel *et al.* (2010)	Shadwick *et al.* (2013)	Dadke *et al.* (2018)	Galdhar *et al.* (2022)
1	TT_4 (nmol/ l)	Mean ± SE	20.81-0.79	15-50	12.9-51.6	29.67± 1.43	28.17 ± 1.18
		25th and 75th percentile	16.73-25				15.70-35.90

Table 19 fT_4 values reported by other authors

Sr. No.	Parameters	Recorded values of present study		Shiel *et al.* (2010)	Shadwick *et al.* (2013)	Dadke *et al.* (2018)	Galdhar *et al.* (2022)
1	fT_4 (pmol/ l)	Mean ± SE	12.59 ± 0.35	6.6-40	8-40	9.07 ± 0.52	13.03 ± 0.68
		25th and 75th percentile	11-14.4				7.8-14.5

Table 20 cTSH values reported by other authors

Sr. No.	Parameters	Recorded values of present study		Kaur *et al.* (2021)	Gulzar *et al.* (2014)	Boretti *et al.* (2022)	Kemppainen and Behrend, (2001)
1	cTSH (µIU/ml)	Mean ± SE	2.03-0.01	0.09-1.08 (ng/ml)	0.30-0.60 (ng/ml)	< 0.6 (ng/ml)	< 0.5 (ng/ml)
		25th and 75th percentile	1.96-2.12				

4.1.4.8 Age-wise thyroid profile:

The healthy dogs (n=67) were grouped into eight groups according to age. Group 1 made up of 9 dogs between 1 to 2.6 years of age. Group 2 made up of 27 dogs between 2.6-4.3 years of age. Group 3 made up of 15 dogs between 4.3 to 5.9 years of age. Group 4 made up of 8 dogs between 5.9 to 7.5 years of age. Group 5 made up of 6 dogs between 7.5 to 9.1 years of age. Group 6 made up of 0 dogs between 9.1 to 10.8 years of age, Group 7 made up of 0 dogs between 10.8 to 12.4 years of age and Group 8 made up of 2 dogs above 12 years of age. In Figure 13, the frequency distribution of age in the case of healthy dogs reported. Most of the dogs are between the ages of 2.6 to 4.3 years. Frequency distribution of TT_3, TT_4, fT_4, and cTSH concentration are presented in Fig.14, Fig.15, Fig 16, and Fig 17 respectively.

The age-wise alteration in TT_3, TT_4, fT_4, and cTSH values of healthy dogs (n=67) is presented in Table 23, Table 24, Table 25 and Table 26 and Fig. 14, Fig.15, Fig. 16, and Fig. 17 respectively.

4.1.4.8.1 Total triiodothyronine TT3 (nmol/l)

For TT3, Mean ± SE values of group 1 (n=9), group 2 (n= 27), group3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8(n= 2) were 0.95 ±0.04nmol/ l, 0.65 ± 0.07 nmol/l, 0.37 ± 0.08 nmol/l, 0.77 ± 0.09 nmol/l, 0.81 ± 0.11 nmol/l, 0 nmol/l, 0 nmol/l and 0.60 ± 0.23 nmol/l respectively. A statistically non-significant ($p< 0.05$) difference was observed in TT3 concentration in all eight age groups Table 21.

As per 2SD evaluation for TT3 (nmol/l), Mean ± 2SD of group 1 (n=9), group 2 (n= 27), group3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8(n= 2) were 0.95 ± 0.25 nmol/l, 0.65 ± 0.70 nmol/l, 0.87 ± 0.62 nmol/l, 0.77 ± 0.51 nmol/l , 0.81 ± 0.53 nmol/l , 0 nmol/l ,0nmol/l and 0.60 ± 0.64 nmol/l respectively.

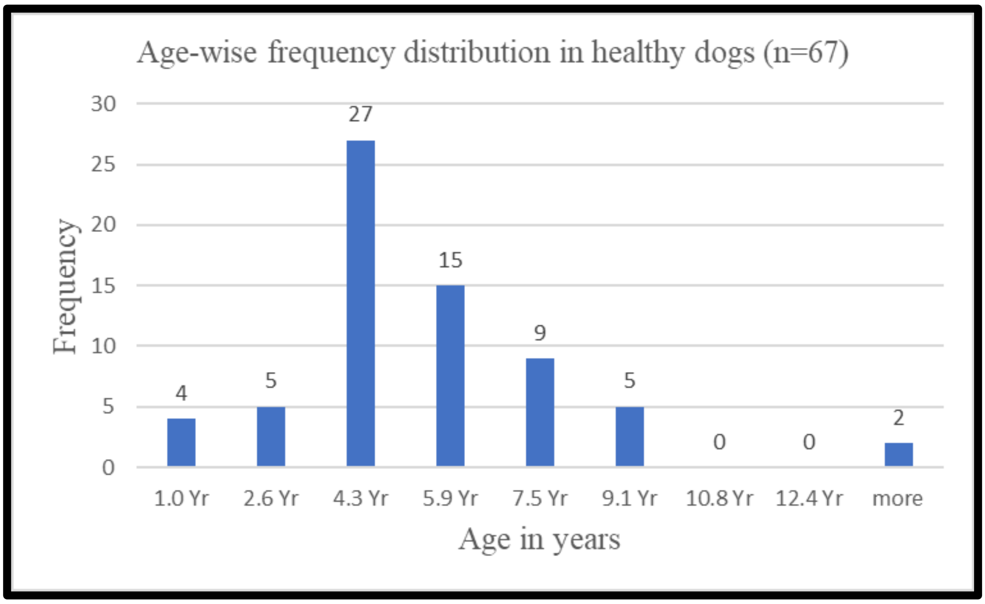

Figure 13 Age-wise frequency distribution in healthy dogs (n=67)

4.1.4.8.2 Total Thyroxine TT_4 (nmol/l)

The mean TT_4 values of group 1 (n=9), group 2 (n=27), group 3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8 (n= 2) were 21.82 ± 1.98 nmol/l, 25.44 ± 2.33 nmol/l, 18.09 ± 1.33 nmol/l, 19.04 ± 1.43 nmol/l, 26.69 ± 2.68 nmol/l,0 nmol/l,0 nmol/l and 23.76 ± 2.71 nmol/l respectively, Statistically non-significant ($p< 0.05$) difference was observed in TT_4 concentration in all four age groups (Table 21).

As per 2SD evaluation for TT4 (nmol/l), Mean ± 2SD of group 1 (n=9), group 2 (n= 27), group3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8(n= 2) were 21.82 ± 1.91 nmol/l, 25.44 ± 14.00 nmol/l, 18.09 ± 8.01 nmol/l, 19.14 ± 8.60 nmol/l , 26.69 ± 16.08 nmol/l , 0 nmol/l ,0 nmol/l and 23.76 ± 16.24 nmol/l respectively.

4.1.4.8.3 Free Thyroxine fT_4 (pmol/l)

For fT_4, Mean ± SE values of group 1 (n=9), group 2 (n=27), group 3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8 (n= 2) were 13.17 ± 0.63 pmol/l, 11.73 ± 0.55pmol/l, 12.45 ± 0.53 pmol/l, 14.10 ± 1.45 pmol/l , 13.18 ± 1.43 pmol/l , 0 pmol/l , 0 pmol/l and 15 ± 2.50 pmol/l respectively. A statistically non-significant ($p< 0.05$) difference was observed in fT_4 concentration in all four age groups. (Table 22).

As per 2SD evaluation for fT4 (pmol/l), Mean ± 2SD of group 1 (n=9), group 2 (n= 27), group3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8(n= 2) were 13.17 ± 3.76 pmol/l, 11.73 ± 5.67 pmol/l, 12.45 ± 4.08 pmol/l, 14.10 ± 8.18 pmol/l , 13.18 ± 7.01 pmol/l , 0 pmol/l ,0 pmol/l and 15 ± 7.07 pmol/l respectively.

4.1.4.8.4 Thyroid Stimulating Hormone cTSH (µIU/ml)

The mean cTSH values of group 1 (n=9), group 2 (n=27), group 3 (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8 (n= 2)were 2.12 ± 0.02 µIU/ml, 1.96 ± 0.02 µIU/ml, 2.02 ± 0.02 µIU/ml, 1.99 ± 0.03 µIU/ml, 2.01 ± 0.05 µIU/ml, 0 µIU/ml,0 µIU/ml and 2.12 ± 0.05 µIU/ml respectively. A statistically non-significant ($p< 0.05$) difference was observed in concentration in all four age groups (Table 22).

As per 2SD evaluation for cTSH (µIU/ml), Mean ± 2SD of group 1 (n=9), group 2 (n= 27), group (n=15), group 4 (n=8), group 5 (n=6), group 6 (n=0), group 7 (n=0) and group 8 (n= 2) were 2.12 ± 0.10 µIU/ml, 1.96 ± 0.21µIU/ml, 2.02 ± 0.17 µIU/ml, 1.99 ± 0.19 µIU/ml, 2.01 ± 0.23 µIU/ml, 0 µIU/ml, 0 µIU/ml and 2.12 ± 0.13 µIU/ml respectively.

When we categorized age-wise groups as per histogram analysis, we found no animals (nil) for group 6 (9.1 to 10.8 years) and group 7 (10.8 to 12.4 years) therefore age-wise thyroid profiles were categorized into four groups between the ages of 1 to 3 years, 3 to 6 years, 6 to 9 years, and more than 9 years are as follows:

The healthy dogs (n=67) were grouped into four groups according to age. Group 1 made up of 19 dogs between 1 to 3 years of age. Group 2 made up of 38 dogs between 3-6 years of age. Group 3 made up of 6 dogs between 6-9 years of age. Group 4 made up of 2 dogs above 9 years. In Figure 13, the frequency distribution of age in the case of healthy dogs reported. Most of the dogs are between the age of 3-6 years. Frequency distribution of TT_3, TT_4, fT_4, and cTSH concentration are presented in Fig.18, Fig.19, Fig 20, and Fig 21 respectively.

The age-wise alteration in TT_3, TT_4, fT_4, and cTSH values of healthy dogs (n=67) is presented in Table 28, Table 29, Table 30, and Table 31 and Fig. 18, Fig.19, Fig. 20, and Fig. 21 respectively.

4.1.4.8.5 Total triiodothyronine TT_3 (nmol/l) (4 Group classification of age)

For TT_3, the Mean ± SE values of group 1 (n=19), group 2 (n= 38), group3 (n=8) and group 4 (n= 2) were 0.89 ± 0.07 nmol/ l, 0.82 ± 0.05 nmol/l, 0.75 ± 0.09 nmol/l and 0.60 ± 0.23 nmol/l respectively. A statistically non-significant ($p< 0.05$) difference was observed in TT_3 concentration in all four age groups Table 27. It was seen that the TT_3 value gradually decreased up to 14 years of age. This observation was in correlation with the observation reported by Dadke (2018) and Galdhar (2020).

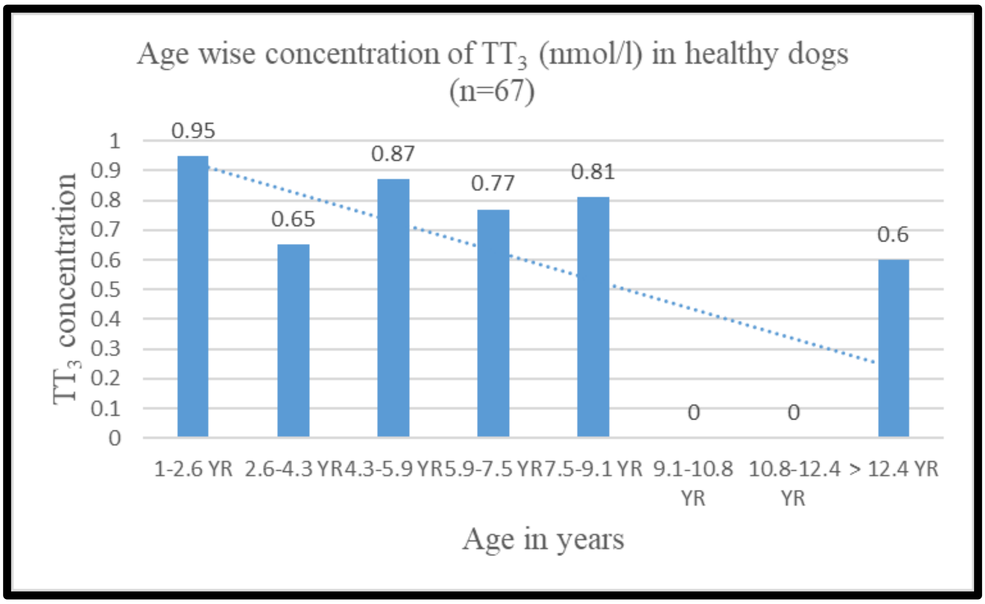

Figure 14 Age wise concentration of TT_3(nmol/l) in healthy dogs (n=67)

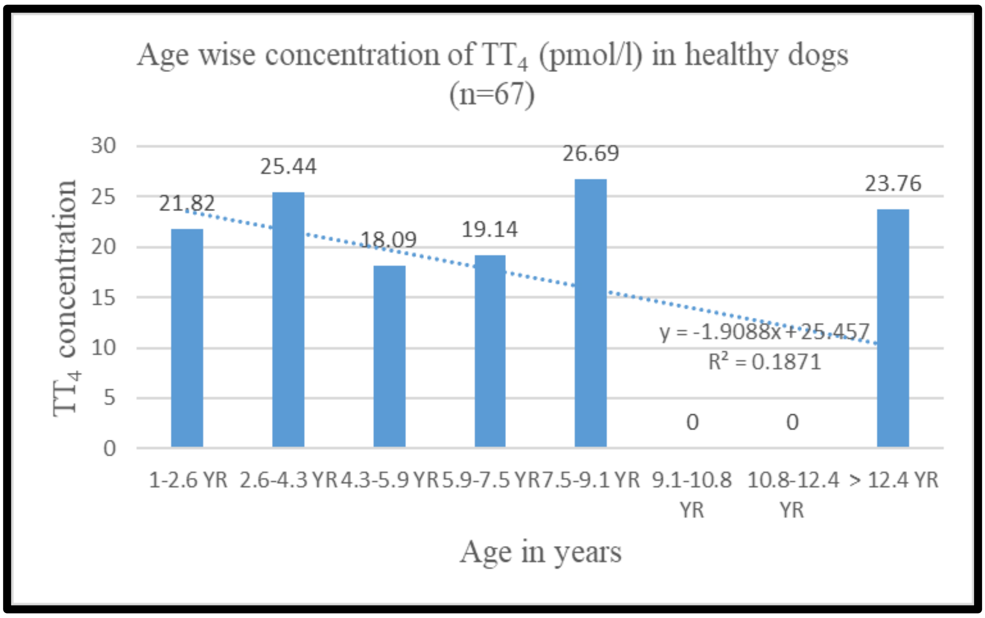

Figure 15 Age wise concentration of TT_4(nmol/l) in healthy dogs (n=67)

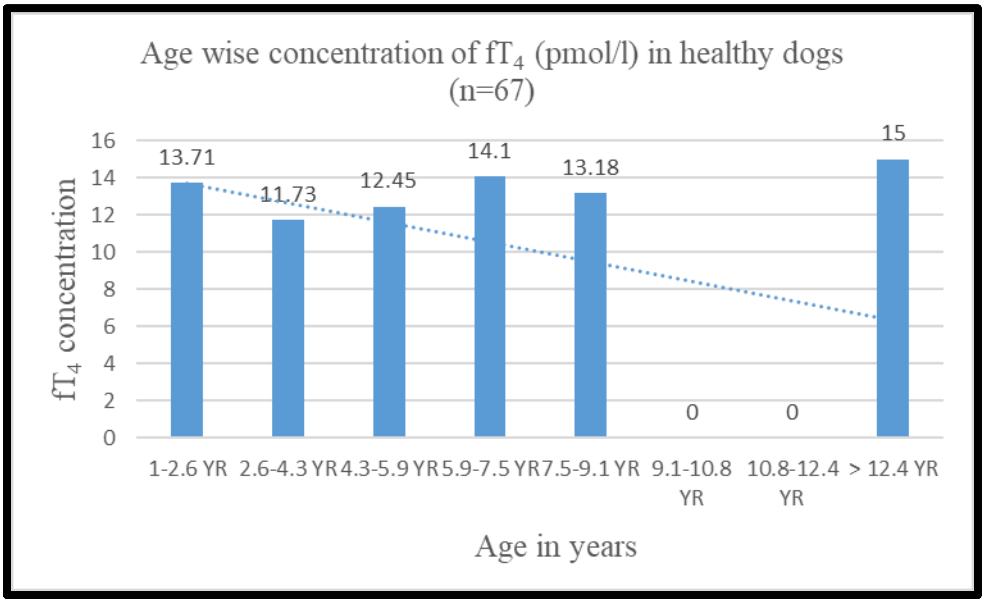

Figure 16 Age wise concentration of fT_4(pmol/l) in healthy dogs (n=67)

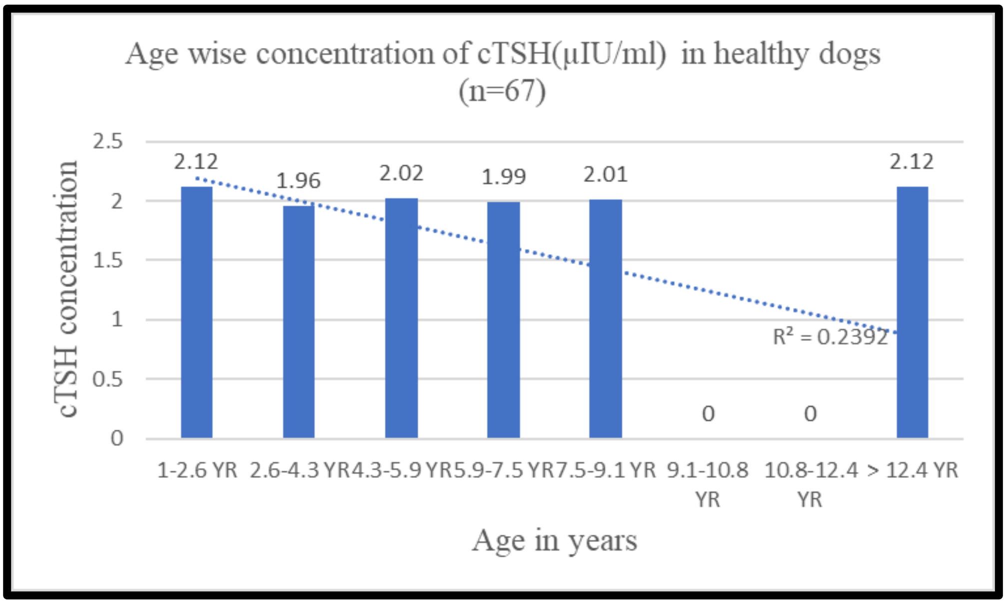

Figure 17 Age wise concentration of cTSH(µIU/ml) in healthy dogs (n=67)

Table 21 Age wise comparison of thyroid hormone in healthy dogs (n=67) (For TT_3 and TT_4)

Sr. No.	Parameter	Age Group	Mean ± SE	F value	F critical
1	TT_3 (nmol/l)	Group 1(n=9) (1 Yr- 2.6Yrs)	0.95 ± 0.04	0.44 N. S	2.16
		Group 2 (n=27) (2.6 Yrs-4.3Yrs)	0.65 ± 0.07		
		Group 3(n=15) (4.3 Yrs-5.9 Yrs)	0.87 ± 0.08		
		Group 4(n=8) (5.9 Yrs-7.5 Yrs)	0.77 ± 0.09		
		Group 5(n=6) (7.5 Yrs- 9.1 Yrs)	0.81 ± 0.11		
		Group 6(n=0) (9.1 Yrs- 10.8 Yrs)	0		
		Group 7(n=0) (10.8 Yrs- 12.4 Yrs)	0		
		Group 8(n=19) (Above 12.4Yrs)	0.60 ± 0.23		
2	TT_4 (nmol/l)	Group 1(n=9) (1 Yr- 2.6Yrs)	21.82 ± 1.98	1.56 N. S	
		Group 2 (n=27) (2.6 Yrs-4.3Yrs)	25.44 ± 2.33		
		Group 3(n=15) (4.3 Yrs-5.9 Yrs)	18.09 ± 1.33		
		Group 4(n=8) (5.9 Yrs-7.5 Yrs)	19.04 ± 1.43		
		Group 5(n=6) (7.5 Yrs- 9.1 Yrs)	26.69 ± 2.68		
		Group 6(n=0) (9.1 Yrs- 10.8 Yrs)	0		
		Group 7(n=0) (10.8 Yrs- 12.4 Yrs)	0		
		Group 8(n=19) (Above 12.4Yrs)	23.76 ± 2.71		

Table 22 Age wise comparison of thyroid hormone in healthy dogs (n=67) (For fT_4 and cTSH)

Sr. No.	Parameter	Age Group	Mean ± SE	F value	F critical
1	fT_4 (nmol/l)	Group 1(n=9) (1 Yr- 2.6Yrs)	13.17 ± 0.63	0.96 N. S	2.16
		Group 2 (n=27) (2.6 Yrs-4.3Yrs)	11.73 ± 0.55		
		Group 3(n=15) (4.3 Yrs-5.9 Yrs)	12.45 ± 0.53		
		Group 4(n=8) (5.9 Yrs-7.5 Yrs)	14.10 ± 1.45		
		Group 5(n=6) (7.5 Yrs- 9.1 Yrs)	13.18 ± 1.43		
		Group 6(n=0) (9.1 Yrs- 10.8 Yrs)	0		
		Group 7(n=0) (10.8 Yrs- 12.4 Yrs)	0		
		Group 8(n=19) (Above 12.4Yrs)	15 ± 2.50		
2	cTSH (µIU/ml)	Group 1(n=9) (1 Yrs- 2.6Yrs)	2.12 ± 0.02	1.73 N. S	
		Group 2 (n=27) (2.6 Yrs-4.3Yrs)	1.96 ± 0.02		
		Group 3(n=15) (4.3 Yrs-5.9 Yrs)	2.02 ± 0.02		
		Group 4(n=8) (5.9 Yrs-7.5 Yrs)	1.99 ± 0.03		
		Group 5(n=6) (7.5 Yrs- 9.1 Yrs)	2.01 ± 0.05		
		Group 6(n=0) (9.1 Yrs- 10.8 Yrs)	0		
		Group 7(n=0) (10.8 Yrs- 12.4 Yrs)	0		
		Group 8(n=19) (Above 12.4Yrs)	2.12 ± 0.05		

Table 23 Age wise concentration of TT_3 (nmol/l) in healthy dogs (n=67)

Sr. No.	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
	1 -2.6 yrs (n=9)	2.6-4.3 yrs (n=27)	4.3-5.9 yrs (n=15)	5.9-7.5 yrs (n=8)	7.5-9.1 yrs (n=6)	9.1- 10.8yrs (n=0)	10.8- 12.4 yrs (n=0)	> 12.4 yrs (n=2)
1	0.72	0.72	0.69	0.6	0.9			0.37
2	0.85	0.64	0.63	0.56	0.57			0.82
3	0.9	0.72	0.49	0.89	0.52			
4	0.96	0.64	1.11	0.55	0.7			
5	1.11	0.49	1.04	0.92	1.23			
6	0.92	0.7	1.04	1.23	0.92			
7	1.04	0.6	0.94	0.92				
8	0.93	0.62	0.92	0.5				
9	1.1	0.7	0.92					
10		0.7	1.04					
11		1.23	0.92					
12		0.92	1.53					
13		1.11	0.92					
14		0.77	0.14					
15		1.69	0.9					
16		0.95						
17		1.99						
18		0.41						
19		0.61						
20		0.86						
21		0.86						
22		1.04						
23		0.78						
24		1.22						
25		0.82						
26		0.56						
27		0.82						
28								
Mean	0.95	0.65	0.87	0.77	0.81	0.00	0.00	0.60
SD	0.12	0.35	0.31	0.26	0.26			0.32
SE	0.04	0.07	0.08	0.09	0.11	0.00	0.00	0.23
Mean ± SE	0.95 ± 0.04	0.65 ± 0.07	0.87 ± 0.08	0.77 ± 0.09	0.81 ± 0.11			0,60 ± 0.23

Table 24 Age -wise concentration of TT_4(nmol/l) in healthy dogs (n=67)

Sr. No.	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
	1 -2.6 yrs (n=9)	2.6-4.3 yrs (n=27)	4.3-5.9 yrs (n=15)	5.9-7.5 yrs (n=8)	7.5-9.1 yrs (n=6)	9.1-10.8yrs (n=0)	10.8-12.4 yrs (n=0)	> 12.4 yrs (n=2)
1	19	14	18	17	35			29.5
2	21	25	21	19	26			18.02
3	17	24	14	21.88	36			
4	25	30.5	21	15.44	25			
5	29.60	14	19.31	15.44	24			
6	19.31	20.5	18.02	19.31	14.16			
7	19.31	30	18.02	28.31				
8	32.18	37	16.73	16.73				
9	14	34	16.73					
10		20.5	18.67					
11		19.31	15.44					
12		15.44	25.74					
13		19.31	16.73					
14		15.44	15.96					
15		16.73	7.2					
16		21.88						
17		28.31						
18		18.02						
19		19.31						
20		18.01						
21		25.10						
22		25.74						
23		19.31						
24		21.88						
25		24.45						
26		5.14						
27		11.58						
Mean	21.82	25.44	18.09	19.14	26.69	00	00	23.76
SD	5.95	7.00	4.00	4.30	8.04	00	00	8.12
SE	1.98	2.33	1.33	1.43	2.68	00	00	2.71
Mean ± SE	21.82 ± 1.98	25.44 ± 2.33	18.09 ± 1.33	19.14 ± 1.43	26.69 ± 2.68	00	00	23.76 ± 2.71

Table 25 Age-wise concentration of fT_4 (pmol/l)in healthy dogs (n=67)

Sr. No.	Group 1 (1 -2.6 yrs) (n=9)	Group 2 (2.6-4.3 yrs) (n=27)	Group 3 (4.3-5.9 yrs) (n=15)	Group 4 (5.9-7.5 yrs) (n=8)	Group 5 (7.5-9.1 yrs) (n=6)	Group 6 (9.1-10.8yrs) (n=0)	Group 7 (10.8-12.4 yrs) (n=0)	Group 8 (> 12.4 yrs) (n=2)
1	14	11.5	6.6	6.1	19			12.5
2	13.5	9.4	13	16	15.2			17.5
3	11.6	13.5	14.5	16	9.6			
4	14	10.8	12.9	16	11.2			
5	11.5	13.5	11.5	14.5	10.6			
6	10	2.8	11	10	13.5			
7	15	14	14	19				
8	12.9	10.9	13.5	15.2				
9	16	12.7	11					
10		12	12.4					
11		10.5	14					
12		11.5	13.9					
13		14	13					
14		14	14.4					
15		12.5	11					
16		10						
17		10.5						
18		15						
19		7.8						
20		14.9						
21		13.5						
22		11.9						
23		15						
24		13.5						
25		15						
26		7.8						
27		8.1						
Mean	13.17	11.73	12.45	14.10	13.18	00	00	15.00
SD	1.88	2.83	2.04	4.09	3.51			3.54
SE	0.63	0.55	0.53	1.45	1.43	00	00	2.50
Mean ± SE	13.17 ± 0.63	11.73 ± 0.55	12.45 ± 0.53	14.10 ± 1.45	13.18 ± 1.43	00	00	15 ± 2.50

Table 26 Age wise concentration of cTSH (µIU/ml)in healthy dogs (n=67)

Sr. No.	Group 1 (1 -2.6 yrs) (n=9)	Group 2 (2.6-4.3 yrs) (n=27)	Group 3 (4.3-5.9 yrs) (n=15)	Group 4 (5.9-7.5 yrs) (n=8)	Group 5 (7.5-9.1 yrs) (n=6)	Group 6 (9.1-10.8yrs) (n=0)	Group 7 (10.8-12.4 yrs) (n=0)	Group 8 (> 12.4 yrs) (n=2)
1	2.03	1.95	1.90	1.79	1.86			2.07
2	2.1	1.97	2.07	2.03	1.96			2.16
3	2.06	1.99	1.97	2.04	1.95			
4	2.1	1.96	2.12	1.97	2.08			
5	2.15	1.84	2	2.07	2.04			
6	2.17	1.87	1.93	2.09	2.18			
7	2.18	2.03	1.99	1.95				
8	2.14	2.14	2.08	1.96				
9	2.13	1.9	2.09					
10		2.08	2.11					
11		1.99	2.17					
12		2	1.94					
13		1.94	2.18					
14		2.02	2.01					
15		1.9	2.02					
16		2.17						
17		2.07						
18		2.03						
19		1.86						
20		2.11						
21		2.14						
22		2.18						
23		2.06						
24		2.02						
25		2.19						
26		2.06						
27		1.87						
Mean	2.12	1.96	2.02	1.99	2.01	00	00	2.12
SD	0.05	0.10	0.09	0.10	0.11			0.06
SE	0.02	0.02	0.02	0.03	0.05	00	00	0.05
Mean ± SE	2.12 ± 0.02	1.96 ± 0.02	2.02 ± 0.02	1.99 ± 0.03	2.01 ± 0.05	00	00	2.12 ± 0.05

Table 27 Age wise comparison of thyroid hormones in healthy dogs (n=67) (4 Group classification)

Sr. No.	Parameter	Age Group	Mean ± SE	F value	F critical
1	TT_3 (nmol/l)	Group 1(n=19) (1 Yr- 3 Yrs)	0.89±0.07	0.97 N.S.	2.75
		Group 2 (n=38) (3 Yrs-6 Yrs)	0.82 ±0.05		
		Group 3(n=8) (6 Yrs-9 Yrs)	0.75 ± 0.09		
		Group 4(n=2) (Above 9 Yrs)	0.60 ± 0.23		
2	TT_4 (nmol/l)	Group 1(n=19) (1 Yr- 3 Yrs)	20.85±1.60	1.06 N.S.	
		Group 2(n=38) (3 Yrs-6 Yrs)	21.47 ±0.91		
		Group 3(n=8) (6 Yrs-9 Yrs)	24.07 ± 2.96		
		Group 4(n=2) (Above 9 Yrs)	23.76 ± 5.74		
3	fT_4 (pmol/l)	Group 1(n=19) (1 Yr- 3 Yrs)	11.84±0.73	0.99 N.S.	
		Group 2(n=38) (3 Yrs-6 Yrs)	12.40 ±0.38		
		Group 3(n=8) (6 Yrs-9 Yrs)	12.65 ± 1.45		
		Group 4(n=2) (Above 9 Yrs)	15.00 ± 2.50		
4	cTSH (µIU/ml)	Group 1(n=19) (1 Yr- 3 Yrs)	2.05 ± 0.02	1.66 N.S.	
		Group 2(n=38) (3 Yrs-6 Yrs)	2.03 ± 0.01		
		Group 3(n=8) (6 Yrs-9 Yrs)	1.98 ± 0.04		
		Group 4(n=2) (Above 9 Yrs)	2.12 ± 0.05		

Mean should be read column wise. (N.S. =non-significant)

Table 28 Age wise concentration of TT_3 (nmol/l) in healthy dogs (n=67)

(4 Group classification)

Sr. No.	Group 1 (n=19) (1-3 Years)	Group 2 (n=38) (3-6 Years)	Group 3 (n=8) (6-9Years)	Group 4 (n=2) (Above 9 Years)
1	0.72	0.64	0.9	0.37
2	0.72	0.72	0.6	0.82
3	0.70	0.64	0.57	
4	0.70	0.69	0.52	
5	0.85	0.63	0.70	
6	0.90	0.49	1.23	
7	0.96	0.60	0.55	
8	0.92	0.62	0.92	
9	1.11	0.49		
10	1.99	0.56		
11	0.92	0.70		
12	0.41	1.11		
13	0.61	1.23		
14	1.04	1.11		
15	1.04	0.77		
16	0.93	0.89		
17	0.78	1.69		
18	0.56	0.95		
19	1.10	1.04		
20		1.04		
21		0.94		
22		0.92		
23		0.92		
24		0.86		
25		1.23		
26		0.92		
27		1.04		
28		0.86		
29		0.92		
30		1.53		
31		0.92		
32		0.92		
33		0.14		
34		1.22		
35		0.82		

36		0.50		
37		0.90		
38		0.82		
MEAN	0.89	0.82	0.75	0.60
SD	0.33	0.29	0.25	0.32
SE	0.07	0.05	0.09	0.32
MEAN ± SE	0.89±0.07	0.82±0.05	0.75 ± 0.09	0.60 ± 0.07

Table 29 Age wise concentration of TT_4 (nmol/l) in healthy dogs (n=67)

(4 Group classification)

Sr. No.	Group 1 (n=19) (1-3 Years)	Group 2 (n=38) (3-6 Years)	Group 3 (n=8) (6-9 Years)	Group 4 (n=2) (Above 9 Years)
1	14.00	25.00	35.00	29.50
2	19.00	24.00	17.00	18.01
3	20.5	30.50	26.00	
4	34.00	18.00	36.00	
5	21.00	21.00	25.00	
6	17.00	14.00	24.00	
7	25.00	30.00	15.44	
8	15.44	37.00	14.15	
9	29.60	14.00		
10	28.31	19.00		
11	19.31	20.50		
12	18.02	21.00		
13	19.31	19.31		
14	19.31	19.31		
15	25.74	15.44		
16	32.18	21.88		
17	19.31	16.73		
18	5.14	21.88		
19	14.00	19.31		
20		18.02		
21		18.02		
22		15.44		
23		16.73		
24		18.01		
25		19.31		
26		16.73		
27		18.67		
28		25.10		
29		15.44		
30		25.74		
31		28.31		
32		16.73		
33		15.96		
34		21.88		
35		24.45		
36		16.73		
37		7.20		

38		11.58		
MEAN	20.85	21.47	24.07	23.76
SD	6.99	5.60	8.36	8.12
SE	1.60	0.91	2.96	5.74
Mean ± SE	20.85 ± 1.60	21.47 ± 0.91	24.07 ± 2.96	23.76 ± 5.74

Table 30 Age wise concentration of fT_4 (pmol/l) in healthy dogs (n=67)

(4 Group classification)

Sr. No.	Group 1 (n=19) (1-3 Years)	Group 2 (n=38) (3-6 Years)	Group 3 (n=8) (6-9Years)	Group 4 (n=2) (Above 9 Years)
1	11.5	9.40	19.00	12.50
2	14.00	13.50	6.10	17.50
3	2.80	10.80	15.20	
4	12.70	6.60	9.60	
5	13.50	13.00	11.20	
6	11.60	13.50	10.60	
7	14.00	14.00	16.00	
8	11.50	10.90	13.50	
9	11.50	14.50		
10	10.50	16.00		
11	10.00	12.00		
12	15.00	12.90		
13	7.80	10.50		
14	15.00	14.00		
15	11.90	14.00		
16	12.90	16.00		
17	15.00	12.50		
18	7.80	10.00		
19	16.00	11.50		
20		11.00		
21		14.00		
22		14.50		
23		13.50		
24		14.90		
25		10.00		
26		11.00		
27		12.40		
28		13.50		
29		14.00		
30		13.90		
31		19.00		
32		13.00		
33		14.40		
34		13.50		
35		15.00		
36		15.20		
37		11.00		

38		8.10		
MEAN	11.84	12.40	12.65	15.00
SD	3.17	2.36	4.09	3.54
SE	0.73	0.38	1.45	2.50
Mean ± SE	11.84 ± 0.73	12.40 ± 0.38	12.65 ± 1.45	15 ± 2.50

Table 31 Age wise concentration of cTSH (µIU/ml) in healthy dogs (n=67)

(4 Group classification)

Sr. No.	Group 1 (n=19) (1-3 Years)	Group 2 (n=38) (3-6 Years)	Group 3 (n=8) (6- 9 Years)	Group 4 (n=2) (Above 9 Years)
1	1.95	1.97	1.86	2.07
2	2.03	1.99	1.79	2.16
3	1.87	1.96	1.96	
4	1.90	1.90	1.95	
5	2.10	2.07	2.08	
6	2.06	1.84	2.04	
7	2.10	2.03	1.97	
8	2.00	2.14	2.18	
9	2.15	1.97		
10	2.07	2.03		
11	2.17	2.08		
12	2.03	2.12		
13	1.86	1.99		
14	2.18	1.94		
15	2.18	2.02		
16	2.14	2.04		
17	2.06	1.90		
18	2.06	2.17		
19	2.13	2.00		
20		1.93		
21		1.99		
22		2.07		
23		2.08		
24		2.11		
25		2.09		
26		2.09		
27		2.11		
28		2.14		
29		2.17		
30		1.94		
31		1.95		
32		2.18		
33		2.01		
34		2.02		
35		2.19		
36		1.96		

37		2.02		
38		1.87		
Mean	2.05	2.03	1.98	2.12
SD	0.10	0.09	0.12	0.06
SE	0.02	0.01	0.04	0.05
Mean ± SE	2.05 ± 0.02	2.03 ± 0.01	1.98 ± 0.04	2.12 ± 0.05

4.1.4.8.6 Total Thyroxine TT_4 (nmol/l) (4 Group classification of age)

The mean TT_4 values of group 1 (n=19), group 2 (n= 38), group3 (n=8) and group 4 (n= 2) were 20.85 ± 1.60 nmol/l , 21.47 ± 0.91 nmol/l , 24.07 ± 2.96 nmol/l and 23.76 ± 5.76 nmol/l respectively. A statistically non-significant ($p< 0.05$) difference was observed in TT_4 concentration in all four age groups (Table 27). Here, T_4 concentrations were increased up to 7 years of age, and in old dogs (above 9 years) TT_4 concentrations were decreased by 1.28 % compared to dogs with 6-9 years of age. This observation is in correlation with in support of Gonzalez and Quadri (1988).

4.1.4.8.7 Free Thyroxine fT_4 (pmol/l) (4 Group classification of age)

For fT_4, the Mean ± SE values of group 1 (n=19), group 2 (n= 38), group3 (n=8), and group 4 (n= 2) were 11.84 ± 0.73 pmol/ l, 12.40 ± 0.38 pmol/l, 12.65 ± 1.45 pmol/l and 15.00 ± 2.50 pmol/l respectively. A statistically non-significant ($p< 0.05$) difference was observed in fT_4 concentration in all four age groups (Table 27). Here, the fT_4 value was increased up to 14 years of age.

4.1.4.8.8 Thyroid Stimulating Hormone cTSH (µIU/ml) (4 Group classification of age)

The mean cTSH values of group 1 (n=19), group 2 (n= 38), group3 (n=8) and group 4 (n= 2) were 2.05 ± 0.02 µIU/ml, 2.03 ± 0.01 µIU/ml,1.98 ± 0.04 µIU/ml and 2.12 ± 0.05 µIU/ml respectively. A statistically non-significant ($p< 0.05$) difference was observed in concentration in all four age groups (Table 27). Here, the cTSH value was increased up to 14 Years of age.

Increased concentration of fT_4 and cTSH in older age (above 9 years) could be due to decreased thyroid hormone clearance as aging can affect the metabolism and clearance rates for thyroxine or due to increased thyroid hormone resistance where body tissue becomes less responsive to the effect of thyroxine, result into the increased release of TSH also.

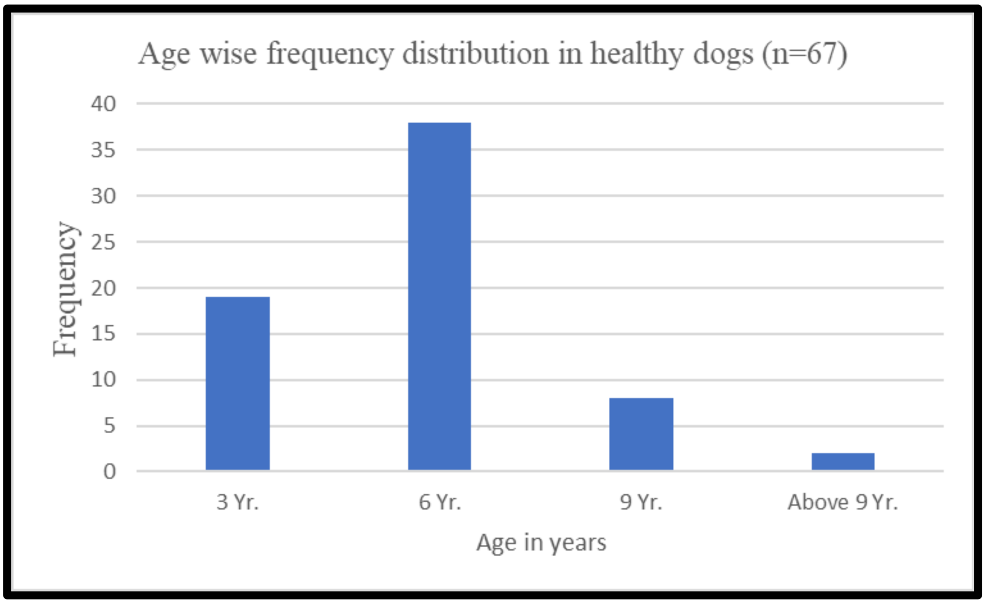

Figure 18 Age-wise frequency distribution in healthy dogs (n=67)

(4 Group classification)

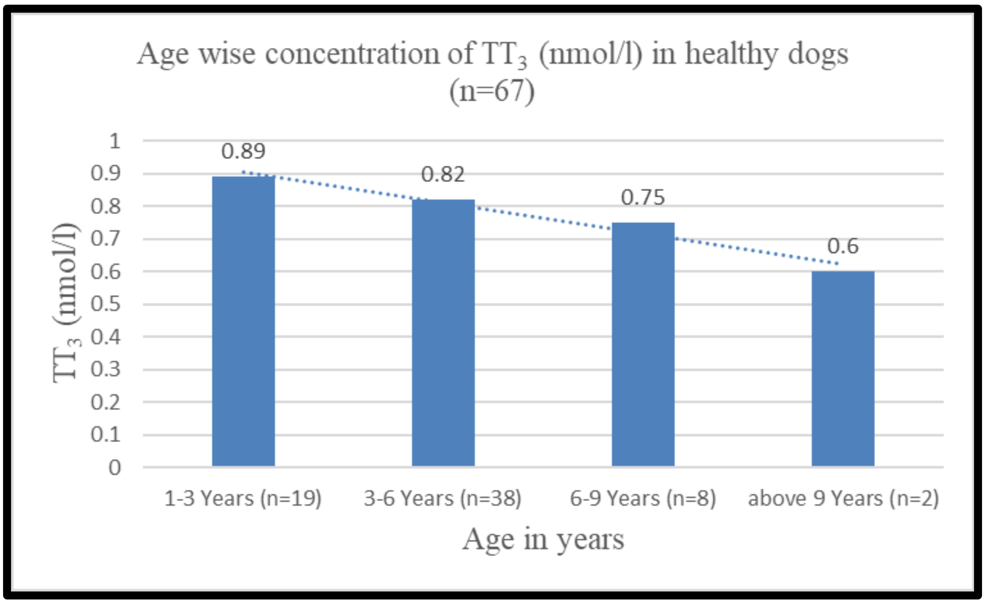

Figure 19 Age wise concentration of TT_3(nmol/l) in healthy dogs (n=67)

(4 Group classification)

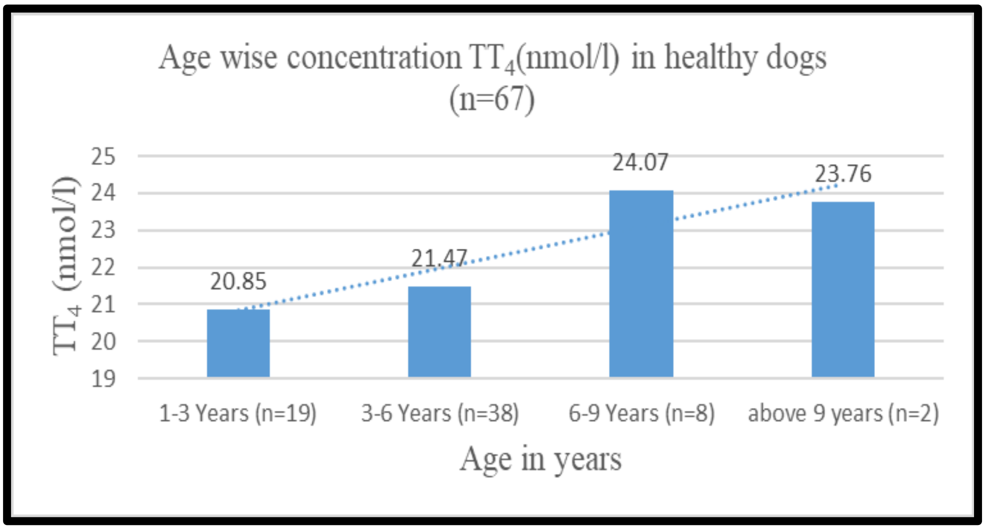

Figure 20 Age wise concentration of TT_4(nmol/l) in healthy dogs (n=67)

(4 Group classification)

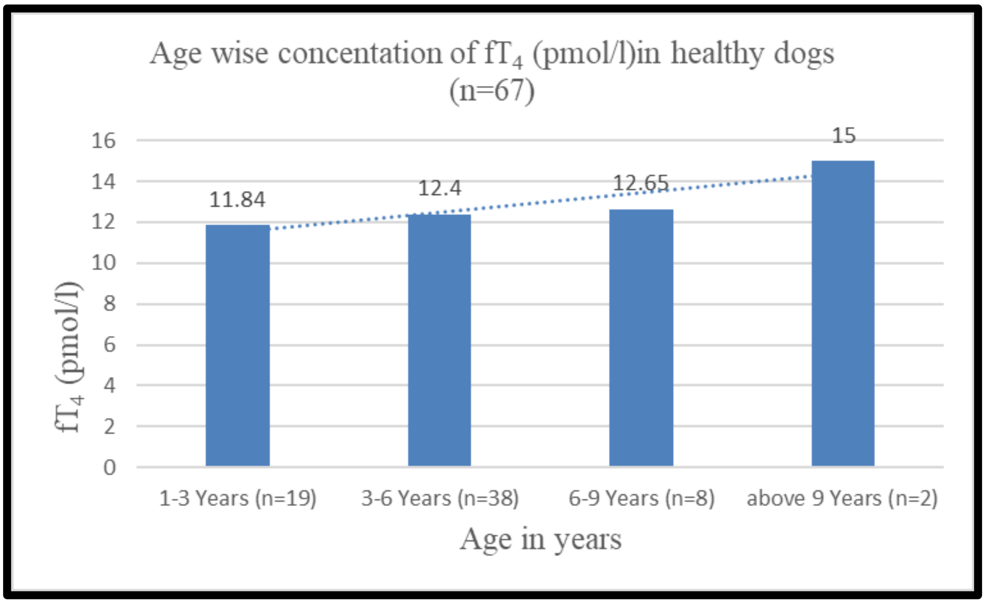

Figure 21 Age wise concentration of fT_4(pmol/l) in healthy dogs (n=67)

(4 Group classification)

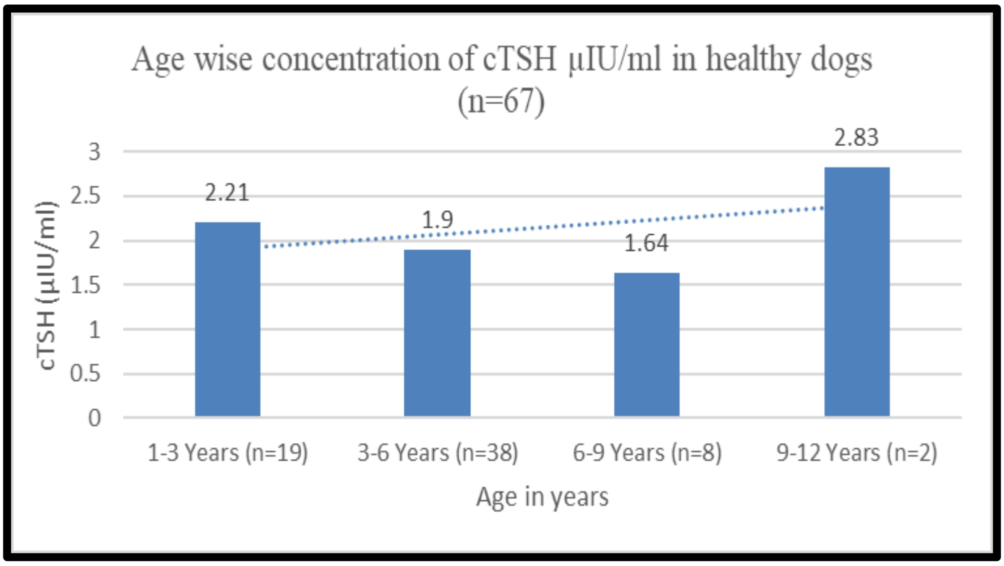

Figure 22 Age wise concentration of cTSH(µIU/ml) in healthy dogs (n=67)

(4 Group classification)

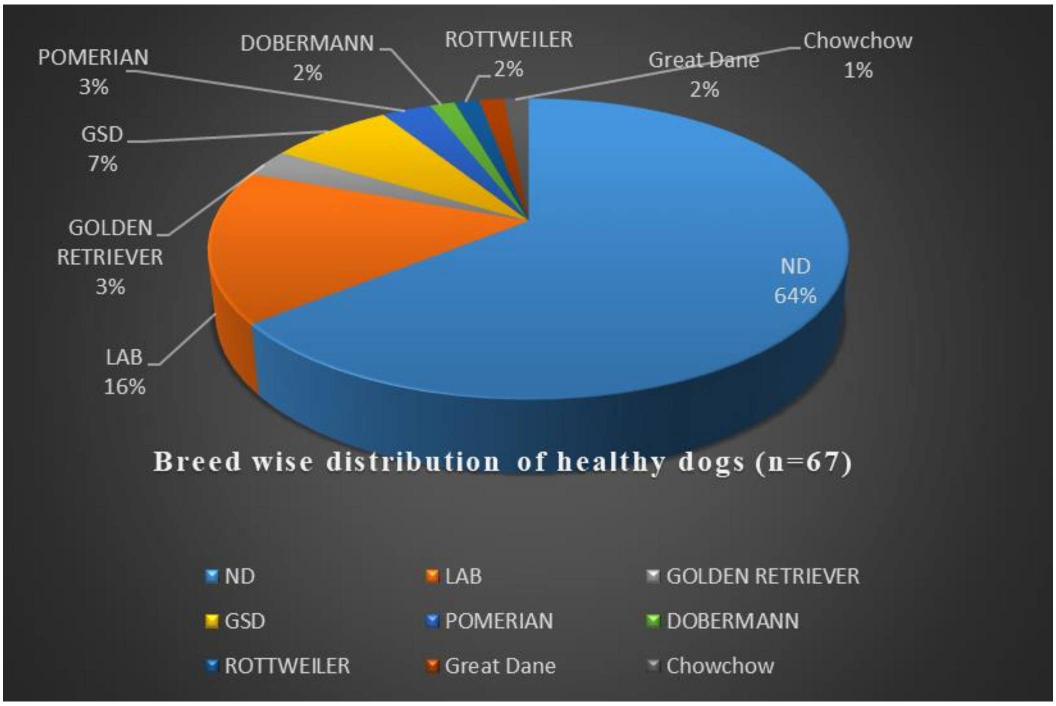

Figure 23 Breed-wise distribution of healthy dogs (n=67)

4.1.4.9 Breed-wise thyroid profile:

A total of 67 healthy dogs were categorized with respect to their breeds into 10 groups. The present study of healthy dogs contained ND (n=43), Labradors (n=11), Golden Retriever (n=2), German Shepherd (n=5), Pomeranian (n=2), Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) breeds of dogs for thyroid profile data. The percentage of different breeds of dogs included in the study is shown in Figure 22

The breed-wise concentration of TT_3, TT_4, fT_4, and cTSH values of healthy dogs is presented in Table 32, Table 34, Table 35, and Table 36, and Fig.23, Fig. 24, Fig. 25, and Fig. 26 respectively. For TT_3 (nmol/l), the Mean ± SE values in ND (n=43), Labradors (n=11), Golden Retriever (n=2), German Shepherd (n=5), Pomeranian (n=2), Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) were 0.80 ± 0.04 nmol/l, 1 ± 0.08 nmol/l, 0.83 ± 0.13 nmol/ l, 1.12 ± 0.16 nmol/l, 0.67 ± 0.27 nmol/ l, 1.04 nmol/l, 0.61 nmol/l, 0.55 nmol/l and 1.04 nmol/l respectively which is summarized in Table 33.

For TT_4 (nmol/l) , the mean concentration observed in ND (n=43) , Labradors (n=11) ,Golden Retriever (n=2) , German Shepherd (n=5), Pomeranian (n=2) , Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) were 20.63 ± 1.07 nmol/l , 22.87 ± 1.92 nmol/l , 22.75 ± 2.25 nmol/l , 20.42 ± 2.49 nmol/l , 18.08 nmol/l , 18.02 nmol/l , 19.31 nmol/l , 15.44 nmol/l and 19.31 nmol/l respectively . A summary of the same is shown in Table 33.

For fT4 (pmol/l), the Mean ± SE values in ND (n=43), Labradors (n=11), Golden Retriever (n=2), German Shepherd (n=5), Pomeranian (n=2), Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) were 12.83 ± 0.43 pmol/l, 12.30 ± 0.63 pmol/l, 8.40 ± 5.60 pmol/l, 12.18 ± 0.68 pmol/l, 14.50 ± 0.50 pmol/l, 11 pmol/l, 7.8 pmol/l, 16 pmol/l and 15 pmol/l respectively. Summary of the same is shown in Table 33.

Table 32 Breed-wise concentration of TT_3 (nmol/l) in healthy dogs (n=67)

Sr. No.	ND (n=43)	Labrador (n=11)	Golden Retriever (n=2)	German Shepherd (n=5)	Pomeranian (n=2)	Dobermann (n=1)	Rottweiler (n=1)	Great Dane (n=1)	Chowchow (n=1)
1	0.90	0.72	0.70	1.11	0.94	1.04	0.61	0.55	1.04
2	0.72	0.70	0.96	0.92	0.41				
3	0.60	0.70		0.77					
4	0.64	1.23		1.69					
5	0.64	1.11		1.11					
6	0.69	1.23							
7	0.63	1.53							
8	0.37	0.93							
9	0.72	0.78							
10	0.49	1.22							
11	0.57	0.82							
12	0.60								
13	0.62								
14	0.52								
15	0.49								
16	0.56								
17	0.70								
18	0.85								
19	0.90								

20	1.23								
21	0.92								
22	0.89								
23	0.95								
24	1.04								
25	1.99								
26	0.92								
27	0.92								
28	0.86								
29	0.92								
30	1.04								
31	0.86								
32	1.04								
33	0.92								
34	0.92								
35	0.92								
36	0.92								
37	0.14								

38	0.82								
39	0.82								
40	0.50								
41	0.56								
42	1.10								
43	0.90								
Mean	0.80	1.00	0.83	1.12	0.67	1.04	0.61	0.55	1.04
SD	0.29	0.28	0.18	0.13	0.37				
SE	0.04	0.08	0.13	0.16	0.27				
Mean ± SE	0.80 ± 0.04	1.00 ± 0.08	0.83 ± 0.13	1.12 ± 0.16	0.67 ± 0.27	1.04 ± 0	0.61 ± 0	0.55 ± 0	1.04 ± 0

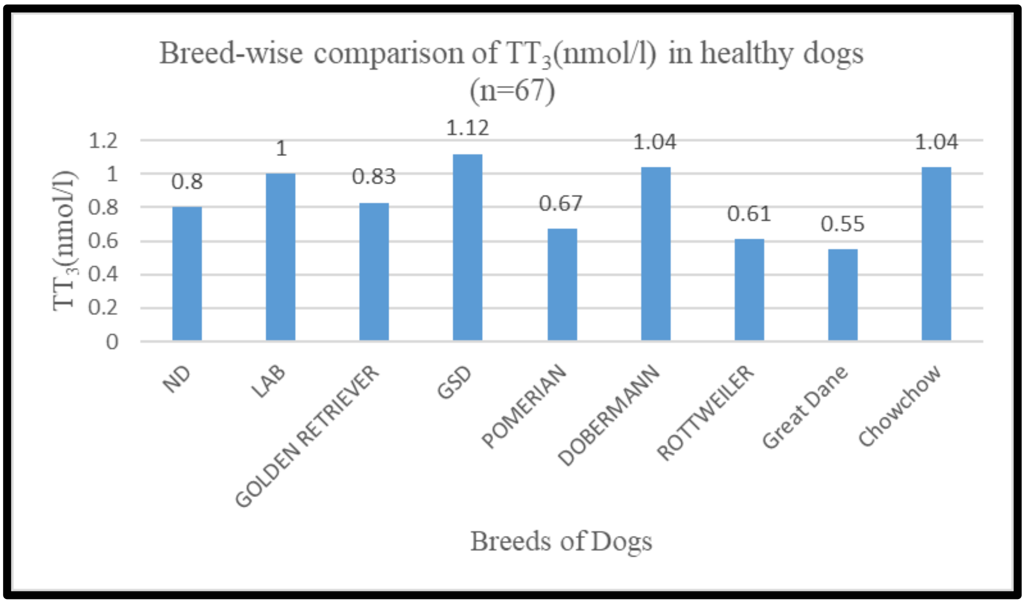

Figure 24 Breed-wise comparison of TT_3 (nmol/l) in healthy dogs

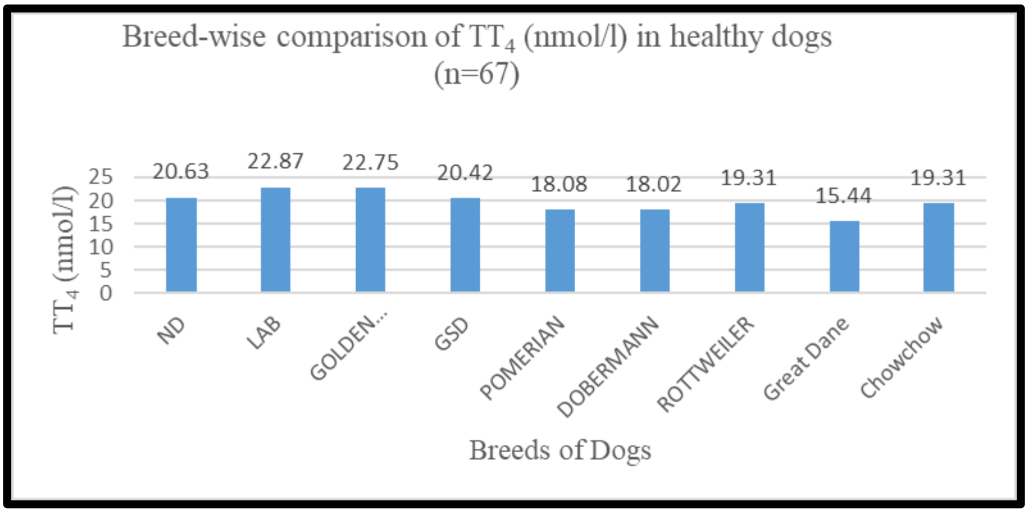

Figure 25 Breed-wise comparison of TT_4 (nmol/l) in healthy dogs(n=67)

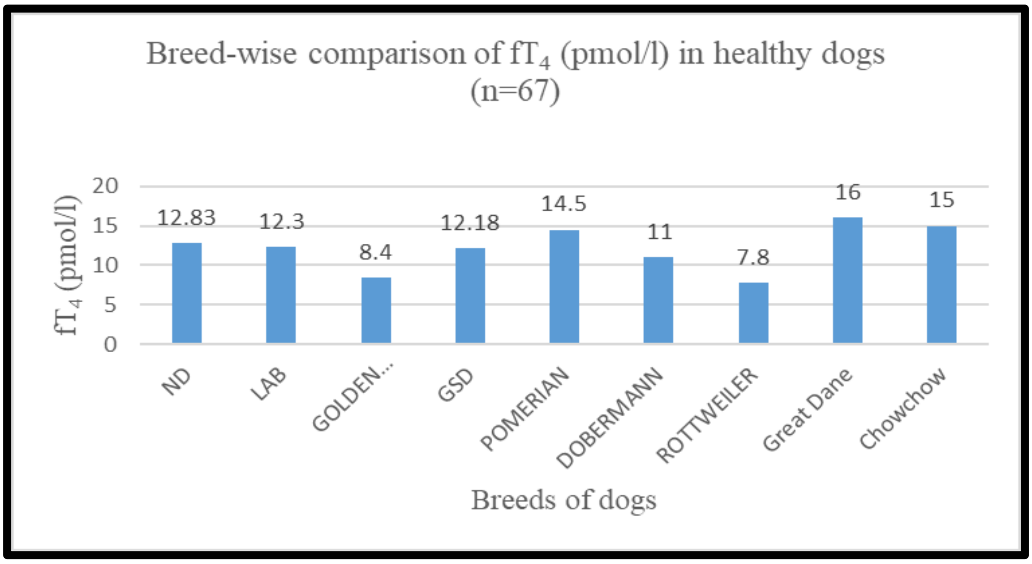

Figure 26 Breed-wise comparison of fT_4 (pmol/l) in healthy dogs(n=67)

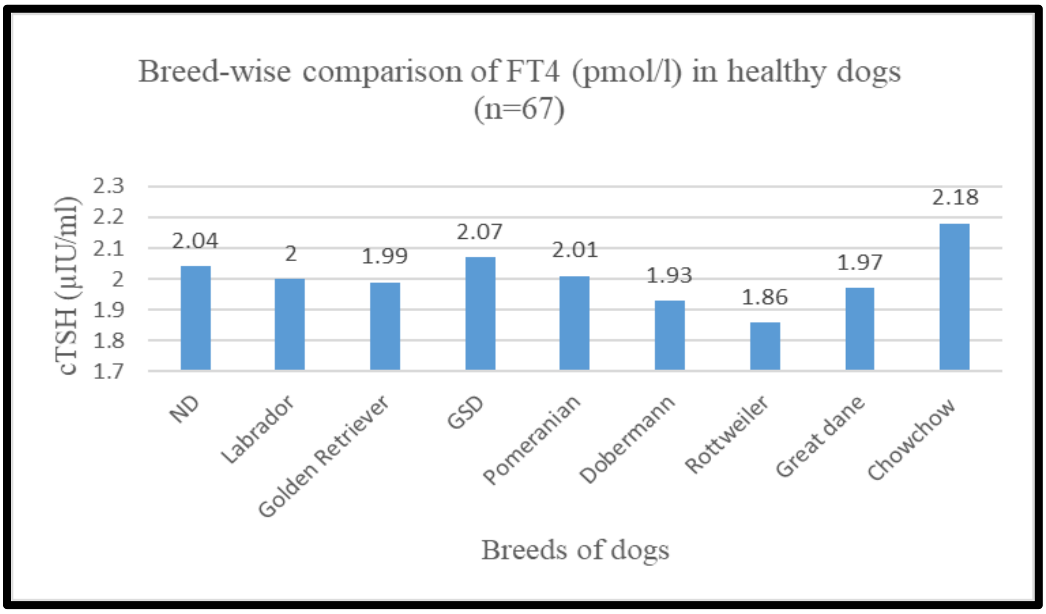

Figure 27 Breed-wise comparison of cTSH (µIU/ml) in healthy dogs (n=67)

Table33 Breed-wise comparison of thyroid profile in healthy dogs (n=67)

Sr. No	Parameter	Breed	Concentration (Mean ± S.E)
1	TT_3 (nmol/l)	ND (n=43)	0.80 ± 0.04
		Labrador (n=11)	1.00 ± 0.08
		Golden Retriever (n=2)	0.83 ± 0.13
		German Shepherd (n=5)	1.12 ± 0.16
		Pomeranian (n=2)	0.67 ± 0.27
		Dobermann (n=1)	1.04 ± 0
		Rottweiler (n=1)	0.61 ± 0
		Geat Dane (n=1)	0.55 ± 0
		Chow Chow (n=1)	1.04 ± 0
2	TT_4 (nmol/l)	ND (n=43)	20.63 ± 1.07
		Labrador (n=11)	22.87 ± 1.92
		Golden Retriever (n=2)	22.75 ± 2.25
		German Shepherd (n=5)	20.42 ± 2.49
		Pomeranian (n=2)	18.08 ± 0
		Dobermann (n=1)	18.02 ± 0
		Rottweiler (n=1)	19.31 ± 0
		Great Dane (n=1)	15.44 ± 0
		Chow Chow (n=1)	19.31 ± 0
3	fT_4 (pmol/l)	ND (n=43)	12.83 ± 0.43
		Labrador (n=11)	12.30 ± 0.63
		Golden Retriever (n=2)	8.40 ± 5.60
		German Shepherd (n=5)	12.18 ± 0.68
		Pomeranian (n=2)	14.50 ± 0.50
		Dobermann (n=1)	11.00 ± 0
		Rottweiler (n=1)	7.80 ± 0
		Geat Dane (n=1)	16.00 ± 0
		Chow Chow (n=1)	15.00± 0
4	cTSH (µIU/ml)	ND (n=43)	2.04 ± 0.01
		Labrador (n=11)	2.00 ± 0.03
		Golden Retriever (n=2)	1.99 ± 0.12
		German Shepherd (n=5)	2.07 ± 0.05
		Pomeranian (n=2)	2.01 ± 0.02
		Dobermann (n=1)	1.93 ± 0
		Rottweiler (n=1)	1.86 ± 0
		Geat Dane (n=1)	1.97 ± 0
		Chow Chow (n=1)	2.18 ± 0

Table 34 Breed-wise concentration of TT_4 (nmol/l) in healthy dogs (n=67)

Sr. No.	ND (n=43)	Labrador (n=11)	Golden Retriever (n=2)	German Shepherd (n=5)	Pomeranian (n=2)	Dobermann (n=1)	Rottweiler (n=1)	Great Dane (n=1)	Chow chow (n=1)
1	35.00	24.00	20.50	21.00	18.08	18.02	19.31	15.44	19.31
2	14.00	34.00	25.00	19.31	18.08				
3	17.00	25.00		15.44					
4	25.00	19.31		16.73					
5	30.50	19.31		29.60					
6	18.00	19.31							
7	21.00	25.74							
8	29.50	32.18							
9	19.00	19.31							
10	14.00	21.88							
11	26.00	11.58							
12	30.00								
13	37.00								
14	36.00								
15	14.00								
16	19.00								

17	20.5								
18	21.00								
19	17.00								
20	24.00								
21	15.44								
22	21.88								
23	21.88								
24	19.31								
25	28.31								
26	15.44								
27	16.73								
28	18.01								
29	16.73								
30	18.67								
31	25.10								
32	25.74								
33	15.44								
34	28.31								
35	16.73								
36	14.16								
37	15.96								
38	18.02								

39	24.45								
40	16.73								
41	5.14								
42	14.00								
43	7.20								
Mean	20.63	22.87	22.75	20.15	18.08	18.02	19.31	15.44	19.31
SD	7.03	6.35	3.18	1.20	0.00				
SE	1.07	0.97	0.49	0.18					
Mean ± SE	20.63 ± 1.7	22.87 ± 1.92	22.75 ± 2.25	20.42 ± 2.49	18.08 ± 0	18.02 ± 0	19.31 ± 0	15.44 ± 0	19.31 ± 0

Table 35 Breed- wise concentration of fT_4 (pmol/l) in heathy dogs (n=67)

Sr. No.	ND	Labrador	Golden Retriever	German Shepherd	Pomeranian	Doberman	Rottweiler	Great Dane	Chow chow
1	19.00	13.50	2.80	12.90	14.00	11.00	7.80	16.00	15.00
2	11.50	12.70	14.00	10.00	15.00				
3	6.10	11.20		14.00					
4	9.40	10.50		12.50					
5	10.80	14.00		11.50					
6	6.60	10.00							
7	13.00	13.90							
8	12.50	12.90							
9	14.00	15.00							
10	13.50	13.50							
11	15.20	8.10							
12	14.00								
13	10.90								
14	9.60								
15	14.50								

16	16.00								
17	12.00								
18	13.50								
19	11.60								
20	10.60								
21	11.50								
22	16.00								
23	10.00								
24	11.50								
25	10.50								
26	14.50								
27	13.50								
28	14.90								
29	11.00								
30	12.40								
31	13.50								
32	11.90								
33	14.00								
34	19.00								

35	13.00								
36	13.50								
37	14.40								
38	17.50								
39	15.00								
40	15.20								
41	7.80								
42	16.00								
43	11.00								
Mean	12.83	12.30	8.40	11.45	14.50	11.00	7.80	16.00	15.00
SD	2.83	2.09	7.92	2.05	0.71				
SE	0.43	0.32	1.21	0.31	0.11				
Mean ± SE	12.83 ± 0.43	12.30 ± 0.63	8.40 ± 5.60	12.18 ± 0.68	14.50 ± 0.50	11 ± 0	7.8 ± 0	16 ± 0	15 ± 0

Table 36 Breed-wise concentration of cTSH (µIU/ml) of healthy dogs (n=67)

Sr. No.	ND (n=43)	Labrador (n=11)	Golden Retriever (n=2)	German Shephard (n=5)	Pomeranian (n=2)	Dobermann (n=1)	Rottweiler (n=1)	Great Dane (n=1)	Chow chow (n=1)
1	1.86	1.99	1.87	2.12	1.99	1.93	1.86	1.97	2.18
2	1.95	1.90	2.10	2.02	2.03				
3	1.79	2.08		1.90					
4	1.97	1.99		2.15					
5	1.96	1.94		2.17					
6	1.90	2.09							
7	2.07	1.94							
8	2.07	2.14							
9	2.03	2.06							
10	1.84	2.02							
11	1.96	1.87							
12	2.03								
13	2.14								
14	1.95								
15	1.97								

16	2.03								
17	2.08								
18	2.10								
19	2.06								
20	2.04								
21	2.00								
22	2.04								
23	2.17								
24	2.00								
25	2.07								
26	2.07								
27	2.08								
28	2.11								
29	2.09								
30	2.11								
31	2.14								
32	2.18								
33	2.17								
34	1.95								

35	2.18								
36	2.18								
37	2.01								
38	2.16								
39	2.19								
40	1.96								
41	2.06								
42	2.13								
43	2.02								
Mean	2.04	2.00	1.99	2.07	2.01	1.93	1.86	1.97	2.18
SD	0.10	0.09	0.16	0.11	0.03				
SE	0.01	0.03	0.12	0.05	0.02				
Mean ± SE	2.04 ± 0.01	2.00 ± 0.03	1.99 ± 0.12	2.07 ± 0.05	2.01 ± 0.02	1.93 ± 0	1.86 ± 0	1.97 ± 0	2.18 ± 0

For fT4 (pmol/l), the Mean ± SE values in ND (n=43), Labradors (n=11), Golden Retriever (n=2), German Shepherd (n=5), Pomeranian (n=2), Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) were 12.83 ± 0.43 pmol/l, 12.30 ± 0.63 pmol/l, 8.40 ± 5.60 pmol/l, 12.18 ± 0.68 pmol/l, 14.50 ± 0.50 pmol/l, 11 pmol/l, 7.8 pmol/l, 16 pmol/l and 15 pmol/l respectively. Summary of the same is shown in Table 33.

For cTSH (µIU/ml), the Mean ± SE values in ND (n=43), Labradors (n=11), Golden Retriever (n=2), German Shepherd (n=5), Pomeranian (n=2), Dobermann (n=1), Rottweiler (n=1), Great Dane (n=1) and Chowchow (n=1) were 2.04 ± 0.01 µIU/ml, 2.00 ± 0.03 µIU/ml, 1.99 ± 0.12 µIU/ml, 2.07 ± 0.05 µIU/ml, 2.01 ± 0.02 µIU/ml, 1.99 ± 0 µIU/ml, 1.86 ± 0 µIU/ml, 1.97 ± 0 µIU/ml and 2.18 ± 0 µIU/ml respectively. A summary of the same is shown in Table 33. It was observed that the German Shepherd dog had a higher concentration of thyroid hormone while the Rottweiler had a relatively low concentration of thyroid hormone.

In healthy German shepherd dogs, higher concentrations of thyroid hormone may be attributed to their breed-specific metabolic characteristics and genetic predispositions.

4.1.4.10 Sex-wise thyroid profile:

Sex sex-wise comparison of the thyroid profile of healthy dogs is shown in Table 39. Among total healthy dogs (n=67), 43 were males (64 %) and 24 were females (36 %). A summary of the same is shown in Fig.27. Sex-wise comparison of TT_3, TT_4, fT_4, and cTSH was performed in healthy dogs (n=67) is presented in Table 37, Table 38, Table 40 and Table 41 and Fig. 28, Fig. 29, Fig. 30 and Fig. 31 respectively.

For TT_3, Mean ± SE values in male and female dogs were 0.82 ± 0.03 nmol/l and 0.92 ± 0.06 nmol/l respectively. For TT_4, the mean concentration values in males and females were 20.12 ± 0.99 nmol/l and 22.04 ± 0.96 nmol/l respectively. For fT_4, Mean ± SE values in male and female dogs were 12.66 ± 0.44 pmol/l and 12.48 ± 0.43 pmol/l respectively. For TSH, the mean values in male and female dogs were 2.04 ±0.01 µIU/ml and 2.02 ± 0.02 µIU/ml respectively. A statistically non-significant ($p < 0.05$) difference was seen in TT_3, TT_4, fT_4, and cTSH concentration between males and females (Table 39). There was no history of a female on heat in the referred study.

Here, the reported study was in correlation with the observation of Raja *et al.* (2021) and Dadke (2018) who also found no significant difference in thyroid hormones between males and females.

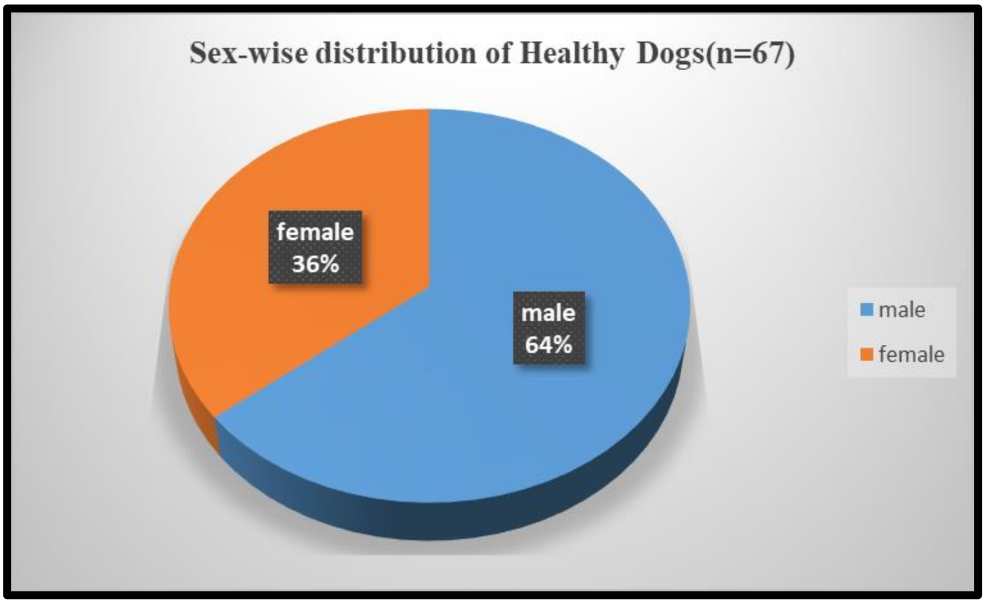

Figure 28 Sex-wise distribution of healthy dogs (n=67)

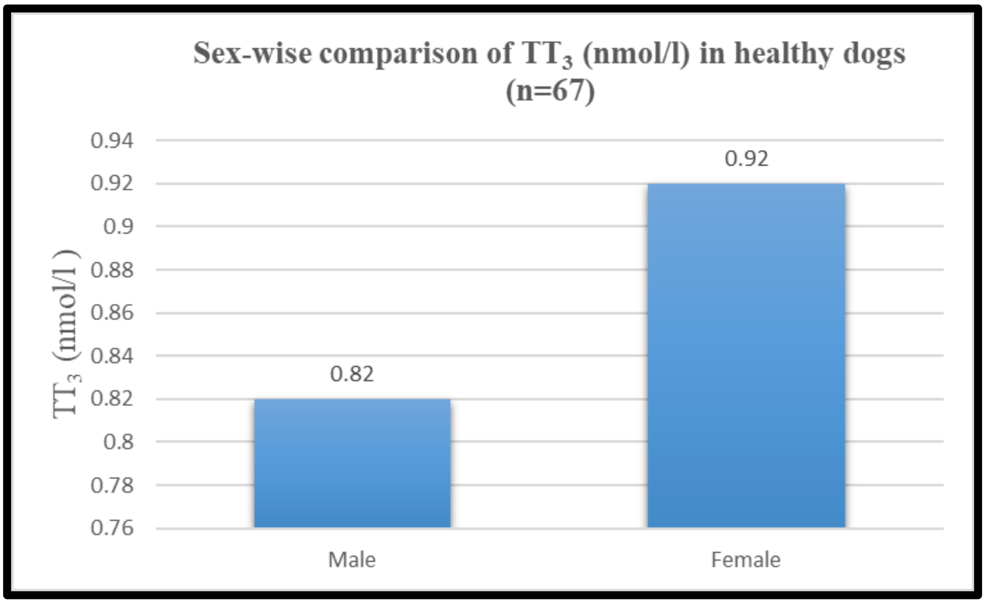

Figure 29 Sex-wise comparison of TT_3 (nmol/l) in healthy dogs (n=67)

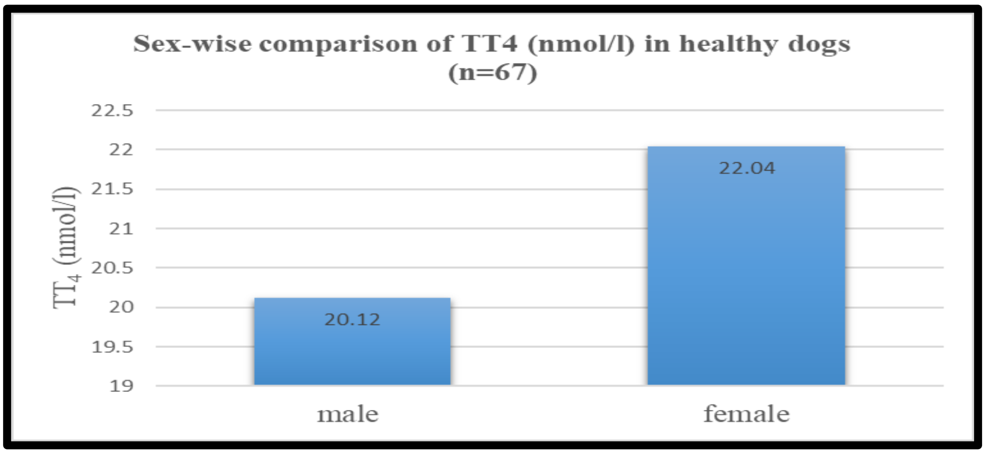

Figure 30 Sex-wise comparison of TT_4 (nmol/l) in healthy dogs (n=67)

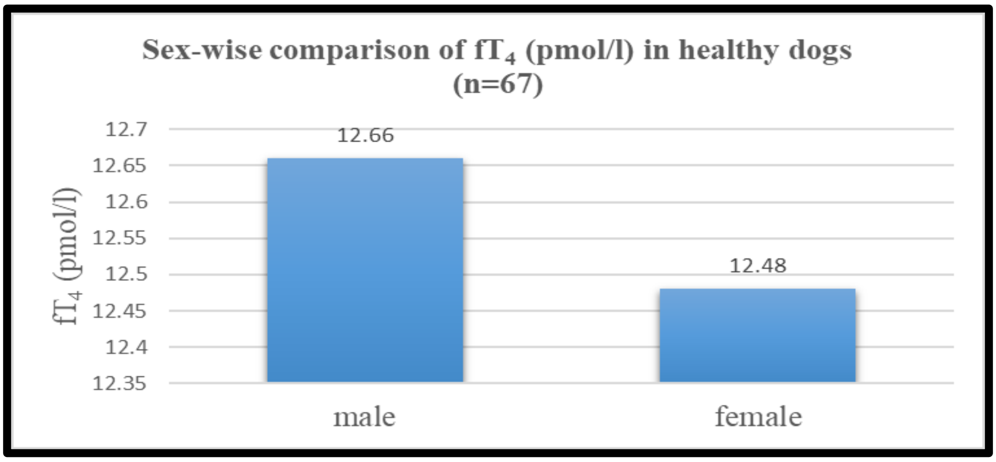

Figure 31 Sex-wise comparison of fT_4 (pmol/l) in healthy dogs (n=67)

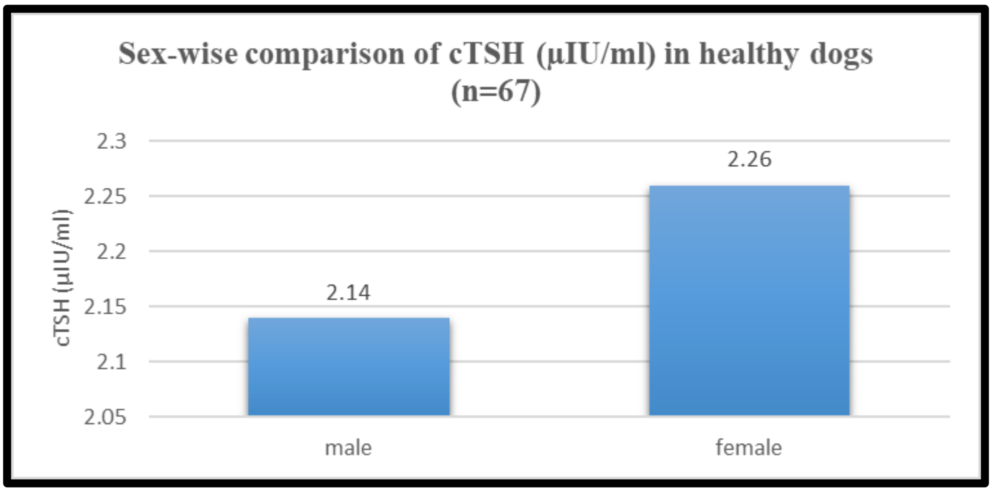

Figure 32 Sex-wise comparison of cTSH (µIU/ml) in healthy dogs (n=67)

Table 37 and 38 Sex – wise TT_3 (nmol/l) and TT_4 (nmol/l) concentration of healthy Dogs (n=67)

For TT_3 (nmol/l)

Sr. No.	Male (n=43)	Female (n=24)
1	0.72	0.90
2	0.64	0.60
3	0.64	0.72
4	0.63	0.69
5	0.37	0.57
6	0.72	0.60
7	0.49	0.85
8	0.70	0.70
9	0.62	0.90
10	0.70	1.23
11	0.52	0.96
12	0.49	1.69
13	0.56	1.99
14	0.70	0.61
15	1.11	0.86
16	1.23	1.04
17	0.92	0.92
18	1.11	1.53
19	0.77	0.93
20	0.89	0.92
21	0.95	0.14
22	1.04	1.22
23	1.11	0.50
24	1.04	0.90
25	0.94	
26	0.92	
27	0.41	
28	0.55	
29	0.92	
30	1.04	
31	0.92	
32	0.86	
33	1.23	
34	0.92	
35	1.04	
36	0.92	
37	0.95	

For TT_4 (nmol/l)

Sr. No.	Male (n=43)	Female (n=24)
1	14.00	35.00
2	25.00	17.00
3	30.50	24.00
4	21.00	18.00
5	29.50	26.00
6	19.00	30.00
7	14.00	21.00
8	20.50	25.00
9	37.00	17.00
10	34.00	24.00
11	36.00	25.00
12	14.00	16.73
13	19.00	28.31
14	20.50	19.305
15	21.00	25.0965
16	19.31	25.74
17	15.44	15.44
18	19.31	25.74
19	15.44	32.175
20	21.88	16.731
21	21.88	15.95
22	19.31	21.87
23	29.60	16.73
24	18.02	7.20
25	18.02	
26	19.31	
27	18.02	
28	15.44	
29	15.44	
30	19.31	
31	16.73	
32	18.01	
33	19.31	
34	16.73	
35	18.67	
36	28.31	
37	14.16	

38	0.78	
39	0.82	
40	0.82	
41	0.56	
42	1.10	
43	0.82	
Mean	0.82	0.92
SD	0.22	0.40
SE	0.03	0.06
Mean ± SE	0.82±0.03	0.92±0.06

38	19.31	
39	18.02	
40	24.45	
41	5.14	
42	14.00	
43	11.58	
Mean	20.12	22.04
SD	6.47	6.30
SE	0.99	0.96
Mean ± SE	20.12±0.99	22.04±0.96

Table 39 Sex-wise comparison of thyroid profile in healthy dogs (n= 67)

Sr. No	Parameter	Sex	Concentration (Mean ± S.E)	t Value	t table
1	TT_3 (nmol/l)	Male (n=43)	0.82±0.03	1.29 N. S	1.99
		Female (n=24)	0.92±0.06		
2	TT_4 (nmol/l)	Male (n=43)	20.12±0.99	1.17 N. S	
		Female (n=24)	22.04±0.96		
3	fT_4 (pmol/l)	Male (n=43)	12.66±0.44	0.24 N. S	
		Female (n=24)	12.48±0.43		
4	cTSH (µIU/ml)	Male (n=43)	2.04 ± 0.01	0.23 N. S	
		Female (n=24)	2.02 ± 0.02		

Mean should be read column -wise (N.S.=non-significant)

Table 40 Sex-wise concentration of fT_4 (nmol/l) in healthy dogs(n=67)

Sr. no.	Male (n=43)	Female (n=24)
1	11.50	19.00
2	9.40	6.10
3	10.80	13.50
4	13.00	6.60
5	12.50	15.20
6	14.00	14.00
7	13.50	13.50
8	2.80	11.20
9	10.90	11.60
10	12.70	10.60
11	9.60	14.00
12	14.50	12.50
13	16.00	10.50
14	12.00	7.80

Table 41 Sex-wise concentration of cTSH (µIU/ml) in healthy dogs (n= 67)

Sr. no.	Male (n=43)	Female (n=24)
1	1.95	1.86
2	1.97	1.79
3	1.96	1.99
4	2.07	1.90
5	2.07	1.96
6	2.03	2.03
7	1.84	2.10
8	1.87	2.08
9	2.14	2.06
10	1.90	2.04
11	1.95	2.10
12	1.97	1.90
13	2.03	2.07
14	2.08	1.86

15	12.90	13.50
16	10.50	11.90
17	11.50	14.00
18	14.00	13.90
19	14.00	12.90
20	16.00	13.00
21	10.00	14.40
22	11.50	13.50
23	11.50	15.20
24	11.00	11.00
25	14.00	
26	10.00	
27	15.00	
28	16.00	
29	14.50	
30	15.00	
31	13.50	
32	14.90	
33	10.00	
34	11.00	
35	12.40	

15	2.12	2.14
16	1.99	2.18
17	2.00	2.17
18	1.94	1.94
19	2.02	2.14
20	2.04	2.18
21	2.17	2.01
22	2.00	2.02
23	2.15	1.96
24	1.93	2.02
25	1.99	
26	2.17	
27	2.03	
28	1.97	
29	2.07	
30	2.18	
31	2.08	
32	2.11	
33	2.09	
34	2.09	
35	2.11	

36	19.00	
37	13.50	
38	15.00	
39	17.50	
40	15.00	
41	7.80	
42	16.00	
43	8.10	
Mean	12.66	12.48
SD	2.92	2.83
SE	0.44	0.43
Mean±SE	12.66±0.44	12.48±0.43

36	1.95	
37	2.18	
38	2.06	
39	2.16	
40	2.19	
41	2.06	
42	2.13	
43	1.87	
Mean	2.04	2.02
SD	0.09	0.11
SE	0.01	0.02
Mean±SE	2.04 ± 0.01	2.02 ± 0.02

4.1.4.11. Diet-wise thyroid profile:

Diet-wise comparison of the thyroid profile of healthy dogs is presented in Table 42. Healthy dogs were classified into three groups such as dogs having commercial diet (n=23), homemade diet (n=9), and mixed diet (n=35) on the basis of the type of food given to the dogs. Percentage distribution (Figure 32) of commercial, homemade, and mixed diets was 34 %, 14 %, and 52 % respectively. Diet-wise comparison of TT_3, TT_4, fT_4, and cTSH was performed in healthy dogs (n=63) is presented in Table 43, Table 44, Table 45, and Table 46 and Fig. 33, Fig.34, Fig. 35, and Fig. 36 respectively.

For $TT_{3,}$ Mean ± SE values of dogs having commercial diet (n=23), homemade diet (n=9), and mixed diet (n= 35) were 0.62 ± 0.03 nmol/l, 0.86 ± 0.08 nmol/l and 0.66 ± 0.02 nmol/l respectively. Mean TT_4 values of dogs having commercial diet (n=23), homemade diet (n=9) and mixed diet (n= 35) were 22.98 ± 1.55 nmol/l, 15.22 ± 2.14 nmol/l and 23.78 ± 1.18 nmol/l respectively. For fT_4, Mean ± SE values of dogs having commercial diet (n=23), homemade diet (n=9), and mixed diet (n= 35) were 12.48 ± 0.55 pmol/l, 12.79 ± 1.16 pmol/l and 11.38 ± 0.66 pmol/l respectively. For cTSH, the Mean concentration of dogs having commercial diet (n=23), homemade diet (n=9), and mixed diet (n= 35) were 2.05 ± 0.02 µIU/ml 2.04 ± 0.04 µIU/ml and 2.02 ± 0.02 µIU/ml respectively.

Statistically non-significant ($p < 0.05$) difference was seen in TT_3, $fT_{4,}$ and cTSH concentration for dogs having commercial diet (n=23), homemade diet (n=9), and mixed diet (n= 35) on the basis of type of food given to the dogs. In the case of TT_4, a statistically significant ($p < 0.05$) difference was observed between dogs on commercial and mixed food while dogs on a homemade diet differed significantly (Table 42).

TT_4 concentration was observed to be less for homemade diet than dogs on commercial and mixed diet whereas a non-significant difference was reported in dogs on homemade, commercial, and mixed for TT_3, $fT_{4,}$ and cTSH. This finding is in correlation with the findings of Dadke (2018). Relatively low concentrations of minerals, protein, and iodine in a homemade diet could be responsible for a lower concentration of thyroid concentration in a homemade diet.

Table 42 Diet-wise comparison of thyroid profile in healthy dogs (n=67)

Sr. no	Parameter	Type of diet	Concentration (Mean ± SE)	F value	F Table
1	TT_3 (nmol/l)	Homemade (n=9)	0.86 ± 0.08	0.05 N.S.	3.14
		Commercial (n=23)	0.62 ± 0.03		
		Mixed (n=35)	0.66 ± 0.02		
2	TT_4 (nmol/l)	Homemade (n=9)	15.22 ± 2.14^{b}	4.31^{*}	
		Commercial (n=23)	22.98 ± 1.55^{a}		
		Mixed (n=35)	23.78 ± 1.18^{a}		
3	fT_4 (pmol/l)	Homemade (n=9)	12.79 ± 1.16	1.13 N.S.	
		Commercial (n=23)	12.48 ± 0.55		
		Mixed (n=35)	11.38 ± 0.66		
4	cTSH (µIU/ml)	Homemade (n=9)	2.04 ± 0.04	0.91 N.S.	
		Commercial (n=23)	2.05 ± 0.02		
		Mixed (n=35)	2.02 ± 0.02		

Mean should be read column wise. Means showing dissimilar superscripts differ significantly ($p < 0.05$) (N.S.=non-significant) (* = significant)

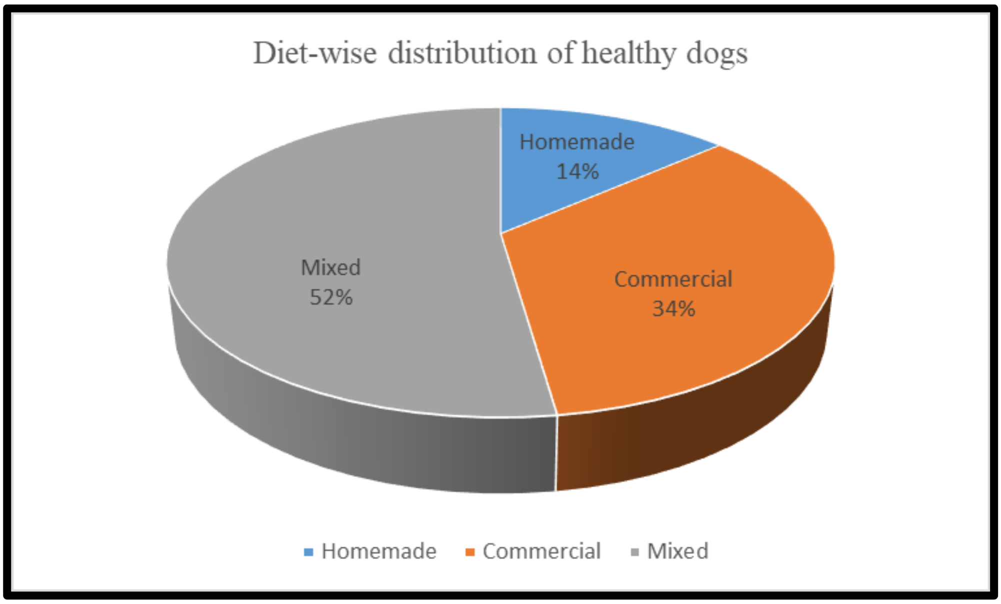

Figure 33 Diet-wise distribution of healthy dogs (n=67)

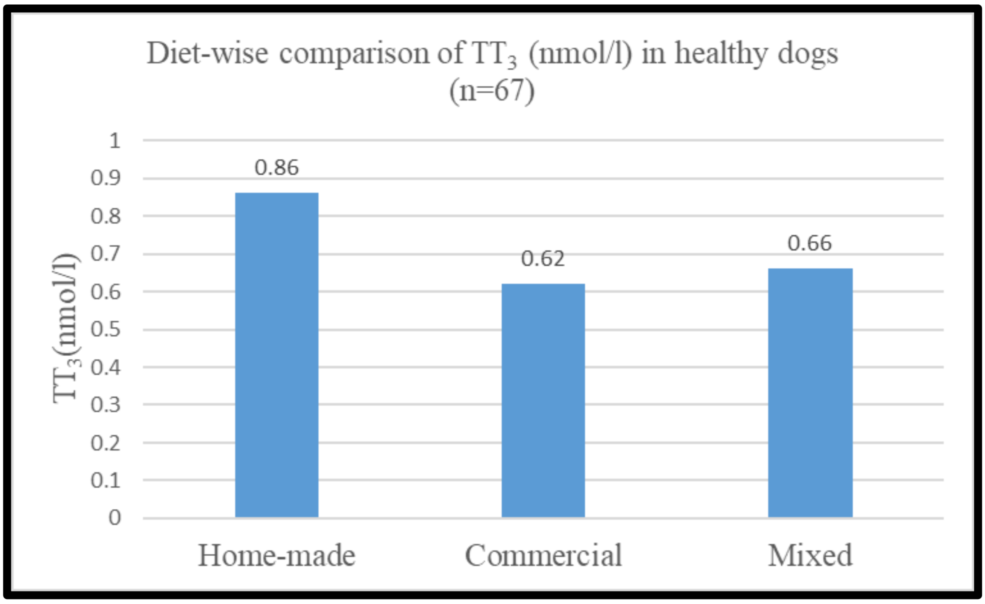

Figure 34 Diet-wise comparison of TT_3 (nmol/l) in health dogs (n=67)

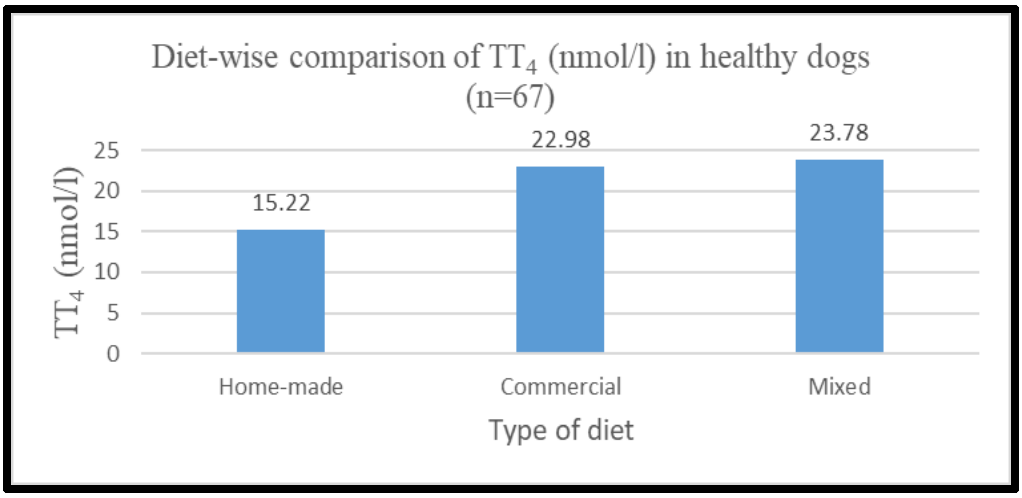

Figure 35 Diet-wise comparison of TT_4 (nmol/l) in health dogs (n=67)

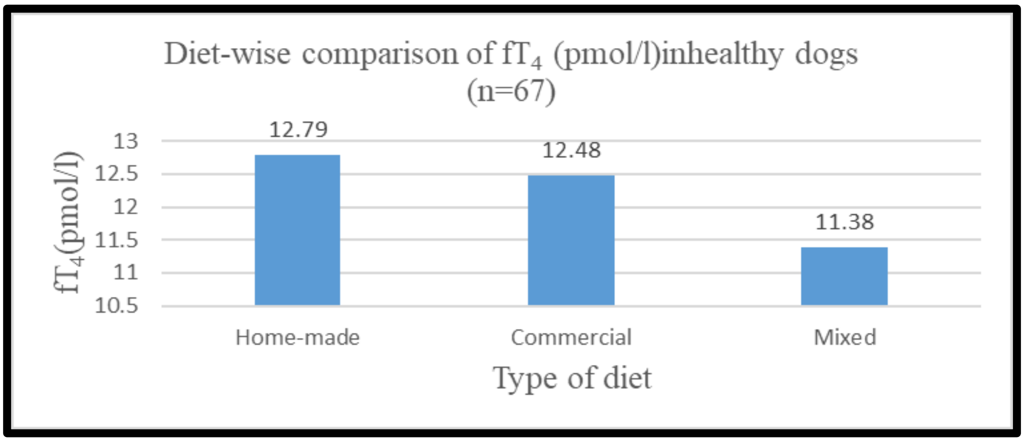

Figure 36 Diet-wise comparison of fT_4 (pmol/l) in health dogs (n=67)

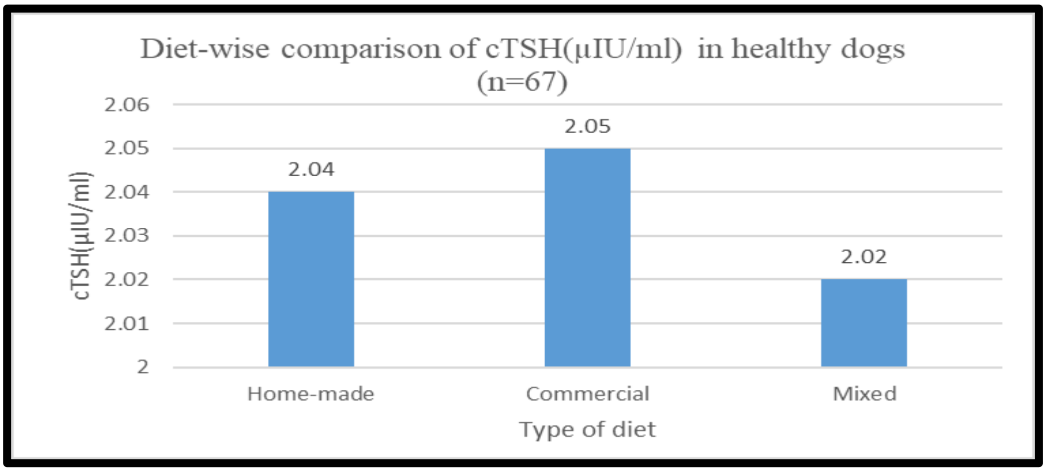

Figure 37 Diet-wise comparison of cTSH (µIU/ml) in health dogs (n=67)

Table 43 Diet-wise concentration of TT_3 (nmol/l) in in healthy dogs (n=67)

Sr. No.	Home-made (n=9)	Commercial (n=23)	Mixed (n=35)
1	1.04	0.70	0.90
2	1.22	0.52	0.72
3	0.82	0.49	0.60
4	0.82	0.56	0.64
5	0.50	0.70	0.72
6	0.56	0.85	0.64
7	1.10	0.70	0.69
8	0.90	0.41	0.63
9	0.82	0.61	0.37
10		0.55	0.72
11		0.92	0.49
12		1.04	0.57
13		0.92	0.70
14		0.86	0.60
15		1.23	0.62
16		0.92	0.90
17		1.04	1.23
18		0.86	0.96

Table 44 Diet-wise concentration of TT_4 (nmol/l) healthy dogs (n=67)

Sr. No.	Home-made (n=9)	Commercial (n=23)	Mixed (n=35)
1	18.02	34.00	35.00
2	21.88	36.00	14.00
3	18.02	14.00	17.00
4	24.45	19.00	25.00
5	16.73	20.50	24.00
6	5.14	21.00	30.50
7	14.00	25.00	18.00
8	7.20	18.02	21.00
9	11.58	19.31	29.50
10		15.44	19.00
11		15.44	14.00
12		19.31	26.00
13		16.73	20.50
14		18.01	30.00
15		19.31	37.00
16		16.73	17.00
17		18.67	24.00
18		25.10	25.00

19		1.04	1.11
20		0.92	1.23
21		1.53	0.92
22		0.93	1.11
23		0.92	0.77
24			0.89
25			1.69
26			0.95
27			1.04
28			1.11
29			1.99
30			0.94
31			0.92
32			0.92
33			0.92
34			0.14
35			0.78
Mean	0.86	0.62	0.66
SD	0.24	0.14	0.14
SE	0.08	0.03	0.02
M± SE	0.86 ± 0.08	0.62 ± 0.03	0.66 ± 0.02

19		25.74	21.00
20		15.44	19.31
21		25.74	15.44
22		32.18	19.31
23		28.31	15.44
24			21.88
25			16.73
26			21.88
27			19.31
28			29.60
29			28.31
30			18.02
31			19.31
32			16.73
33			14.16
34			15.96
35			19.31
Mean	15.22	22.98	23.78
SD	6.41	7.41	6.96
SE	2.14	1.55	1.18
M± SE	15.22 ± 2.14	22.98 ± 1.55	23.78 ± 1.18

Table 45 Diet-wise concentration of fT_4 (pmol/l) (μIU/ml) in healthy dogs (n=67)

Sr. No.	Home-made (n=9)	Commercial (n=23)	Mixed (n=35)
1	11.00	12.70	19.00
2	13.50	9.60	11.50
3	17.50	14.50	6.10
4	15.00	16.00	9.40
5	15.20	12.00	13.50
6	7.80	13.50	10.80
7	16.00	11.20	6.60
8	11.00	15.00	13.00
9	8.10	7.80	12.50
10		16.00	14.00
11		14.50	13.50
12		15.00	15.20
13		13.50	2.80
14		14.90	14.00
15		10.00	10.90
16		11.00	11.60
17		12.40	10.60

Table 46 Diet-wise concentration of cTSH in healthy dogs (n=67)

Sr. No.	Home-made (n=9)	Commercial (n=23)	Mixed (n=35)
1	1.93	1.90	1.86
2	2.02	1.95	1.95
3	2.16	1.97	1.79
4	2.19	2.03	1.97
5	1.96	2.08	1.99
6	2.06	2.10	1.96
7	2.13	2.08	1.90
8	2.02	2.03	2.07
9	1.87	1.86	2.07
10		1.97	2.03
11		2.07	1.84
12		2.18	1.96
13		2.08	1.87
14		2.11	2.03
15		2.09	2.14
16		2.09	2.06
17		2.11	2.04

18		13.50	14.00
19		11.90	12.90
20		14.00	10.50
21		13.90	11.50
22		12.90	14.00
23		19.00	14.00
24			16.00
25			12.50
26			10.00
27			11.50
28			11.50
29			10.50
30			14.00
31			10.00
32			13.00
33			13.50
34			14.40
35			15.00
Mean	12.79	12.48	11.38
SD	3.48	2.65	3.90
SE	1.16	0.55	0.66
Mean ± SE	12.79 ± 1.16	12.48 ± 0.55	11.38 ± 0.66

18		2.14	2.10
19		2.18	2.12
20		2.17	1.99
21		1.94	2.00
22		2.14	1.94
23		1.95	2.02
24			2.04
25			1.90
26			2.17
27			2.00
28			2.15
29			2.07
30			1.99
31			2.17
32			2.18
33			2.18
34			2.01
35			2.06
Mean	2.04	2.05	2.02
SD	0.11	0.09	0.10
SE	0.04	0.02	0.02
Mean ± SE	2.04 ± 0.04	2.05 ± 0.02	2.02 ± 0.02

4.2 To study alteration in canine TSH, total triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) in suspected cases of thyroid dysfunction.

In the present study, we have established data about cTSH by using a canine TSH-specific ELISA kit and its association with total Triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) in healthy dogs. The thyroid profile data regarding TT_3, TT_4, and fT_4 were established by using Radio Immuno Assay. The mean concentration of TT_3, TT_4, fT_4 and cTSH were 0.85 ± 0.04 nmol/l, 20.81 ± 0.79 nmol/l, 12.59 ± 0.35 pmol/l and 2.03 ± 0.01 µIU/ml respectively. The study utilized a canine-specific TSH ELISA kit to estimate the reference range for canine thyroid stimulating hormone due to the unavailability of a canine TSH RIA kit and structural differences between canine and human TSH binding proteins.

4.2.1 Thyroid profile in healthy and hypothyroid dogs

A total of 67 healthy dogs and 10 hypothyroid dogs were enrolled in the present study. The complete thyroid profile of hypothyroid dogs is shown in Table 47. Comparison between concentrations of TT_3, TT_4, fT_4, and cTSH of healthy and hypothyroid dogs are shown in Fig.37, Fig.38. Fig. 39, and Fig. 40 respectively. For each box plot T bar represents the data (range), the box represents the middle half of the data, and the horizontal bar in the line represents the median of the data.

4.2.2 Alterations in TT_3 (nmol/l)

The mean concentration of TT_3 in hypothyroid dogs was 0.93 ± 0.07 nmol/l while in the case of healthy dogs, the mean concentration of TT_3 was 0.85 ± 0.04 nmol/l. The comparison of concentration TT_3 in healthy and hypothyroid dogs is presented in Figure 37. Figure 37 shows a box plot of TT_3 concentration in healthy (n=67) and hypothyroid dogs (n=10). For each box plot, the T bar represents the range of TT_3 (0.61-1.32 nmol/l), the box represents the middle half of the data (0.73-1.12nmol/l) and the horizontal bar in the line represents the median of data (0.92 nmol/l) in hypothyroid dogs. Non-significant difference ($p<0.05$) was found in the mean concentration of TT_3 value of healthy (n=67) and hypothyroid dogs

Table 47 Thyroid profile of hypothyroid dogs (n=10)

Sr.No.	TT_3 (nmol/l)	TT_4 (nmol/l)	fT_4 (pmol/l)	cTSH (µIU/ml)
1	0.77	8.75	6	2.21
2	1.22	7.72	6.6	2.22
3	1.32	14.16	2.1	2.1
4	0.92	7.21	2.7	1.89
5	1.08	10.30	5	2.22
6	0.94	8.49	10	2.17
7	0.62	7.72	5	2.26
8	0.86	10.29	10	2.21
9	0.92	9.01	6.4	2.08
10	0.61	6.44	4.9	2.19
Mean	0.93	9.01	5.87	2.16
SD	0.23	2.19	2.62	0.11
SE	0.073	0.694	0.828	0.03
Range	0.61-1.32	0.43-14.15	2.10-10	1.79-2.19

Table 48 Alteration in thyroid profile of healthy and hypothyroid dogs.

<table>
<tr><th>Sr. No.</th><th>Parameter</th><th>Group</th><th>Concentration</th><th>T value</th><th>T table</th></tr>
<tr><td rowspan="2">1</td><td rowspan="2">TT_3 (nmol/l)</td><td>Healthy (n=67)</td><td>0.85 ± 0.04</td><td rowspan="2">0.74 N.S.</td><td rowspan="8">1.99</td></tr>
<tr><td>Hypothyroid (n=10)</td><td>0.93 ±0.07</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">TT_4 (nmol/l)</td><td>Healthy (n=67)</td><td>20.81± 0.79</td><td rowspan="2">5.73*</td></tr>
<tr><td>Hypothyroid (n=10)</td><td>9.01 ± 0.69</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">fT_4 (pmol/l)</td><td>Healthy (n=67)</td><td>12.59 ± 0.35</td><td rowspan="2">6.99*</td></tr>
<tr><td>Hypothyroid (n=10)</td><td>5.87 ± 0.82</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">cTSH (µIU/ml)</td><td>Healthy (n=67)</td><td>2.03 ± 0.01</td><td rowspan="2">3.62*</td></tr>
<tr><td>Hypothyroid (n=10)</td><td>2.16 ± 0.03</td></tr>
</table>

Mean should be read column wise. (N.S. = non-significant) (*= significant)

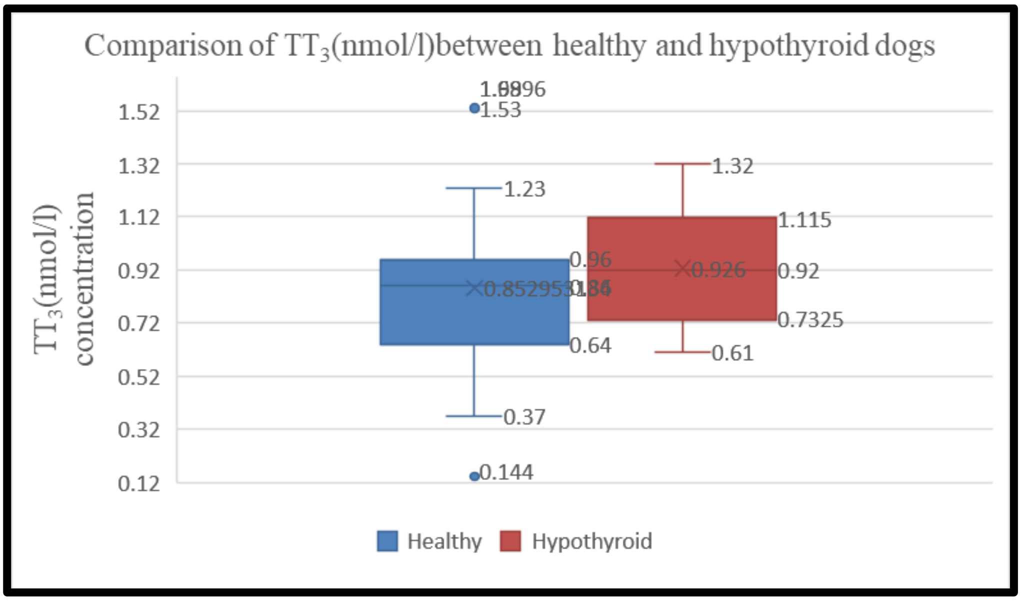

Figure 38 Comparison of TT_3(nmol/l) between healthy and hypothyroid dogs (n=67)

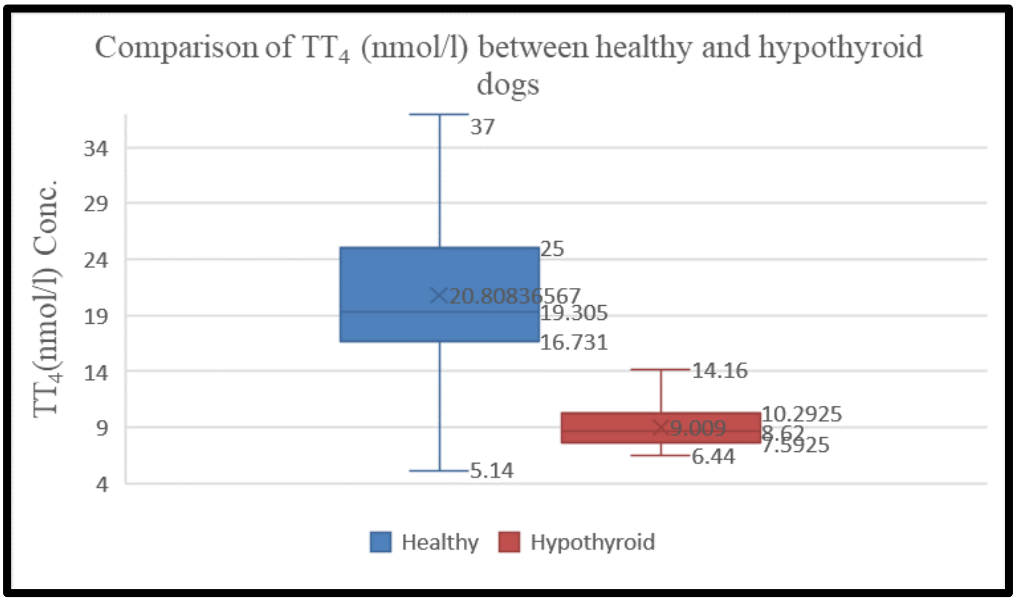

Figure 39 Comparison of TT_4(nmol/l) between healthy and hypothyroid dogs (n=67)

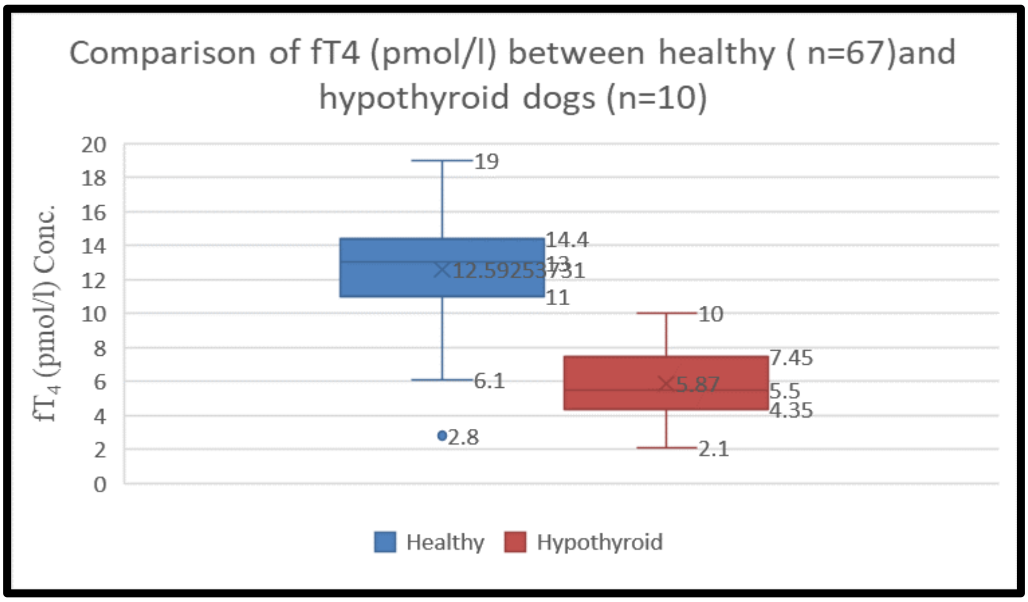

Figure 40 Comparison of fT_4(pmol/l) between healthy and hypothyroid dogs (n=67)

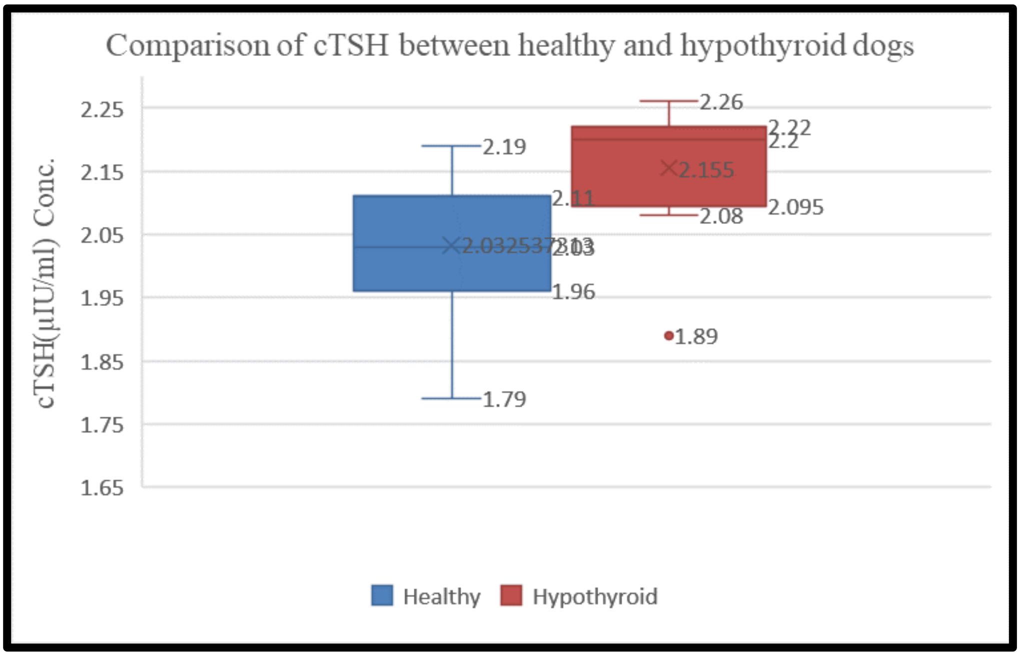

Figure 41 Comparison of cTSH(µIU/ml) between healthy and hypothyroid dogs (n=67)

(Table 48). This finding was in correlation with observation reported by Panciera (1999) who explained that under stimulation by TSH, the thyroid gland preferentially secrets T_3, and much of the circulating T_3 is derived from peripheral deiodination of T_4.

4.2.3 Alterations in TT_4 (nmol/l)

The mean concentration of TT_4 in hypothyroid and healthy dogs was 9.01 ± 0.69 nmol/l and 20.81 ± 0.79 nmol/l respectively. The comparison of concentration TT_4 in healthy and hypothyroid dogs is presented in Figure 38. Figure 38 shows a box plot of TT_4 concentration in healthy (n=67) and hypothyroid dogs (n=10). For each box plot T bar represents the range (6.44-14.16 nmol/l), the box represents the middle half of the data (7.5-10.29nmol/l) and the horizontal bar in the line represents the median of data (8.62 nmol/l) in hypothyroid dogs.

The concentration of TT_4 in hypothyroid dogs had a lower value as compared to ranges established by other researchers Shadwick *et al.* (2013), Shiel *et al.* (2015), Dadke *et al.* (2018), and Galdhar *et al.* (2022). A significant difference ($p<0.05$) was found in the mean concentration TT_4 value of hypothyroid and healthy dogs (Table 48).

4.2.4 Alterations in fT_4 (pmol/l)

The mean concentration of fT_4 in hypothyroid dogs was 12.59 ± 0.35 pmol/l while in the case of healthy dogs, the mean concentration of fT_4 was 5.87 ± 0.82 pmol/l. The comparison of concentration fT_4 in healthy and hypothyroid dogs is presented in Figure 39. Figure 39 shows a box plot of TT_3 concentration in healthy (n=67) and hypothyroid dogs (n=10). For each box plot T bar represents the range (2.1-10 pmol/l), the box represents the middle half of the data (4.35-7.45 pmol/l) and the horizontal bar in the line represents the median of data (5.5 pmol/l) in hypothyroid dogs.

The concentration of fT_4 in hypothyroid dogs was lower than the reference range of healthy dogs reported by previous workers (Shadwick *et al.* 2013, Shiel *et al.* 2015, Dadke *et al.* 2018, and Galdhar *et al.* 2022). A significant difference ($p<0.05$) was found in the mean concentration fT_4 value of hypothyroid and healthy dogs (Table 48).

4.2.5 Canine thyroid stimulating hormone (µIU/ml)

The mean concentration of cTSH in healthy and hypothyroid dogs was 2.03 ± 0.01 µIU/ml and 2.16 ± 0.03 µIU/ml respectively**.** The comparison of concentration cTSH in healthy and hypothyroid dogs is presented in Figure 40. Figure 40 shows a box plot of cTSH concentration in healthy (n=67) and hypothyroid dogs (n=10). For each box plot T bar represents the range (2.08-2.26 µIU/ml), the box represents the middle half of the data (2.09-2.22 µIU/ml) and the horizontal bar in the line represents the median of data (2.09 µIU/ml) in hypothyroid dogs.

A significant difference ($p<0.05$) was found in the mean concentration cTSH value of hypothyroid and healthy dogs (Table 48).

4.2.6 Alteration in canine TSH of healthy and hypothyroid dogs.

In the case of healthy dogs (n=67), the reference range (25^{th} to 75^{th} percentile) was 1.96 -2.11 µIU/ml while in the case of hypothyroid dogs (n=10), the percentile range (25^{th} to 75^{th} percentile) was 2.09-2.22. Out of 10 hypothyroid dogs, 7 (70%) dogs had an orientation range above that of the reference range of healthy dogs, and 3 (30%) dogs had a reference range the same as that of the reference range of healthy dogs**.** These findings support the observation reported by Dixon (2001). According to him, the combination of reduced TT_4 and increased canine TSH values is highly specific for hypothyroidism. However, approximately 20% of hypothyroid dogs have reference range canine TSH values.

William *et al*. (1996), Peterson *et al*. (1997), Ramsey *et al.* (1997), and Dixon (2001) observed that some naturally occurring cases of canine hypothyroidism had TSH concentration within the reference range due to later diagnosis or prolonged periods of low thyroid hormones might result in disruption of feedback pathway by down-regulation or exhaustion of TSH production by pituitary thyrotropes.

The study evaluated the sensitivity and specificity of canine thyroid stimulating hormone concentration as a diagnostic tool for hypothyroidism. Sensitivity in dogs refers to the accuracy of a diagnostic test in detecting hypothyroidism, such as low fT_4 or low TT_4 or high cTSH concentration, and specificity in dogs, indicating the test's ability to exclude dogs without the condition, based on serum hormone concentration within a range.

True positive (TP): Number of hypothyroid dogs correctly identified as hypothyroid by the cTSH ELISA kit (TSH levels above the reference range). False negative (FN): Number of hypothyroid dogs incorrectly identified as healthy by the TSH ELISA kit (TSH levels within the reference range). True Negative (TN): Number of healthy dogs correctly identified as healthy by TSH ELISA kit (TSH level within the reference range). False positive (FP): Number of healthy dogs incorrectly identified as hypothyroid by the cTSH ELISA kit (TSH levels above reference range). Number of True Positive (TP) , False Negative (FN), True Negative (TN) and False Positive (FP) dogs were 5 ,3, 50 and 19 respectively.

Sensitivity = TP/(TP+FN) and Specificity =TN/(TN+FP); the sensitivity and specificity of canine TSH in the present study were 63% and 72% respectively. These findings are in support of Borreti (2022). His study suggests that thyroid stimulating hormone (TSH) levels increase during low T4 states, but normal cTSH concentrations cannot exclude hypothyroidism in dogs. The low sensitivity of 63-87% is due to factors like physiological variations, underestimated secondary hypothyroidism, pituitary gland exhaustion, and distinct TSH isoforms not detected by current cTSH assays. However, the specificity of increased cTSH is high, with values up to 98%. Alteration in the thyroid profile of healthy and hypothyroid dogs is shown in Table 42.

4.2.7 Correlation of TT_4 and fT_4, TT_4 and TSH and fT_4 and TSH in healthy and hypothyroid dogs.

In the present study, the Correlation between TT_4 and fT_4, TT_4 and TSH, and fT_4 and TSH were studied in healthy and hypothyroid dogs (Table 49).

Table 49 Correlation of TT_4 and fT_4, TT_4 and TSH, and fT_4 and TSH in healthy and hypothyroid dogs.

Sr. No.	Name of parameter	Correlation coefficient	
		Healthy	Hypothyroid
1	TT_4 vs fT_4	0.099	0.17
	fT_4 vs TT_4		
2	TT_4 vs TSH	0.143	0.007
	TSH vs TT_4		
3	fT_4 vs TSH	0.357	0.45
	TSH vs fT_4		

Correlation between TT_4 and fT_4, fT_4 and TSH, and fT_4 and TSH was done based on concentration from n=67 healthy dogs. There was a moderate positive correlation between TT4 and fT_4 with (r^2=0.44), a very weak positive correlation between TT_4 and TSH with (r^2=0.143), and a weak positive correlation fT_4 and TSH with (r^2=0.357) magnitude of the correlation coefficient.

Correlation between TT_4 and fT_4, fT_4 and TSH, and fT_4 and TSH was done based on concentration from n=10 hypothyroid dogs. There was a very weak positive correlation between TT4 and fT_4 with (r^2=0.17), a very weak positive correlation between TT_4 and TSH with (r^2=0.007), and a moderate positive correlation between fT_4 and TSH with (r^2=0.45) magnitude of the correlation coefficient.

4.2.8 Assessment of clinical and haemato-biochemical alterations in hypothyroid dogs (n=10)

10 dogs suspected of hypothyroidism were included in the present study. The clinical and hemato-biochemical alteration in these dogs is presented in this study.

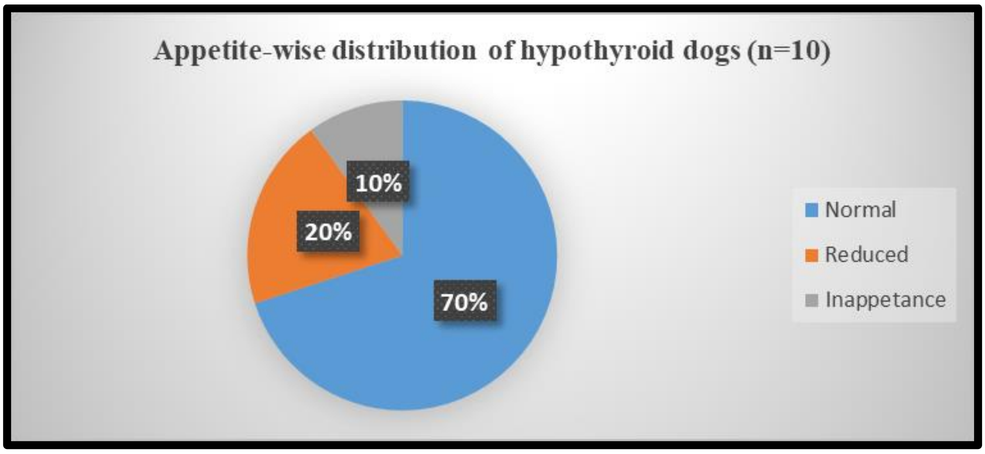

Figure 42 Appetite-wise distribution of hypothyroid dogs (n=10)

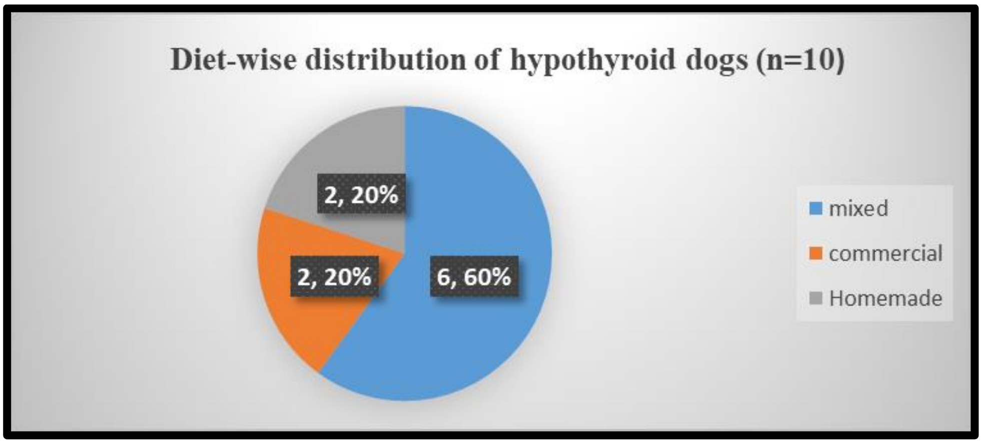

Figure 43 Diet-wise distribution of hypothyroid dogs(n=10)

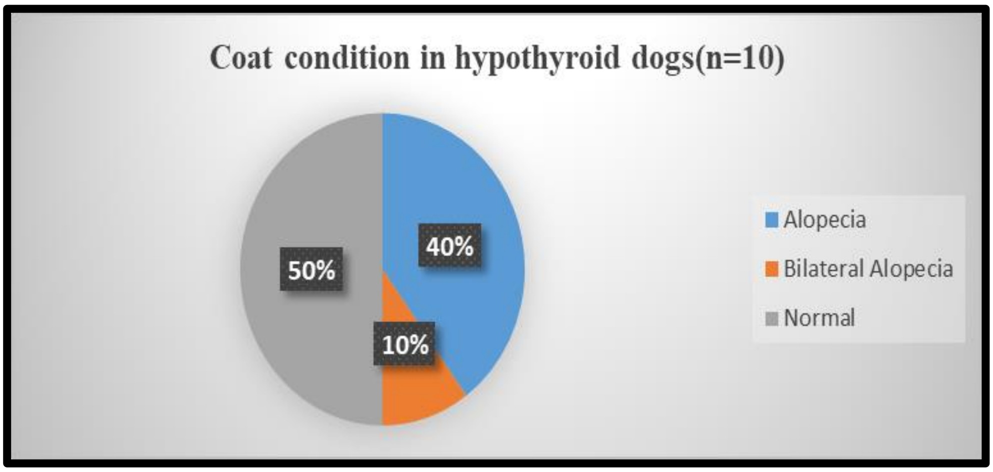

Figure 44 Coat condition in hypothyroid dogs (n=10)

4.2.8.1 Anamnesis and clinical finding of dogs with hypothyroidism (n=10)

The detail of anamnesis of dogs suspected of hypothyroidism is mentioned in Table 50. The clinical examination of each dog is summarized in Table 52. In case dogs were suspected of hypothyroidism, 4 (24%) had pale pink mucous membranes and 13 (76 %) had pink mucous membranes.

Out of 10 dogs suspected of hypothyroidism, 7 (70 %) dogs had a normal appetite, 1(10 %) dog had slight inappetence, and 2 (20 %) dogs had reduced appetite (Figure 41). Among 10 dogs, 2 (20 %) were on a commercial diet, 2 (20%) dogs and 6 (60 %) dogs were on a homemade and mixed diet respectively (Figure 42). 4 (40 %) were showed alopecia, 5 (50 %) were showed normal hair coat and 1 (10 %) were showed bilateral alopecia in dogs (Figure 43).

4.2.8.2 Clinical findings in hypothyroid dogs (n=10):

Out of total hypothyroid dogs (n=10), 10% of cases had otitis externa, 20% of cases had the lethargic sign, 10% of cases had hair thinning, 50 % of cases had hyperpigmentation, 50% of cases had alopecia, 20 % of cases had rat-tail appearance,10 % of cases had itching. The summary of the same is presented in Table 51. From the total number of hypothyroid dogs, 80 % of dogs had dermatological affection and 20 % of dogs had other signs.

4.2.8.3 Hematological findings in hypothyroid dogs (n=10):

Dogs suspected of hypothyroidism are presented in Appendix C. Mean ± SE values of hemoglobin, packed cell volume, and TEC of hypothyroid dogs were 12.10 ± 0.83 gm %, 34.88 ± 2.70 % and 5.34 ± 0.44 x 10^3/cmm respectively. The mean concentrations of MCV, MCH, and MCHC were 64 ± 0.95 fl, 22.65 ± 0.56 pg and 35.06 ± 0.87 gm/dl respectively. Mean ± SE of total leucocyte count, DLC of neutrophils, lymphocyte, monocyte, eosinophils basophils, and plateles

Table 50 Anamnesis of hypothyroid dogs (n=10)

Diet	Coat	Appetite
Mixed	Alopecia	Normal
Mixed	Alopecia	Reduced
Mixed	Normal	Normal
Mixed	Normal	Normal
Commercial	Alopecia	Reduced
Commercial	Bilateral alopecia	Normal
Mixed	Normal	Inappetence
Homemade	Alopecia	Normal
Mixed	Normal	Normal
Homemade	Normal	Normal

Table 51 Clinical findings in hypothyroid dogs (n=10)

Sr.No.	Sample Code	Clinical signs of hypothyroid dogs
1	HT-1	Alopecia, itching
2	HT-2	Alopecia, rat-tail appearance, hyperpigmentation
3	HT-3	Otitis externa
4	HT-4	Hair thinning, hyperpigmentation
5	HT-5	Alopecia, lethargic
6	HT-6	Bilateral alopecia, rat-tail appearance, hyperpigmentation
7	HT-7	Hyperpigmentation
8	HT-8	Alopecia
9	HT-9	lethargic
10	HT-10	Hyperpigmentation

Clinical findings in hypothyroid dogs

Plate 11. Before treatment (HT-2)

Plate 12. After treatment (HT-2)

Plate 13. Before and after treatment (HT-4)

Plate 14. Before treatment (HT-6)

Plate 15. After treatment (HT-6)

Plate 16. Before treatment (HT-7)

Plate 17. After treatment (HT-7)

were 17.77 ± 2.62 x 10^3/cmm, 72.40 ± 2.93 %, 21.00 ± 3.19 %, 1.00 ± 0, 5.70 ±2.02 %, 0 % and 2.31 ± 0.16 lacs/cu mm respectively. The present study reported significant ($p<0.05$) alterations in Hemoglobin and packed cell volume between healthy and hypothyroid dogs. statistical analysis of hemato-biological alterations in healthy and hypothyroid dogs is summarized in Table 53.

Reported studies were in correlation with the observation of Sullivan *et al.* (1993) and Kour *et al.* (2021). According to Sullivan, anemia reported in hypothyroid dogs resulted from reduced thyroxine concentration as it can directly affect erythroid stimulation and indirectly by increasing the cellular oxygen demands of erythropoietic cells.

4.2.8.4 Biochemical findings in hypothyroid dogs (n=10):

Biochemical findings of all dogs with hypothyroidism are presented in Appendix D. The mean concentration of BUN and creatinine of hypothyroid dogs were 17.82 ± 2.30 mg/dl and 1.00 ± 0.08 mg/dl respectively. Mean ± SE values of total bilirubin, direct bilirubin, and indirect bilirubin were 0.24 ± 0.02 mg/dl, 0.10 mg/dl, and 0.14 ± 0.02 mg/dl respectively. The mean concentration of ALP, AST, and ALT were 199.40 ± 38.88 IU/L, 47.65 ± 6.02 IU/L, and 47.13 ± 6.57 IU/L respectively. The mean values of total protein, albumin, and globulin were 7.94 ± 0.47 gm/dl, 2.72 ± 0.19 gm/dl, and 5.22 ± 0.47 gm/dl respectively.

The mean concentrations of serum cholesterol, triglyceride, and glucose were 293.54 ± 39.35 mg/dl, 129.59 ± 25.35 mg/dl, and 73.60 ± 5.11 mg/dl respectively. The present study showed a non-significant ($p <0.05$) difference in biochemical parameters between healthy and hypothyroid dogs except for random blood glucose, ALP, globulin, and cholesterol. A summary of statistical analysis of biochemical alterations in healthy and hypothyroid dogs is shown in Table 54.

The study found hypercholesterolemia in dogs due to decreased peripheral lipoprotein lipolysis, low-density lipoprotein receptors, and hepatic utilization.

Elevated glucose, cholesterol, and ALP levels were found in dogs with hypothyroidism. Elevated serum alkaline phosphatase could be due to degenerative myopathies in canine hypothyroidism or hepatic dysfunction. Increased globulin levels could be due to chronic inflammation or increased protein metabolism. Elevated glucose levels could be due to insulin resistance, decreased glucose utilization, or impaired glycogenolysis and gluconeogenesis (Panceria 2000, Dixon, 1999, Muller et al. 1989).

Table 52 Clinical examination of hypothyroid dogs (n=10)

Sr. No.	Respiratory rate (beats/min)	Heart rate (beats/min)	Mucous membrane	Rectal Temperature (°F)	Capillary refill time (sec)	Blood pressure (mm Hg)	B.C.S.
1	24	108	PINK	102	2	110	3
2	22	90	PALE PINK	100	2	110	3
3	25	85	PINK	102	3	90	3
4	29	120	PINK	101	2	120	3
5	28	140	PINK	102.5	2	140	4
6	28	110	PINK	101	2	105	3
7	20	90	PINK	101	2	120	3
8	27	125	PINK	99.9	2	100	2
9	25	90	PALE PINK	101	2	100	3
10	25	110	PINK	102	2	110	2
Mean	**25.30**	**106.80**	**----**	**101.24**	**2.10**	**110.5**	**2.90**
SD	**2.83**	**18.11**	**----**	**0.81**	**0.32**	**13.83**	**0.57**
SE	**0.90**	**5.73**	**---**	**0.28**	**0.10**	**4.37**	**0.18**
Mean± SE	**25.30 ± 0.90**	**106.80 ± 5.73**	**----**	**101.24 ± 0.28**	**2.10 ± 0.10**	**110.50 ± 4.37**	**2.90 ± 0.18**

Table 53 Comparison of hematological parameters of healthy (n=67) and hypothyroid dogs (n=10)

Sr. no.	Parameter	Group	Concentration (Mean ± SE)	T value	T table
1	Hemoglobin (gm %)	Healthy (n=67)	14.01 ± 0.28	**2.42***	1.99
		Hypothyroid (n=10)	12.10 ± 0.83		
2	Packed Cell Volume (%)	Healthy (n=67)	40.52 ± 0.72	**2.70***	1.99
		Hypothyroid (n=10)	34.88 ± 2.45		
3	Total Erythrocyte Count (10^6/cmm)	Healthy (n=67)	8.13 ± 1.31	0.82 N.S.	1.99
		Hypothyroid (n=10)	5.34 ± 0.44		
4	MCV (fl)	Healthy (n=67)	64.67 ± 0.94	0.16 N.S.	1.99
		Hypothyroid (n=10)	64.27 ± 0.95		
5	MCH (pg)	Healthy (n=67)	23.03 ± 0.64	0.22 N.S.	1.99
		Hypothyroid (n=10)	22.65 ± 0.56		
6	MCHC (gm/dl)	Healthy (n=67)	34.10 ± 0.30	1.150 N.S.	1.99
		Hypothyroid (n=10)	35.06 ± 0.87		
7	TLC (10^3/cmm)	Healthy (n=67)	15.12 ± 0.65	1.36 N.S.	1.99
		Hypothyroid (n=10)	17.77 ± 2.62		
8	DLC % Neutrophils (%)	Healthy (n=67)	70.72 ± 1.23	0.49 N.S.	1.99
		Hypothyroid (n=10)	72.40 ± 2.93		
	Lymphocyte (%)	Healthy (n=67)	24.36 ± 1.03	1.14 N.S.	1.99
		Hypothyroid (n=10)	21.00 ± 3.19		

	Monocyte (%)	Healthy (n=67)	1.18 ± 0.06	1.149 N.S.	1.99
		Hypothyroid (n=10)	1 ± 00		
	Eosinophils (%)	Healthy (n=67)	3.03 ± 0.41	1.03 N.S.	1.99
		Hypothyroid (n=10)	5.70 ± 2.02		
	Basophils (%)	Healthy (n=67)	0.01 ± 0.01	0.384 N.S.	1.99
		Hypothyroid (n=10)	0		
9	Platelets (/cu.mm)	Healthy (n=67)	2.52 ± 0.09	0.836 N.S.	1.99
		Hypothyroid (n=10)	2.31 ± 0.16		
Means should be read column wise. (N. S= Non-significant) (* = significant) Means showing dissimilar superscripts differ significantly (p<0.05).					

Table 54 Comparison of biochemical parameters of healthy (n=67) and hypothyroid dogs (n=10)

Sr. No	Parameter	Group	Concentration (Mean ± SE)	T value	T table
1	Total bilirubin (mg/dl)	Healthy (n=67)	0.29 ± 0.01	1.59 N.S.	1.99
		Hypothyroid (n=10)	0.24 ± 0.02		
2	Direct bilirubin (mg/dl)	Healthy (n=67)	0.12 ± 0.0	1.51 N.S.	1.99
		Hypothyroid (n=10)	0.10 ± 0		
3	Indirect bilirubin (mg/dl)	Healthy(n=67)	0.17 ± 0.01	1.15 N.S.	1.99
		Hypothyroid (n=10)	0.14 ± 0.02		
4	BUN (mg/dl)	Healthy (n=67)	16.02 ± 0.87	0.75 N.S.	1.99
		Hypothyroid (n=10)	17.82 ± 2.30		
5	Creatinine (mg/dl)	Healthy (n=67)	0.96 ± 0.03	0.45 N.S.	1.99
		Hypothyroid (n=10)	1 ± 0.08		
6	ALP (IU/L)	Healthy (n=67)	120.11 ± 7.43	**3.28***	1.99
		Hypothyroid (n=10)	199.40 ± 38.88		
7	AST (IU/L)	Healthy (n=67)	42.24 ± 2.44	0.80 N.S.	1.99
		Hypothyroid (n=10)	47.65 ± 6.02		

8	ALT (IU/L)	Healthy (n=67)	39.92 ± 2.15	1.18 N.S.	1.99
		Hypothyroid (n=10)	47. 13 ± 6.57		
9	Total Protein (gm/dl)	Healthy (n=67)	7.30 ± 0.12	1.75 N.S.	1.99
		Hypothyroid (n=10)	7.94 ± 0.47		
10	Albumin (gm/dl)	Healthy (n=67)	2.90 ± 0.05	1.09 N.S.	1.99
		Hypothyroid (n=10)	2.72 ± 0.19		
11	Globulin (gm/dl)	Healthy (n=67)	4.40 ± 0.13	**2.19***	1.99
		Hypothyroid (n=10)	5.22 ± 0.47		
12	Triglyceride (mg/dl)	Healthy (n=67)	121.30 ± 5.76	0.47 N.S.	1.99
		Hypothyroid (n=10)	129.59 ± 25.35		
13	Cholesterol (mg/dl)	Healthy (n=67)	191.17 ± 6.85	**4.44***	1.99
		Hypothyroid (n=10)	293.54 ± 39.35		
14	Radom Blood Glucose (mg/dl)	Healthy (n=67)	83.61 ± 1.04	**3.03***	1.99

Mean should be read column wise. (N.S=Non- significant)(*= significant) Mean showing dissimilar superscripts differ significantly ($p < 0.05$).

Summary and Conclusions

5. SUMMARY AND CONCLUSIONS

The current study was conducted at Mumbai Veterinary College, Parel, Mumbai-12, at the Department of Veterinary Clinical Medicine, Department of Veterinary Nuclear Medicine, and Radio Isotope Laboratory (Level-II: Research Purpose). The objectives of the study were to establish the data about canine TSH (by using canine specific ELISA kit) and its association with total triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine(fT_4) (by using RIA) in healthy dogs. It also includes the study of alterations in canine TSH, total triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) in healthy and hypothyroid dogs.

A total of 77 dogs were ethically enrolled in the study after receiving ethical approval from the Mumbai Veterinary College's Institutional Ethics Committee for Veterinary Clinical Research and Institutional Biosafety Committee, (VCM/7/2023. Dated: 25/07/2023, Ethics Committee No.MVC/IEC-VCR2023/VCM-M-3) as well as consent from pet owners. Dogs that were referred by Bai Sakarbai Dinshaw Petit Hospital for Animals (BSDPHA) were included in the study. Basedon clinical symptoms, the study population of interest was divided into two groups:healthy dogs (n = 67) and hypothyroid dogs (n = 10).

The Mean serum concentration of Total Thyroxine (TT_3 nmol/l), Total triiodothyronine (TT_4, nmol/l), Free thyroxin (fT_4, pmol/l) and cTSH (µIU/ml) in healthy dogs were 0.85 ± 0.04 nmol/l, 20.81 ± 0.79 nmol/l, 12.59 ± 0.35 pmol/l and 2.03 ± 0.01 µIU/ml respectively. A significant difference (P <0.05) was recorded in TT_4, fT_4, and cTSH of healthy and hypothyroid dogs.

The mean, interquartile range (i.e. 25th to 75th percentile) for thyroid hormone (TT3, TT4, fT4, and cTSH) in healthy dogs were 0.64-0.96 nmol/l, 16.73-25 nmol/l, 11-14.4 pmol/l and 1.96-2.11 µIU/ml and for hypothyroid doges were 0.73-1.12 nmol/l, 7.5-10.29 nmol/l, 4.35-7.45 pmol/l and 2.09-2.22 µIU/ml respectively.

In the present study hematological and biochemical parameters were compared between healthy and hypothyroid dogs, and significant ($p < 0.05$) alterations were seen in hemoglobin (Hb, mg/dl) and packed cell volume (PCV %) of hematological parameters and alkaline phosphatase, globulin, cholesterol and random blood glucose of biochemical parameters between healthy and hypothyroid dogs.

Mean TT_3, TT_4 fT_4, and cTSH, concentration of hypothyroid dogs was found to be 0.93 ± 0.07 nmol/l, 9.01 ± 0.69 nmol/l, 5.87 ± 0.82 pmol/l and 2.16 ±0.03 µIU/ml respectively. The mean TT_4 and fT_4 concentrations of hypothyroid dogs decreased from healthy dogs by 56.70 % and 53.37 % respectively.

In the case of healthy dogs (n=67), the reference range of cTSH (25^{th} to 75^{th} percentile) was 1.96 -2.11 µIU/ml while in the case of hypothyroid dogs (n=10), the reference range (25^{th} to 75^{th} percentile) was 2.09-2.22 µIU/ml respectively. The study found that 70% of hypothyroid dogs had a reference range above that of healthy dogs, while 30% had a reference range the same as healthy dogs. The sensitivity and specificity of measuring canine TSH were 63% and 72%, respectively.

Based on the findings in the present study, the following conclusions were drawn from the present study:

1. The Mean serum concentration of Total triiodothyronine (TT_3, nmol/l), Total thyroxine (TT_4, nmol/l), Free thyroxin (fT_4, pmol/l) and cTSH in healthy dogs were, 0.85 ± 0.04 nmol/l, 20.81 ± 0.79 nmol/l, 12.59 ± 0.35 pmol/l and 2.03 ± 0.01 µIU/ml respectively.
2. The mean, interquartile range (i.e. 25^{th} to 75^{th} percentile) for thyroid hormone (TT_3, TT_4, fT_4, and cTSH) in healthy dogs were 0.64-0.96 nmol/l, 16.73-25 nmol/l, 11-14.4 pmol/l and 1.96-2.11 µIU/ml and for hypothyroid dogs were 0.73-1.12 nmol/l, 7.5-10.29 nmol/l, 4.35-7.45 pmol/l and 2.09-2.22 µIU/ml respectively.
3. The study found significant differences ($p<0.05$) in mean concentrations of thyroid hormones (TT_4, fT_4, and cTSH) in healthy and hypothyroid dogs, but non-significant differences ($p<0.05$) in TT_3.
4. The sensitivity and specificity of measuring canine TSH were 63% and 72%, respectively.

Bibliography

6. BIBLIOGRAPHY

Badawi, N. M., & A. A. Yousif (2020). ESTIMATION OF SOME HEMATOLOGICAL AND BIOCHEMICAL REFERENCES VALUES OF CLINICALLY HEALTHY DOGS IN BAGHDAD PROVINCE, IRAQ. *Biochemical & Cellular Archives*, *20*(2).

Beaver, B. V., & L. I. Haug (2003). Canine behaviors associated with hypothyroidism. *Journal of the American Animal Hospital Association*, *39*(5), 431-434.

Boretti, F. S. (2022). Canine hypothyroidism: diagnosis and treatment.

Bruyette, D. (Ed.). (2020). *Clinical Small Animal Internal Medicine*. John Wiley & Sons.

Cettour-Rose, P., C. Theander-Carrillo, C. Asensio, M. Klein, T. J. Visser, A. G. Burger, & F. Rohner-Jean Renaud (2005). Hypothyroidism in rats decreases peripheral glucose utilization, a defect partially corrected by central leptin infusion. *Diabetology*, *48*, 624-633.

Cote E. (2015). Clinical Veterinary Advisor-E-Book: Dogs and Cats. Elsevier Health Sciences.

Dadke A. R. (2018). Studies on thyroid profile in dogs with special reference to radioimmunoassay. M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University.

Daminet, S., & D. C. Ferguson (2003). Influence of drugs on thyroid function in dogs. *Journal of Veterinary Medicine*, *17*(4), 463-472.

Daminet, S., M. Paradis, K. R. Refsal, & C. Price (1999). Short-term influence of prednisone and phenobarbital on thyroid function in euthyroid dogs. *The Canadian Veterinary Journal*, *40*(6), 411.

David, L. P. (2001). Conditions associated with canine hypothyroidism. *Veterinary Clinics of North America: Small Animal Practice*, *31*(5), 935-950.

Dixon, M., S. W. J. Reid, & C. T. Mooney (1999). Epidemiological, clinical, hematological and biochemical characteristics of canine hypothyroidism. *Veterinary Record*, *145*(17), 481-487.

Dixon, R. (2001). Recent developments in the diagnosis of canine hypothyroidism. *In Practice*, *23*(6), 328-335.

Engvall, E., & P. Perlmann, (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry*, *8*(9), 871-874.

Feldman E. C., R. W. Nelson and J. Catharine R. Scott-Moncrieff (2015) Canine and feline endocrinology. Saunders.

Galdhar C. N and R. V. Gaikwad (2017) presented lead paper on "Radio Immune Assay (RIA): concept and application in veterinary clinical practice and research" in 35th annual convention of ISVM and National Symposium on Innovative Techniques, Emerging Issues and Advancement in Veterinary Medicine to Meet the Challenges, Present and the Future. pp. 321-324.

Galdhar, C. N. and R. V. Gaikwad (2015). Radioisotopes in veterinary medicine and biomedical research. Lead paper presented in 33rd National symposium of ISVM 15 atCollege of Veterinary and Animal Science, Pookode, Wayanad from 22.01.2015 to 24.01.2015

Galdhar, C. N., R. V. Gaikwad, P. S. Masare, K. V. Garud, S. Vaidya, G. R. Bhojne,... & A. G. Parkhe (2022). Triidothyronine (TT3), total thyroxine (TT4) and free thyroxine (FT4) reference range in healthy dogs by radioimmunoassay (RIA). *Indian Journal of Animal Research*, *58*(1), 46-50.

Galdhar, C. N., S. M. Vaidya, &, R. V. Gaikwad (2021). Serum triiodothyronine (T3) and thyroxine (T4) levels in healthy goats. *Indian Journal of Small Ruminants (The)*, *28*(2), 403-404.

Gommeren, K., I. Van Hoek, H. P. Lefebvre, G. Benchekroun, P. Smets, & S. Daminet (2009). Effect of thyroxine supplementation on glomerular filtration rate in hypothyroid dogs. *Journal of veterinary internal medicine*, *23*(4), 844-849.

Gonzalez, E., &, S. K. Quadri (1988). Effects of aging on the pituitary-thyroid axis in the dog. *Experimental Gerontology*, *23*(3), 151-160.

Gori, E., P. Gianella, I., Lippi & V. Marchetti (2023). Retrospective Evaluation of Gastrointestinal Signs in Hypothyroid Dogs. *Animals: an open access journal from MDPI*, *13*(16), 2668. https://doi.org/10.3390/ani13162668

Gulzar, S., R. Khurana, D. Agnihotri, A. Aggarwal & G. Narang (2014). Prevalence of hypothyroidism in dogs in Haryana. *Indian Journal of Veterinary Research (The)*, *23*(1), 1-9.

Gupta, H., G.K. Sawale, C.N. Galdhar and S.D. Moregaonkar (2023).Pathology and diagnosis of hypothyroidism in dogs. Indian J. Vet. Pathol., 47(3): 201-205.

Haseltine, J. (Ed.). (2019). *Clinical small animal internal medicine*. John Wiley & Sons.

Inteeworn, N. (2008). *The Effect of Hypothyroidism on Glucose Tolerance in Dogs* (Doctoral dissertation, Virginia Tech).

Jaggy, A., J. E. Oliver, D. C. Ferguson, E. A. Mahaffey, & T. G. Jun (1994). Neurological manifestations of hypothyroidism: a retrospective study of 29 dogs. *Journal of Veterinary Internal Medicine*, *8*(5), 328-336.

Jaiswal, M., P. C. Shukla, A. Tiwari, D. Gupta, B. Singh, P. Maravi,... & A. A. Sheikh. (2018). Recent approaches in diagnosis and management of canine hypothyroidism: A review. *The Pharma Innovation Journal*, *7*, 90-94.

Jayabhaye, R. M. (2019). Thyroid Profile in Pigs with Special Reference to Radio Immuno Assay. M. V. Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University.

Jayabhaye, R. M., C. N., Galdhar , A. R. Dadke, R. V.Gaikwad , S. Banik, & S. Rajkhowa (2021). Radio immune assay (RIA) enabled thyroid profile (TT. *Indian J. Vet. Med. Vol*, *41*(2), 9-15.

Jensen, A. L., L. Iversen, & R. Høier (1999). Evaluation of analytical performance assisted by total error criteria of a commercial enzyme immunometric assay for canine serum thyrotropin. *Veterinary Clinical Pathology*, *28*(2), 53-56.

Kawa, M. P., K. Grymuła, E. Paczkowska, M. Baśkiewicz-Masiuk, E. Dąbkowska, M. Koziołek and A. Syrenicz (2010). Clinical relevance of thyroid dysfunction in human hematopoiesis: biochemical and molecular studies. European Journal of Endocrinology, **162**(2), 295-305

Kemppainen, R. J., & E. N. Behrend (2001). Diagnosis of canine hypothyroidism.

Klaassen, J. K. (1999). Reference values in veterinary medicine. *Laboratory medicine*, *30*(3), 194-197.

Kooistra, H. S., M. Diaz-Espineira, J. A. Mol, W. E. Van Den Brom, & A. Rijnberk (2000). Secretion pattern of thyroid-stimulating hormone in dogs during euthyroidism and hypothyroidism. *Domestic Animal Endocrinology*, *18(1)*, 19-29.

Kour, H., S. Chhabra, & C. S. Randhawa (2021). Clinical and Haemato-biochemical Characteristics of Hypothyroidism in Canines. *Indian Journal of Veterinary Sciences & Biotechnology*, *17*(3), 1-5.

Kraft, W., U. M. Dürr & M. Fürll (1999). Referenzbereiche [Reference intervals]. *Klinische Labordiagnostik in der Tiermedizin. 5th ed. Stuttgart: Schattauer Verlag*.

Kumar, D. (1978). Thyroid gland: An anatomical perspective. *Blood*, 4.

Lathan, P. (2012). Canine Hypothyroidism. Clinician's Brief Mississippi State University. pp. 25-28.

Li, G., P. Lee, N. Mori, I. Yamamoto, K. Kawasumi, H. Tanabe, & T. Arai (2012). Supplementing five-point body condition score with body fat percentage increases the sensitivity for assessing overweight status of small to medium sized dogs. *Veterinary Medicine: Research and Reports*, 71-78.

Liyanage, A. T., N. B. Ramesh, & H. Ariyarathna (2022). Owner-misperception of Canine Body Condition Reduces After Using a Five-point Body Condition Score Chart: A Study of 95 Large-Sized Purebred Dogs. *Topics in Companion Animal Medicine*, *50*, 100677.

Mazaki-Tovi, M., Y. Feuermann, G. Segev, E. Klement, E. Yas-Natan, A. Farkas,... & A. Shamay (2010). Increased serum leptin and insulin concentrations in canine hypothyroidism. *The veterinary journal*, *183*(1), 109-114.

Mills, D. S., & P. Lathan (2018). Canine Primary Hyperparathyroidism.

Milne, K. L., & H. M. Hayes Jr. (1981). Epidemiologic features of canine hypothyroidism. *The Cornell Veterinarian*, *71*(1), 3-14.

Moritz, A., Y. Fickenscher, K. Meyer, K. Failing, & D. J. Weiss (2004). Canine and feline hematology reference values for the ADVIA 120 hematology system. *Veterinary clinical pathology*, *33*(1), 32–38. https://doi.org/10.1111/j.1939-165x.2004.tb00347.x

Morré, W. A., D. L. Panciera, G. B. Daniel, K. R. efsal, M. M. Rick, & K. Arrington (2017). Thyrotoxicosis induced by excessive 3, 5, 3′-triiodothyronine in a dog. *Journal of the American Veterinary Medical Association*, *250*(12), 1427-1431.

Naik P. 2016. Studies on thyroid profile in cats with special reference to radioimmunoassay. M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University.

Panciera, D. L. (1994). Hypothyroidism in dogs: 66 cases (1987-1992). *Journal of the American Veterinary Medical Association*, *204*(5), 761-767.

Panciera, D. L. (2001). Thyroid Function Tests—What Do They Really Tell Us?. *Journal of Veterinary Internal Medicine*, *15*(2), 86-88.

Parry, N. M. (2013). Hypothyroidism in dogs: laboratory findings. *UK Vet Companion Animal*, *18*(3), 101-105.

Pawar (2009). Study of Hypothyroidism in canines. M.V.Sc. Thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur (M.S).

Pearce, E. N. (2012). Update in lipid alterations in subclinical hypothyroidism. *The Journal of Clinical Endocrinology & Metabolism*, *97*(2), 326-333.

Peterson, M. E. C., Melián, & R. ,Nichols (1997). Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. *Journal of the American Veterinary Medical Association*, *211*(11), 1396-1402.

Raja, R., D. Mondal, J. M. Velayudhan, R. S. K. Mandal, S. Bhatt, V. Joshi, & U. Dimri (2021). Studies on role of Thyroperoxidase (TPO) Enzyme in Primary Hypothyroidism Affected Dogs.

Ramsey, I. (1997). Diagnosing canine hypothyroidism. *In Practice*, *19*(7), 378-383.

Randolph J. F., S. V. Lamb, J. L. Cheraskin, B. J. Schanbacher, V. J. Salerno, K. M. Mack, J. M. Scarlett and N. J. Place (2015). Free Thyroxine Concentrations by Equilibrium Dialysis and Chemiluminescent Immunoassays in 13 Hypothyroid Dogs Positive for Thyroglobulin Antibody. J Vet Intern Med. 29: 877–881.

Rochon, C., I. Tauveron, C. Dejax, P. Benoit, P. Capitan, A. Fabricio,... & J. Grizard (2003). Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism. *Clinical science*, *104*(1), 7-15.

Roopali, B., S. Roy, M. Roy, & C. N. Galdhar. (2020). Epidemiological study of canine hypothyroidism in Chhattisgarh state, India. *Int J Curr Microbiol Appl Sci*, *9*, 1432-1439.

Ryad, N. M., E. S. Ramadan, N. Y. Salem, & I. A. E. S. Saleh (2021). Oxidative biomarkers and lipid alterations in euthyroid and hypothyroid dogs. *Internal Comparative Clinical Pathology*, *30*, 571-576.

Salutgi, P., C. Galdhar, R. Sonigra, K. Natu, N. Mumbarkar, S. Mathkar,... & R. Gaikwad. (2023). Radio Immune Assay (RIA) Enabled Total Triiodothyronine (TT3) and Total Thyroxine (TT4) in Canine Trypanosomiasis: First Case Report from Maharashtra (India). *Iranian Journal of Parasitology*, *18*(1), 107.

Salvatore, D., T. F. Davies, M. J., Schlumberger, I. D. Hay, & P. R. Larsen. (2016). Thyroid physiology and diagnostic evaluation of patients with thyroid disorders. In *Williams textbook of endocrinology* (pp. 333-368). Elsevier.

Sanderson, S. L. (2010). Body condition score techniques for dogs.

Schenk, A. P., R. F. Nachreiner and K. R. Refsal. 2017. Michigan State University, Veterinary Diagnostic Laboratory, Endocrinology Conversion Factors. Available at: https://cvm.msu.edu/assets/documents/VDL/Endocrinology-Conversion-Factors.pdf (material and method)

Scott-Moncrieff, J. C., & R. W. Nelson. (1998). Change in serum thyroid-stimulating hormone concentration in response to administration of thyrotropin-releasing hormone to healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *Journal of the American Veterinary Medical Association*, *213*(10), 1435–1438.

Shadwick, S. R., M. D. Ridgway, & A. Kubier (2013). Thyrotoxicosis in a dog induced by the consumption of feces from levothyroxine-supplemented housemate. *The Canadian Veterinary Journal*, *54*(10), 987.

Shiel, R. E., M. Sist, R. F. Nachreiner, C. P., Ehrlich, &, C. T. Mooney (2015). Assessment of criteria used by veterinary practitioners to diagnose hypothyroidism in sighthounds and investigation of serum thyroid hormone concentrations in healthy Salukis. *Journal of the American Veterinary Medical Association*, *236*(3), 302-308.

Snedecor, G. M. and W.C. Cochran (2004) Statistical methods, 8TH
edition, Iowa State University Press, Ames, Iowa.

Srikala, D., & K. S. Kumar. (2014). Hypothyroidism associated systemic and peripheral disorders in dogs. *Animal science reporter*, *8*(1), 31-40.

Sullivan, P., R. Gompf, L. Schmeitzel, R. Clift, M. Cottrell, & T. P. McDonald (1993). Altered platelet indices in dogs with hypothyroidism and cats with hyperthyroidism. *American journal of veterinary research*, *54*(12), 2004-2009.

Vala, H., J. R. Mesquita, F. Esteves, C. Santos, R. Cruz, C. Mega, & C. Nóbrega (2013). The Endocrine Glands in the Dog: From the Cell to Hormone. In *Insights from Veterinary Medicine*. Intech Open.

Waters DJ. Unlocking the science behind exceptional longevity in dogs. In: *North American Veterinary Conference.* Orlando, FL (2015). p. 15-17.

Yalow, R. S., & S. A. Berson (1959). Assay of plasma insulin in human subjects by immunological methods. *Nature*, *184*(4699), 1648-1649.

Appendices

7. APPENDICES

Appendix A Hematological findings of healthy dogs (n=67)

Sr. No.	HB (gm%)	PCV %	TEC (10^6/c)	MCV (fl)	MCH (pg)	MCHC (gm/dl)	TLC (10^3/c)	Neu %	Lym %	Eosin %	MONO %	BASO %	Platelets (/cu.mm)
1.	14.6	41.9	6.42	65.26	22.74	34.84	12.2	34	51	5	1	0	2.88
2.	11.1	34.1	5.29	64.46	20.98	32.55	9.2	67	26	6	1	0	2.06
3.	9.6	28.8	4.68	61.54	20.51	33.33	14.6	88	9	2	1	0	2.25
4.	12	40	5.5	72.22	24.15	33.44	9.2	81	11	7	1	0	1.85
5.	11.7	34.3	5.4	63.52	21.67	34.11	16.5	57	38	3	2	0	3.5
6.	13.9	38.9	5.59	69.7	24.9	35.8	9.1	78	20	0	2	0	1.7
7.	11.2	31.9	5.1	68.11	23.23	34.11	9.4	53	46	0	1	0	1.9
8.	15.5	44.2	6.52	67.79	23.77	35.07	21	84	14	1	0	1	4.1
9.	13	39	8.5	70	26	35	20	77	32	0	2	0	4
10.	11.8	33.9	5.53	61.3	21.34	34.81	36	16	83	16	1	0	2.12
11.	12.9	38.5	5.47	70.6	23.58	33.42	23.6	72	19	8	1	0	2.55
12.	12.4	37.9	6.24	60.74	19.87	32.72	17.5	73	26	0	1	0	4.1
13.	13.2	38.5	5.54	69.49	23.83	34.29	20	71	26	2	1	0	2
14.	13.9	38.8	5.78	67.99	24.05	35.37	19.9	57	40	2	1	0	3
15.	14	35	80	70	25	35	20	77	25	0	2	0	4
16.	16	44.7	7.19	62.17	22.25	35.79	12.3	67	27	5	1	0	1.82
17.	13	37.2	5.65	65.83	23.01	34.95	19	85	14	0	1	0	2.14
18.	12.6	36	5.58	64.52	22.58	35	21	90	9	0	1	0	4.79
19.	11.3	37.8	7.14	52.94	15.83	29.89	15.8	79	18	2	1	0	4.2

20.	15.7	46.86	7.7	60.15	20.15	33.5	15.7	61	27	11	1	0	2.3
21.	14.6	41.8	5.7	72.82	23.44	34.93	20.8	74	24	1	1	0	2
22.	13	37	6.3	72.24	23.97	33.19	18	76	20	2	2	0	2.1
23.	13.9	42.5	8.5	64.3	21.51	33.46	14.6	57	37	5	1	0	1.8
24.	13.2	30.9	4.71	65.61	21.87	33.33	20	76	21	2	1	0	4.1
25.	12.6	37.3	5.56	67.09	22.66	33.78	10.8	30	69	0	1	0	2
26	13	38	8	70	20	35	30	70	25	0	2	0	3
27	11.8	35.5	5.65	62.83	20.88	33.24	19	62	28	9	1	0	3.97
28	11	32.3	4.23	76.36	26	34.06	8.4	66	32	1	1	0	2.1
29	14.2	40.2	5.58	69.84	24.24	34.7	20	80	19	0	1	0	2.23
30	13.5	38.9	5.57	65.73	22.31	33.95	17	69	26	4	1	0	2.06
31	15.7	45.4	6.63	68.48	23.68	34.58	11.6	68	26	4	2	0	1.92
32	13	38	5.8	65.46	25	33.95	17	69	26	4	1	0	2.08
33	16.3	46.8	7.48	62.57	21.79	34.83	11.8	72	23	4	1	0	2.3
34	15	43.6	6.65	65.56	22.56	34.4	7.7	69	28	2	1	0	1.98
35	13.5	40	6	69.84	24.24	34.7	14	75	24	0	1	0	2.23
36	14	7	5.7	62	19	33	6.1	80	17	2	1	0	2.5
37	15.1	44.5	7.28	61.13	20.74	33.93	12.3	79	11	9	1	0	2.5
38	13.9	42.5	8.5	50	16.35	32.71	12.8	43	47	9	1	0	2.67

39	14	40	6.2	71.16	21	29.52	8.6	84	13	2	1	0	2.52
40	17.6	50.3	7.44	67.61	23.66	34.99	19	65	29	5	1	0	2.03
41	17.2	47.8	7.19	66.59	24	36.1	18	77	20	0	3	0	2.24
42	18	50	7.64	66.36	24.48	36.88	15.1	68	31	0	1	0	2
43	14.2	40.5	6.25	64.8	22.72	35.06	12.2	67	23	9	1	0	2.09
44	12.7	38.3	6.01	63.73	21.13	33.16	14.2	67	19	13	1	0	3.01
45	18	51.2	8.24	62.14	22.94	36.91	10.7	60	38	1	1	0	2.01
46	12.6	38	5.5	64.45	21.34	33.13	14.2	79	18	2	1	0	2.2
47	17.8	49.3	7.37	66.89	24.15	36.11	9.7	56	22	21	1	0	2.01
48	15	42.1	6.52	64.5	23	35.7	13	80	18	0	2	0	2.1
49	12.6	37.6	6.26	60.06	20.13	33.51	15.7	64	34	1	1	0	3.14
50	17.8	49.3	7.37	66.89	24.15	36.11	9.7	56	22	21	1	0	2.01
51	17.6	50.3	7.44	67.61	23.66	34.99	19.2	65	29	5	1	0	2.03
52	17.7	52.46	7.35	71	24	33.7	14.6	65	32	2	1	0	2
53	13.2	37.1	5.59	66.37	23.61	33.58	12.4	69	17	13	1	0	3.8
54	10.2	27.3	4.99	54.6	20.5	37.5	16.3	89	10	2	1	0	2.3
55	20	54	54.1	61.2	23.7	38.7	15	83	16	0	1	0	1.89

56	14.8	46.2	7.47	14.6	61.85	19.81	14.6	76	21	2	1	0	3.23
57	18.7	50.7	7.64	66.36	24.48	36.88	15.1	68	31	0	1	0	2.09
58	13.5	38.9	5.57	69.84	24.24	34.7	11.6	75	24	0	1	0	2.2
59	11.8	35.5	5.65	62.83	20.88	33.24	21.2	62	28	9	1	0	3.9
60	13.7	39.8	6.43	61.9	21.31	34.42	19	61	26	4	1	0	2.4
61	9.4	30.9	5.08	60.86	18.5	30.42	10.7	60	38	1	1	0	2.07
62	17.2	47.8	7.19	66.5	24	36.1	18	77	20	0	3	0	2.25
63	13.9	38.9	5.59	69.7	24.9	35.8	9	78	20	0	2	0	1.7
64	15.5	43	6.63	64.86	23.23	35.81	6.6	62	30	6	1	0	2.8
65	13.5	39.8	6.05	65.79	22.31	33.92	10.5	80	13	6	1	0	1.8
66	12.1	37.1	5.84	63.53	20.72	32.61	14.4	76	20	3	1	0	1.95
67	11.9	37	5.4	60	18.5	30.42	10.7	61	32	2	1	0	2.07
Mean	**14.01**	**40.02**	**8.13**	**64.67**	**23.03**	**34.10**	**15.12**	**70.72**	**24.36**	**3.03**	**1.18**	**0.01**	**2.52**
SD	**2.28**	**7.19**	**10.70**	**7.71**	**5.24**	**2.42**	**5.29**	**13.28**	**12.59**	**4.79**	**0.49**	**0.12**	**0.77**
SE	**0.28**	**0.72**	**1.31**	**0.94**	**0.64**	**0.30**	**0.65**	**1.23**	**1.03**	**0.41**	**0.06**	**0.01**	**0.09**
Mean ± SE	**14.01± 0.28**	**40.02± 0.72**	**8.13± 1.31**	**64.67 ± 0.94**	**23.03 ± 0.64**	**34.10± 0.30**	**15.12 ±0.65**	**70.72 ±1.23**	**24.36 ±1.03**	**3.03 ±0.41**	**1.18± 0.06**	**0.01± 0.01**	**2.52± 0.09**

Appendix B Biochemical findings of healthy dogs (n=67)

Sr. No	BUN (mg/dl)	Creat (mg/dl)	TB (mg/dl)	DB (mg/dl)	IB (mg/dl)	ALP (U/L)	AST (IU/L)	ALT (IU/L)	ALB (gm/dl)	GLO (gm/dl)	TP (gm/dl)	TGL (mg/dl)	CHOL (mg/dl)	BGL (mg/dl)
1	18.1	0.7	0.2	0.1	0.1	57	23	22	3.3	3.8	7.1	126	236.9	72
2	15.3	0.7	0.2	0.1	0.1	129	26	37	2.7	3.4	6.1	51.58	124.9	86
3	15.1	0.9	0.2	0.1	0.1	107	27	61	2.4	5.8	8.2	133.7	374.4	96
4	11.2	1.1	0.2	0.1	0.1	73	31	43	2.6	2.4	6.6	155.6	168.2	81
5	19.1	1.1	0.2	0.1	0.1	72	31	46	2.7	4.8	7.5	86.65	216.7	80
6	7.45	0.88	0.44	0.12	0.32	37.61	38.44	40	2.58	4.65	7.23	74.7	164.6	85
7	18.1	0.7	0.2	0.1	0.1	54	41	51	2.6	5.5	8.1	162.6	232.2	87
8	22.1	1.1	0.3	0.2	0.1	173	41	39	3.1	5.2	8.3	146	209.8	90
9	25	1.2	0.4	0.1	0.2	100	55	40	4.2	4.5	6.1	257	150	78
10	11.2	1.2	0.2	0.1	0.1	106	29	28	2.1	4	6.1	98.86	248.8	70
11	21.1	1.1	0.3	0.1	0.2	47	64	61	3.9	4.9	8.8	97.81	215.7	96
12	19.1	1.4	0.3	0.1	0.2	87	44	52	3.1	5.8	8.9	64.32	105.6	93
13	46.1	1.8	0.2	0.1	0.1	112	51	41	2.6	5.7	8.3	90	132.7	80
14	11.2	1	0.3	0.1	0.2	161	75	31	2.6	5.5	8.1	209	134	99
15	27	0.9	0.4	0.1	0.2	112	81	41	4	3	7.5	145	120	74
16	15.1	1	0.3	0.1	0.2	121	45	51	3.4	4.3	7.7	169	189	90
17	13.1	0.9	0.4	0.1	0.3	122	54	61	3.3	3.2	6.5	79.27	225.6	90
18	40	1.29	0.4	0.14	0.3	23.5	82	67	2.6	3.87	6.47	73.07	146	75
19	18.1	0.8	0.3	0.1	0.2	187	81	21	2.8	3.7	6.5	99.84	105.5	80
20	7.9	0.6	0.3	0.1	0.2	200	60	70	2.2	2.5	4.7	68.3	173.2	93
21	20.1	1.2	0.2	0.1	0.1	83	42	26	2.4	4.7	7.1	80.18	218.1	98
22	28	1	0.3	0.1	0.2	160	55	57	2.4	4.5	6.9	220	150	90

23	10.1	0.9	0.3	0.1	0.2	170	61	60	2.6	4.9	7.5	198	200	100
24	12.1	0.9	0.4	0.1	0.2	150	68	31	2.6	5.7	8.3	176	231	80
25	8.1	0.8	0.2	0.1	0.1	149	25	51	3.1	5.2	8.3	87.95	154	76
26	25	1	0.2	0.1	0.2	105	60	60	3.8	4	7.8	136	210	96
27	16.1	0.8	0.3	0.1	0.2	154	29	38	2.8	3.3	6.1	80.31	157.2	81
28	16.1	0.9	0.2	0.1	0.1	104	37	31	2.8	4	6.8	92.26	158.3	77
29	14.2	0.8	0.2	0.1	0.1	78	21	26	3.1	5.8	8.9	115.6	214	68
30	14.1	0.9	0.2	0.1	0.1	84	18	61	2.6	5.9	8.5	93.18	185.1	73
31	11.2	0.8	0.2	0.1	0.1	92	24	31	2.9	4.5	7.4	156.4	162.2	78
32	16	0.8	0.2	0.1	0.1	78	21	26	3.1	5.8	8.9	136.4	288.8	79
33	11.2	0.8	0.3	0.1	0.2	128	45	73	2.8	5.9	8.7	140.8	190.8	70
34	13.1	0.9	0.3	0.2	0.1	156	65	84	3.3	4.8	8.1	158	159	96
35	16	1.4	0.2	0.1	0.1	78	21	26	3.1	5.8	8.9	173	162.6	68
36	11.2	0.8	0.3	0.1	0.2	145	18	21	2.4	4.2	6.6	113	166.9	65
37	14.1	0.8	0.3	0.1	0.2	215	34	31	3.3	3.3	6.6	93	126.9	82
38	14.3	1	0.2	0.1	0.1	121	38	44	3.1	3.6	6.7	108	153.1	83
39	8.3	0.8	0.3	0.2	0.1	150	94	81	2.6	4.5	7.1	244.8	315.8	91
40	14.3	1.1	0.3	0.1	0.2	197	32	24	3.5	4.6	8.1	216	230	87
41	8.96	0.82	0.57	0.21	0.36	141	70.82	27.79	2.14	4.77	6.91	130.3	99.65	90
42	16.2	0.8	0.3	0.1	0.2	161	31	25	2.6	4.3	6.9	94.94	187.4	81
43	24.1	1.6	0.3	0.1	0.2	131	23	31	2.7	3.9	6.6	110.5	151.7	85
44	8.1	0.7	0.2	0.1	0.1	343	41	21	2.7	3	5.7	101.2	159.1	90
45	9.2	1.1	0.3	0.1	0.2	95	43	32	3.3	3.5	6.8	66.6	89.31	92
46	20.1	0.6	0.4	0.2	0.2	160	38	55	3.1	4.6	7.7	158	310	79
47	12.1	0.8	0.2	0.1	0.1	34	22	15	3.2	3.6	6.8	87	278.9	73
48	10.28	0.95	0.45	0.17	0.26	36.51	31.53	25.59	2.77	4.08	6.85	71.69	213	81

49	23.1	0.8	0.3	0.2	0.1	178	46	33	2.9	3.8	6.7	93	210	85
50	12.1	0.8	0.2	0.1	0.1	34	22	15	3.2	3.6	6.8	101	230	80
51	14.3	1.1	0.3	0.1	0.2	197	32	24	3.5	4.6	8.1	152	143	86
52	15.1	0.7	0.3	0.1	0.2	159	89	87	2.2	4.5	6.7	104.7	173.2	84
53	13.1	0.8	0.3	0.1	0.2	74	38	36	3.1	2.2	5.3	98	198	78
54	32.42	1.6	0.3	0.2	0.2	170	51.26	35.34	2.6	2.64	5.34	145	125	84
55	14.89	1.46	0.46	0.12	0.34	44.5	56	58	3.42	3.98	7.4	191.8	181	88
56	8.7	0.9	0.2	0.1	0.1	164	21	29	2.6	6.3	8.9	174	148	96
57	16.2	0.8	0.3	0.2	0.2	161	31	25	2.6	4.3	6.9	98.92	183	85
58	14.2	0.8	0.2	0.1	0.1	78	21	26	3.1	5.8	8.9	109	190	92
59	16.1	0.8	0.3	0.1	0.1	301	29	38	2.8	3.3	6.1	106.4	192.1	90
60	17.2	0.9	0.3	0.1	0.2	150	79	62	3.2	3.9	7.1	100	229.1	88
61	16.1	1.1	0.2	0.1	0.1	39	26	22	2.6	6.1	8.7	100	184.6	85
62	8.96	0.82	0.57	0.21	0.36	141	70.82	27.79	2.14	4.77	6.91	129.1	184	76
63	7.45	0.88	0.44	0.12	0.32	37.61	38.44	33.86	2.58	4.65	7.23	40	324.5	78
64	12.1	1	0.3	0.2	0.1	87	19	21	3.1	2.8	5.9	59	215.7	82
65	14.1	1.1	0.2	0.1	0.1	65	25	39	3.4	3.3	6.7	55.7	274.6	73
66	16.1	0.8	0.3	0.1	0.2	37	22	23	2.9	5.3	8.2	116.9	240	96
67	17	1.2	0.2	0.1	0.1	39	26	22	2.6	6.1	8.7	125	186	72
Mean	**16.02**	**0.96**	**0.29**	**0.12**	**0.17**	**120.11**	**42.24**	**39.92**	**2.87**	**4.40**	**7.30**	**121.30**	**191.17**	**83.61**
SE	**7.12**	**0.24**	**0.09**	**0.04**	**0.07**	**60.82**	**20.01**	**17.56**	**0.42**	**1.03**	**1.02**	**47.17**	**56.07**	**8.51**
SD	**0.87**	**0.03**	**0.01**	**0.00**	**0.01**	**7.43**	**2.44**	**2.15**	**0.05**	**0.13**	**0.12**	**5.76**	**6.85**	**1.04**
Mean± SE	**16.02± 0.87**	**0.96 ± 0.03**	**0.29± 0.01**	**0.12± 0.00**	**0.17± 0.01**	**120.11 ±7.43**	**42.24± 2.44**	**39.92± 2.15**	**2.87± 0.05**	**4.40± 0.13**	**7.30± 0.12**	**121.30± 5.76**	**191.17± 6.85**	**83.61± 1.04**

Appendix C Hematological findings of hypothyroid dogs (n=10)

Sr. No.	HB (gm%)	PCV%	TEC (10^6/cmm)	MCV (fl)	MCH (pg)	MCHC (gm/dl)	TLC (10^3/cmm)	Neu %	Lym %	Eosin %	MONO %	BASO %	Platelets (/cu.mm)
1.	16	43.9	7.09	61.92	22.57	36.45	8	63	31	6	1	0	2.08
2.	8.5	20.6	3.23	63.8	26.3	41.2	26.28	82	15	2	1	0	1.38
3.	13	51	8.24	62.14	22.94	36.91	21	60	38	1	1	0	2.01
4.	12	36.9	5.2	60	20	32	6.1	80	17	2	1	0	2.5
5.	8.8	26.6	4.04	65.84	21.78	33.08	25.5	87	10	2	1	0	2.1
6.	12.8	37.4	5.36	69.78	23.88	34.22	8	70	22	7	1	0	2.3
7.	15.2	42.3	6.5	65.08	23.38	35.93	14	74	4	21	1	0	3.09
8.	13.1	39.8	6.43	61.9	21.31	34.42	20	61	26	12	1	0	2.5
9.	8.9	25.9	3.32	67.98	23.36	34.36	19.8	77	20	2	1	0	2.11
10.	12.7	3.8	6	64.3	21	32	29	70	27	2	1	0	3
Mean	**12.10**	**34.88**	**5.34**	**64.27**	**22.65**	**35.06**	**17.77**	**72.40**	**21.00**	**5.70**	**1.00**	**0.00**	**2.31**
SD	**2.62**	**13.77**	**1.64**	**3.01**	**1.77**	**2.75**	**8.29**	**9.25**	**10.08**	**6.38**	**0.00**	**0.00**	**0.50**
SE	**0.83**	**2.45**	**0.44**	**0.95**	**0.56**	**0.87**	**2.62**	**2.93**	**3.19**	**2.02**	**0.00**	**0.00**	**0.16**
Mean± SE	**12.10 ± 0.83**	**34.88 ± 2.45**	**5.34 ± 0.44**	**64.27 ± 0.95**	**22.65 ± 0.56**	**35.06 ± 0.87**	**17.77 ±2.62**	**72.40 ± 2.93**	**21.00 ±3.19**	**5.70 ±2.02**	**1.00± 0**	**0**	**2.31 ± 0.16**

Appendix D Biochemical findings of hypothyroid dogs (n=10)

Sr. No.	BUN	Creatinine	B. Total	B. Direct	B. Indirect	A. P.	S.G.O.T.	S.G.P.T.	Albumin	Globulin	T.P.	TRIG	CHOLES	GLUCOSE
1.	13.8	1.4	0.2	0.1	0.1	148	42	69	3.1	4.6	7.7	78	402	60
2.	15	0.6	0.3	0.1	0.2	323	74.5	87.3	2.12	6.3	8.42	90.3	121	73
3.	9.2	1.1	0.3	0.1	0.2	172	43	32	3.3	3.5	6.8	90.37	174.3	61
4.	25.1	1.3	0.2	0.1	0.1	169	32	23	2.7	5.4	8.1	255.4	344.5	100
5.	32	1.2	0.2	0.1	0.1	71	61	44	1.9	4	5.9	300	350	61
6.	11.2	0.7	0.2	0.1	0.1	102	16	34	3.8	5.8	9.6	99.71	164.8	90
7.	25.2	0.9	0.2	0.1	0.1	145	42	55	2.2	5.6	7.8	116	154.3	98
8.	17.2	0.9	0.3	0.1	0.2	197	79	62	3.2	4.9	8.1	103.2	431	68
9.	13.1	0.9	0.2	0.1	0.1	173	46	39	2.3	8.5	10.8	61.44	368	65
10.	16.4	1	0.3	0.1	0.2	494	41	26	2.6	3.6	6.2	101.44	425.5	60
Mean	**17.82**	**1.00**	**0.24**	**0.10**	**0.14**	**199.40**	**47.65**	**47.13**	**2.72**	**5.22**	**7.94**	**129.59**	**293.54**	**73.60**
SD	**7.27**	**0.25**	**0.05**	**0.00**	**0.05**	**122.94**	**19.04**	**20.77**	**0.61**	**1.49**	**1.48**	**80.15**	**124.43**	**16.17**
SE	**2.30**	**0.08**	**0.02**	**0.00**	**0.02**	**38.88**	**6.02**	**6.57**	**0.19**	**0.47**	**0.47**	**25.35**	**39.35**	**5.11**
Mean ± SE	**17.82 ± 2.30**	**1.00 ± 0.08**	**0.24 ± 0.02**	**0.10 ± 0**	**0.14 ± 0.02**	**199.40 ± 38.88**	**47.65 ± 6.02**	**47.13 ± 6.57**	**2.72 ± 0.19**	**5.22 ± 0.47**	**7.94 ± 0.47**	**129.59 ± 25.35**	**293.54 ± 39.35**	**73.60 ± 5.11**

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Department of Veterinary Clinical Medicine, Ethics and Jurisprudence

Date: / /

STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO RADIO IMMUNO ASSAY

CONSENT FORM

APPENDIX E

Owner's Name: ____________________________ Contact: Email ID:

Address:

Pet: Dog **Name:** **Breed:** **Age**
Sex: M/F

Color: **No. of dogs:**

Referred By:

CONSENT FORM

I, , hereby authorize the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, T.V.C.C and/or O.P.D to collect blood samples from my dog/s for routine hemato-biochemical and hormonal analysis to include in "**Studies on thyroid profile in dogs with special reference to assessment of canine TSH"**

The procedure, its requirements and the risks associated with the procedure, if any; have been explained to me. I understand that this is a part of the routine diagnostic protocol and will aid in diagnosis, prognosis and treatment of my dog/s.

I do not object to the use of data generated through these procedures for education, research and/or publication purposes.

(Sign of Veterinarian) (Signature of Owner)

Abstract

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	**:**	**STUDIES ON THYROID PROFILE IN DOGS WITH SPECIAL REFERENCE TO ASSESSMENT OF CANINE TSH**
b)	Full name of student	**:**	Alone Pallavi Dipakrao
c)	Name and address of Major Advisor	**:**	Dr. C. N. Galdhar Assistant Professor and Radiological Safety Officer (VNM), Veterinary Clinical Medicine, Ethics and Jurisprudence
d)	Degree to be awarded	**:**	M. V. Sc.
e)	Year of award of degree	**:**	2024
f)	Major subject	**:**	Veterinary Clinical Medicine, Ethics and Jurisprudence
g)	Total number of pages in the thesis	**:**	121
h)	Number of words in the abstract	**:**	299
i)	Signature of Student	**:**	
j)	Signature, Name, and address of forwarding authority (HOD / SH)	**:**	Dr. R. V. Gaikwad Professor and Head, Dept. of Veterinary Clinical Medicine, Ethics and Jurisprudence
	Signature of the Associate Dean		

ABSTRACT

The present study was undertaken to establish data about canine TSH and its association with Total triiodothyronine (TT_3), Total thyroxine (TT_4), and Free thyroxine (fT_4) in healthy dogs; and to study alteration in canine TSH, Total triiodothyronine (TT_3), Total thyroxine (TT_4) and Free thyroxine (fT_4) in suspected cases of thyroid dysfunction, if any. The present study enrolled a total of 77 dogs, with 67 healthy and 10 hypothyroid.

The canine-specific TSH ELISA kit was used to measure the serum concentration of cTSH, while a RIA kit was used to measure the serum concentration of Total triiodothyronine, Total thyroxine, and Free thyroxine. The mean concentration of Total triiodothyronine (TT_3), Total thyroxine (TT_4), Free thyroxine (fT_4), and Thyroid stimulating hormone (cTSH) in healthy dogs were 0.85 ± 0.04 nmol/l, 20.81 ± 0.78 nmol/l, 12.59 ± 0.35 pmol/l and 2.03 ± 0.01 µIU/ml respectively. In hypothyroid dogs, the mean concentration of Total triiodothyronine (TT_3), Total thyroxine (TT_4), Free thyroxine (fT_4), and Thyroid stimulating hormone (cTSH) was 0.93 ± 0.07 nmol/l, 9.01 ± 0.69 nmol/l, 5.87 ± 0.82 pmol/l and 2.16 ± 0.03 µIU/ml respectively. The study reported significant changes in the concentration of TT_4, fT_4, and cTSH between healthy and hypothyroid dogs.

In the case of healthy dogs, the reference range (25^{th} to 75^{th} percentile) of cTSH was 1.96 -2.11 µIU/ml while in the case of hypothyroid dogs, the reference range (25^{th} to 75^{th} percentile) of cTSH was 2.09-2.22. Out of 10 hypothyroid dogs, 70% had a reference range above the healthy range, while 30% had a reference range equal to the healthy range. The sensitivity (%) and specificity (%) of the measurement of cTSH were 63 % and 72 % respectively. The study revealed discrepancies in clinical findings, hematobiochemical results, and thyroid profiles in both healthy and hypothyroid dogs.

प्रबंध सारांश

a)	प्रबंधाचे शीर्षक	:	**श्वानामधील थायरॉईड प्रोफाईलवरील थायरॉईड उत्तेजक संप्रेरकाच्या मूल्यांकनासाठी विशेष संदर्भासह अभ्यास**
b)	विद्यार्थ्याचे पूर्णनाव	:	अलोणे पल्लवी दिपकराव
c)	प्रमुख मार्गदर्शकाचे नाव व पत्ता	:	डॉ. चंद्रकांत न. गलधर सहाय्यक प्राध्यापक आणि रेडिओलॉजिकल सुरक्षा अधिकारी, चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र विभाग मुंबई पशुवैद्यकीयमहाविद्यालय, मुंबई
d)	पदवी	:	पदव्युत्तर पदवी
e)	पदवीप्रदान करण्याचे वर्ष	:	२०२४
f)	मुख्यविषय	:	चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र
g)	प्रबंधतील एकूण पृष्टांची संख्या	:	121
h)	सारांशाचे एकूण शब्द	:	१६0
i)	विद्यार्थ्याची सही	:	
j)	प्रबंध पाठविणाऱ्या अधिकाऱ्याचे संपूर्ण नाव, पत्ता व सही	:	डॉ .राजीव व. गायकवाड प्राध्यापक आणि प्रमुख चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र विभाग मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई
k)	सहयोगी अधिष्ठाता, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई ४०००१२		

सारांश

सध्याचा अभ्यास कॅनाइन टीएसएच बद्दलचा डेटा आणि निरोगी श्वानामधील टोटल ट्रायओडोथायरोनिन ((TT_3), टोटल थायरॉक्सिन (TT_4), आणि फ्री थायरॉक्सिन (fT_4) शी संबंध स्थापित करण्यासाठी आणि कॅनाइन टीएसएच, टोटल ट्रायओडोथायरोनिनमधील बदलांचा अभ्यास करण्यासाठी केला गेला. (TT_3), टोटल थायरॉक्सिन (TT_4) आणि फ्री थायरॉक्सिन (fT_4) थायरॉईड चे बिघडलेले कार्य अशा संशयास्पद प्रकरणांमध्ये, जर असेल तर. सध्याच्या अभ्यासात एकूण 77 श्वानांची नोंदणी करण्यात आली होती, ज्यामध्ये 67 निरोगी आणि 10 हायपोथायरॉईड होते.

कॅनाइन-विशिष्ट TSH ELISA किटचा वापर TSH च्या सीरम एकाग्रता मोजण्यासाठी केला गेला, तर RIA किटचा वापर एकूण ट्रायओडोथायरोनिन, टोटल थायरॉक्सिन आणि फ्री थायरॉक्सिनच्या सीरम एकाग्रता मोजण्यासाठी केला गेला. निरोगी श्वानांमध्ये एकूण ट्रायओडोथायरोनिन (TT_3), एकूण थायरॉक्सिन (TT_4), फ्री थायरॉक्सिन (fT_4.) आणि थायरॉईड उत्तेजक संप्रेरक (cTSH) ची सरासरी एकाग्रता 0.85 ± 0.04 nmol/l, 20.81± 0.78 nmol/l, 12.59 ± 0.35 pmol/l आणि 2.03 ± 0.01 µIU/ml अनुक्रमे. हायपोथायरॉईड श्वानांमध्ये एकूण ट्रायओडोथायरोनिन (TT_3), एकूण थायरॉक्सिन (TT_4), फ्री थायरॉक्सिन (fT_4), आणि थायरॉईड उत्तेजक संप्रेरक (cTSH) ची सरासरी एकाग्रता 0.93 ± 0.07 nmol/l, 9.01 ± 0.69 nmol/l होती. , 5.87 + 0.82 pmol/l आणि 2.16 ± 0.03 µIU/ml अनुक्रमे. अभ्यासात निरोगी आणि हायपोथायरॉईड श्वानांमधील TT_3, TT_4,fT_4 आणि cTSH च्या एकाग्रतेमध्ये लक्षणीय बदल नोंदवले गेले.

निरोगी श्वानांचा बाबतीत cTSH ची संदर्भ श्रेणी (25 ते 75 टक्केवारी) 1.96-2.11 µIU/ml होती तर हायपोथायरॉईड श्वानांचा बाबतीत, cTSH ची संदर्भ श्रेणी (25 ते 75 टक्के) 2.09-2.22 होती. 10 हायपोथायरॉईड श्वानांपैकी 70% ची संदर्भ श्रेणी निरोगी श्रेणीपेक्षा जास्त होती, तर 30% ची संदर्भ श्रेणी निरोगी श्रेणीच्या समान होती. cTSH च्या मोजमापाची संवेदनशीलता (%) आणि विशिष्टता (%) अनुक्रमे 63% आणि 72% होती. अभ्यासात निरोगी आणि हायपोथायरॉईड दोन्ही श्वानांमध्ये क्लिनिकल निष्कर्ष, हेमॅटोबायोकेमिकल परिणाम आणि थायरॉईड प्रोफाइलमध्ये विसंगती दिसून आली.

Vita

VITA

The Author Dr. Alone Pallavi Dipakrao was born on 24th OCT 1997 in DARWHA, Dist: Yavatamal of Maharashtra State. She passed S.S.C. under the Amaravati Board, securing 95.07% with distinction in the year 2013, and H.S.C. under the Amaravati Board of Secondary and Higher Secondary Education in 2015.

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