STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS

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)

2025

Appendix-B

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS"** 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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Appendix-C(i)

DECLARATION OF ADVISORY COMMITTEE

KAWADE JAYDEEP RAMDAS has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, **"STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS"** submitted by him 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.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree from any other University.

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ADVISORY COMMITTEE

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ii)	Dr. S. D. Ingole	Associate Dean, Professor and Head Department of Veterinary Physiology	
iii)	Dr. D. P. Kadam	Professor and Head Department of Veterinary Pathology	

Appendix-C(ii)

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS"** submitted by Mr. **KAWADE JAYDEEP RAMDAS** 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.

Name and Signature of External Examiner	Signature with Seal Head of Department (Dr. R. V. Gaikwad)	Signature of Guide Dr. C. N. Galdhar

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Dedicated to my beloved family

Acknowledgements

ACKNOWLEDGEMENT

This research is the culmination of contributions from many individuals, and while my name appears on the cover, its completion would not have been possible without their invaluable support. It is both a pleasure and a privilege to express my gratitude to them.

First and foremost, I am deeply grateful to ***Chhatrapati Shivaji Maharaj*** *and* ***Chhatrapati Sambhaji Maharaj****, whose vision, courage, and wisdom continue to inspire generations. Their ideals have been a guiding force in my journey, providing direction and purpose.*

I consider it a great honor to have worked under the exceptional guidance of my esteemed mentor, ***Dr. C. N. Galdhar****, Professor, Veterinary Clinical Medicine, Ethics, and Jurisprudence at Mumbai Veterinary College. His vast knowledge and insightful perspectives have continuously motivated me to learn and grow. His critical feedback has shaped my thinking, and I sincerely appreciate his invaluable suggestions, creative insights, and unwavering support in refining this research.*

I extend my heartfelt gratitude to ***Dr. R. V. Gaikwad****, Professor & Head of the Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, for his invaluable guidance and supervision. His expert evaluations have provided direction and motivation throughout my research.*

My sincere thanks go to ***Dr. D. P. Kadam****, Professor & Head of the Department of Veterinary Pathology, and* ***Dr. S. D. Ingole****, Professor & Head of the Department of Physiology, for their professional guidance, practical solutions, and support, which have been instrumental in shaping my work. I also appreciate the invaluable assistance of Dr. Chaudhari from Unique Lab.*

I am deeply thankful to ***Dr. P. B. Hase****, Assistant Professor in the Department of Clinical Medicine, Ethics, and Jurisprudence, for his keen observations, practical expertise, and unwavering support throughout this journey. My sincere gratitude also goes to* ***Dr. D. A. Pawalkar****, Associate Professor in the Department of Veterinary Epidemiology and Preventive Medicine, and* ***Dr. J. U.***

***Patil**, Assistant Professor in the Department of Veterinary Epidemiology and Preventive Medicine, for their patience in addressing my queries and for their valuable insights before and during my research.*

*I extend my special thanks to **Dr. S. U. Gulvane**, Associate Dean of Mumbai Veterinary College, for allowing me the opportunity to conduct this research at this esteemed institution.*

This work would not have been possible without the support of the Anand Brothers who provided Radio Immuno Assay kits as needed and RMC, Mumbai for their timely cooperation.

I also acknowledge the support staff of the department and the ward assistants of Bai Sakarbai Dinshaw Petit Hospital for Animals for their enthusiasm and hands-on assistance with my cases.

A special mention goes to Dr. Pranali for her constant support and for being a source of encouragement and stress relief throughout this journey.

I am immensely grateful to my seniors— Dr. Nikita Pradhan, Dr. Abhishek Gaikwad, Dr. Pallavi Alone, Dr. Arjun Das, Dr. Mahima Gulati, Dr. Sujata Mote, Dr. Suraj Koujalagi, Dr. Shubham Vyas and Dr. Anoop Kumar —for their guidance and encouragement throughout this journey.

I also express my sincere appreciation to my colleagues—Dr. Shradha, Dr. Nisha, Dr. Priyanka and Dr. Prathamesh —for their constant support, discussions, and motivation, all of which enriched my research experience.

My gratitude extends to my juniors—Dr. Prasad Mane, Dr. Rhea Ghiara, Dr. Anushka Advani, Dr. Akshay Raut, Dr. Mohit Dadheech and Dr. Pankaj Hatila— for their assistance in completing my thesis work.

I am also thankful to the non-teaching staff, including Dr. Kesarkar, Sanchita Mavashi, Adsul Kaka, Mangesh Salvi Kaka, Mr. Patange, Mr. Nilesh, Satish and Sanket for their support throughout my research.

I am grateful to Pranav and Mavashi for their delicious and timely food throughout my journey.

I also extend my appreciation to my dear friends—Dr. Pavan Telharkar, Dr. Omkar Mishra, Dr. Vikas Yamgar, Dr. Kiran Mali, Dr. Kiran Jogdand, Dr. Sanket Jadhav, Dr. Anushka Gadhari and Dr. Gauri Ghangale —for their unwavering support and joyful companionship.

This work is dedicated to my beloved parents, ***"Mummy-Baba"****, my brother* ***Er. Lakshadeep Dada****, Sister-in-law* ***Rutuja****, Grandparents* ***"Aai- Bappu"*** *and my cousins* ***Rutuja*** *and* ***Rajdeep*** *whose love, encouragement, and moral support have been the foundation of my success. Their unwavering belief in me has been a driving force in my academic journey.*

I am sincerely grateful to all my teachers, friends, seniors, and juniors who contributed to shaping this research into its final form.

Finally, I extend my heartfelt thanks to everyone who, in any way, directly or indirectly, supported and contributed to this research.

Date:

Place: Mumbai

(Dr. Kawade Jaydeep Ramdas)

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LIST OF ABBREVIATIONS

Sr. No.	Abbreviation	Name
1	%	Percent
2	% B/B0	Percent binding at zero standard
3	<	Lesser than
4	>	Greater than
5	≤	Lesser than or equal to
6	≥	Greater than or equal to
7	±	Plus or minus
8	µl	Microliter
9	0_F	Degree Fahrenheit
10	125_I	Iodine – 125 (Radioisotope)
11	ALP	Alkaline phosphatase
12	ALT	Alanine Aminotransferase
13	AST	Aspartate transaminase
14	B_0/T	Zero binding to total binding ratio
15	BRIT	Board of Radiation and Isotope Technology
16	BSDPHA	Bai Sakarbai Dinshaw Petit Hospital for Animals
17	BUN	Blood Urea Nitrogen
18	cmm	Cubic millimeter
19	CPM	Count per minute
20	CV	Coefficient of variation
21	dl	Deciliter
22	EDTA K3	Tripotassium ethylenediaminetetraacetic acid
23	ELISA	Enzyme-linked Immunosorbent Assay
24	*et al.*	Et alia (and others)
25	Fig	Figure
26	fT_4	Free Thyroxine
27	gm	Gram
28	Hb	Hemoglobin
29	HCT	Hematocrit
30	I	Iodine

31	IU/L	International Units per liter
32	kg	Kilogram
33	MCH	Mean Corpuscular Hemoglobin
34	MCHC	Mean Corpuscular Hemoglobin Concentration
35	MCV	Mean Corpuscular Volume
36	µIU/ml	Micro international unit per ml
38	mg	Milligram
39	ml	Milliliter
40	n	Sample size
41	ND	Nondescript
42	ng	Nanogram
43	ng/mL	Nanogram per milliliter
44	nmol/L	Nanomole per Liter
45	P	Calculated probability (p-value)
46	PLT	Platelets
47	pmol/L	Picomole per Liter
48	cTSH	Canine thyroid stimulating hormone
49	Q. C.	Quality Control
50	RBC	Red Blood Cells
51	RIA	Radio Immuno Assay
52	rpm	Rotations per minute
53	SE	Standard Error
54	Sr. No.	Serial Number
55	T_3/ TT_3	Total Triiodothyronine
56	T_4/ TT_4	Total Thyroxine
57	TEC	Total Erythrocyte Count
58	TLC	Total Leucocyte Count
59	TP	Total Proteins
60	TRH	Thyrotropin Releasing Hormone
61	TSH	Thyroid Stimulating Hormone
62	TVCC	Teaching Veterinary Clinical Complex
64	WBC	White Blood Cells
65	µg	Microgram
66	* and **	Significant at 0.05 and 0.01
67	##	Significant at 0.01

Introduction

1. INTRODUCTION

For centuries, dogs have been cherished as loyal companions, earning the affectionate title of "man's best friend." Like all living beings, however, they are vulnerable to numerous diseases stemming from diverse factors, including infections, nutritional deficiencies, and hormonal imbalances.

Amongst hormonal imbalances, hypothyroidism is the most encountered endocrinopathy in dogs (Dixon, 2001). The global prevalence of canine hypothyroidism is reported to range from 0.20% to 0.80% (Catherine *et al.,* 2005). In India, the prevalence was recorded 0.206% in Mumbai (Pawar, 2009), 0.40% in Hisar (Gulzar *et al.,* 2014) and 0.174 % in Punjab (Kour *et al.,* 2020).

A deficiency or excess of thyroid hormones can indirectly impact most bodily systems (McCann, 2015). Thyroid hormones play a vital role in regulating various metabolic processes. During fetal development, calorigenic thyroid hormones are crucial for properly developing the neural and skeletal systems. The primary function of the thyroid gland is to produce the active thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) in response to thyroid stimulating hormone (TSH). These hormones are predominantly bound to proteins in circulation, with around 60% of T_4 bound to thyroxine-binding globulin, 17% to transthyretin, 12% to albumin, and 11% to various lipoproteins. T3 binding follows a similar pattern. Compared to humans, dogs exhibit lower binding affinities for these hormones, which results in lower overall hormone concentrations, higher free hormone fractions (approximately 0.1-0.3% for T_4 and 1% for T_3), and shorter serum half-lives (10-16 hours for T4 and 5-6 hours for T3) (Ettinger and Feldman 2000).

Primary hypothyroidism, responsible for more than 95% of cases, is most commonly caused by lymphocytic thyroiditis or idiopathic thyroid atrophy. In rare cases, hypothyroidism may arise from a thyrotropin (thyroid-stimulating hormone, TSH) deficiency caused by reduced pituitary gland activity. Less common forms include tertiary hypothyroidism, resulting from a deficiency of thyrotropin-releasing hormone (TRH), and congenital hypothyroidism ((Lathan, 2012).

Hypothyroidism in dogs is manifested by various clinical signs. The most common and typical signs are 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).

The animal body is one of nature's most intricate and efficient systems. Despite its complexity and remarkable functionality, its fundamental processes are regulated by just two systems: the nervous system and the endocrine system. The nervous system uses rapid electrical and chemical signals to initiate swift organic responses. In contrast, the endocrine system relies on the synthesis and release of chemical messengers, enabling it to regulate various functions at a slower pace but with longer-lasting effects (Vala *et al.,* 2013).

Under normal bodily conditions, thyroxine plays a crucial role in boosting the respiratory function of mitochondria, thereby aiding in the generation of adenosine triphosphate (ATP) during aerobic metabolism. Thyroxine appears to enhance the activity of ATPase, which in turn amplifies the function of the ATP-dependent Na+/K+ pump. This heightened ATPase activity is linked to an increased transport of ATP across mitochondrial membranes. In cases of hypothyroidism, the deficiency of ATP and the reduced activity of ATPase hinder the functioning of the Na+/K+ pump, resulting in a disruption of pump-driven axonal transport. Recent findings have shown a decrease in axonal transport within the sciatic nerves of hypothyroid rats, indicating that hypothyroidism may lead to axonal degeneration and peripheral neuropathy (Jaggy *et al.,* 1994).

Hence, hypothyroidism is linked to a range of neurological disorders, which can involve issues with individual or multiple cranial nerves, peripheral neuropathies, and diseases affecting the central nervous system. Key neurological signs in dogs with hypothyroidism include facial nerve paralysis, megaoesophagus, peripheral vestibular disease, and lower motor neuron dysfunction. The latter can present as generalized weakness, deficits in proprioception, and reduced segmental reflexes, which may progress to paraparesis or tetraparesis (Ettinger and Feldman 2000; Lathan 2012).

The nonspecific and vague clinical signs of canine hypothyroidism make its diagnosis challenging. The factors like non-thyroidal illnesses, medications, and natural physiological variations may affect the accuracy and precision of thyroid

function tests Additionally, the accuracy of thyroid function tests may be affected which can lead to misdiagnoses in clinical settings (Ferguson, 1994).

Among the various diagnostic methods, radioimmunoassay (RIA) provides higher specificity and sensitivity. The first immunoassay was documented by Yalow and Berson in 1959.

The application of RIA in hormone measurement for veterinary clinical purposes has been widely studied in India (Dadke *et al.,* 2018, Roopali *et al.,* 2020, Galdhar *et al.,* 2021, Jayabhaye *et al.,* 2021, Galdhar *et al.,* 2022 and Salutgi *et al.,* 2023). The Department of Veterinary Clinical Medicine and Veterinary Nuclear Medicine, including the radioisotope laboratory at Mumbai Veterinary College, has established reference ranges for canine thyroid hormones such as T_3, T_4, and fT_4 using Radio Immunoassay.

However, despite wide research on hypothyroidism in dogs, there is some research gap between the linkage of hypothyroidism with neuromuscular manifestations especially in India and Maharashtra. Given this need and the presence of a hormonal assay laboratory at Mumbai Veterinary College, the proposed research aims to achieve the following objectives:

1) To study the neurological manifestations associated with hypothyroidism.
2) To study alterations in the clinico-hematobiochemical profile in neuromuscular disorders associated with hypothyroidism.
3) To assess therapeutic efficacy of replacement therapy.

Review of Literature

2. REVIEW OF LITERATURE

The present study entitled '**Studies on hypothyroidism in dogs with special reference to neuromuscular disorders'** was reviewed for literature under the following headings:

2.1 Hypothyroidism in dogs.

2.2 Clinical and neuromuscular manifestations of hypothyroidism in dogs.

2.3 Diagnosis

2.3.1 Haemato-biochemical alterations in hypothyroidism (Complete Blood Count, Liver and Kidney Function, Lipid Profile, Serum Electrolytes, Calcium and Phosphorus).

2.3.2 Thyroid Profile

2.3.3 Radioimmunoassay

2.4 Therapeutic Management

2.4.1 Levothyroxine

2.4.2 Response to replacement therapy

2.1 Hypothyroidism in dogs

Peterson (1988) defined hypothyroidism in dogs as the clinical condition resulting from inadequate production of thyroid hormones by the thyroid gland, most commonly due to primary glandular dysfunction, such as lymphocytic thyroiditis or thyroid atrophy.

Jaggy *et al.* (1994) conducted a retrospective study on 29 dogs to examine the neurological manifestations of hypothyroidism. They found that the affected dogs were primarily older (mean age = 9.5 years) and of large breeds, with no predisposition related to sex or breed.

Panciera (1994) stated that hypothyroidism was the most common hormone imbalance of dog and dogs beyond 8 years were more susceptible and spayed females and castrated males were at greater risk. Further in the study he screened 132 dogs for hypothyroidism and the prevalence was 0.2%. Author explained that the lower prevalence rate compared to reported earlier 0.64% was because of more restrictive criteria for diagnosing hypothyroidism.

Chastain and Panciera (1995) highlighted that hypothyroidism was the most common hormone imbalance of dogs with the incidence rate at about 1: 150 to 1: 500.

Williams *et al.* (1996) stated that hypothyroidism is one the most common endocrine disorders of the dog. They noted its significant impact on a dog's overall health, with the disease affecting multiple organ systems due to the widespread role of thyroid hormones in regulating metabolism. This work highlighted the importance of accurate diagnosis, as hypothyroidism can mimic other conditions or be masked by concurrent illnesses.

Ettinger and Feldman (2000) reported prevalence of canine hypothyroidism to range from 0.20 % to 0.80 %. Further, they found that the prevalence of hypothyroidism was in between the range of 0.5 -15 years and the mean age at diagnosis of hypothyroidism was 7 years. Further, author defined hypothyroidism in dogs as a hormonal deficiency disorder, usually caused by primary hypothyroidism, which disrupts the normal metabolic activities regulated by thyroid hormones.

Dixon (2001) reported hypothyroidism as a common endocrine disorder of dogs, with a reported prevalence between 0.20 and 0.64 % compared with the estimates of 0.0005 to 1.50 % for canine diabetes mellitus. Author mentioned that spontaneous hypothyroidism is most frequently recognised in medium to large breeds, particularly labrador retrievers, spaniels, dobermans, shetland sheepdogs and setters. It typically affects middle-aged dogs but tends to present at a younger age in the predisposed breeds. It is rare in dogs less than two years of age although

the underlying pathology obviously starts before clinical signs become apparent. Both entire and neutered males and females are affected.

Bonagura and Twedt (2013) stated that hypothyroidism is defined as an endocrine disorder characterized by insufficient production of thyroid hormones, predominantly thyroxine (T_4) and triiodothyronine (T_3), which results in a reduction in basal metabolic rate and various systemic clinical manifestations.

Feldman *et al.* (2014) defined canine hypothyroidism as a deficiency in the synthesis or secretion of thyroid hormones, primarily caused by primary thyroid disease, leading to metabolic, dermatologic, and neuromuscular abnormalities.

Gulzar *et al.* (2014) conducted a study among the pets presented to Teaching Veterinary Clinical Complex (TVCC), LUVAS, Hisar between September 2010 and March 2011 and found that the maximum occurrence of hypothyroidism was observed in Labrador breed with five positive cases out of 34 (14.70%), followed by two hypothyroidism cases in German shepherd dogs (15.38%) and one in Pomeranian dog.

Nelson and Couto (2019) stated that hypothyroidism is defined as a state of thyroid hormone deficiency resulting from thyroid gland dysfunction, most commonly due to immune-mediated lymphocytic thyroiditis or idiopathic atrophy.

Fernandez and Seth (2016) opined that hypothyroidism was the most common endocrinopathy in dogs and 95% of cases were caused by lymphocytic thyroiditis or idiopathic atrophy of the thyroid gland, leading to a decrease in thyroid hormone production.

Kour *et al.* (2020) conducted a study on 20,108 dogs aged one year or greater presented for various health reasons from June 2018 to February 2020 to small animal OPD, Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab with signs of endocrinopathies. On the basis of low levels of T_4 and higher levels of TSH, a total of 35 dogs were diagnosed with hypothyroidism. In the present study the hospital prevalence of hypothyroidism was 0.174 % (n=35). The highest prevalence was in

Labrador retriever (n=18 dogs, 51.43%) followed by Pug (n=7, 20.0%), Samoyed (n=3 dogs, 8.57%) Dachshund and German shepherd (n=2 dogs, 5.71%) and Pitbull, Shih Tzu and Mongrel (n= 1 each, 2.86%). Results of the study concluded that majority of the cases were from middle age group (21 cases, 60%) followed by young age group (12 cases, 34.3%).

Ryad *et al.* (2020) stated that hypothyroidism is one of the most common endocrinopathies in dogs, it is defined as a decrease in production of thyroid hormones.

Ghallab *et al.* (2021) conducted epidemiological study on hypothyroidism in dogs in Egypt. Hypothyroidism was mostly recorded in middle aged dogs (1-5 year) (10.66%), followed by older dogs' group (>5 year) (7.94%). Males (10.91%) showed higher incidence to hypothyroidism compared to females (9.42%).

Raja *et al.* (2021) screened dogs for hypothyroidism presented at Referral Veterinary Polyclinic, Indian Veterinary Research Institute during 2016-17. Twenty (20) dogs were found positive for hypothyroidism during the study period. Adult dogs aged more than 5 years were commonly affected. Male (n=10) and female (n=10) dogs were equally susceptible to hypothyroidism and there was no sexual susceptibility in this study. Labrador retriever dogs were exceedingly (n=15) diagnosed with hypothyroidism which may be because of increased adoption of such breed by owners.

Naveen (2024) conducted research on the topic studies on hypothyroidism in dogs and its therapeutic management at the Veterinary Clinical Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. During the study in March 2023 to November 2023, a total of 5957 dogs were registered. Out of these, 27 dogs were diagnosed with hypothyroidism thus representing an occurrence of 0.45%. The occurrence of hypothyroidism was higher in dogs aged above 8 years (40.74%) followed by 18.52 per cent in dogs between 2-4, 4-6 and 6-8 years and was less (3.70%) in dogs below 2 years of age. The study reported that occurrence of hypothyroidism was higher in male dogs (70.37%) when compared to female dogs (29.63%). Further, researcher mentioned that higher

occurrence of hypothyroidism in male dogs in the current study could be attributed to the over representation of male dogs, possibly influenced by the preference of pet owners towards male dogs.

2.2 Clinical and neuromuscular manifestations of hypothyroidism in dogs

Bichsel *et al.* (1988) studied neurologic manifestations associated with hypothyroidism in four dogs. Hypothyroidism was believed responsible for peripheral and central neurologic abnormalities. Clinical signs consisted of abnormalities of gait and postural reactions and dysfunction of multiple cranial nerves in all 4 dogs. Circling, hypermetria, and spontaneous vertical nystagmus were recorded in some of the dogs. Further, authors mentioned that in neurological manifestations of hypothyroidism, the dog may not show any of the classical symptoms such as lethargy and dermatological changes.

Jaggy *et al.* (1994) conducted a retrospective study on 29 dogs to examine the neurological manifestations of hypothyroidism. They found that eleven dogs exhibited lower motor neuron signs, nine had peripheral vestibular deficits, four had megaesophagus, and five had laryngeal paralysis. The study also suggested that vestibular or lower motor neuron signs, megaesophagus, or laryngeal paralysis might be the only clinical indications of a more generalized polyneuropathy associated with hypothyroidism.

Panciera (1994) studied hypothyroidism in 66 dogs during 1987 to 1992 at department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin. In his study the most common clinical findings were obesity (41%), seborrhoea (39%), alopecia (26%), weakness (21%), lethargy (20%), bradycardia (14%), and pyoderma (11%). Further, they stated less commonly reported abnormalities are central and peripheral nervous system, cardiovascular system, and other organ systems. Neurologic abnormalities were recorded in 19 dogs which included unilateral and bilateral facial nerve paralysis, decreased or absent palpebral reflexes, lip droop, ear droop, decreased tear production, peripheral

vestibular nerve palsy, megaesophagus, cervical spondylomyelopathy, laryngeal paralysis and generalised weakness.

Scarlett (1994) stated that neurologic, reproductive, ocular, hematologic, and cardiovascular abnormalities have been reported in hypothyroid dogs and highlighted the neurologic implications of hypothyroidism, noting its association with peripheral neuropathies, including weakness, ataxia, and proprioceptive deficits. These abnormalities arise from the disruption of metabolic processes necessary for nerve function.

David (2001) asserted that facial nerve paralysis is present in up to 70% of dogs with hypothyroidism and nerve dysfunction. The condition typically manifests as drooping of the ear, lip, and eyelid, as well as an inability to blink, and is a hallmark of cranial nerve involvement in hypothyroidism.

Dixon (2001) stated that less common signs include neuromuscular, reproductive, ocular, gastrointestinal and cardiovascular abnormalities. Laryngeal paralysis, megaoesophagus, peripheral vestibular disease, cranial nerve disorders, behavioural abnormalities and seizures have all previously been attributed to hypothyroidism in the literature.

Huber *et al.* (2001) mentioned that megaesophagus, an important cause of regurgitation, may be associated with hypothyroidism.

McKeown (2002) reported excessive drooling; drooping of the left ear, eyelid, and lip; and a pronounced head tilt to the left in a 6-year-old, 30-kg, intact male boxer dog was presented for evaluation of acute onset of a profound head tilt and unilateral facial nerve paralysis.

Higgins *et al.* (2006) stated that neurologic manifestations of canine hypothyroidism are relatively uncommon compared with other clinical signs such as lethargy, weight gain, and dermatologic abnormalities. Clinical signs observed in hypothyroid dogs exhibiting neurologic dysfunction often are referable to the peripheral nervous or neuromuscular systems and include facial nerve paralysis, polyneuropathy, peripheral vestibular disease, and laryngeal paralysis.

Fors (2007) reported that in addition to the most commonly occurring symptoms such as dermatological changes and signs of general metabolic disturbances, a number of neurological manifestations have been reported to occur in hypothyroidism in dogs. Neurological symptoms of hypothyroidism can originate from the central and peripheral nervous systems as well as from the muscles. Symptoms from the peripheral nervous system which have been described were exercise intolerance, general weakness, paraparesis and tetraparesis, and symptoms of the cranial nerves such as vestibular syndrome and facial paralysis. Laryngeal paralysis and megaoesophagus have also been reported in hypothyroid dogs. Hypothyroidism is an important differential diagnosis for generalised peripheral neuropathy and peripheral vestibular syndrome since it is a treatable disease and the prognosis for complete recovery is good.

Kumar *et al.* (2007) suspected 18 dogs for hypothyroidism at Hyderabad, India. They highlighted classical collection of clinical signs in suspected dogs, Viz., some kind of skin abnormality (94%), generalized hair loss (88%), typical rat tail (83%), skin lesions, pigmentation and pruritus (27%), brittle, dry and lustreless coat (83%), obesity (88%), anaemic (33%), puppy – like coat (22%) and corneal lipidosis (11%). Further authors mentioned that in hypothyroidism 2-4% dogs exhibit some behavioural changes and neurological signs such as unprovoked aggression, head tilt, seizures, ataxia, circling and facial nerve paralysis.

Pettigrew *et al.* (2007) reported instances of stunted physical growth and neurological abnormalities in 9-week-old terrier puppies. The condition was diagnosed as hypothyroidism, characterized by low serum T_4 and f T_4 levels, along with elevated TSH levels.

Vitale and Olby (2007) observed that hypothyroid Labrador Retrievers exhibited a variety of neurological signs, including seizures, coma, brainstem signs, tetraparesis, central and peripheral vestibular signs, facial paralysis, and paraparesis.

Blois *et al.* (2008) documented a case of primary hypothyroidism leading to central nervous system atherosclerosis in a dog. The affected Australian shepherd

exhibited chronic mild ataxia, obesity, and lethargy. Despite treatment with levothyroxine, the ataxia progressed, and cranial nerve abnormalities emerged, ultimately resulting in euthanasia. A postmortem examination revealed significant thyroid gland atrophy and extensive, severe central nervous system atherosclerosis. Further, authors also mentioned that neurologic signs occur in 7.5% of hypothyroid dogs.

Suraniti *et al.* (2008) observed hypothyroid-related polyneuropathy in six Doberman Pinschers aged 6 to 8 years. They found that this condition was common in large breeds of middle to mature age, even when typical signs of hypothyroidism were not present.

Johnson *et al.* (2009) mentioned that hypothyroidism historically has been cited as a possible cause of megaesophagus. Citing the study by Gaynor, Shofer, and Washabau (1997) of 136 dogs with acquired megaesophagus, 272 control dogs from the general hospital population, and 151 control dogs that underwent thyroid-stimulating hormone response tests, and one retrospective study of 29 hypothyroid dogs, in which four had megaesophagus; one dog showed clinical improvement in esophageal symptoms when treated with thyroid supplement but radiographic evidence of a dilated esophagus persisted in all four dogs author stated that a definitive association between hypothyroidism and megaesophagus has not been proved.

Rossmeisl *et al.* (2009) stated that skeletal muscular weakness, cramping, exercise intolerance, and pain or signs of pain have been reported as potential sequelae of hypothyroidism in humans and dogs.

Srikala and Kumar (2011) studied hypothyroidism associated systemic and peripheral disorders in 42 dogs over the period spanning March 2009 to February 2011. They reported neuromuscular disorder in 25.53% of the cases. Seizures, paraplegia, and facial paralysis were observed in 12 cases (25.53%). Musculoskeletal disorders like lameness, limping and reluctant to move, and unable to bear weight was noticed in 2 (4.26%) dogs. Further authors concluded that hypothyroidism not only causes pruritic skin and coat abnormalities, but it is also associated with secondary skin infections, dilated cardiomyopathy, facial paralysis

and musculoskeletal disorders. His findings are in opinion with **Jaggy *et al.* (1994)** who stated that in hypothyroidism, nerves do not carry electrical impulses normally which may lead to weakness, exercise intolerance and lethargy.

Romão *et al.* (2012) highlighted obesity, lethargy, alopecia, “rat tail” and reproductive abnormalities as the classical signs of hypothyroidism in dog. Further, the authors asserted that neurological signs are rare in primary hypothyroidism and include seizures, circling, disorientation, coma, polyneuropathy and focal neuropathy.

Bertalan *et al.* (2013) stated that in some instances , neurologic deficits may be the sole manifestation of hypothyroidism. Further, author also mentioned that isolated or multiple cranial neuropathies involving the vestibular branch of the trigeminal nerve may be observed in dogs with hypothyroidism.

Reusch *et al.* (2015) mentioned that a subclinical myopathy has also been reported in hypothyroid dogs. Cranial nerve dysfunction (facial, trigeminal and vestibulocochlear), with or without abnormal gait, has also been reported. Neurological signs may be multifocal, acute or chronic, static or progressive, and may occur without other clinical signs of hypothyroidism.

Chaves *et al.* (2016) in a case report of a 7-year-old male canine, boxer dog referred to the Veterinary Medical Teaching Hospital of the UFSM (Universidade Federal de Santa Maria) stated that neurological disorders due to hypothyroidism was rare in dogs but, when manifested, these signs refer to the peripheral or central nervous system and the most common include: vestibular disease, seizures, laryngeal paralysis, poly neuropathy and paralysis of the facial nerve.

Fernandez and Seth (2016) stated that central nervous system and peripheral nervous systems can be affected in hypothyroid dogs. The most common neurological manifestation of hypothyroidism is diffuse peripheral neuropathy causing generalised weakness, ataxia and decreased reflexes.

Nelson and Couto (2019) asserted that Neuromuscular signs may be the predominant problem in some dogs with hypothyroidism. Signs include seizures,

ataxia, circling, weakness, and proprioceptive and postural reaction deficits. These signs are often seen in conjunction with vestibular signs (e.g., head tilt, nystagmus) or facial nerve paralysis. Peripheral neuropathies identified in hypothyroid dogs include facial nerve paralysis, generalized weakness associated with diffuse lower motor neuron tetraparesis, and knuckling or dragging of the feet, with excessive wear of the dorsal part of the toenail. Hypothyroid myopathy is characterized by type II myofiber atrophy, myofiber degeneration, and depletion in skeletal muscle carnitine, and may result in skeletal muscle wasting, weakness, exercise intolerance, and increased serum of creatine kinase, aspartate aminotransferase, and lactate dehydrogenase activities. Thyroxine-responsive unilateral forelimb lameness has also been observed in dogs.

De Oliveira *et al.* (2022) reported a case of hypothyroid-related neuropathy in a 4-year-old intact male Dogo Argentino weighing 64 kg. The dog presented with symptoms including obesity, anorexia, prostration, circling behaviour, absence of proprioception in all four limbs, and chronic dermatopathy.

Woelfel *et al.* (2023) in a study on presumed pituitary apoplexy in 26 dogs, found that 6 (23%) dogs were previously diagnosed with hypothyroidism with neurological signs.

Naveen (2024) conducted research on the topic studies on hypothyroidism in dogs and its therapeutic management at the Veterinary Clinical Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. During the study in March 2023 to November 2023, a total of 5957 dogs were registered. Out of these, 27 dogs were diagnosed with hypothyroidism. It was evident from the present study that 22.22 % per cent of the hypothyroid dogs exhibited neuromuscular weakness, particularly hind leg weakness. Out of these six dogs, three of them (11.11%) were diagnosed with megaesophagus upon radiographic examination.

2.3 Diagnosis

2.3.1 Haemato-biochemical alterations in hypothyroidism

(Complete Blood Count, Liver and Kidney Function, Lipid Profile, Serum Electrolytes, Calcium and Phosphorus)

Panciera (1994) studied hypothyroidism in 66 dogs during 1987 to 1992 at department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin. In his study the most common clinicopathologic abnormalities included hypercholesterolemia (73%), nonregenerative anaemia (32%), high serum alkaline phosphatase activity (30%) and high serum creatinine kinase activity (18%).

Fors (2007) noted that various diagnostic aids can help substantiate hypothyroidism, including mild normocytic, normochromic, nonregenerative anemia, and serum biochemical abnormalities such as hypercholesterolemia, hyperlipidemia, and hypertriglyceridemia.

Higgins *et al.* (2006) investigated central vestibular disease associated with hypothyroidism in ten dogs. Non-neurologic diagnostic investigations revealed mild, nonregenerative anemia in one dog. Hypercholesterolemia and hypertriglyceridemia were noted in 7 of 10 and 2 of 4 dogs, respectively. In the 7 dogs with hypercholesterolemia, the median cholesterol concentration was 791 mg/dL. In the 2 dogs with hypertriglyceridemia, triglyceride concentrations were 588 and 1,609 mg/dL. Two dogs with a history of glucocorticoid administration had mild increases in serum alkaline phosphatase (ALP) activity presumed to be secondary to steroid isoenzyme induction.

Kumar *et al.* (2007) reported hypothyroidism in dogs at Hyderabad, India. 18 cases were suspected for hypothyroidism out of which 6 (33%) cases had non-regenerative type of anemia. Biochemical findings revealed mild to moderate hike in mean serum ALT (170 u/l), ALP (99.8 u/l) and Cholesterol (14.6 nmol/ l) levels in suspected dogs.

Vitale and Olby (2007) studied six Labrador dogs with neurological signs linked to hypothyroidism. These dogs exhibited mild, nonregenerative anemia, with a mean red blood cell count of 4.67 × 10^6/mL (range: 4.22 × 10^6/mL to 5.27 × 10^6/mL; reference range: 4.78–8.62 × 10^6/mL). Biochemistry profiles revealed increased creatine kinase (CK) activity, with a mean CK of 1,549 U/L (range: 337–3,182 U/L). All dogs had severe hypercholesterolemia, with a mean serum cholesterol concentration of 1,568 mg/dL (range: 995 mg/dL to 2,180 mg/dL). The fasting triglyceride concentration in dogs 2–4 had a mean of 742 mg/dL (range: 397–1,152 mg/dL).

Blois *et al.* (2008) documented a case of primary hypothyroidism leading to central nervous system atherosclerosis in a dog. In the present case a complete blood count (CBC) revealed mild normocytic, normochromic, nonregenerative anemia, with a haematocrit of 0.30 L/L (reference interval, 0.39 to 0.56 L/L) and the serum biochemical profile showed marked hypercholesterolemia (25.38 mmol/L; reference interval, 3.6 to 10.2 mmol/L).

Suraniti *et al.* (2008) found in a study on hypothyroid-associated polyneuropathy in dogs that serum cholesterol levels, creatine kinase, and alkaline phosphatase activities were mildly to markedly elevated.

Fracassi and Tamborini (2011) studied hypothyroidism associated megaesophagus in a seven-year-old entire female German shepherd dog. The blood results of the dog showed a moderate non-regenerative normochromic and normocytic anaemia and mild increase in serum cholesterol concentrations.

Mooney (2011) remarked that a complete blood count may reveal nonregenerative anemia as a result of reduced erythropoietin production consequent to reduced T_4 levels in hypothyroidism.

Srikala and Kumar (2011) studied the clinico-therapeutic management of thyroxine-responsive polyneuropathies in 42 dogs, finding normocytic, normochromic, nonregenerative anemia and elevated levels of serum cholesterol and triglycerides.

Romão *et al.* (2012) stated that major laboratory findings in hypothyroidism are non-regenerative anaemia and hypercholesterolemia. Other findings found in lower frequency include hyponatremia, increase on alanine transferase and alkaline phosphatase activity. According to the authors the results of hemogram, biochemistry panel and urinalysis can confirm the hypothyroidism diagnosis and rule out other disorders.

Bharti *et al.* (2015) Assessed serum minerals and electrolytes in human thyroid patients. The study included 75 patients and 30 controls. Patients with subclinical hypothyroidism and overt hypothyroidism showed significant decrease in serum calcium and sodium levels and significant increase in serum phosphorous, magnesium, potassium and chloride levels.

Dambal *et al.* (2016) in a controlled human case study on Serum Calcium and Electrolytes in Hypothyroidism found significant decrease in serum calcium, sodium and potassium in patients compared to controls ($p<0.0001$). A significant increase in serum chlorides was observed in cases ($p=0.03$) compared to controls. When correlated with TSH, serum calcium, sodium, potassium and chlorides showed negative correlation in subjects with hypothyroidism. The study concluded that serum calcium, sodium and potassium levels were decreased whereas serum chloride levels were increased in hypothyroidism in comparison to euthyroid subjects.

Fernandez and Seth (2016) opined that haematology reveal a non-regenerative anaemia in up to 50% of cases. The most common abnormality observed in the serum biochemistry is hyperlipidaemia due to hypercholesterolaemia and hypertriglyceridemia seen in 75% of hypothyroid dogs. Mild increases in alkaline phosphatase and creatinine kinase are not uncommon.

Nelson and Couto (2019) stated that the most consistent clinicopathologic findings in dogs with hypothyroidism are hypercholesterolemia and hypertriglyceridemia. Hypercholesterolemia is identified in approximately 75% of hypothyroid dogs, and the cholesterol concentration can exceed 1000 mg/dL. Although fasting hypercholesterolemia and hypertriglyceridemia can be associated

with several other disorders, their presence in a dog with appropriate clinical signs is strong evidence for hypothyroidism. A mild normocytic, normochromic, nonregenerative anemia (packed cell volume [PCV] of 28%-35%) is a less consistent finding, identified in approximately 30% of dogs.

Kour *et al.* (2020) conducted a study on 20,108 dogs aged one year or greater presented for various health reasons from June 2018 to February 2020 to small animal OPD, Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab with signs of endocrinopathies. The biochemical findings of the study showed a significant reduction of T_4, increased TSH, hypercholesterolemia, hyper-triglyceridemia, elevated ALP, hypocalcaemia, and hypophosphatemia.

Ryad *et al.* (2020) conducted study in cosmopolitan governorate, Cairo multicentre and Giza for crude determination of hypothyroidism in dog. In period of December-2019 to May-2020, 220 dogs with various dermatologic signs represented to different clinics in October city, EL-Rehab city, Giza, Nasr city and Maddi district were selected. Author reported that significant decrease in RBCs and PCV with non-significant decrease in HB and non-significant increase in cholesterol with no statistical changes detected in ALT, ALP in hypothyroid patient were recorded compared to control group

De Oliveira *et al.* (2022) reported in a case study on canine hypothyroidism with neurological disorders that the serum cholesterol value was above the reference limit.

Alone (2024) studied thyroid profile in dogs with special reference to assessment of canine TSH 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. Author compared hematological and biochemical parameters between healthy (n=67) and hypothyroid dogs (n=10), and reported significant ($p < 0.05$) alterations in hemoglobin (Hb, mg/dl) and packed cell volume

(PCV %) of hematological parameters and alkaline phosphatase, and cholesterol of biochemical parameters.

Naveen (2024) conducted research on the topic studies on hypothyroidism in dogs and its therapeutic management at the Veterinary Clinical Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. During the study in March 2023 to November 2023, a total of 5957 dogs were registered. Out of these, 27 dogs were diagnosed with hypothyroidism. Author recorded significantly ($P < 0.01$) lower values in the means of haemoglobin, packed cell volume, total erythrocyte count and total leukocyte count in hypothyroid dogs compared to healthy control.

2.3.2 Thyroid profile

Bichsel *et al.* (1988) studied neurologic manifestations associated with hypothyroidism in four dogs. Hypothyroidism was diagnosed in all 4 dogs on the basis of low resting serum thyroxine concentration and inadequate response to thyroid stimulating hormone.

Baker (1986) concluded that, if the dog shows clinical signs of the disease, a lab test that shows lower than normal values of T_3, T_4 (both bound and free forms) with or without elevated levels of TSH is pretty diagnostic for hypothyroidism.

Baker (1997) found that many of the dogs with true hypothyroidism did not have elevated TSH levels, as one would expect. Still the test is often helpful in making the diagnosis of hypothyroidism. Further, author concluded that if the dog shows clinical signs of the disease, a lab test that shows lower than normal values of T3, T4 (bound and free) with or without elevated levels of TSH is pretty diagnostic for hypothyroidism.

Jaggy *et al.* (1994) conducted a retrospective study on 29 dogs to examine the neurological manifestations of hypothyroidism. In the present study diagnosis was based on the response to levothyroxine supplementation, lack of response to thyroid-stimulating hormone, and persistently elevated serum cholesterol levels.

Cuddon (2002) reported that in primary hypothyroidism, the levels of free T_4 and total T_4 are low and TSH elevated or normal.

Higgins *et al.* (2006) investigated central vestibular disease associated with hypothyroidism in ten dogs. In the study, dogs were diagnosed with primary hypothyroidism if the f T_4 and T T_4 concentrations were less than the lower limit of the reference range (6–42 pmol/L and 15 67 nmol/L, respectively) and either the TSH concentration was greater than the upper limit of the reference range (0–30 mU/L) or other clinical or clinicopathologic signs of hypothyroidism were present.

Fors (2007) stated that treatment with thyroxine supplementation is successful in most cases of polyneuropathy and peripheral vestibular syndrome, but less so in laryngeal paralysis and megaoesophagus.

Kumar *et al.* (2007 mentioned that Free T_4 levels are less subject to fluctuate into a falsely low range in response to non-thyroidal diseases or drugs than is a total T_4 level.

Mooney (2011) stated that measurement of free T_4 concentration, which is not affected by nonthyroidal illness, is recommended for patients with clinical signs of hypothyroidism. A definitive diagnosis of hypothyroidism is established by demonstrating a serum f T_4 concentration below 90% of the reference range, in addition to clinical suspicion of the disease. Endogenous TSH levels can help strengthen the diagnosis and are 80% diagnostic for hypothyroidism however measurement of endogenous TSH concentration is more specific in presence of a low f T_4 level than if interpreted alone.

Romão *et al.* (2012) asserted that serum thyrotropin (TSH) concentration usually increases in cases of primary hypothyroidism. However, 20% of hypothyroid animals present normal TSH levels. Further they stated that basal serum total T_4 has traditionally been the main element for the diagnosis of canine hypothyroidism and remains an excellent line of diagnostic test for the disease. The free T_4 concentration is considered the most reliable diagnostic test among available options.

Fernandez and Seth (2016) stated that in a dog showing clinical signs and clinicopathological findings with a low total T_4 or free T_4, but a TSH in the normal limits, TSH stimulation test can be performed. TSH is difficult to source and expensive. Alternatively, a therapeutic trial can be the most practical approach to confirm a diagnosis of hypothyroidism. If therapy leads to an improvement of clinical signs in an appropriate time frame, treatment should be temporarily discontinued to determine a recurrence of clinical signs, which would be compatible with hypothyroidism.

Nelson and Couto (2019) All serum T_4, both protein bound and free, comes from the thyroid gland. Therefore, tests that measure serum total and fT_4 concentrations, in conjunction with the serum TSH concentration, are currently recommended for assessment of thyroid gland function in dogs suspected of having hypothyroidism. . A low serum T_4 concentration (ideally <0.5 µg/dL [6 nmol/L]) in conjunction with hypercholesterolemia and clinical signs strongly suggestive of the disease supports the diagnosis of hypothyroidism, especially if systemic illness is not present. The definitive diagnosis must then rely on response to trial therapy with synthetic levothyroxine. Additional tests of thyroid gland function (i.e., TSH and f T_4) are warranted if the serum T_4 concentration is equivocal.

Ryad *et al.* (2020) conducted study in cosmopolitan governorate, Cairo multicentre and Giza for crude determination of hypothyroidism in dog. In period of December-2019 to May-2020, 220 dogs with various dermatologic signs represented to different clinics in October city, EL-Rehab city, Giza, Nasr city and Maddi district were selected. Researcher found that there is, significant decrease in TT_4 and fT_4 along with non- significant elevation in TSH in hypothyroid patients compared to control data recorded. After administration of levothyroxine, TT_4, fT_4 and TSH were brought to normal levels. The study concluded that diagnosis of hypothyroidism in dogs is not always straightforward and its one of the most over diagnosed endocrine disorder in dogs. Therefore, depending on T4 alone cannot confirm hypothyroidism but can be ruled out. A normal or low TSH does not rule out hypothyroidism. fT4 measurement is ideal to help confirm the diagnosis.

Gupta *et al.* (2023) conducted a study to know the occurrence of canine hypothyroidism in dogs of Mumbai region. Total of twenty-five cases were considered for this study based on the clinical signs and serum hormone profile (T3 , T4 and TSH). On the basis of ECLIA result, all the twenty-five dogs investigated had low T3 level than the normal value, while T4 levels in 12 dogs was below 13 nmol/L and 13 dogs had T4 level between 13-18.01 nmol/L (border line) and were included in hypothyroid category based on clinical signs and response to levothyroxine treatment. Serum values of TSH by ECLIA were either normal (24) or subnormal (01) for all the twenty-five canine hypothyroid cases.

Alone (2024) studied thyroid profile in dogs with special reference to assessment of canine TSH 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. Author concluded that the mean concentration of Total triiodothyronine (TT_3), Total thyroxine (TT_4), Free thyroxine (fT_4), and Thyroid stimulating hormone (cTSH) in healthy (n=67) 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 (n=10) 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.

Naveen (2024) conducted research on the topic studies on hypothyroidism in dogs and its therapeutic management at the Veterinary Clinical Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. During the study in March 2023 to November 2023, a total of 5957 dogs were registered. Out of these, 27 dogs were diagnosed with hypothyroidism. In the study hypothyroidism was confirmed based on total T4 and free T4 estimation. The study reported mean total T4 concentration in healthy dog 34.86 ± 4.30 nmol/L, whereas in hypothyroid dogs, it was significantly lower at 7.74 ± 0.60 nmol/L. A highly significant

difference ($P < 0.01$) was observed in the mean serum total T4 concentrations between the two groups. Further, in hypothyroid dogs, the mean free T4 concentration was 0.32 ± 0.04 ng/dL, which was significantly lower ($P<0.01$) than the concentration in healthy dogs (0.96 ± 0.05 ng/dL). The autor reported non-significant difference in total T3 and TSH concentrations between the groups.

2.3.3 Radioimmunoassay

Berson and Yalow (1960) described labelled antigen-antibody complex is formed by binding of radiolabelled hormone to specific antibody. RIA works on the principal of competitive binding between labelled hormone and unlabelled hormone in biological fluids. As a result of competitive inhibition, a ratio of bound to free is calculated and concentration of unknown sample is obtained by interpolation with standard curve.

Goldsmith (1975) described RIA as antigen-antibody complexes formed by competition between radiolabelled and unlabelled antigen for specific antibody sites. At equilibrium, bound radiolabelled is separated from free radiolabelled antigen. Varying concentration of standards are used to plot standard curve. Ratio of bound to free is determined in order to extract the values of unknown sample.

Pawar (2009) investigated six clinical cases of canine hypothyroidism using history, clinical examination, hematology, biochemical parameters, thyroid profiling, electrocardiography (E.C.G.), and gamma scintigraphy. He recommended these tests for assessing thyroid function, noting a positive correlation in confirming hypothyroidism with CLIA thyroid profiles and 99mTc-pertechnetate uptake percentages.

Gnanasekar et al. (2010) described two-step RIA procedure for the measurement of fT_4 in human serum. Validation was done by spiking serum sample with tracer and estimation of extraction efficacy. Concentration of f T_4 with varying amount of sample volume was checked.

Galdhar and Gaikwad (2015) highlighted the lack of veterinary-specific RIA kits in India, noting the reliance on human-based kits, which may not always provide clinically justifiable results. They emphasized the need to validate these kits for veterinary use.

Naik (2016) conducted an eight-month study on fifty-nine cats, measuring the serum concentrations of total Triiodothyronine (TT_3), total Thyroxine (TT_4), and free Thyroxine (fT_4) using RIA. While TT_3 estimation followed standard methods, TT_4 and fT_4 measurements involved modifications to the standard procedures. The study also examined the variations in thyroid hormone levels in healthy cats based on age, breed, sex, diet, collection time, and litter usage. The mean serum concentrations of TT_3, TT_4, and fT_4 in healthy cats were 0.45±0.08 ng/ml, 21.98±2.94 ng/ml, and 24.4±2.24 pg/ml, respectively. Significant differences in fT_4 levels were observed between age groups (1-3 years, 3-6 years, and 6-9 years), while no significant differences were found across breeds, sexes, or diets.

Galdhar and Gaikwad (2017) detailed the principles and applications of RIA in veterinary practice and research, including assay terminologies and quality control parameters. They also discussed radiation exposure, biological effects, and safety measures in veterinary practice.

Dadke (2018) screened 107 dogs for thyroid hormone levels using BRIT-manufactured RIA kits designed for human samples, with some modifications for fT_4 estimation to improve tracer binding and protein removal. The mean serum concentrations of TT_4, TT_3, and fT_4 in healthy dogs were 29.67±1.43 nmol/l, 1.03±0.02 nmol/l, and 9.07±0.52 pmol/l, respectively. Significant differences were noted in the thyroid profiles of healthy, non-thyroidal illness, and hypothyroid dogs, providing reference data for assessing thyroid health in the Indian canine population.

Jayabhaye (2019) analysed the thyroid hormone profiles of 100 pigs in India using BRIT-manufactured RIA kits. The mean serum concentrations of TT_3, TT_4, and fT_4 in healthy pigs were 0.81±0.04 nmol/l, 41.58±1.23 nmol/l, and

5.31±0.18 pmol/l, respectively. While the kits were suitable for TT_3 and TT_4 estimation in swine, partial modifications were necessary for fT_4 measurement.

Galdhar *et al.* (2022) examined thyroid function in 192 hypothyroid dogs from MAFSU constituent colleges. Using RIA, they 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 intervals (25th to 75th percentile) were 0.88-1.51 nmol/l for TT_3, 15.70-35.29 nmol/l for TT_4, and 7.80-14.75 pmol/l for fT_4, with medians of 1.13 nmol/l, 24.54 nmol/l, and 10.00 pmol/l, respectively. This baseline data is valuable for identifying thyroid dysfunction in dogs.

Alone (2024) studied thyroid profile in dogs with special reference to assessment of canine TSH on a total of seventy-seven (n=77) dogs at Radio Isotope Laboratory, Mumbai Veterinary College, Parel, Mumbai-400012, (MAFSU). In the study estimation of Total triiodothyronine (TT_3), Total thyroxine (TT_4) and Free thyroxine (fT_4) was carried out using radioimmunoassay. The study reported significant changes in the concentration of TT_4 and fT_4 between healthy and hypothyroid dogs.

2.4 Therapeutic Management

2.4.1 Levothyroxine

Panciera *et al.* (1994) observed that starting doses of 0.02 mg/kg every 12 hours effectively restored euthyroid states in hypothyroid dogs. Adjustments based on serum T4 levels helped avoid under- or over-treatment.

Fenner *et al.* (1995) emphasizes that individualized dosing is critical, particularly in breeds like Greyhounds, which have naturally lower baseline T4 levels. Further, author recommends rechecking total T4 levels 4–6 hours post-administration 4 weeks after starting therapy. The target is to achieve T4 levels in the upper half of the reference range or slightly above.

Dixon *et al.* (2002) recommended measuring serum T4 concentrations 4–6 hours after dosing to evaluate peak levels and adjust therapy accordingly.

Feldman *et al.* (2014) stated that the oral bioavailability of levothyroxine in dogs is highly variable, ranging from 10–20%. It is significantly affected by food intake, with better absorption observed when the medication is given on an empty stomach. Monitoring peak serum levels 4–6 hours after dosing helps assess absorption and efficacy.

Van *et al.* (2014) Levothyroxine has variable bioavailability in dogs, influenced by factors such as gastrointestinal absorption and food intake. Administering the medication on an empty stomach improves absorption, but if given with food, higher doses may be necessary to maintain therapeutic levels. Plasma thyroxine concentrations reach a peak approximately 4–6 hours post-administration, with a half-life ranging between 12 to 16 hours depending on the formulation.

Fernandez and Seth (2016) asserted that the treatment of choice for hypothyroidism is synthetic sodium levothyroxine (L-thyroxine). L thyroxine has an oral bioavailability of greater than 50%, which is further decreased if administered with food, hence it is preferred to be administered on an empty stomach.

Papich *et al.* (2016) noted that food delays absorption but does not significantly reduce the overall bioavailability if consistent feeding schedules are maintained during treatment. Levothyroxine's elimination half-life in dogs is shorter (~12–16 hours) compared to humans. This pharmacokinetic profile explains the need for twice-daily dosing in many cases to maintain steady-state levels.

Heseltine (2019) mentioned that the standard initial dosage of levothyroxine for dogs is typically 0.02 mg/kg administered orally twice daily. In some cases, after stabilization and monitoring, dosing may be adjusted to once daily if clinical signs and thyroid hormone levels remain within the therapeutic range. For obese dogs, dosage calculation should be based on estimated lean body weight

to avoid overmedication. While twice-daily dosing ensures stable hormone levels, many dogs can transition to once-daily administration after initial stabilization. Monitoring through pre- and post-pill blood samples helps determine the adequacy of once-daily dosing. Adjustments are made based on thyroid hormone levels (total T4 or free T4) and clinical response

Nelson and Couto (2019) mentioned that synthetic levothyroxine is the treatment of choice for hypothyroidism. The initial dosage is 0.01 to 0.02 mg/kg body weight. The plasma half-life of sodium levothyroxine ranges from 9 to 14 hours and depends, in part, on the dosage and frequency of administration.

2.4.2 Response to Replacement Therapy

Bichsel *et al.* (1988) studied neurologic manifestations associated with hypothyroidism in four dogs. After thyroid hormone supplementation, resolution of neurologic abnormalities was complete in 2 dogs and partial in the other 2 dogs.

Jaggy *et al.* (1994) conducted a retrospective study on 29 dogs to examine the neurological manifestations of hypothyroidism. Following two months of levothyroxine treatment, 90.9% of dogs with lower motor neuron signs and 88.8% of those with vestibular signs showed recovery. Dogs with megaoesophagus and laryngeal paralysis improved within 4 to 5 months.

Panciera (1994) studied hypothyroidism in 66 dogs during 1987 to 1992 at department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin. Dogs were supplemented with 0.022 mg/kg, PO, q 12 h. Out of the dogs suffering from neuromuscular disorders vestibular nerve abnormalities resolved in 3 of 4 dogs, facial nerve abnormalities resolved in 2 dogs and function improved in 2 of 5 dogs, megaoesophagus resolved after 3 months of supplementation in 1 of 4 dogs and did not reoccur after cessation levothyroxine supplementation. 2 dogs with laryngeal paralysis did not showed improvement when only on levothyroxine supplementation.

Panciera (1997) asserted that response to treatment is frequently noted within 1 week of initiating treatment. Often, the first clinical sign noticed is that the

dog's activity level increases. Although peripheral neuropathies often improve rapidly, some may persist indefinitely. Normally, a lack of a response after 6 to 8 weeks should be reason to investigate reasons for treatment failure, such as another underlying medical condition.

Panciera (1999) stated that when a dog shows classic clinical signs, a subnormal serum T_4 level may be sufficient to make a tentative diagnosis and begin trial treatment with levothyroxine. However, if the dog has atypical signs, further testing is indicated.

McKeown (2002) studied hypothyroidism in a boxer dog with head tilt and facial nerve paralysis. Author mentioned that two weeks after the onset of clinical signs and initiation of treatment, the owner reported an improvement in the head tilt and drooping facial features, except when the dog panted.

Higgins *et al.* (2006) investigated central vestibular disease associated with hypothyroidism in ten dogs. Treatment with levothyroxine resulted in clinical improvement of vestibular ataxia, abnormal nystagmus, or paresis in all dogs. The median time from initiation of levothyroxine supplementation to improvement in clinical signs was 4 days (range, 2–7 days). Within 4 weeks, complete resolution of vestibular signs occurred in 9 dogs.

Fracassi and Tamborini (2011) studied hypothyroidism associated megaesophagus in a seven-year-old entire female German shepherd dog. The dog was treated with 20 µg/kg body weight levothyroxine administered orally every 12 hours. The dog showed complete resolution of the clinical and radiographic signs.

Srikala and Kumar (2011) studied hypothyroidism associated systemic and peripheral disorders in 42 dogs over the period spanning March 2009 to February 2011. Treatment with levothyroxine was administered to 13 of the dogs, resulting in noticeable improvements in physical activity after 30 days and complete recovery within 120 days.

Romão *et al.* (2012) in a case report on 9-year-old female dog presented at the School of Veterinary Medicine and Animal Science of Sao Paulo State

University – FMVZ Botucatu mentioned that the synthetic levothyroxine is the standard treatment for hypothyroidism. The initial dose is 0.02 mg/kg/day; this should be raised or decreased based on the clinical response and evolution of laboratorial tests results.

Bertalan *et al.* (2013) mentioned that response to thyroid supplementation is often rapid in dogs with polyneuropathy. Affected dogs may show improvement in 24 hours, with complete resolution occurring within 1 to 2 months.

Chaves *et al.* (2016) in a case report of a 7-year-old male canine, boxer dog referred to the Veterinary Medical Teaching Hospital of the UFSM stated that most dogs with neurological disorders associated with hypothyroidism will present partial or total improvement of clinical signs between two and four months, generally being observed improvement within the first week of treatment. Therefore, with appropriate treatment, hypothyroidism is a disease with an excellent prognosis.

Nelson and Couto (2019) reported that improvement in neurologic manifestations associated with hypothyroidism is usually evident within days of treatment initiation with levothyroxine. However, complete resolution of neurologic signs is unpredictable and may require treatment for 4 to 8 weeks or longer before it occurs.

De Oliveira *et al.* (2022) reported remarkable improvement, three days after initiating thyroid hormone replacement therapy, the neurological symptoms regressed, and the dog became more active and responsive in a case of hypothyroid-related neuropathy in a 4-year-old intact male Dogo Argentino weighing 64 kg.

Materials &Methods

3. MATERIALS AND METHODS

The present study entitled '**Studies on hypothyroidism in dogs with special reference to neuromuscular disorders'** was carried out on a total of twenty-eight (n=28) 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

The present study was initiated after permission from the Board of Studies in Veterinary Clinical Medicine, Ethics and Jurisprudence, Resolution no: VCM/5/2024. Dated: 14/06/2024, Ethics Committee No.- IEC-VCR/sub-committee/01/2024 IEC-VCR Dated: 28/12/2024 and IBSC no. MVC/IBSC/02/2024 Dated: 03/05/2024.

3.2 Design of the study

The study included two distinct groups: apparently healthy dogs (Group I) and dogs with hypothyroidism associated neuromuscular disorders (Group II). The tentative diagnosis of hypothyroidism was based on the dog's history and clinical symptoms. However, the condition was confirmed through thyroid hormone assessment. An extensive evaluation was conducted on the day of presentation in dogs with hypothyroidism associated neuromuscular disorders which involved detailed clinical and neurological examination; haemato-biochemical assessments to rule out associated conditions and non-thyroidal illness if any. The status of hypothyroidism in dogs was studied with respect to age, breed, gender, and body weight. The affected dogs were subjected to treatment and the efficacy of therapy was monitored.

3.3 Selection of dogs

In the present study, a total of twenty-eight (n=28) dogs were selected from different breeds in Mumbai. All dogs were included with due consent of the owner (Appendix 1). The dogs included in the study were from TVCC (Teaching Veterinary Clinical Complex), Mumbai Veterinary College, Mumbai, Bai Sakarbai Dinshaw Petit Hospital for Animals (BSDPHA), Parel, Mumbai, and the referred cases by field veterinarians in Mumbai. The selected dogs were grouped as apparently healthy dogs (Group I; n=7) and dogs with neuromuscular disorders associated with hypothyroidism (Group II; n=21).

3.3.1 Selection of healthy dogs

Seven apparently healthy dogs (n=7) were screened for thyroid profile and enrolled in the study for comparison.

3.3.2 Criteria for the enrollment of hypothyroid dogs

Dogs of either sex, all age groups and any breed including nondescript dogs showing signs of neuromuscular disorders (viz. ataxic gait, foot dragging or knuckling, paraparesis, tetraparesis, head tilt or leaning, loss of balance, facial asymmetry, regurgitation, etc.) or undergoing treatment for it were screened for thyroid profile and enrolled in the study if found hypothyroid by thyroid profile (TT_3, TT_4 and fT_4).

Dogs with history of previous thyroid supplementation and concomitant drug administration which interfere thyroid function (viz. steroid, methimazole and L-thyroxine, perchlorate or iodine therapy, sulphonamide, etc.) were excluded.

The summary of the selected healthy dogs and hypothyroid dogs are shown in Table 3.1 and Table 3.2 respectively.

3.4 Diagnosis

3.4.1 Anamnesis

On presentation of the cases history was taken regarding age, sex, breed, deworming, vaccination, appetite and present complaints. The pet owners were also enquired about any ongoing medications or recent treatment being done. The suspected cases then were subjected to clinical and laboratory examination.

Table 3.1 Summary details of healthy dogs(n=7)

Sr. No.	Sample Code	Age (Yr.)	Breed	Sex	Body Weight (Kg)
1	H-1	7.00	ND	Female	16.40
2	H-2	8.50	ND	Male	28.00
3	H-3	4.00	ND	Female	14.00
4	H-4	9.00	ND	Female	19.50
5	H-7	11.00	Indian Spitz	Male	11.00
6	H-8	3.00	ND	Male	21.70
7	H-9	10.00	Labrador Retriever	Male	32.00
Mean		7.50			20.37
SD		3.01			7.53
SE		1.14			2.85
Mean ± SE		7.50 ± 1.14			20.37 ± 2.85

Table 3.2 Summary details of hypothyroid dogs (n=21)

Sr. No.	Sample Code	Age (Yr.)	Breed	Sex	Body Weight (Kg)
1	D-1	5	Labrador Retriever	Female	28.00
2	D-2	11	ND	Female	10.30
3	D-3	11	Labrador Retriever	Female	32.00
4	D-4	8	Labrador Retriever	Female	29.00
5	D-5	2.5	Pakistani Bully	Male	29.00
6	D-6	13	Shitzu	Male	8.25
7	D-7	2	Cocker Spaniel	Female	11.40
8	D-8	2	Golden Retriever	Male	26.00
9	D-9	8	Labrador Retriever	Female	24.50
10	D-10	10	ND	Female	21.20
11	D-11	6	Cavalier K. C.	Male	6.60
12	D-12	6	German Shephard	Male	16.50
13	D-13	7.5	Doberman	Male	29.40
14	D-14	7	Rottweiller	Male	40.00
15	D-15	7	Labrador Retriever	Female	45.00
16	D-18	8	ND	Male	11.50
17	D-19	2	Lhasa	Female	5.40
18	D-20	8.5	Lhasa	Female	11.00
19	D-21	4	ND	Female	13.30
20	D-22	8	ND	Male	22.00
21	D-24	7	Boxer	Male	26.00
Mean	--	6.83	--	--	21.26
SD	--	3.11	--	--	11.08
SE	--	0.68	--	--	2.42
Mean ± SE	--	6.83 ± 0.68	--	--	21.26 ± 2.42

3.4.2 Clinical and neurological examination

After history taking all the dogs were examined clinically for hydration status, heart rate (/min), respiration rate (/min), rectal temperature(°F), and auscultation of lungs and heart was carried out for abnormal lung sounds, alteration in rate and rhythm of heart beat and evidence of murmurs.

The dogs were subjected to thorough neuromuscular examination including

mentation, gait analysis, postural reactions, spinal reflexes, cranial nerve examination and muscle tone and strength analysis (Plate 3.1, 3.2 and 3.3). The observations recorded were documented in the examination sheet prepared (Appendix 2).

The facial nerve paresis dogs were categorized into various classes of severity based on Botman and Jongkees Scale (Botman and Jongkees, 1995) for human facial nerve function grading. The scale was partially modified according to the need of canine medicine and is mentioned in table

Table 3.3 Modified Botman and Jongkees Scale

Class	Signification
0	Normal facial activity: symmetrical ear, eyelid, and lip movements at rest and during stimulation (e.g., vocalization or food anticipation).
I	Mild paresis: normal at rest, mild asymmetry during actions like barking, blinking, or ear movements; partial ability to close the eyelids,
II	Moderate paresis: asymmetry noticeable at rest; reduced ear and lip movements; incomplete eyelid closure (lagophthalmos).
III	Severe paralysis: marked asymmetry at rest; minimal to absent voluntary ear, lip, and eyelid movements; drooping of lips and ears on the affected side.
IV	Total paralysis: complete loss of voluntary facial movements on the affected side; absent blink reflex; severe drooping of lips and ears; possible muscle contracture or atrophy over time.

3.4.3 Blood Collection and Storage

About 6 ml of blood sample was collected from each dog initially on the day of presentation and then next time again to monitor the response to therapy. Immediately after collection of a blood sample, 2 ml was transferred to EDTA vials, and the remaining 4 ml was kept for harvesting of serum for estimation of sero-biochemistry and thyroid profile. Serum samples for thyroid profile estimation were stored at -20 °C until the time of analysis.

(a) Hematological analysis

Immediately after the collection of blood, 2.0 ml of blood was transferred to 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. Total Erythrocyte Count (TEC, $\times 10^6$/ µl)
3. Packed Cell Volume (PCV, %)
4. Mean Corpuscular volume (MCV, fl)
5. Mean Corpuscular Hemoglobin (MCH, pg)
6. Mean Corpuscular Hemoglobin Concentration (MCHC, gm/dl)
7. Total Leucocyte Count (TLC, $\times 10^3$/ µl)
8. Neutrophils (N, %)
9. Lymphocyte (L, %)
10. Basophils (B, %)
11. Monocyte (M, %)
12. Eosinophils (E, %)
13. Platelets (PLT, $\times 10^3$/µl)

(b) <u>Biochemical Analysis</u>

Out of 6 ml collected blood sample remaining 4 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 3000 rpm for 10 minutes. The clear supernatant (serum) was aspirated using pipettes into sterile serum tubes and analyzed for biochemical parameters. After biochemical analysis, 4 aliquots of the remaining serum sample were made and stored at -20ºC until the estimation of thyroid hormones. Serum biochemical parameters were analyzed by using Em 200 which uses the principle of spectrophotometry and Bars law. The following parameters were analyzed:

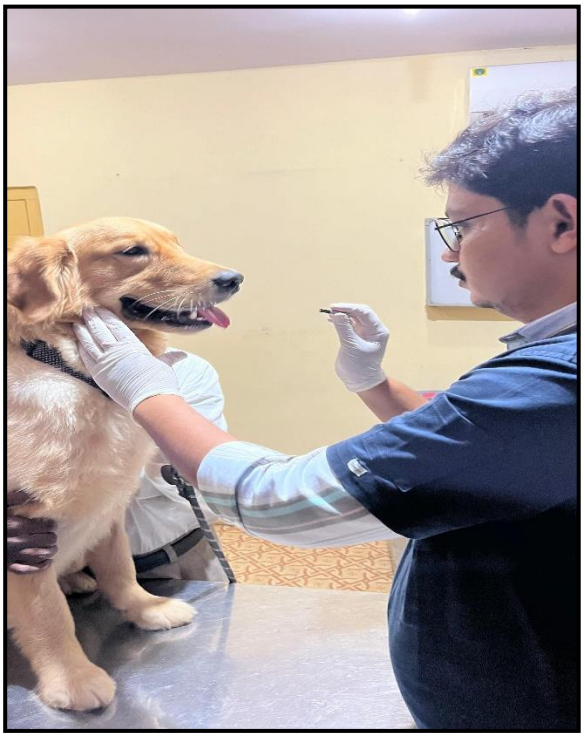


Plate 3.1 Neurological Examination

Plate 3.2 Neurological Examination

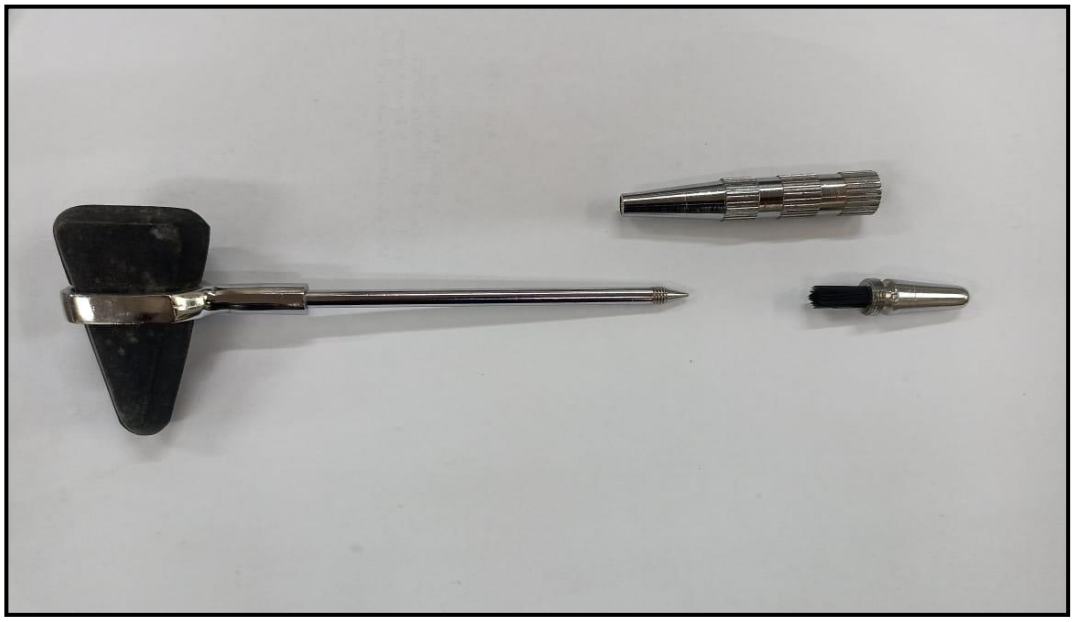


Plate 3.3 Pleximeter

Plate 3.4 Setting up hormonal assay

(i) 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) and Globulin (G, gm/dl).

(ii) Kidney profile tests

Blood urea nitrogen (BUN, mg/dl)

Creatinine (mg/dl)

Sodium (mEq/L), Potassium (mEq/L) and Chloride (mEq/L).

Calcium (mg/dl) and phosphorus (mg/dl)

(iii) Lipid Profile

Serum cholesterol (mg/dl)

Serum triglycerides (mg/dl)

(c) Thyroid profile

(i) Estimation of Total Triiodothyronine (TT_3), Thyroxine (TT_4) and free Thyroxine (fT_4) using Radio Immuno Assay (RIA)

Thyroid hormones (fT_4, TT_4 and TT_3) were estimated using RIA and the procedure was carried out at the Radio Isotope Laboratory, Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai, and Radiation Medicine Centre, BARC, Mumbai. Commercial RIA kits for human purposes, manufactured by Immunotech s.r.o., Radiova 1122/1, 102 00 Prague 10, Czech Republic, Beckman Coulter Company, were procured for thyroid profile estimation in dogs.The details of the kits procured for thyroid profiling are presented in Table 3.4 and RIA was undertaken as per safety guidelines recommended by Radiological Safety Officer. The representative image of setting up an assay is presented in plate 3.4.

Table 3.4 Details of RIA kits procured

Sr. No.	RIA Kit Code No.	Description of kits
1	IM1699 – Total T 3 BECKMAN COULTER	Competitive Assay with ^{125}I-labelled T_3 as a tracer, monoclonal antibody coated tubes (Plate 3.5)
2	IM1447 – Total T 4 BECKMAN COULTER	Competitive Assay with ^{125}I-labelled TT_4 as a tracer, monoclonal antibody coated tubes (Plate 3.6)
3	IM1363 – Free T 4 BECKMAN COULTER	Competitive Assay with ^{125}I-labelled monoclonal antibody, as a tracer (Plate 3.7)

Total Triiodothyronine (TT_3, nmol/L), Total Thyroxin (TT_4, nmol/L) and free Thyroxine (fT4, pmol/L) concentrations were measured using the Genesys Geni series, a NaI-based six-well detector by Laboratory Technologies Inc (Plate 3.9). The standard procedure outlined by the manufacturers as mentioned in the table 3.5, Table 3.6 and table 3.7 for Total Triiodothyronine (TT_3, nmol/L), Total Thyroxin (TT_4, nmol/L) and free Thyroxine (fT4, pmol/L) respectively was followed. The results were obtained based on the standard graph generated based on % B/Bo.

Plate 3.5 Total T_3 RIA kit setup

Plate 3.6 Total T_4 RIA kit setup

Plate 3.7 Free T_4 RIA kit setup

Plate 3.8 Canine TSH ELISA kit setup

Plate 3.9 Single well and Six well Gamma Counter

Plate 3.10 ELISA Reader

Table 3.5 Assay Flow Chart of Total Triiodothyronine (TT_3)

1	2	3
Tube No.	**Standards/ Samples (µl)**	**Tracer**
C0	25 µl	200 µl
C1	25 µl	
C2	25 µl	
C3	25 µl	
C4	25 µl	
C5	25 µl	
Control A	25 µl	
Recovery	25 µl (12.5 µl STD + 12.5 µl STD)	
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	--
	Tracer	200µl	200µl	200µl
Mix (Vortex gently 1-2 sec)				
2. Incubation: Incubate for 1 hour with shaking at 18-25ºC (≥280 rpm)				
3. Counting: Aspirate carefully the contents of tubes (expect two tubes for total the count). Count bound CPM (B) and total CPM (T) for 1 min.				

*Procedure was undertaken as per Steps.

Table 3.6 Assay Flow Chart of Total Thyroxine (TT_4)

1	2	3
Tube No.	**Standards/ Samples (µl)**	**Tracer**
C0	20 µl	500 µl
C1	20 µl	
C2	20 µl	
C3	20 µl	
C4	20 µl	
C5	20 µl	
Control A	20 µl	
Recovery	20 µl (10 µl STD + 10 µl STD)	
Samples	20 µl	

Steps		**Calibrators and control**	**Serum**	**Tubes for total count**
1. Additions To antibody-coated tubes add successively	calibrators or control, serum sample	20µl	20µl	--
	Tracer	500µl	500µl	500µl
Mix (Vortex gently 1-2 sec)				
2. Incubation: Incubate for 1 hour with shaking at 18-25ºC (≥280 rpm)				
3.Counting: Aspirate carefully the contents of tubes (expect two tubes for total the count). Count bound CPM (B) and total CPM (T) for 1 min.				

*Procedure was undertaken as per Steps.

Table 3.7 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 (12.5 µl STD + 12.5 µl STD)		
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	--
	Tracer	400µl	400µl	400µl
	Ligand	100 µl	100µl	--
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 total count). Count bound CPM (B) and total CPM (T) for 1 min.				

*Procedure was undertaken as per Steps.

(ii) <u>Estimation of Thyroid Stimulating Hormone (TSH) using Canine specific ELISA kit</u>

Thyroid Stimulating Hormone (TSH) concentrations were estimated using Enzyme Linked Immunosorbent Assay (ELISA) and the procedure was carried out at the Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai. Commercial Canine TSH specific ELISA kit manufactured by Wuhan Fine Biotech Co., Ltd was procured. Details of the kit are as mentioned in the Table 3.8.

Table 3.8 Details of the ELISA kit procured

Sr. No.	ELISA Kit Code No.	Description of kit
1	ECA0025-Canine TSH (Thyroid Stimulating Hormone)	Competitive-ELISA (Plate 3.8)

Assay Procedure

Step1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells.

Step2: Add 50ul Standard or Sample into each well. Immediately add 50ul Biotin-labeled Antibody into each well, gently tap the plate for 1min to ensure thorough mixing then static incubate for 45 minutes at 37°C. Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul SABC working solution into each well, seal the plate and static incubate for 30 minutes at 37°C. Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.) (Plate 3.11 to 3.14).

Step 5: Add 50ul stop solution. Read at 450nm immediately(Plate 3.10 – ELISA Reader).

3.5 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**.**

3.6 Therapeutic Management

The primary goal of therapeutic management is to normalize thyroid hormone

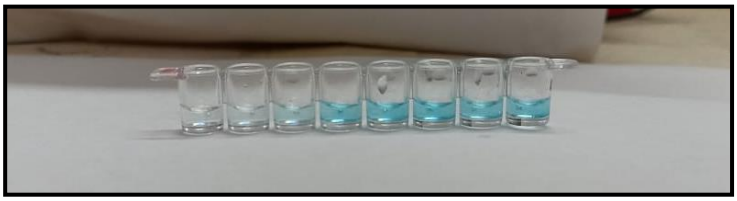


Plate 3.11 ELISA Standards before addition of stop solution

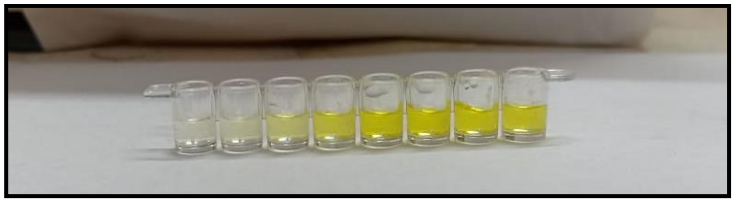


Plate 3.12 ELISA Standards after addition of stop solution

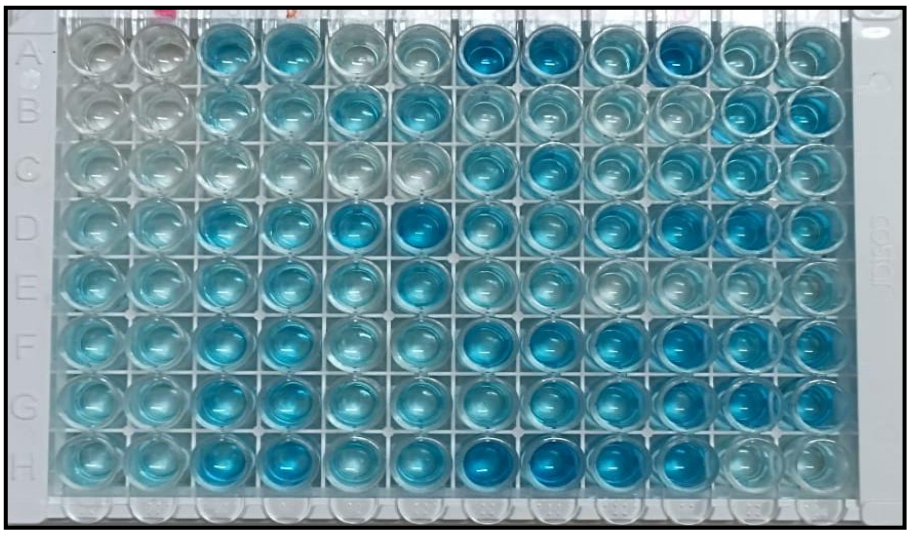


Plate 3.13 ELISA plate before addition of stop solution

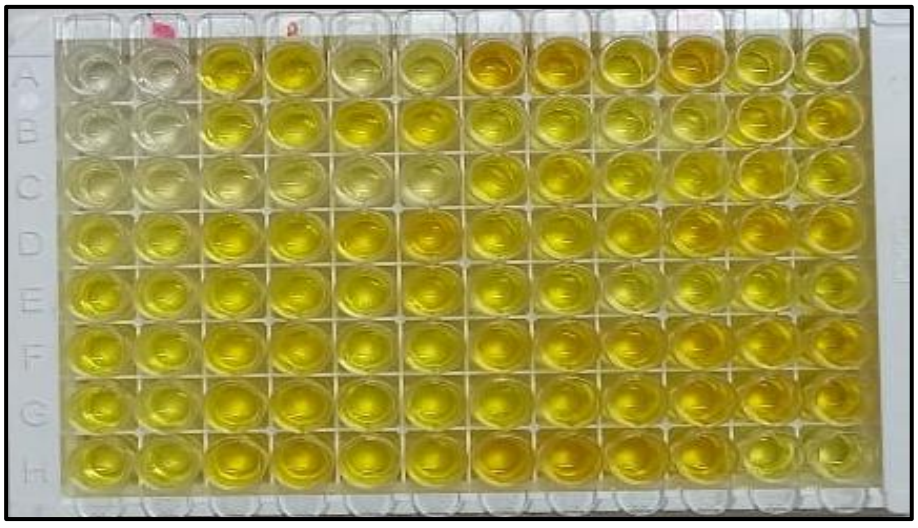


Plate 3.14 ELISA plate after addition of stop solution

levels and alleviate both systemic and neuromuscular clinical signs associated with hypothyroidism. Treatment protocols were developed based on the severity of clinical signs, diagnostic findings, and the individual dog's response to therapy.

Levothyroxine sodium, a synthetic form of thyroxine (T_4), was used as the primary treatment. The initial dose varied based on the signs and laboratory reports ranging from 5 mcg/kg body weight once daily to 10 mcg/kg body weight twice daily. Initially the therapy was monitored by assessment of thyroid profile after a week and then depending upon clinical recovery in individual case.

Along with the primary treatment supportive therapy was also administered to the patients whenever required which included intravenous fluids, antacids, antiemetics and multivitamins.

3.7 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 (2009).

Results & Discussion

4. RESULTS AND DISCUSSION

The current research work entitled '**Studies on hypothyroidism in dogs with special reference to neuromuscular disorders**' was carried out 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.

The study included a total of twenty-eight (n=28) dogs which were grouped as apparently healthy dogs (n=7) (Group I) and the other included dogs with hypothyroidism associated neuromuscular disorders (n=21) (Group II) (henceforth mentioned as hypothyroid group).

4.1 Anamnesis of hypothyroid dogs

In hypothyroid dogs' history about vaccination, deworming and diet was collected and was as presented in Table 4.1.

All the hypothyroid dogs (n=21/21) enrolled in the study were complete with their status of vaccination and deworming. The diet of dogs was categorised as homemade, commercial and mixed including both homemade and commercial feed. In the present study homemade, commercial and mixed feeding history was found in 33.33% (n=7/21), 28.57% (n=6/21) and 38.10% (n=8/21) hypothyroid dogs respectively as depicted in Figure 4.1.

4.2 Age wise distribution of hypothyroid dogs

The hypothyroid (Group II) dogs were categorized into different age groups (histogram auto group- Excel) as mentioned in the Table 4.2 and Figure 4.2.

The age groups were ≤ 2 years, > 2 years to ≤ 4.75 years, > 4.75 years to ≤ 7.5 years, > 7.5 years to ≤ 10.25 years and > 10.25 years and had three, two, seven, six and three dogs enrolled in them respectively.

Table 4.1 Anamnesis of healthy dogs (n=21)

Sr. No.	Sample Code	Vaccination Status	Deworming Status	Diet
1	D-1	Complete	Complete	Homemade
2	D-2	Complete	Complete	Mixed
3	D-3	Complete	Complete	Mixed
4	D-4	Complete	Complete	Homemade
5	D-5	Complete	Complete	Mixed
6	D-6	Complete	Complete	Commercial
7	D-7	Complete	Complete	Homemade
8	D-8	Complete	Complete	Commercial
9	D-9	Complete	Complete	Mixed
10	D-10	Complete	Complete	Mixed
11	D-11	Complete	Complete	Commercial
12	D-12	Complete	Complete	Mixed
13	D-13	Complete	Complete	Homemade
14	D-14	Complete	Complete	Mixed
15	D-15	Complete	Complete	Commercial
16	D-18	Complete	Complete	Homemade
17	D-19	Complete	Complete	Homemade
18	D-20	Complete	Complete	Commercial
19	D-21	Complete	Complete	Commercial
20	D-22	Complete	Complete	Mixed
21	D-24	Complete	Complete	Homemade

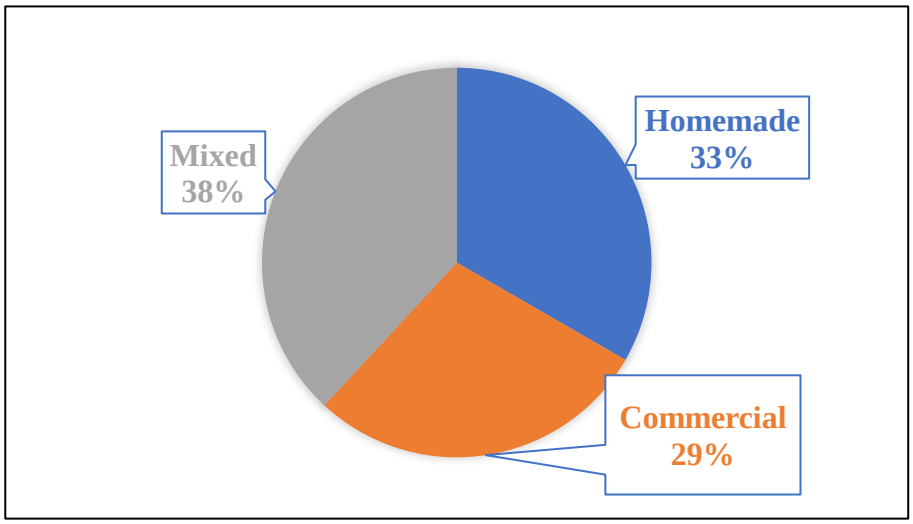


Figure- 4.1 Diet-wise distribution of hypothyroid dog

Table 4.2 Age-wise distribution of hypothyroid dogs (Years) (n=21)

Sr. No.	Age Group (Year)	No. of dogs	% of dogs
1	≤ 2	3	14.29
2	> 2 to ≤ 4.75	2	9.52
3	> 4.75 to ≤ 7.5	7	33.33
4	> 7.5 to ≤ 10.25	6	28.57
5	> 10.25	3	14.29
	Total	21	100

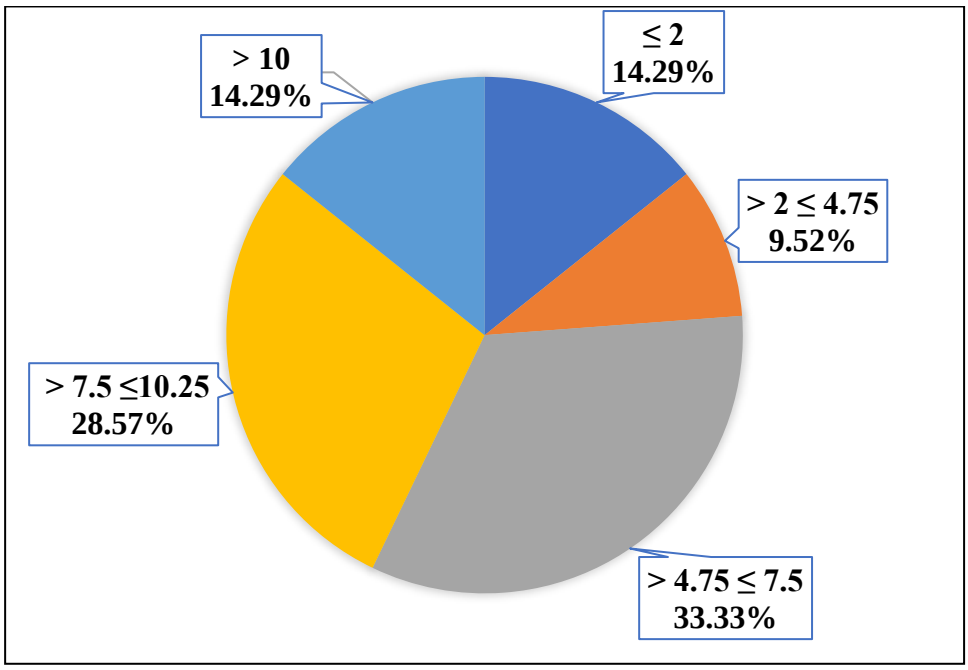


Figure- 4.2 Age-wise distribution of hypothyroid dogs (year) (n=21)

The distribution of the dogs based on age reveals that highest number of affected dogs (33.33%) (n=7/21) falls in the age range of > 4.75 to ≤ 7.5 years, followed by > 7.5 to ≤ 10.25 (28.57%) (n=6/21), ≤ 2 (14.29%) (n=3/21), > 10.25 (14.29%) (n=3/21) and > 2 to ≤ 4.75 (9.52%) (n=2/21) respectively. The overall Mean ± SE of age in hypothyroid group dogs was recorded as 6.83 ± 0.68 years. Panceira (1994) reported Similar findings with mean age of 7.2 years at diagnosis of hypothyroidism. Results on age at diagnosis of hypothyroidism in the present study are in agreement with Ettinger and Feldman (2000), who cited the average

age at diagnosis of hypothyroidism as approximately 7 years and ranges between 0.5 to 15 years. While Dixon (2001) and Kour *et al*. (2020) reported hypothyroidism as a disorder of middle-aged dogs. Similar findings were also reported by Alone (2024), recording 6.48 years as the mean age of hypothyroid dogs. Further, Raja *et al.* (2021) reported higher prevalence of hypothyroidism in dogs above 5 years of age which is also in accordance with findings of present study.

4.3 Body weight wise distribution of hypothyroid dogs

The hypothyroid dogs were categorised across different body weight groups (histogram auto group- Excel) as mentioned in Table 4.3 and Figure 4.3. The body weight groups 5.4 Kg, > 5.4 Kg to ≤ 15.3 Kg, > 15.3 Kg to ≤ 25.2 Kg and > 35.1 Kg had one, seven, four, seven and two dogs enrolled in them respectively.

Table 4.3 Body weight wise distribution of hypothyroid dogs (Kg) (n=21)

Sr. No.	Body Weight (Kg)	No. of dogs	% of dogs
1	5.4	1	4.76
2	> 5.4 to ≤ 15.3	7	33.33
3	> 15.3 to ≤ 25.2	4	19.05
4	> 25.2 to ≤ 35.1	7	33.33
5	> 35.1	2	9.52
	Total	21	100

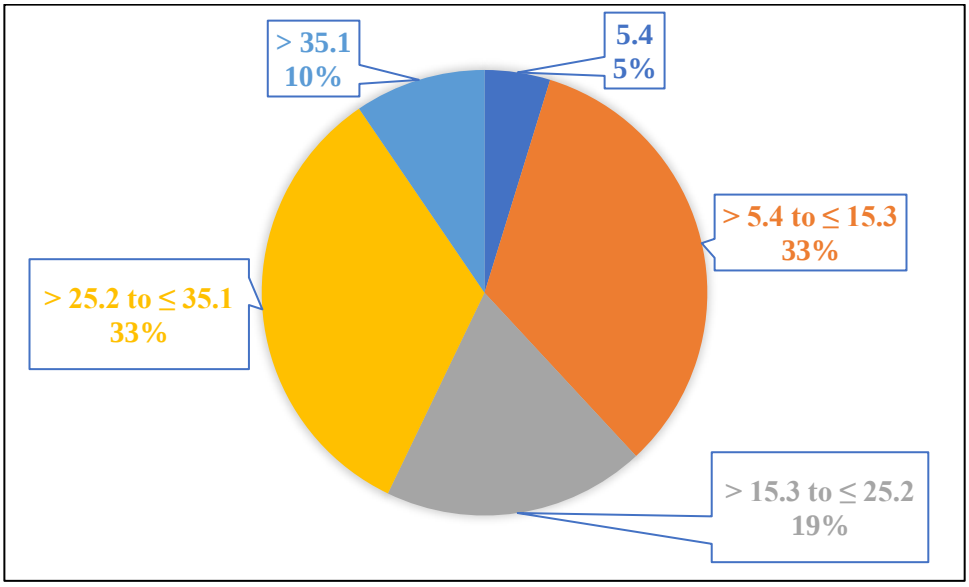


Figure- 4.3 Body weight wise distribution of hypothyroid dogs (Kg) (n=21)

The present study revealed highest number of hypothyroid dogs in the body weight (Kg) group > 25.2 to ≤ 35.1 and > 5.4 to ≤ 15.3 (n=7/21 each) followed by > 15.3 to ≤ 25.2 (n=4/21), > 35.1(n=2/21) and 5.4 (n=1/21) respectively. The mean age of hypothyroid dogs was found 21.26 ± 2.42 kg.

The current study recorded 20 Kg to 30 Kg body weight in nine (n=9/21; 42.86%) and above 30 Kg body weight in four (n=4/21; 19.05%) dogs. Similar findings were recorded by Nachreiner *et al.* (2002); Guglielmini *et al.* (2019), Roopali (2020) and Naveen (2024). Nachreiner *et al.* (2002) reported a significant correlation between body weight and hypothyroidism, emphasizing the need to assess thyroid function in obese dogs.

4.4 Breed wise distribution of hypothyroid dogs

The distribution of hypothyroid dogs (Group II) across different breeds is presented in Table 4.4 and Figure 4.4.

In the current study, the most commonly affected breeds were Labrador Retrievers and non-descript dogs, each accounting for 23.81% (n=5/21). These were followed by Lhasa Apso (9.52%, n=2/21), and some breeds with equal frequency (4.76%, n=1/21), including Doberman, Rottweiler, German Shepherd,

Golden Retriever, Shih Tzu, Pakistani Bully, Cocker Spaniel, Cavalier King Charles Spaniel, and Boxer. Similar observations regarding Labrador Retrievers were reported by Gulzar *et al.* (2014) and Raja *et al.* (2021). However, Kour *et al.* (2020) noted the highest prevalence in Labrador Retrievers but the lowest in mongrels (mixed-breed dogs). The increased occurrence in Labrador Retrievers and non-descript dogs in this study could be attributed to their higher population in the area due to owner preference.

Table 4.4 Breed-wise distribution of hypothyroid dogs (n=21)

Sr. No.	Breed	No. of animals	% of animals
1	Labrador Retriever	5	23.81
2	ND	5	23.81
3	Lhasa	2	9.52
4	Doberman	1	4.76
5	Rottweiller	1	4.76
6	German Shephard	1	4.76
7	Golden Retriever	1	4.76
8	Shitzu	1	4.76
9	Pakistani Bully	1	4.76
10	Cocker Spaniel	1	4.76
11	Cavalier K. C.	1	4.76
12	Boxer	1	4.76
Total		21	100

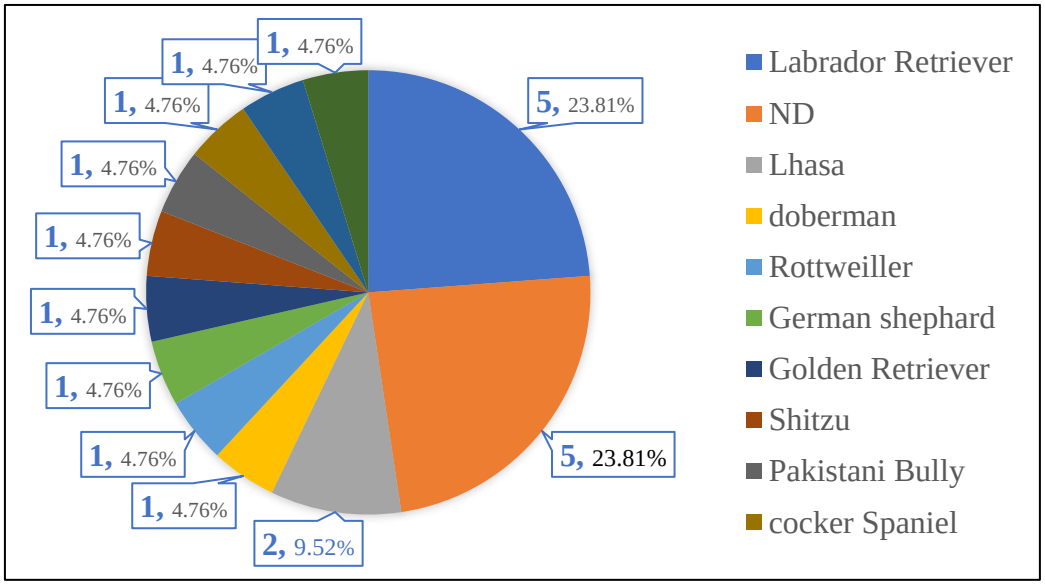


Figure 4.4 Breed-wise distribution of hypothyroid dogs (n=21)

4.5 Sex-wise distribution of hypothyroid dogs

The Sex-wise distribution of hypothyroid dogs (Group II) is presented in Table 4.5 and Figure 4.5.

Present study included 10 (n=10/21) males and 11 (n=11/21) females out off total 21 dogs.

Table 4.5 Sex-wise distribution of hypothyroid dogs (n=21)

Sr. No.	Sex	No. of animals	% of animals
1	Male	10	47.62
2	Female	11	52.38
Total		21	100

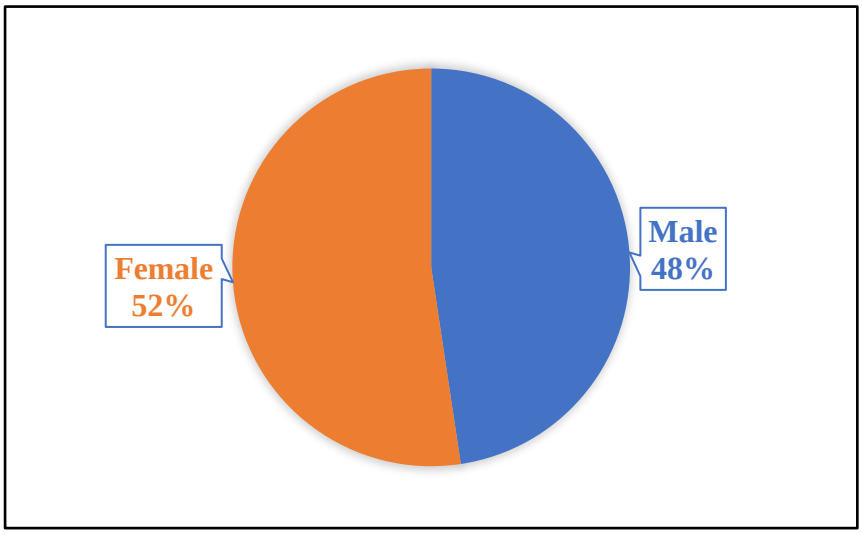


Figure 4.5 Sex-wise distribution of hypothyroid dogs (n=21)

In the present study, females showed a higher but not significant prevalence of hypothyroidism (52.38%; n=11/21) compared to males (47.62%; n=10/21). Bell (2003) and Raja *et al.* (2021) also observed non-noteworthy gender difference in susceptibility to hypothyroidism and the findings of current study are in accordance with these findings. In contrast Gulzar *et al.* (2014) and Guglielmini *et al.* (2019) reported higher susceptibility of females compared to males and studies by Kour *et al.* (2020) and Ghallab *et al.* (2021) identified a greater occurrence in males.

In the present study nine (n=9) males were intact and one (n=1) was castrated whereas four (n=4) females were intact and seven (n=7) were spayed. The findings with respect to males and females are in accordance to Kour *et al.* (2020) and Panceira (1994) respectively. Panceira (1994) stated that castrated males and spayed females are more predisposed to hypothyroidism'

4.6 Neurological Signs in Hypothyroid dogs

Neurological signs recorded in hypothyroid dogs (Group II) in the present study are presented in Table 4.6 and Figure 4.6.

The neurological signs documented in the current study were nystagmus (n=1/21, 4.76%), seizures (n=2/21, 9.52%), proprioceptive ataxia (n=3/21, 14.29%), head tilt (n=3/21, 14.29%), paraparesis (n=3/21, 14.29%), facial asymmetry (n=5/21, 23.81%), regurgitation due to megaesophagus (n=5/21, 23.81%) and tetraparesis (n=6/21, 28.57%).

Table 4.6 Neurological signs in hypothyroid dogs

Sr. No.	Neurological Sign	No. of Dogs	% of Dogs
1	Nystagmus	1	4.76
2	Seizures	2	9.52
3	Proprioceptive Ataxia	3	14.29
4	Head tilt	3	14.29
5	Paraparesis	3	14.29
6	Facial asymmetry	5	23.81
7	Regurgitation/ megaoesophagus	5	23.81
8	Tetraparesis	6	28.57

The study documented neurological signs, including proprioceptive ataxia, paraparesis, tetraparesis, head tilt, nystagmus, facial asymmetry, seizures, and regurgitation (associated with megaesophagus). Similar neurological manifestations have been reported in studies by Bichsel *et al.* (1988), Jaggy *et al.* (1994), Panciera (1994), McKeown (2002), Vitale and Olby (2007), Suraniti *et al.* (2008), Fracassi and Tamborini (2011), Srikala and Kumar (2011), and De Oliveira *et al.* (2022).

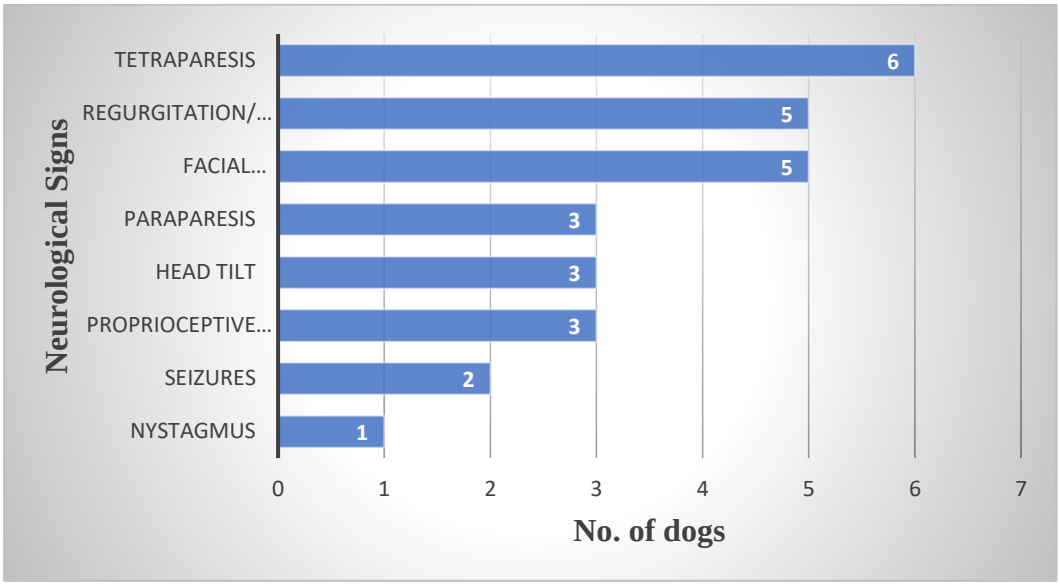


Figure 4.6 Neurological signs in hypothyroid dogs

The study recorded clinical signs associated with hypothyroidism in dogs only in five cases (n=5/21), indicating that neuromuscular signs in association with hypothyroidism could be the sole manifestation. These signs included lethargy and obesity in one dog (n=1/21) with facial paresis, lethargy and a rough hair coat in another dog (n=1/21) with megaesophagus, a sparse hair coat in one dog (n=1/21) with facial paresis and tetraparesis, a rough hair coat in one dog (n=1/21) with head tilt, and a skin infection in another dog (n=1/21) also exhibiting head tilt.

Although the exact pathophysiology of hypothyroidism associated neuromuscular disorders has not been fully elucidated there are various hypotheses proposed. According to Ettinger and Feldman (2000), mucopolysaccharide accumulation, impaired axonal transport, or atherosclerosis could be the possible reason for the hypothyroidism associated neurological signs. While, Nelson and Couto (2019), mentioned that neurological manifestations linked to hypothyroidism could be attributed to segmental demyelination and axonopathy, affecting both the central and peripheral nervous systems and could be the sole manifestation of the condition without the typical signs of hypothyroidism.

Neuromuscular disorders associated with hypothyroidism may occur with or without the characteristic cutaneous signs of the condition. The neurological manifestations of hypothyroidism are often linked to myxedematous or

atherosclerotic changes resulting from hyperlipidemia (Suraniti *et al.* 2008; Romão *et al.* 2012). Although the precise mechanism of hypothyroid neuropathy remains unclear, it is thought to involve deficits in energy metabolism, leading to disruptions in axonal transport and Schwann cell dysfunction. Additionally, cranial nerve involvement may be attributed to the compression caused by myxedematous deposits in nerves or surrounding tissues in the head and neck. (Jaggy *et al.* (1994); Panciera (1994); Cuddon (2002); Daminet *et al.* (2003); Fors (2007); Romão *et al.,* 2012). Reduced vascular perfusion to the inner ear may contribute to the development of facial neuropathies in dogs with hypothyroidism(Vitale and Olby 2007).

4.7 Comparison between apparently healthy dogs (Group I) and dogs with hypothyroidism associated neuromuscular disorders (Group II).

4.7.1 Clinical Parameters

The Mean ± SE values for the clinical parameters including heart rate (beats/min), respiratory rate (breaths/min) and rectal temperature (^{0}F) are presented in Table 4.7.

The present study reported mean count of heart rate, respiration rate and rectal temperature as 108.57 ± 5.11 (beats/min), 24.86 ± 1.82 (breaths/min) and 101.36 ± 0.44 (^{0}F) in healthy group dogs, and 93.62 ± 3.78 (beats/min), 26.43 ± 1.04 (breaths/min) and 101.01 ± 0.24 (^{0}F) for hypothyroid dogs respectively.

Table 4.7 Comparative analysis of clinical parameters in apparently healthy (n=7) (Group I) and dogs with hypothyroidism associated neuromuscular disorders (n=21) (Group II)

Sr. No.	Parameter	Healthy Mean ± SE	Hypothyroid Mean ± SE	t Value
1	Heart Rate (beats/min)	108.57 ± 5.11	93.62 ± 3.78	2.07*
2	Respiration Rate (breaths/min)	24.86 ± 1.82	26.43 ± 1.04	0.75
3	Rectal Temp (^{0}F)	101.36 ± 0.44	101.01 ± 0.24	0.70
*=p≤0.05 (Table t= 2.05)				

The study reported significant ($p \leq 0.05$) difference in Mean ± SE values of heart rate between Group I and Group II dogs which are 108.57 ± 5.11 and 93.62 ± 3.78 respectively. The heart rate in group I (beats/min) ranged from 90 to 132 whereas in Group II it ranged from 52 (beats/min) to 120 (beats/min). While there was non-significant ($p \leq 0.05$) difference in respiratory rate and rectal body temperature between the groups.

Stephan (2003) reported similar findings in 10 hypothyroid dogs with heart rate ranging from 70 to 120 (beats/min). Panciera (1994), Gaalova *et al.* (2008), Kour *et al.* (2021) and Naveen (2024) all reported significant bradycardia and bradypnea in hypothyroid dogs. Thyroid hormones have positive ionotropic and chronotropic effect on the heart (Nelson and Couto 2019) and therefore, comparatively lower heart rate in the hypothyroid group could be attributed to reduced ionotropic and chronotropic effect due to hypothyroidism.

4.7.2 Hematological Parameters

The Mean ± SE values for the hematological parameters of Group I and Group II are presented in Table 4.8. and (Appendix 3 and 4).

The Mean ± SE values reported in the current study in healthy dogs for hemoglobin, total erythrocyte count and packed cell volume were 14.77 ± 0.90 (gm%), 6.55 ± 0.40 ($\times 10^6$/µl) and 43.23 ± 2.63(%) whereas, the mean concentrations of these parameters in hypothyroid dogs were 11.11 ± 0.58 (gm%), 4.80 ± 0.25 ($\times 10^6$/µl) and 32.29 ± 1.71 (%), respectively.

The recorded mean concentrations of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in healthy dogs were 66.23 ± 1.51 (fl), 22.61 ± 0.44 (pg) and 34.17 ± 0.30 (g/dl) while, hypothyroid dogs had Mean ± SE values of 67.45 ± 0.67 (fl), 23.27 ± 0.30 (pg) and 34.54 ± 0.29 (g/dl) respectively.

Total leucocyte count in healthy and hypothyroid dogs was 13.16 ± 1.68 ($\times 10^3$/ µl) and 17.66 ± 2.75 ($\times 10^3$/ µl) respectively and differential counts of neutrophils, eosinophils, lymphocyte, monocyte, and basophils for healthy dogs were 68.57 ± 3.34 %, 3.29 ± 0.68 %, 27.00 ± 3.44 %, 1.14 ± 0.14 % and 0 %,

while these were reported as 74.81 ± 2.47 %, 2.05 ± 0.50 %, 21.81 ± 2.54 %, 1.33 ± 0.13 % and 0 % in hypothyroid dogs, respectively.

The Mean ± SE value of platelets in healthy and hypothyroid dogs was reported as 287.71 ± 41.66 ($\times 10^3/\mu l$) and 274.90 ± 28.46 ($\times 10^3/\mu l$) respectively.

Table 4.8 Comparative analysis of hematological parameters in apparently healthy (n=7) (Group I) and dogs with hypothyroidism associated neuromuscular disorders (n=21) (Group II)

Sr. No.	Parameter	Healthy Mean ± SE	Hypothyroid Mean ± SE	t Value
1	Hb (gm%)	14.77 ± 0.90	11.11 ± 0.58	3.24**
2	TEC($\times 10^6/\mu l$)	6.55 ± 0.40	4.80 ± 0.25	3.50**
3	PCV (%)	43.23 ± 2.63	32.29 ± 1.71	3.28**
4	MCV (fl)	66.23 ± 1.51	67.45 ± 0.67	0.84
5	MCH (pg)	22.61 ± 0.44	23.27 ± 0.30	1.11
6	MCHC (g/dl)	34.17 ± 0.30	34.54 ± 0.29	0.69
7	TLC($\times 10^3/\mu l$)	13.16 ± 1.68	17.66 ± 2.75	0.92
8	Neutrophils %	68.57 ± 3.34	74.81 ± 2.47	1.32
9	Eosinophils %	3.29 ± 0.68	2.05 ± 0.50	1.30
10	Lymphocytes %	27.00 ± 3.44	21.81 ± 2.54	1.06
11	Monocytes %	1.14 ± 0.14	1.33 ± 0.13	0.81
12	Platelets ($\times 10^3/\mu l$)	287.71 ± 41.66	274.90 ± 28.46	0.23
**=$p \leq 0.01$ (Table t= 2.77)				

The current study reported highly significant ($p \leq 0.01$) difference in haemoglobin, total erythrocyte count and packed cell volume between apparently healthy dogs (Group I) and dogs with hypothyroidism associated neuromuscular disorders (Group II). The Mean ± SE values of mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were in the normal reference range whereas for haemoglobin and packed cell volume the values were below the reference range indicating normocytic, normochromic anaemia in group II dogs. These findings are in agreement with Jaggy *et al.* (1994), Kumar *et al.* (2007), Blois (2008), Fracassi and Tamborini (2011), Romão *et al.* (2012), Kour *et al.* (2021) and de Oliveira (2022).

Previously it has been reported by Mooney (2011), Bertalan *et al.* (2013) and Kour *et al.* (2020) that thyroid hormones were found to stimulate erythropoiesis by promoting the rapid proliferation of immature erythroid progenitors and

enhancing erythropoietin (EPO) production through the activation of erythropoietin gene expression. Whereas Kumar *et al.* (2007) stated bone marrow suppression as a possible cause of anaemia in hypothyroidism

Few other studies by Kour *et al.* (2020) and Naveen (2024) reported significant ($p \leq 0.05$) alterations in the total leucocyte count between healthy and hypothyroid, with later showing higher values.

4.7.3 Biochemical Parameters

The Mean ± SE values for the biochemical parameters of Group I and Group II are presented in Table 4.9. and (Appendix 5 and 6).

The mean concentrations of total bilirubin, direct bilirubin and indirect bilirubin were 0.27 ± 0.02 (mg/dl), 0.11 ± 0.01 (mg/dl) and 0.16 ± 0.02 (mg/dl) in healthy dogs and 0.28 ± 0.02 (mg/dl), 0.13 ± 0.01 (mg/dl) and 0.15 ± 0.02 (mg/dl) in hypothyroid dogs, respectively.

The concentrations of SGOT, SGPT and ALP were 35.43 ± 5.66 (IU/L), 46.57 ± 14.35 (IU/L) and 204.86 ± 35.37 (IU/L) in healthy dogs and 47.47 ± 6.17 (IU/L), 81.68 ± 19.29 (IU/L) and 234.53 ± 32.89 (IU/L) in hypothyroid dogs, respectively.

Total proteins, albumin and globulin had mean concentrations of 5.84 ± 0.30 (gm/dl), 2.40 ± 0.11 (gm/dl) and 3.44 ± 0.34 (gm/dl) in healthy dogs and 6.14 ± 0.22 (gm/dl), 2.43 ± 0.05 (gm/dl) and 3.73 ± 0.22 (gm/dl) in hypothyroid dogs, respectively.

The Mean ± SE values for blood urea nitrogen and creatinine were 14.63 ± 1.69 (mg/dl) and 0.91 ± 0.14 (mg/dl) in healthy dogs and 20.59 ± 3.56 (mg/dl) and 1.08 ± 0.13 (mg/dl) in hypothyroid dogs, respectively.

The lipid profile including serum cholesterol and serum triglycerides, the Mean ± SE values of 154.71 ± 17.95 (mg/dl) and 75.00 ± 9.91 (mg/dl) in healthy dogs and 219.14 ± 13.76 (mg/dl) and 85.43 ± 11.21 (mg/dl) in hypothyroid dogs, respectively.

The mean concentrations of sodium, potassium and chloride were 146.99 ± 0.47 (mEq/L), 4.64 ± 0.12 (mEq/L) and 108.86 ± 1.41 (mEq/L) in healthy dogs

and 147.61 ± 1.15 (mEq/L), 4.41 ± 0.10 (mEq/L) and 110.91 ± 1.10 (mEq/L) in hypothyroid dogs, respectively.

The mean concentrations for calcium and phosphorus were 10.67 ± 0.25 (mg/dl) and 4.49 ± 0.22 (mg/dl) in healthy dogs and 10.05 ± 0.29 (mg/dl) and 4.86 ± 0.42 (mg/dl) in hypothyroid dogs, respectively.

Table 4.9 Comparative analysis of biochemical parameters in apparently healthy (n=7) (Group I) and dogs with hypothyroidism associated neuromuscular disorders (n=21) (Group II)

Sr. No.	Parameter	Healthy Mean ± SE	Hypothyroid Mean ± SE	t Value
1	Total bilirubin (mg/dl)	0.27 ± 0.02	0.28 ± 0.02	0.22
2	Direct bilirubin (mg/dl)	0.11 ± 0.01	0.13 ± 0.01	0.81
3	Indirect bilirubin (mg/dl)	0.16 ± 0.02	0.15 ± 0.02	0.23
4	SGOT (IU/L)	35.43 ± 5.66	47.47 ± 6.17	1.06
5	SGPT (IU/L)	46.57 ± 14.35	81.68 ± 19.29	1.01
6	ALP (IU/L)	204.86 ± 35.37	234.53 ± 32.89	0.49
7	Total protein (gm/dl)	5.84 ± 0.30	6.14 ± 0.22	0.72
8	Albumin(gm/dl)	2.40 ± 0.11	2.43 ± 0.05	0.27
9	Globulin (gm/dl)	3.44 ± 0.34	3.73 ± 0.22	0.69
10	BUN (mg/dl)	14.63 ± 1.69	20.59 ± 3.56	0.94
11	Creatinine (mg/dl)	0.91 ± 0.14	1.08 ± 0.13	0.72
12	Sr. Cholesterol (mg/dl)	154.71 ± 17.95	219.14 ± 13.76	2.46*
13	Sr. Triglycerides (mg/dl)	75.00 ± 9.91	85.43 ± 11.21	0.51
14	Sodium (mEq/L)	146.99 ± 0.47	147.61 ± 1.15	0.30
15	Potassium (mEq/L)	4.64 ± 0.12	4.41 ± 0.10	1.25
16	Chlorides (mEq/L)	108.86 ± 1.41	110.91 ± 1.10	0.98
17	Sr. Calcium (mg/dl)	10.67 ± 0.25	10.05 ± 0.29	1.17
18	Sr. Phosphorus (mg/dl)	4.49 ± 0.22	4.86 ± 0.42	0.51
*=p≤0.05 (Table t= 2.05)				

The present study reported non-significant (p≤0.05) alterations in bilirubin profile. Similar findings were reported by Gori *et al.* (2023) Alone (2024) and Naveen (2024). However, Gupta *et al.* (2010) and Dubova (2023) reported significant alterations in bilirubin profile in induced hypothyroidism in sheep and dogs respectively, which was attributed to hepatic insult and lack of bile clearance.

The current study revealed comparatively higher mean concentrations of SGOT and SGPT in the hypothyroid group compared to apparently healthy but

were not significantly ($p \leq 0.05$) higher. This was in accordance with Suraniti *et al.* (2008), Kour *et al*. (2021), Gori *et al.* (2023), Alone (2024) and Naveen (2024). On the contrary Giza *et al.* (2015) reported mild elevation of SGOT in hypothyroid dogs along with serum creatinine kinase which could be attributed to myopathy in hypothyroidism.

Non-significant ($p \leq 0.05$) difference was reported in Mean ± SE value of ALP between the two groups from the current study. This finding was similiar to the finding reported by Ryad *et al.* (2020). Similar finding was reported by Romão *et al.* (2012) in a hypothyroid dog. On the contrary Higgins *et al*. (2006), Kour *et al*. (2021), Alone (2024) and Naveen (2024) reported increased serum ALP and was attributed to hepatic dysfunction, degenerative myopathy and use of steroids in therapy.

The present study reported significant ($p \leq 0.05$) difference in serum cholesterol between apparently healthy dogs (Group I) and dogs with hypothyroidism associated neuromuscular disorders (Group II). This finding was in accordance with Kumar *et al.* (2007), Vitale and Olby (2007), Blois *et al.* (2008), Suraniti *et al.* (2008), Fracassi and Tamborini (2011), Romão *et al.* (2012), de Oliveira (2022), Alone (2024) and Naveen (2024). The findings of the current study align with Kahn *et al.* (2006) who stated that hypothyroid cases show upper higher normal limits of cholesterol. These researchers highlighted the role of thyroxine in lipid synthesis, mobilization and degradation by its action on hepatic and lipoprotein lipase along with its action on hepatic low-density lipoproteins (LDL) receptors and therefore, attributed comparative hypercholesterolemia to reduced thyroxine levels in hypothyroidism.

The study documented non-significant ($p \leq 0.05$) elevation serum triglycerides in hypothyroid group compared to apparently healthy group. The finding was familiar to that of Alone (2024). Similar finding was reported by Romão *et al.* (2012) in a hypothyroid dog. On the contrary, Higgins *et al*. (2006), Vitale and Olby (2007), Kour *et al*. (2021) and Naveen (2024) recorded significant elevation in triglycerides. This may be attributed to lack of plasma clearance of triglycerides in hypothyroidism.

Although the difference was not significant in the current study but the

increased values compared to healthy dogs in hypothyroid dogs suggest subclinical alterations in the lipid metabolism. The absence of notable difference in the current study could be because of variability in the disease progression in hypothyroid dogs.

On comparing total protein, albumin and globulin between the groups non-significant ($p \leq 0.05$) difference was found in the current study. This was in agreement with Suraniti *et al.* (2008), Kour *et al.* (2021) and Naveen (2024). However, Vitale and Olby (2007) reported increased plasm proteins in the hypothyroid dogs which was attributed to lipemia, as albumin and globulins on serum biochemistry were in reference range. Alone (2024) reported increased serum globulins in hypothyroid subjects and explained it as a cause of chronic inflammation and excessive protein metabolism. Whereas, Pöppl (2015) reported hypoalbuminemia in a hypothyroid dog.

The study reported non-significant ($p \leq 0.05$) alterations in the mean concentrations of blood urea nitrogen and serum creatinine between the two groups. This finding was in accordance with Suraniti *et al.* (2008), Kour *et al.* (2021) and Alone (2024). However, Naveen (2024) reported increased serum creatinine values in hypothyroid dogs and was attributed to decreased GFR suggesting renal impairment.

The study reported non-significant ($p \leq 0.05$) alterations in serum electrolytes (sodium, potassium and chloride) and minerals (calcium and phosphorus) between the healthy and hypothyroid group. The findings got support from Higgins *et al*. (2006) and Suraniti *et al.* (2008) respectively. On the contrary Kour *et al.* (2021) reported lower mean concentrations of calcium and phosphorus in hypothyroid dogs compared to healthy control dogs.

4.7.4 Thyroid profile

(a) Quality control

Assessment of TT_3, TT_4 and fT_4 was done using radioimmunoassay. All assays met the recommended quality control parameters specified by the manufacturer. The control samples provided with the kits were analysed with each assay and compared to the reference ranges specified by the manufacturers to ensure the accuracy and precision of the assays. The recovery percentage for each assay was calculated as a quality control parameter to increase the level of assurance. This estimation involved using half the volume of a standard and half the volume of a dog serum sample. The percentage recovery was determined by comparing the observed value to the expected value. [% Recovery = (Observed value ÷ Expected value) × 100]

A summary of the quality control parameters for TT_3, TT_4, and fT_4 is presented in Table 4.10, Table 4.11, and Table 4.12, respectively. The optical density (OD) and concentrations of calibrators in cTSH assay are presented in Table 4.13. The standard plots for TT_3, TT_4, fT_4 and TSH are presented in Plate 4.1, Plate 4.2 Plate 4.3 and Plate 4.4, respectively.

Table 4.10 Summary of Quality Control parameters of Total Triiodothyronine (TT_3, nmol/L)

Tube Details	Counts/ minute	Average Counts/ minute	Percent CV	Percent B/B0
Total Count a	31768	30890.00	0.31	---
Total Count b	30012			
Calibration 0 a	23349	23178.00	1.00	100.00
Calibration 0 b	23007			
Calibration 1 a	16987	18005.00	8.00	77.70
Calibration 1 b	19024			
Calibration 2 a	12594	12390.00	2.30	53.50
Calibration 2 b	12186			
Calibration 3 a	9233	8725.00	8.20	37.60
Calibration 3 b	8217			
Calibration 4 a	6341	6343.00	0.00	27.40
Calibration 4 b	6345			
Calibration 5 a	4234	4255.00	0.70	18.40
Calibration 5 b	4275			
Control I a	11908	11586.00	3.90	50.00
Control I b	11263			
Control II a	7556	7806.00	4.50	33.70
Control II b	8056			
Recovery Tube A	7739	7689.00	0.90	33.20
Recovery Tube B	7639			

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	75.03 %
3	**80 % Intercept**	0.63 nmol/L
4	**50 % Intercept**	2.32 nmol/L
5	**20 % Intercept**	11.96 nmol/L
6	**QC sample -1**	2.32 nmol/L*(2.13 ± 0.53)
7	**QC sample -2**	4.65 nmol/L*(4.26 ± 1.07)
8	**Recovery**	95.74% (Observed= 4.5 nmol/L; Expected= 4.7 nmol/L)
*Observed Value		

Table 4.11 Summary of Quality Control parameters of Total Triiodothyronine (TT_4, nmol/L)

Tube Details	Counts/ minutes	Average Counts/ minutes	Percent CV	Percent B/B0
Total Count a	16445	17101.00	5.00	--
Total Count b	17757			
Calibration 0 a	10612	10524.00	0.90	100.00
Calibration 0 b	10436			
Calibration 1 a	8855	8739.00	1.20	83.00
Calibration 1 b	8624			
Calibration 2 a	6880	6769.00	1.30	64.30
Calibration 2 b	6658			
Calibration 3 a	4690	4406.00	4.30	41.90
Calibration 3 b	4122			
Calibration 4 a	2929	2833.00	1.80	26.90
Calibration 4 b	2736			
Calibration 5 a	1976	1959.00	0.40	18.60
Calibration 5 b	1942			
Control I a	5749	5735.00	0.20	54.50
Control I b	5712			
Control II a	3269	3273.00	0.10	31.10
Control II b	3278			
Recovery Tube A	3718	3832.00	1.80	36.40
Recovery Tube B	3946			

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	61.54 %
3	**80 % Intercept**	20.75 nmol/L
4	**50 % Intercept**	83.38 nmol/L
5	**20 % Intercept**	346.16 nmol/L
6	**QC sample -1**	72.00 nmol/L*(80 ± 20)
7	**QC sample -2**	166.00 nmol/L*(186 ± 47)
8	**Recovery**	102.54% (Observed= 133.30 nmol/L; Expected=130 nmol/L)
*Observed Value		

Table 4.12 Summary of Quality Control parameters of Total Triiodothyronine (fT_4, pmol/L)

Tube Details	Counts/ minutes	Average Counts/ minutes	Percent CV	Percent B/B0
Total Count a	51612	51693.00	0.40	--
Total Count b	51774			
Calibration 0 a	33592	33859.00	1.50	100.00
Calibration 0 b	34126			
Calibration 1 a	29311	29486.00	1.00	87.10
Calibration 1 b	29661			
Calibration 2 a	22194	21954.00	1.60	64.80
Calibration 2 b	21714			
Calibration 3 a	9392	9370.00	0.20	27.70
Calibration 3 b	9348			
Calibration 4 a	3960	5270.00	4.80	12.60
Calibration 4 b	4581			
Control I a	17306	17601.00	2.20	52.00
Control I b	17895			
Recovery Tube A	8368	8377.00	0.10	24.70
Recovery Tube B	8386			

Sr. No.	Assay Q.C. Parameters and Performance Evaluation	
1	**Percent Non-Specific Binding**	N. A
2	**Percent Zero Binding (Bo/T) in RIA**	65.50 %
3	**80 % Intercept**	4.54 pmol/L
4	**50 % Intercept**	14.17 pmol/L
5	**20 % Intercept**	42.39 pmol/L
6	**QC sample**	13.31 pmol/L*(14 ± 2.80)
7	**Recovery**	91.62% (Observed= 33.90 pmol/L; Expected= 37 pmol/L)
*Observed Value		

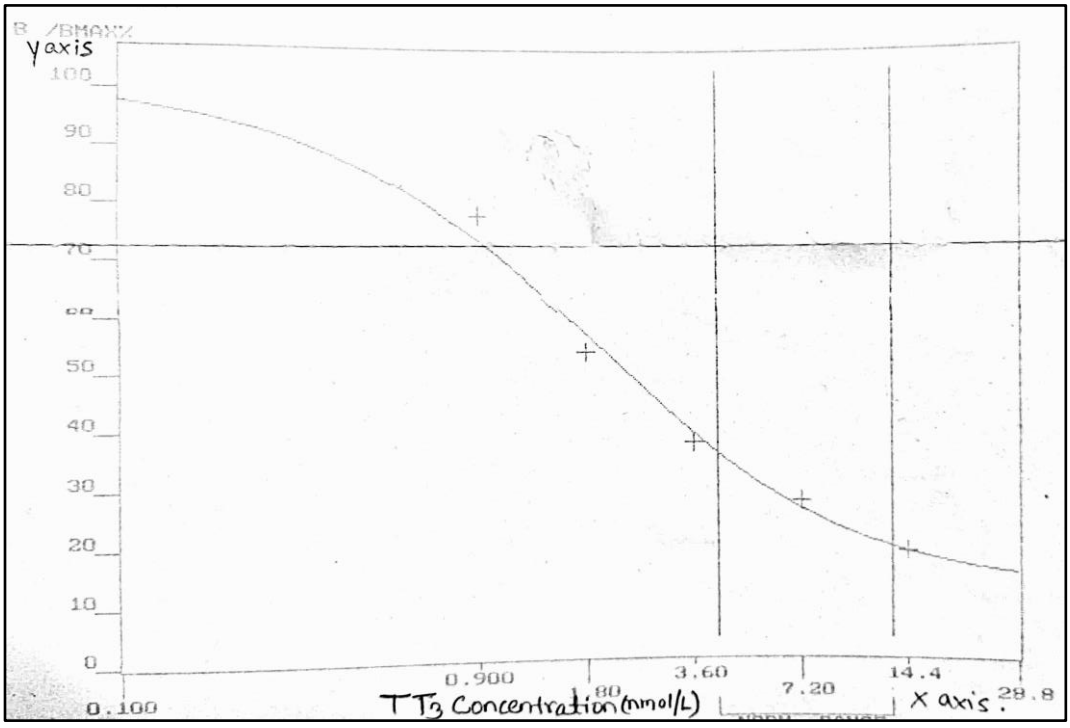


Plate 4.1 Graph of TT_3(nmol/L)

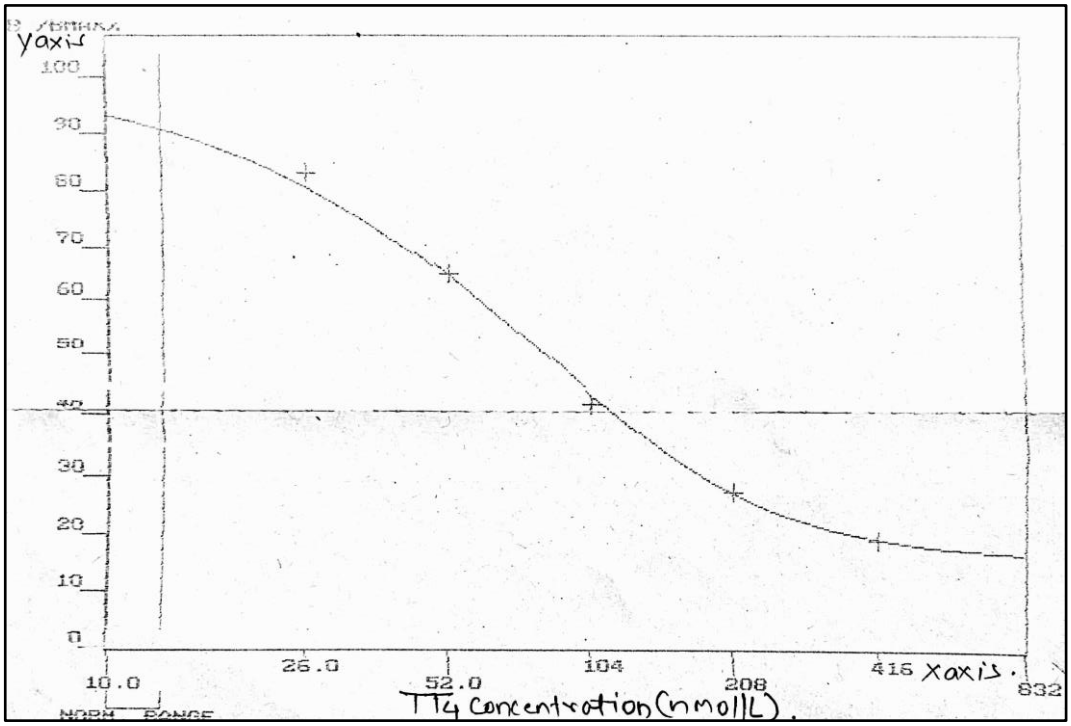


Plate 4.2 Graph of TT_4(nmol/L)

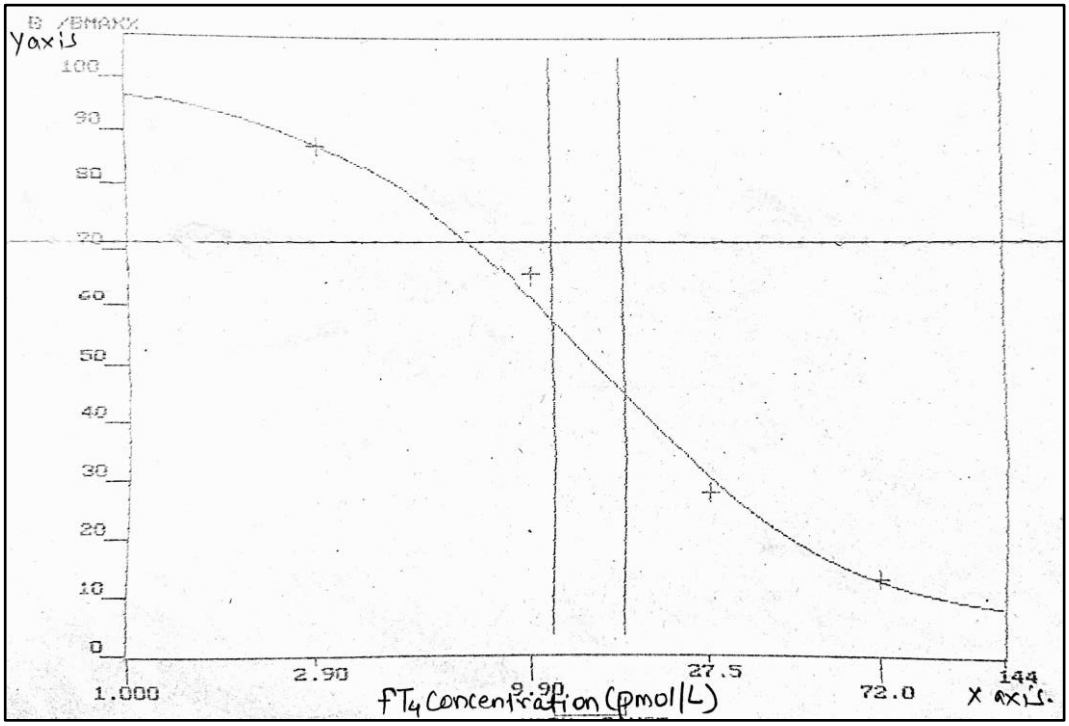


Plate 4.3 Graph of fT_4(pmol/L)

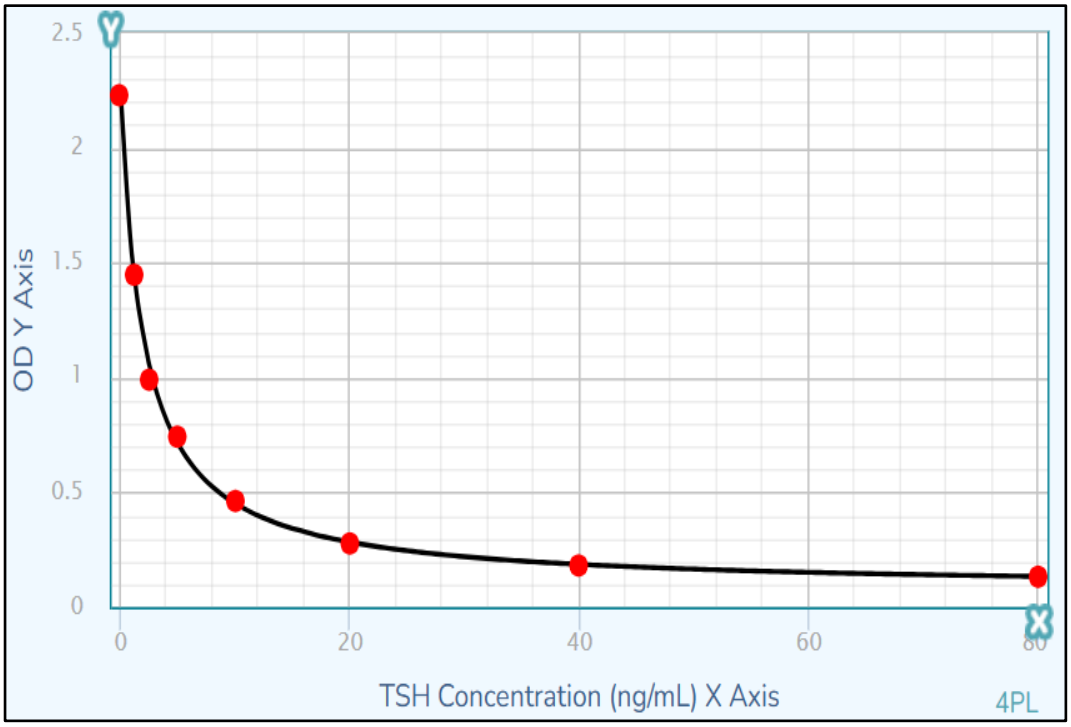


Plate 4.4 Graph of TSH (ng/mL)

Table 4.13 Optical density (OD) and concentrations of calibrators in cTSH assay (ng/mL)

Calibrator	0	1	2	3	4	5	6	7
OD	2.23	1.45	1.00	0.74	0.46	0.28	0.18	0.13
Concentration	0	1.25	2.5	5	10	20	40	80

(b) <u>Comparative analysis of thyroid profile between apparently healthy and hypothyroid dogs</u>

The Mean ± SE values for the thyroid profile of Group I and Group II are presented in Table 4.14. (Appendix 7)

The mean concentrations of TT_3, TT_4, fT_4 and TSH were 1.08 ± 0.14 (nmol/L), 20.12 ± 1.40 (nmol/L), 14.77 ± 0.83 (pmol/L) and 0.25 ± 0.04 (ng/mL) in healthy dogs and 0.89 ± 0.04 (nmol/L), 9.45 ± 0.94 (nmol/L), 7.72 ± 0.37 (pmol/L) and 1.59 ± 0.20 (ng/mL) in hypothyroid dogs respectively.

Table 4.14 Comparative analysis of thyroid profile in apparently healthy (n=7) (Group I) and dogs with hypothyroidism associated neuromuscular disorders (n=21) (Group II)

Sr. No.	Parameter	Healthy Mean ± SE	Hypothyroid Mean ± SE	t Value
1	TT_3 (nmol/L)	1.08 ± 0.14	0.89 ± 0.04	1.89
2	TT_4 (nmol/L)	20.12 ± 1.40	9.45 ± 0.94	5.86**
3	fT_4 (pmol/L)	14.77 ± 0.83	7.72 ± 0.37	8.92**
4	TSH (ng/mL)	0.25 ± 0.04	1.59 ± 0.20	$6.60^{\#\#}$
**=p≤0.01 (Table t= 2.77) and $^{\#\#}$=p≤0.01 (Table t= 2.88)				

A comparison of TT_3 concentrations between healthy and hypothyroid dogs is illustrated in Figure 4.7, which displays a violin plot and a box plot. This plot includes data from healthy dogs (n = 7) and hypothyroid dogs (n = 21). In the box plot, the T-bars represent the range of TT_3 values (0.67–1.22 nmol/L), the boxes indicate the interquartile range (middle 50% of the data, 0.78–0.96 nmol/L), and the horizontal line within the box denote the median TT_3 concentration (0.85 nmol/l) in hypothyroid dogs. Whereas, violin plot shows the

frequency distribution of the data points.

The comparison of TT_4 concentrations between healthy and hypothyroid dogs is illustrated in Figure 4.8, which presents a violin plot and a box plot. The plot is drawn based on data from healthy dogs (n = 7) and hypothyroid dogs (n = 21). In each box plot, the T-bars represent the range of TT_4 values (3.01 –14.40 nmol/L), the boxes indicate the interquartile range (middle 50% of the data, 5.38–13.48 nmol/L), and the horizontal lines within the boxes denote the median TT_4 concentration (9.36 nmol/l) in hypothyroid dogs. A violin plot, on the other hand, represents the frequency distribution of the data points.

Similar comparison was made for fT_4 concentrations between healthy and hypothyroid dogs and is showed in Figure 4.9, which displays a violin plot and a box plot. This plot includes data from healthy dogs (n = 7) and hypothyroid dogs (n = 21). In the box plot, the T-bars represent the range of fT_4 values (5.13–10.60 pmol/L), the boxes indicate the interquartile range (middle 50% of the data, 6.38–8.82 pmol/L), and the horizontal lines within the boxes denote the median fT_4 concentration (7.80 pmol/l) in hypothyroid dogs. A violin plot depicts the frequency distribution of the data points.

The comparison of TSH concentrations between healthy and hypothyroid dogs using both a violin plot and a box plot is illustrated in Figure 4.10. The data includes measurements from healthy dogs (n = 7) and hypothyroid dogs (n = 21). In the box plot, the T-bars indicate the overall range of TSH values (0.11–2.98 ng/mL), while the boxes represent the interquartile range (middle 50% of the data, 1.08–2.53 ng/mL). The horizontal line inside each box marks the median TSH concentration (1.45 ng/mL) in hypothyroid dogs. Meanwhile, the violin plot visually depicts the frequency distribution of the data points.

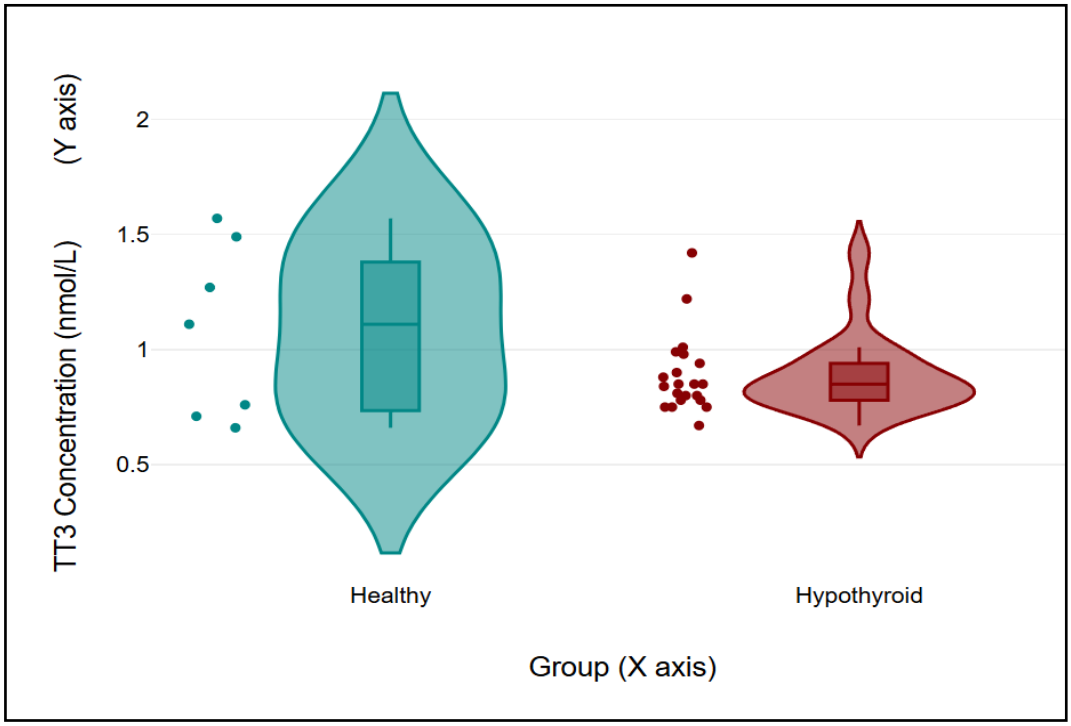
The mean concentrations of TT_3, TT_4 and fT_4 in hypothyroid dogs were lower than the reference range documented by Galdhar *et al*. (2022) for healthy dogs. Highly significant ($p \leq 0.01$) difference was recorded in the serum total thyroxine and serum free thyroxine between groups and non-significant difference was detected in total triiodothyronine concentrations between the groups. Similar findings were reported by Higgins *et al*. (2006), Alone (2024) and Naveen (2024).

Lack of significant difference in TT_3 between the healthy and hypothyroid gained support by Kantrowitz *et al.* (2001) who stated that sensitivity and accuracy of TT_3 was doubtful for diagnosis of hypothyroidism.

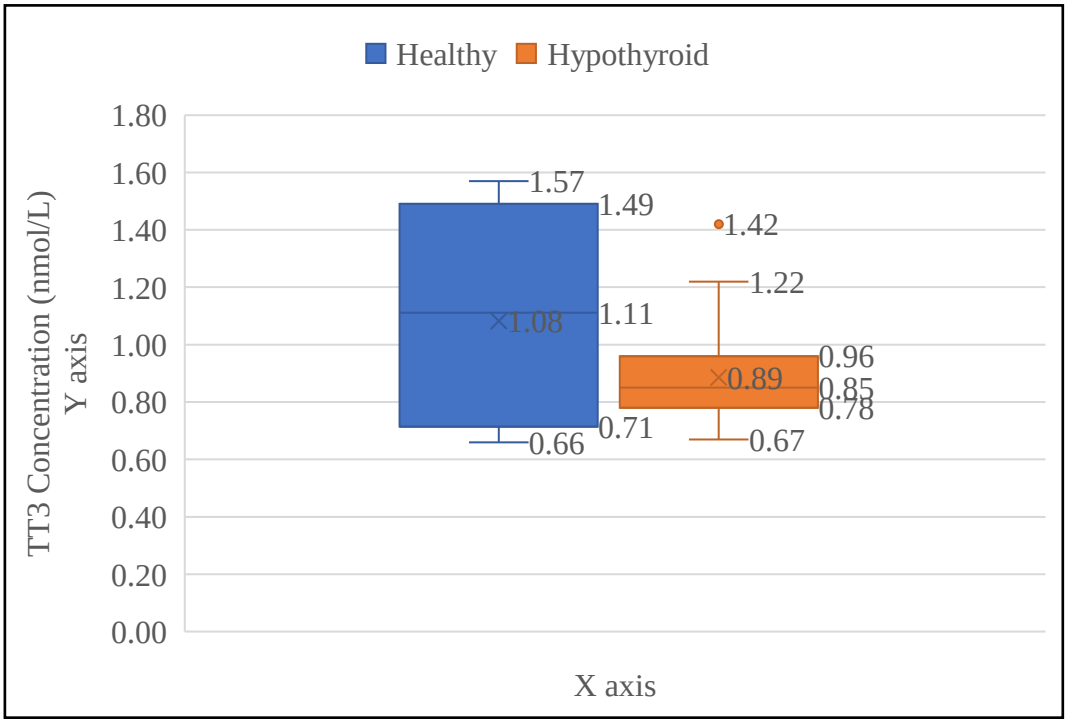
The mean TSH concentration recorded in the hypothyroid group in the current study was higher than the reference range by Kemppainen and Behrend (2001), Gulzar *et al.* (2014), Kaur *et al.* (2021) and Boretti *et al.* (2022) for healthy dogs. The present study recorded significant difference ($p \leq 0.01$) in the TSH concentrations between the healthy and hypothyroid groups, later being higher. This finding is in agreement with Bonagura and Twedt (2009); Pawar (2009) and Fernandez and Seth (2016) who recorded significant elevation of TSH in hypothyroid dogs.

The primary function of thyroid gland is to produce the active thyroid hormones. Any structural or functional deformity in the thyroid gland or hypothalamic-pituitary-thyroid gland complex leading to decreased thyroid hormone production results in a state called hypothyroidism. Depending upon the location of the defect it is classified as primary, secondary or tertiary hypothyroidism. Primary hypothyroidism occurs when the problem is with the thyroid gland whereas, secondary and tertiary hypothyroidism occurs due to the failure of pituitary and hypothalamus functioning respectively (Nelson and Couto, 2019; Ettinger and Feldman, 2000). The subnormal levels of thyroid hormones in the primary hypothyroidism results in decreased negative feedback on the pituitary TSH synthesis and release resulting in elevated circulating TSH concentrations in the hypothyroid subjects (Dixon, 2001).

In the current study in the hypothyroid group 4 dogs had TSH concentrations within the reference range making 19.04% of total hypothyroid dogs enrolled. This finding aligns with Scott-Moncrieff *et al.* (2002) who recorded TSH concentrations within reference range for 30% dogs due to inability of the TSH assay to detect all isoforms of circulating TSH.

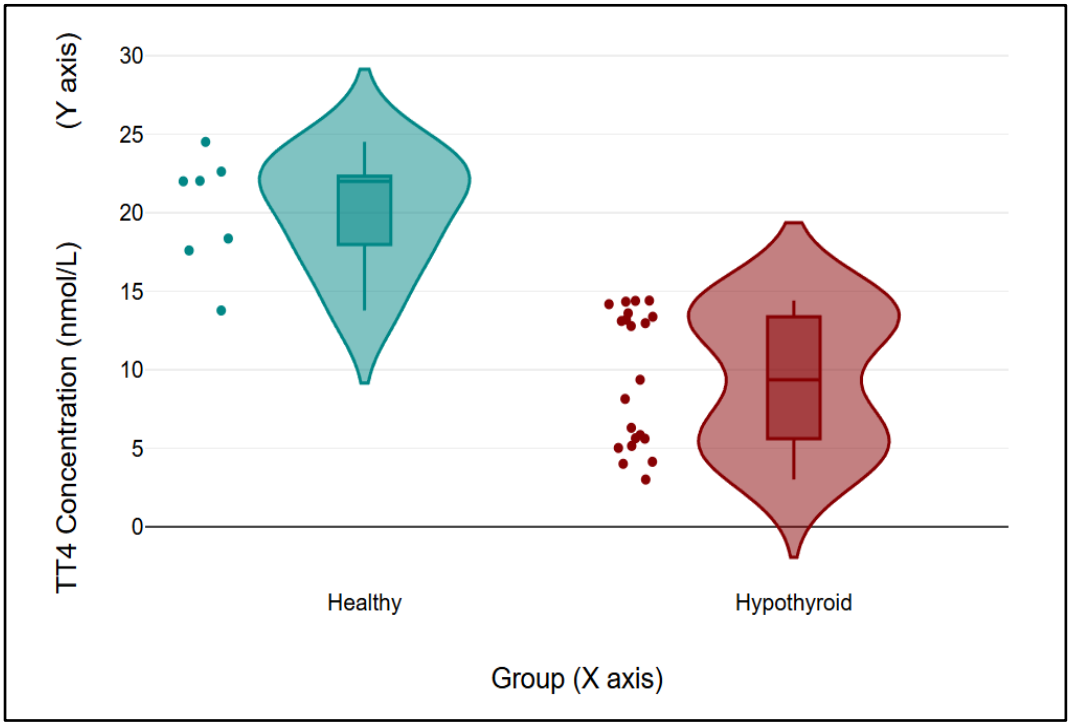


(a)



(b)

Figure 4.7 Comparative analysis of TT_3 between healthy (n=7) and hypothyroid (n=21) dogs (a) Violin plot (b) Box plot



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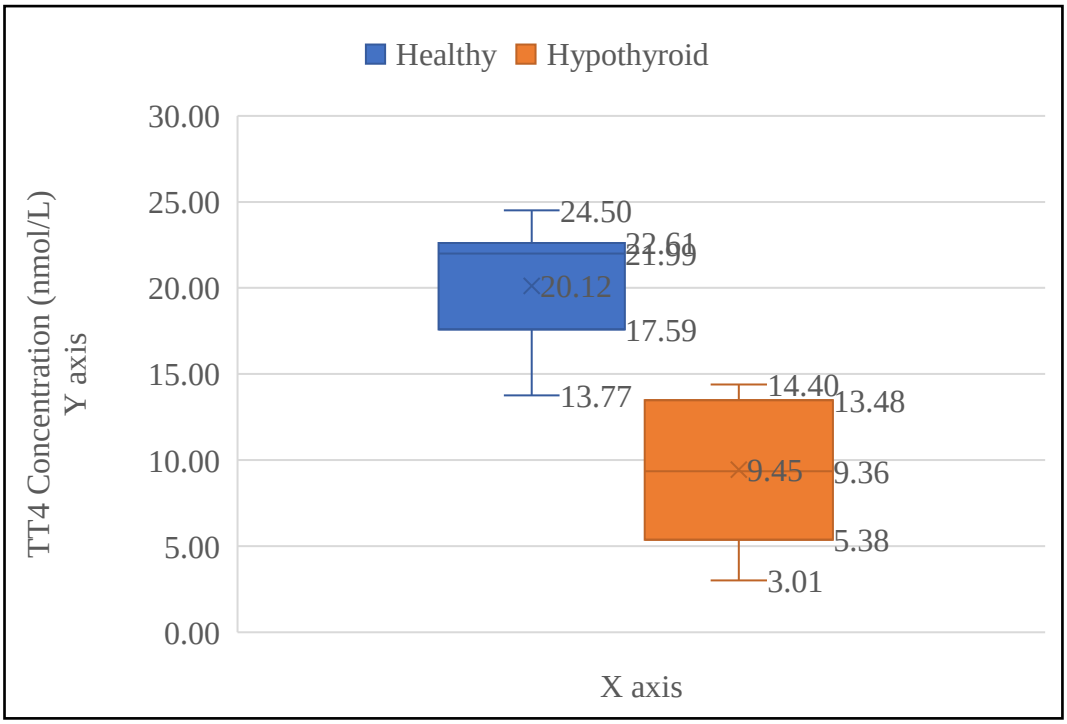
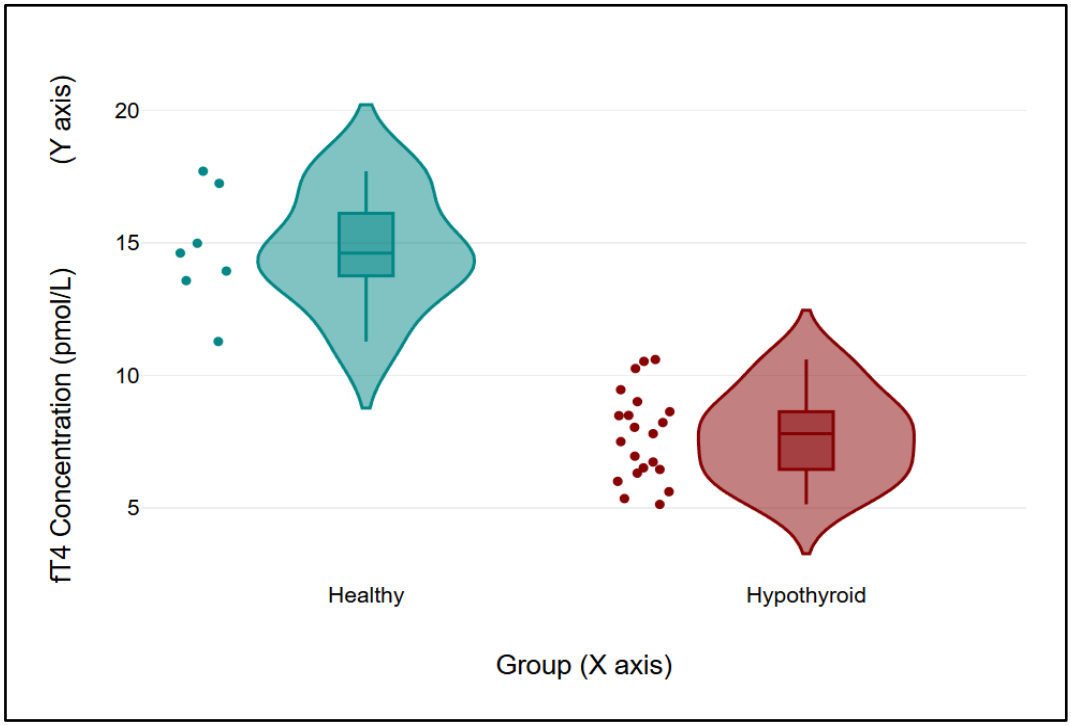


(b)

Figure 4.8 Comparative analysis of TT_4 between healthy (n=7) and hypothyroid (n=21) dogs (a) Violin plot (b) Box plot



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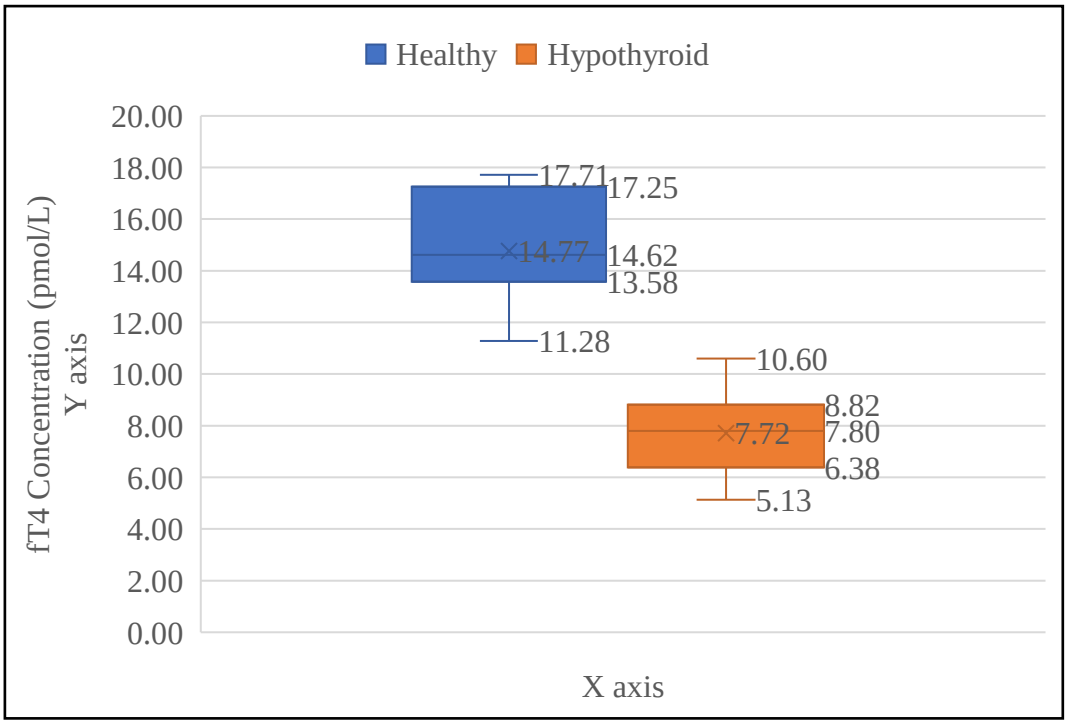
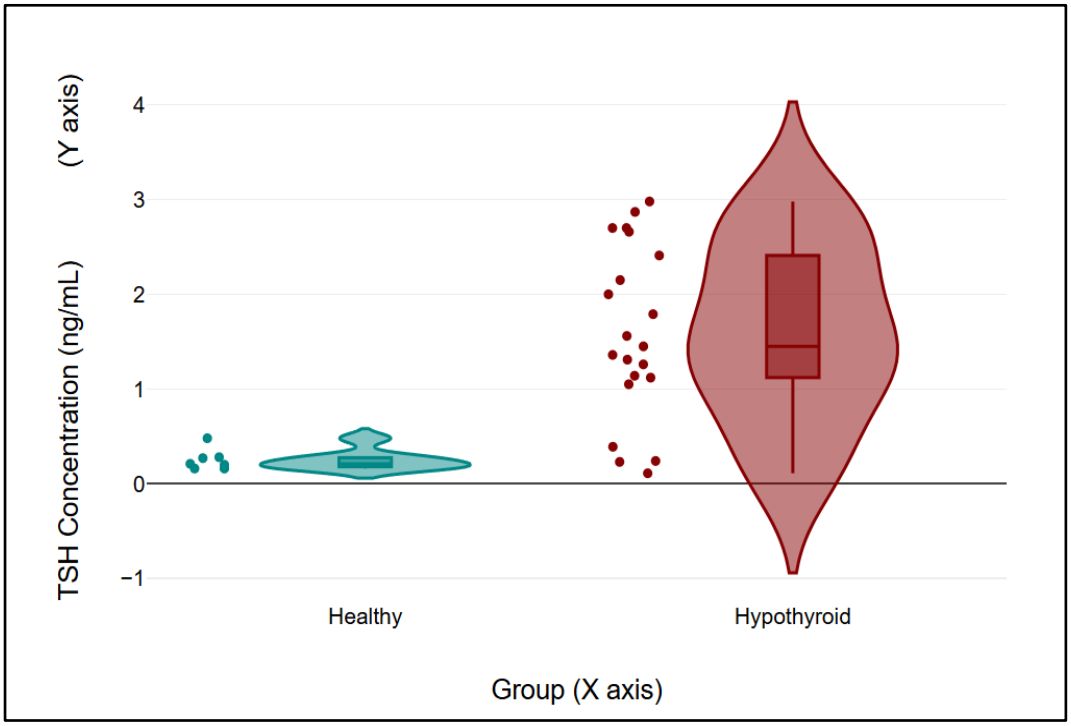
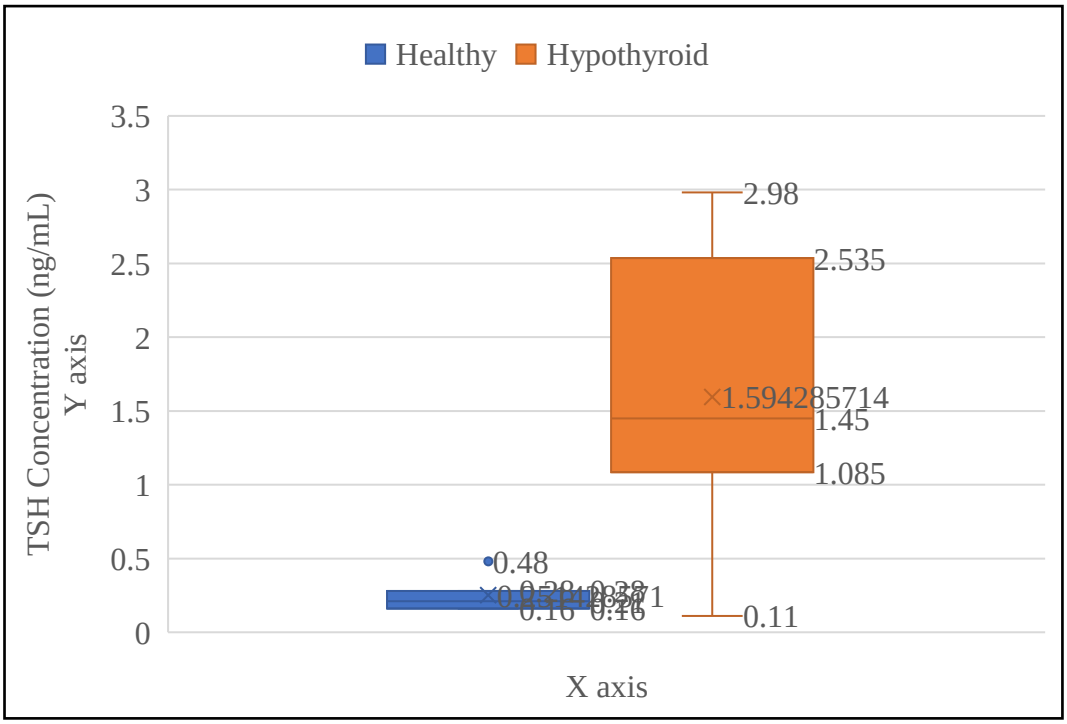


(b)

Figure 4.9 Comparative analysis of fT4 between healthy (n=7) and hypothyroid (n=21) dogs (a) Violin Plot (b) Box Plot



(a)



(b)

Figure 4.10 Comparative analysis of TSH between healthy (n=7) and hypothyroid (n=21) dogs (a) Violin plot (b) Box plot

4.8 Efficacy of Replacement Therapy

In the present study from group II (hypothyroid dogs with neuromuscular disorders post therapy), data was collected in seven cases (n=7). Data from other cases could not be collected due to lack of owner compliance during the treatment or after recovery. Data from these seven cases was used to study the efficacy of replacement therapy in hypothyroid dogs. On the similar grounds like healthy and hypothyroid comparison the before and after therapy comparisons were made based on neurological signs, physiological parameters, hematobiochemical parameters and thyroid profile.

4.8.1 Neuromuscular Signs

Neuromuscular signs before and after replacement therapy in the individual cases enrolled in the post therapy evaluation are as follows-

On presentation 1 case (n=1/7) showed right sided head tilt and proprioceptive ataxia. The dog had history of head tilt for 1 year. Before presentation the dog was treated with antibiotics, multivitamins, thiamine, diuretics and intravenous fluids, which failed to improve the neurological signs. Detailed neurological examination of the dog revealed vertical nystagmus and right sided head tilt. Menace, palpebral and pupillary light response were intact bilaterally. No abnormality was detected in spinal reflexes. Postural reactions were normal except for proprioceptive positioning manifested as ataxic gait. The dog was treated with oral levothyroxine sodium @ 10 mcg/kg body weight twice daily. Along with this dog was also treated with intravenous dextrose and normal saline. Noticeable change (mild reduction) in the head tilt was reported after a week of levothyroxine therapy. Nystagmus was not noticed after the initiation of therapy. Head tilt was substantially (near to complete) reduced over 1 month of replacement therapy and proprioceptive ataxia also resolved (Plate 4.5). The head tilt did not reduce significantly after the reduction noticed after a month.

The present finding gained support from Higgins *et al.* (2006) who reported improvement in the vestibular signs associated with hypothyroidism in

dog. Complete recovery was recorded in nine cases and one dog showed partial improvement. McKeown (2002) also reported similar finding with respect to head tilt in hypothyroid dog. Pathogenesis of vestibular dysfunction in hypothyroidism is not fully understood. Various proposed mechanisms are vascular encephalopathy, multiple functional metabolic derangements of neuronal or glial cells as a sequela to reduced thyroid hormone concentrations and dysfunction of metabolic pathways in brain or reduced local thyroid hormone circulation (Higgins *et al.,* 2006).

Other 3 (n=3/7) dogs reported facial asymmetry. Out of these, 1 (n=1/3) dog had left sided drooping/paresis whereas, other 2 (n=2/3) dogs had right sided drooping/paresis. The facial nerve paresis dogs were categorized into various classes of severity based on Botman and Jongkees Scale for human facial nerve function grading. The scale was partially modified according to the need of canine medicine and is mentioned in materials and methods section. The response to therapy was also graded based on the same scale.

The dog with left sided facial asymmetry was presented 15 days after the occurrence of facial asymmetry. The dog had open wounds on both the forelimbs due to self-mutilation. On neurological examination spinal reflexes were normal. Cranial nerve examination revealed abnormal pupillary light reflex in left eye with contralateral reflex being absent. The dog also showed reduced facial sensation on the left side with left facial side and ear drooping. Based on the severity of signs the dog was categorized into class III- moderate paresis according to modified Botman and Jongkees scale. Bilateral temporal muscle atrophy was recorded with left side being predominant. No abnormality was detected in the X ray imaging of limbs and spine. The dog was started with oral supplementation of levothyroxine sodium @ 10 mcg/kg body weight twice a day. PLR of the left eye and contralateral PLR was improved post 2 days of replacement therapy. Facial asymmetry reduced noticeably (mild paresis) post 6 days of therapy and was significantly reduced post 15 days (normal facial activity) (Plate 4.6). Open wounds on the forelimbs started healing after 5 days and were almost healed by 15 days of therapy.

The other 2 (n=2/3) dogs had right sided facial drooping. One of them

ageing 2 years reported frequent episodes of seizures before presentation and on presentation had right upper lip drooping when barking or panting (Class II- Mild paresis) and excessive drooling of saliva with no other abnormality in cranial nerves and spinal reflexes. Initially the dog was treated with vitamin B complex for a week by a attending vet but no improvement reported in clinical signs. Then the dog was started with levothyroxine sodium replacement therapy @ 10 mcg/kg body weight twice daily. Post 2 doses the dog the asymmetry was reduced and the reduction was significantly noticeable post 3rd dose. On follow-up examination on 13th day facial asymmetry was completely resolved (normal facial activity) (Plate 4.7). No seizures were reported during the course of time. The supplementation then was gradually reduced and stopped. No complaints about asymmetry or seizures were received from the owner till date (post 3 months).

The other one dog with facial asymmetry was presented with complaint of vomiting once on the day before presentation. Detailed history and examination revealed weight gain in previous 2-3 months despite of being on a regular meal. Ptosis of right upper eyelid and right sided drooping of face was noticed (Class - II Moderate paresis). Right temporal muscle atrophy was also recorded. Facial sensation and all other cranial nerve responses evaluated were normal along with spinal reflexes. The replacement therapy with oral levothyroxine sodium tablets @ 10 mcg/kg body weight twice daily was started. Ptosis of right upper eyelid was not reported one day post therapy and facial asymmetry resolved (normal facial activity) post 3 days of therapy (Plate 4.8). The dog lost 2 kg body weight by 30 days of therapy. During the course the dog was initially also treated with antiemetic and no vomiting was reported afterwards.

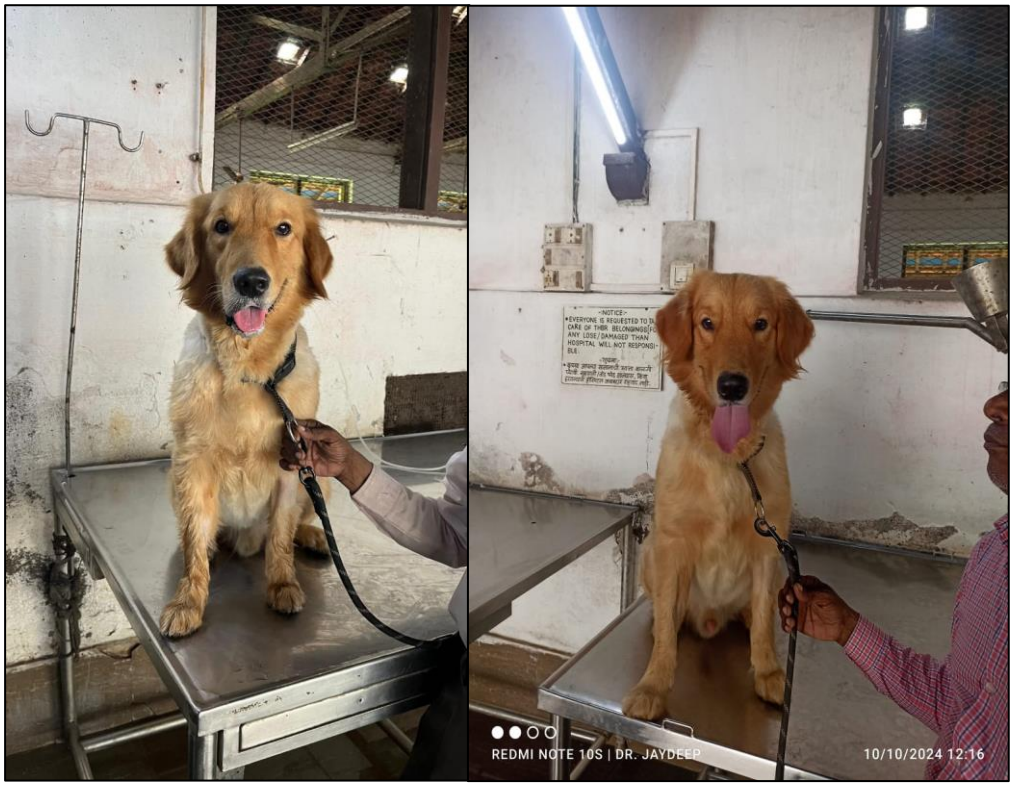
The above findings were in accordance with Panciera (1994) who reported improvement in the facial nerve abnormalities after levothyroxine replacement therapy. Autor also reported functional improvement after the replacement therapy. The exact pathophysiology of hypothyroid associate facial paresis is not fully elucidated. Certain mechanisms believed to be involved are cranial nerve paralysis due to the myxedema deposits around the nerves, impaired

Before **After**

Plate 4.5 Head tilt in a dog (before and after replacement therapy)

Before **After**

Plate 4.6 Left sided Facial asymmetry (before and after replacement therapy)



Before **After**

Plate 4.7 Right sided Facial asymmetry (before and after replacement therapy)

Before **After**

Plate 4.8 Right sided Facial asymmetry (before and after replacement therapy)

metabolism of Schwann cells leading to demyelination, hypercholesterolemia leading to decreased perfusion to inner ear due to increased viscosity of blood or altered axonal transport or axonal loss due to metabolic defects (Chaves *et al.*, 2016).

Evaluation of replacement therapy was also carried out in other 2 (n=2/7) dogs suffering from tetraparesis. One of them was presented with complaint of not being able to support weight on limbs. No abnormality was detected in cranial nerve examination, spinal reflexes, muscle tone and radiography. Initially the dog was treated with mannitol by the primary vet. On finding the dog as hypothyroid the replacement therapy was started with oral levothyroxine sodium tablet @ 10 mcg/kg body weight twice daily. Post seven days of therapy the dog started supporting weight with manual assistance and was able to take few steps. Post 10 days of replacement therapy the dog was able to walk on its own with few incidents of imbalance. After 15 days of therapy the dog was able to walk and run on its own (Plate 4.9).

The other dog with tetraparesis as major sign on presentation suffered gastroenteritis 10-15 days back and was treated for it. After 2 to 3 days of recovery the dog was not able to get up and bear weight on limbs. Detailed examination revealed deficit in withdrawal reflexes in all four limbs and deep nociception. The muscle tone of both thighs was reduced. No other abnormality was detected in spinal reflexes, cranial nerve responses and radiography of spine. Tablet levothyroxine sodium replacement therapy was initiated @ 10 mcg/kg body weight twice daily. Along with intravenous Dextrose and Normal Saline was also administered. Post 2 days of therapy dog started limb movements, withdrawal reflexes were restored to normal and proprioception was recorded on the 3rd day. Post 5 days the dog was able to walk with mild proprioceptive ataxia and by 10 days of replacement therapy the dog was able to walk normally (Plate 4.10). Further after evaluation the thyroid supplementation was stopped gradually and no complaint was received by from owner till date (3 months).

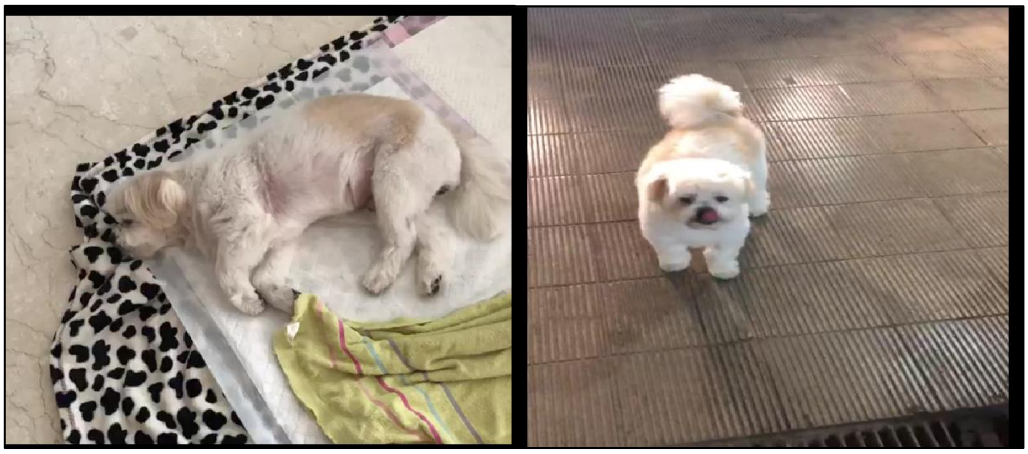
The findings are in accordance with Jaggy *et al.* (1994) who reported improvement in the hypothyroidism associated neuropathies after levothyroxine supplementation.

Tetraparesis in dogs can arise from a variety of etiologies, including spinal or cranial injuries, autoimmune disorders, metabolic disorders, and neuromuscular diseases. In the present study, the clinical signs and diagnostic findings strongly indicate hypothyroid associated polyneuropathy as the underlying cause rather than spinal or cranial trauma.

Possibilities of skeletal injury being the cause of tetraparesis in the present cases was ruled out using radiographic imaging findings revealing no detectable abnormality. Additionally, anamnesis revealed no history of trauma, which is typically related with acute onset of neurological deficits. Spinal cord injuries, including intervertebral disc disease (IVDD) or fractures, caused by acute traumas are generally manifested with a sudden onset of neurological signs. In contrast, the cases reported in the current study exhibited a gradually deteriorating paresis, which is consistent with respect to metabolic neuropathy rather than an acute mechanical or compressive injury. (Nelson and Couto, 2019). Further, on clinical examination there was no pain throughout the spine on palpation. Also, the dogs were treated with mannitol, which is predominantly used to reduce intracranial pressure in cases of central nervous system injury, before presentation, but did not report any improvement in the signs.

The presence of signs associated with lower motor neuron dysfunction in both thoracic and pelvic limbs, along with reduced muscle tone, hyporeflexia, and flaccid paresis, is highly suggestive of a generalized neuromuscular disorder. In contrast, injury in the spinal cord or cranium is presented either with upper motor neuron (UMN) or mixed UMN/LMN signs depending on location of the lesion. An occurrence of solely LMN presentation across both thoracic and pelvic limbs does not seems to be the result of injury at the central nervous system (CNS) lesion but rather points to a metabolic or peripheral nerve disorder (Nelson and Couto, 2019; Ettinger and Feldman, 2000; De Lahunta *et al.,* 2020).

Hypothyroidism in dogs is one of the most common differential diagnoses of neuromuscular complications, including polyneuropathy, which is attributed to impaired nerve conduction and myopathy. The pathophysiology of hypothyroid associated neuromuscular signs is not completely understood. It involves reduced



Before **After**

Plate 4.9 Tetraparesis in a dog (before and after replacement therapy)

Before **After**

Plate 4.10 Tetraparesis in a dog (before and after replacement therapy)

thyroid hormone levels leading to axonal degeneration, demyelination, and secondary muscle weakness (Mooney, 1999; Jaggy *et al.,* 1994).

In the present study a confirmatory indicator of hypothyroidism associated polyneuropathy was the absence of skeletal abnormalities, the presence of lower motor neuron signs in both thoracic and pelvic limbs, the progressive nature of the signs, and the lack of response to treatment with mannitol, spinal or cranial injury. The presented signs being characteristic of a systemic or metabolic cause, the confirmed hypothyroid status based on hormonal analysis, and their favorable response to levothyroxine replacement therapy strongly suggest hypothyroid polyneuropathy as the cause of tetraparesis.

These findings emphasize the importance of thyroid hormone analysis in dogs presenting with lower motor neuron tetraparesis, as timely thyroid hormone replacement therapy can achieve noteworthy clinical improvement.

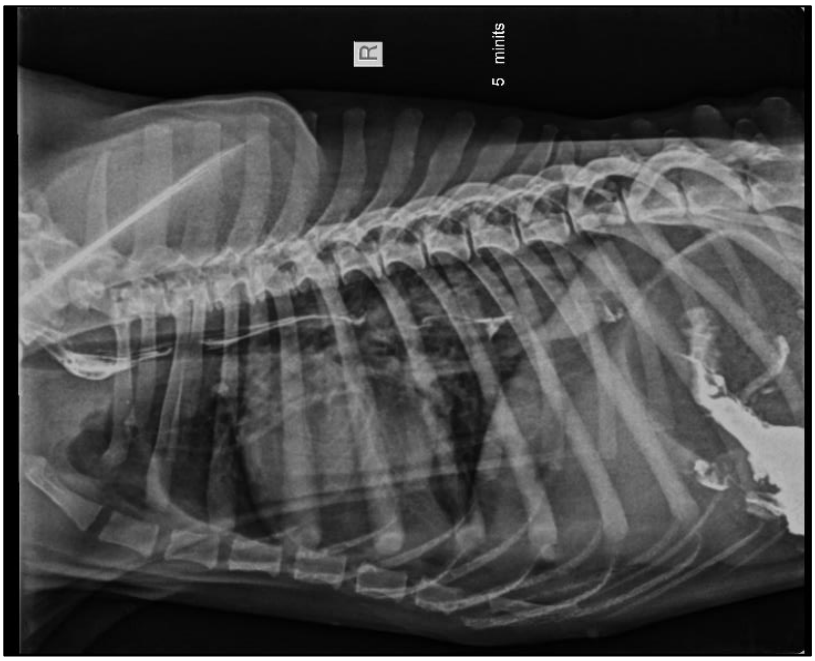
Post therapy evaluation was also carried out in one (n=1/7) case diagnosed with megaesophagus on barium meal study. The spinal and cranial nerve responses were normal. The dog had negative energy balance on presentation with intermittent regurgitation of food after meal as major complaint. The AChER antibody test was normal and deworming schedule was complete. On barium meal study a small oesophageal dilatation (pouch) was recorded at thoracic inlet. The dog was started on oral levothyroxine sodium therapy @ 10 mcg/kg body weight twice daily. Regurgitation completely stopped post 2 days of levothyroxine supplementation. Post 7 days of therapy barium meal study was repeated and the dilatation (pouch) was not visible (Plate 4.11). The dog gained 1 kg weight post 7 days and 6 kg post 25 days of therapy. After re-evaluation of thyroid profile noticing it in reference range, the levothyroxine supplementation was gradually reduced and then stopped. No complaint was received from owner till date (3 months).

This finding gained support from Jaggy *et al.* (1994), Panciera (1994) and Fracassi and Tamborini (2011) who reported improvement in hypothyroidism associated megaesophagus. Their findings also reported that the condition did not reoccur after termination of replacement therapy.

Overall, the concept of improvement in the neuromuscular signs associated with hypothyroidism gained support from the research works of

Bichsel *et al.* (1988), Jaggy *et al.* (1994), Panciera (1994), McKeown (2002), Higgins *et al.* (2006), Fracassi and Tamborini (2011), Srikala and Kumar (2011), and De Oliveira *et al.* (2022). According to them efficiently managed replacement therapy can lead to complete or partial recovery in the neuromuscular signs associated with hypothyroidism in dogs depending on the type of signs and duration of occurrence. Further, they also reported variability in the time period of response to therapy ranging from days to months. Panciera (1997), Bertalan *et al.* (2013), Nelson and Couto (2019) reported that response to replacement therapy can be within 24 hours and complete recovery may take 4 to 6 weeks.

The summary of neuromuscular signs improved after levothyroxine replacement therapy is presented in Table 4.15 and 4.16. Table 4.16 also includes cumulative dose in dogs after 7^{th} and 15^{th} day of therapy. Cumulative dose here indicates the total dose required in each case to improve particular neurological sign.



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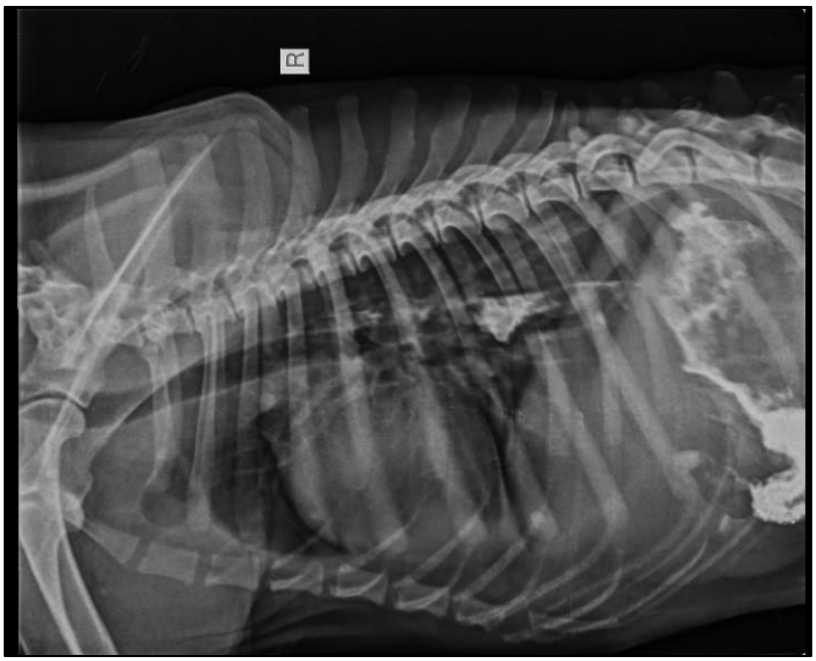

After

Plate 4.11 Megaoesophagus in dog (before and after replacement therapy)

Table 4.15 Summary details of improvement in neuromuscular signs (Hallmarks achieved in days)

Sr. No.	Case No.	Patient details	History	Neurological findings	Ruling out skeletal abnormality/other causes	Post therapy improvement (days)						
						Head tilt	Nystagmus	Facial asymmetry	PLR	Seizures	Tetra-paresis	Regurg-itation
1	2	Age- 11 year B.wt.- 10.3 kg Sex- F Breed- ND	Right Sided head tilt	1) Right sided head tilt 2) Vertical nystagmus bilaterally 3) Proprioceptive ataxia	1) Spinal Reflexes- no abnormality detected 2) X-ray spine- NAD 3) No history of trauma 4) Hypothyroid 5) Response to levothyroxine replacement therapy	30	3	-	-	-	-	-
2	3	Age- 8 year B.wt.- 29 kg Sex- F Breed- LR	Left sided facial asymmetry	1) Left sided facial asymmetry 2) Reduced facial sensation on left side of face 3) PLR – abnormal in left eye 4) Contralateral PLR- absent	1) Spinal reflexes – NAD 2) Hypothyroid 3) Response to levothyroxine replacement therapy	-	-	15	2	-	-	-

3	4	Age- 2 year B.wt.- 26 kg Sex- M Breed- GR	Seizures	1) Right sided facial asymmetry (drooping of right upper lip)	1) Spinal reflexes – NAD 2) Hypothyroid 3) Response to levothyroxine replacement therapy	-	-	7	-	1	-	-
4	5	Age- 11 year B.wt.- 32 kg Sex- F Breed- LR	Weight gain and one episode of vomiting	1) Right sided facial asymmetry (ptosis of right upper eyelid and right sided facial drooping)	1) Spinal reflexes – NAD 2) Hypothyroid 3) Response to levothyroxine replacement therapy	-	-	3	-	-	-	-
5	6	Age- 13 year B.wt.- 8.25 kg Sex- M Breed- Shitzu	Not able to bear weight on limbs (non-ambulatory tetraparesis)	1) Sluggish patellar reflex bilaterally 2) Deep nociception in all the limbs	1) X-ray spine- NAD 2) No history of trauma 3) AChER antibody test- Negative 4) Hypothyroid 5) Response to levothyroxine replacement therapy	-	-	-	-	-	15	-

6	7	Age- 2 year B.wt.- 11.4 kg Sex- F Breed- CS	Not able to bear weight on limbs (non-ambulatory tetraparesis)	1) Deficit in withdrawal reflexes in all the limbs and deep nociception 2) Reduced muscle tone in thighs	1) X-ray spine- NAD 2) No history of trauma 3) AChER antibody test- Negative 4) Response to levothyroxine replacement therapy	-	-	-	-	-	15	-
7	8	Age- 2.5 year B.wt.- 29 kg Sex- M Breed- PB	Regurgitation	1) Megaoesophagus (oesophageal dilatation at thoracic inlet)	1) AChER antibody test- Negative 2) Hypothyroid 3) Response to levothyroxine	-	-	-	-	-	-	2

(B.wt.- Body weight, M- Male, F- Female, ND- Nondescript, LR- labrador Retriever, CS- Cocker Spaniel, PB- Pakistani Bully)

Table 4.16 Summary details of improvement in neuromuscular signs (post 7 and 15 days) and ruling out skeletal abnormality

Sr. No.	Neurological findings	After seven days of therapy		After fifteen days of therapy	
		Neurological findings	Cumulative dose (mcg)	Neurological findings	Cumulative dose (mcg)
1	1) Severe right sided head tilt 2) Vertical nystagmus bilaterally 3) Proprioceptive ataxia	1) No nystagmus reported 2) Proprioceptive ataxic gait resolved 3) Mild reduction in head tilt	1442	1) Moderate reduction in head tilt	3090
2	1) Left sided facial asymmetry (Class-II) 2) Reduced facial sensation on left side of face 3) PLR – abnormal in left eye 4) Contralateral PLR- absent	1) PLR in left eye and contralateral PLR present 2) Noticeable reduction in facial asymmetry (mild asymmetry) 3) Improved sensation on left facial side	1120	1) Facial asymmetry significantly reduced (normal facial activity)	8700
3	1) Right sided facial asymmetry (drooping of right upper lip) (Class – I) 2) Frequent episodes of seizures	1) Drooping of lip resolved (normal facial activity) 2) No seizures reported post initiation of therapy	3640	--	7800

4	1) Right sided facial asymmetry (ptosis of right upper eyelid and right sided facial drooping) (Class - II)	3) Facial asymmetry resolved (normal facial activity)	4480	--	9600
5	1) Sluggish patellar reflex bilaterally 2) Deep nociception in all the limbs 3) Non ambulatory tetraparesis	1) Patellar reflex and nociception restored 2) Ambulatory with manual assistance	1155	1) Ambulatory	2475
6	1) Deficit in withdrawal reflexes in all the limbs and deep nociception 2) Reduced muscle tone in thighs 3) Non ambulatory tetraparesis	1) Withdrawal reflexes restored to normal 2) Ambulatory with manual assistance	1596	1) Ambulatory	3420
7	1) Megaoesophagus (oesophageal dilatation at thoracic inlet)-	1) No regurgitation reported 2) Dilatation was not visible on x-ray	4060	--	8700

4.8.2 Clinical Parameters

The Mean ± SE values for the physiological parameters including heart rate (beats/min), respiratory rate (breaths/min) and rectal temperature (^{0}F) before and after replacement therapy are presented in Table 4.17.

The mean heart rate, respiratory rate and rectal temperature before replacement therapy was recorded as 95.43 ± 4.20 (beats/min), 26.29 ± 1.87 (breaths/min) and 100.87 ± 0.48 (^{0}F) respectively. Whereas, after the replacement therapy the mean values of heart rate, respiratory rate and rectal temperature were 102.71 ± 2.81 (beats/min), 26.57 ± 1.36 (breaths/min) and 101.14 ± 0.42 (^{0}F) respectively.

Table 4.17 Comparative analysis of clinical parameters before and after replacement therapy in dogs with hypothyroidism associated neuromuscular disorders (n=7)

Sr. No.	Parameter	Before Mean ± SE	After Mean ± SE	t Value
1	Heart Rate (beats/min)	95.43 ± 4.20	102.71 ± 2.81	2.71*
2	Respiration Rate (breaths/min)	26.29 ± 1.87	26.57 ± 1.36	0.17
3	Rectal Temp (^{0}F)	100.87 ± 0.48	101.14 ± 0.42	0.43
*=p≤0.05 (Table t = 2.45)				

The study reported significant (p≤0.05) improvement in the heart rate after replacement therapy. This was in agreement with Hare *et al.* (2018) and Guglielmini *et al.* (2019). Thyroid hormones have positive ionotropic and chronotropic effect on the heart which resulted in the increased heart rate after the hormonal supplementation (Nelson and Couto 2019).

4.8.3 Hematological Parameters

The Mean ± SE values for the hematological parameters before and after replacement therapy are presented in Table 4.18 and (Appendix 8).

The Mean ± SE values for hemoglobin, total erythrocyte count, and packed cell volume in hypothyroid dogs were 10.53 ± 1.19 gm%, 4.52 ± 0.46 ($\times 10^6/\mu l$), and 30.86 ± 3.25% before replacement therapy, which increased to 14.07 ± 0.60 gm%, 5.94 ± 0.28 ($\times 10^6/\mu l$), and 40.71 ± 2.25 % after replacement therapy.

Similarly, the Mean ± SE values for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) before replacement therapy were 68.09 ± 1.05 fl, 23.12 ± 0.43 pg, and 33.99 ± 0.57 g/dl, respectively, and after replacement therapy, these values were 68.55 ± 2.06 fl, 23.77 ± 0.81 pg, and 34.75 ± 0.84 g/dl, respectively.

The total leucocyte count (TLC) before and after replacement therapy was 14.34 ± 3.33 ($\times 10^3/\mu l$) and 14.32 ± 2.54 ($\times 10^3/\mu l$), respectively. Differential counts of neutrophils, eosinophils, lymphocytes, monocytes, and basophils before therapy were 66.71 ± 3.53 %, 2.29 ± 0.84 %, 29.71 ± 3.92 %, 1.29 ± 0.18 %, and 0 %, respectively, which changed to 76.21 ± 4.73 %, 4.96 ± 1.29 %, 17.57 ± 4.40 %, 1.26 ± 0.26 %, and 0 %, respectively, after therapy.

Platelet count before and after replacement therapy was 202.28 ± 28.75 ($\times 10^3/\mu l$) and 234.14 ± 24.51 ($\times 10^3/\mu l$) respectively.

Table 4.18 Comparative analysis of hematological parameters before and after replacement therapy in dogs with hypothyroidism associated neuromuscular disorders (n=7)

Sr. No.	Parameter	Before Mean ± SE	After Mean ± SE	t Value
1	Hb (gm%)	10.53 ± 1.19	14.07 ± 0.60	3.89**
2	TEC($\times 10^6/\mu l$)	4.52 ± 0.46	5.94 ± 0.28	2.68*
3	PCV (%)	30.86 ± 3.25	40.71 ± 2.25	3.34*
4	MCV (fl)	68.09 ± 1.05	68.55 ± 2.06	0.26
5	MCH (pg)	23.12 ± 0.43	23.77 ± 0.81	1.31
6	MCHC (g/dl)	33.99 ± 0.57	34.75 ± 0.84	0.76
7	TLC($\times 10^3/\mu l$)	14.34 ± 3.33	14.32 ± 2.54	0.005
8	Neutrophils %	66.71 ± 3.53	76.21 ± 4.73	1.80
9	Eosinophils %	2.29 ± 0.84	4.96 ± 1.29	1.66
10	Lymphocytes %	29.71 ± 3.92	17.57 ± 4.40	2.35
11	Monocytes %	1.29 ± 0.18	1.26 ± 0.26	0.08
12	Platelets ($\times 10^3/\mu l$)	202.28 ± 28.75	234.14 ± 24.51	1.54
*=p≤0.05 (Table t = 2.45) **=p≤0.01 (Table t = 3.70)				

The study reported non-significant ($p \leq 0.05$) difference in the Mean ± SE values in all the hematological parameters except for Hb, TEC and PCV. As mentioned earlier, on comparison of hypothyroid group with apparently healthy group significant ($p \leq 0.01$) alterations were reported for the same parameters.

The findings of the present study reported improvement in the concentrations of Hb, TEC and PCV after replacement therapy when compared to before replacement therapy. Highly significant ($p \leq 0.01$) improvement was reported with respect to hemoglobin, while TEC and PCV showed significant ($p \leq 0.05$) improvement. The notable changes in the concentrations of haemoglobin, PCV, and TEC, trending toward normal levels, indicates a favourable overall response to levothyroxine supplementation.(Le Traon *et al.*, 2009). Similar findings were documented by Dixon *et al.* (2002) and Naveen (2024). Notable improvement occurs in the erythrocyte count 2 weeks after thyroid hormone replacement therapy (Dixon *et al.* 2002). This could be attributed to role of thyroid hormones to stimulate erythropoiesis by promoting the rapid proliferation of immature erythroid progenitors and boosting erythropoietin (EPO) production through the activation of erythropoietin gene expression (Kour *et al.* 2021).

4.8.4 Biochemical Parameters

The Mean ± SE values for the biochemical parameters before and after replacement therapy are presented in Table 4.19 and (Appendix 9).

The Mean ± SE values for total bilirubin, direct bilirubin, and indirect bilirubin in hypothyroid dogs were 0.29 ± 0.04 (mg/dl), 0.14± 0.02 (mg/dl), and 0.14 ± 0.03 (mg/dl) before replacement therapy, which changed to 0.31 ± 0.03 (mg/dl), 0.15 ± 0.02 (mg/dl), and 0.19 ± 0.03 (mg/dl) after replacement therapy.

Similarly, the Mean ± SE values for SGOT, SGPT, and ALP before replacement therapy were 46.57 ± 13.74 (IU/L), 116.43 ± 51.21 (IU/L), and 202.14 ± 44.93 (IU/L), respectively, and after replacement therapy, these values were 26.06 ± 3.94 (IU/L), 136 ± 57.99 (IU/L), and 380.99 ± 98.09 (IU/L), respectively.

The total protein concentration before and after replacement therapy was 6.13 ± 0.41 (gm/dl) and 6.03 ± 0.39 (gm/dl), respectively. Where albumin and globulin concentrations before therapy were 2.49 ± 0.10 (gm/dl), and 3.64 ± 0.45 (gm/dl) respectively, which changed to 2.71 ± 0.10 (gm/dl) and 3.32 ± 0.44 (gm/dl) respectively, after therapy.

The mean concentrations of BUN and creatinine were 20.41 ± 2.53 (mg/dl) and 0.99 ± 0.13 (mg/dl) before therapy, which were reduced to 17.48 ± 2.71 (mg/dl), and 0.81 ± 0.12 (mg/dl) respectively.

The lipid profile including serum cholesterol and triglycerides had mean concentrations of 225.29 ± 28.08 (mg/dl) and 106.29 ± 21.70 (mg/dl) before replacement therapy which changed to 237.86 ± 25.05 (mg/dl) and 97.14 ± 17.36 (mg/dl) after replacement therapy respectively.

The Mean ± SE values for Sodium, Potassium, and Chlorides in hypothyroid dogs were 148.14 ± 0.90 (mEq/L), 4.44 ± 0.14 (mEq/L), and 111.33 ± 0.90 (mEq/L) before replacement therapy, which changed to 147.04 ± 0.42 (mEq/L), 4.96 ± 0.18 (mEq/L), and 108.73 ± 0.71 (mEq/L) after replacement therapy.

The mean concentrations of serum calcium and serum phosphorus were 10.20 ± 0.91 (mg/dl) and 4.91 ± 2.10 (mg/dl) before replacement therapy, which became 10.06 ± 0.76 (mg/dl) and 4.39 ± 1.15 (mg/dl) after replacement therapy respectively.

The current study reported non-significant ($p \leq 0.05$) difference in all the biochemical parameters mentioned above except for serum chlorides.

Earlier comparison between healthy and hypothyroid group had yield significant ($p \leq 0.05$) difference in concentrations of serum cholesterol being elevated in hypothyroid group. Non-significant ($p \leq 0.05$) difference was reported in the mean concentrations of cholesterol before and after replacement therapy. Dixon *et al.* (2002) and Naveen (2024) reported significant decline in the concentrations of cholesterol post treatment in hypothyroid dogs. Lack of decline in the serum cholesterol concentrations after replacement therapy in the current study could be attributed to variability in the after-replacement therapy sample collection period, as the follow-up samples were particularly collected when the neurological signs

were partially improved or completely resolved and hence the time period being different for each case.

The Mean ± SE values of serum chlorides showed significant decline after the replacement therapy but the values were in the reference range itself.

Table 4.19 Comparative analysis of biochemical parameters before and after replacement therapy in dogs with hypothyroidism associated neuromuscular disorders (n=7)

Sr. No.	Parameter	Before Mean ± SE	After Mean ± SE	t Value
1	B. Total (mg/dl)	0.29 ± 0.04	0.31 ± 0.03	0.46
2	B. Direct (mg/dl)	0.14± 0.02	0.15 ± 0.02	0.21
3	B. Indirect (mg/dl)	0.14 ± 0.03	0.19 ± 0.03	1.00
4	SGOT (IU/L)	46.57 ± 13.74	26.06 ± 3.94	1.31
5	SGPT (IU/L)	116.43 ± 51.21	136 ± 57.99	0.23
6	ALP (IU/L)	202.14 ± 44.93	380.99 ± 98.09	1.54
7	Total protein (gm/dl)	6.13 ± 0.41	6.03 ± 0.39	0.51
8	Albumin(gm/dl)	2.49 ± 0.10	2.71 ± 0.10	2.26
9	Globulin (gm/dl)	3.64 ± 0.45	3.32 ± 0.44	1.99
10	BUN (mg/dl)	20.41 ± 2.53	17.48 ± 2.71	0.71
11	Creatinine (mg/dl)	0.99 ± 0.13	0.81 ± 0.12	1.06
12	Sr. Cholesterol (mg/dl)	225.29 ± 28.08	237.86 ± 25.05	0.78
13	Sr. Triglycerides (mg/dl)	106.29 ± 21.70	97.14 ± 17.36	0.42
14	Sodium (mEq/L)	148.14 ± 0.90	147.04 ± 0.42	1.16
15	Potassium (mEq/L)	4.44 ± 0.14	4.96 ± 0.18	2.09
16	Chlorides (mEq/L)	111.33 ± 0.90	108.73 ± 0.71	3.40*
17	Sr. Calcium (mg/dl)	10.20 ± 0.91	10.06 ± 0.76	0.50
18	Sr. Phosphorus (mg/dl)	4.91 ± 2.10	4.39 ± 1.15	0.80
*=p≤0.05 (Table t = 2.45)				

4.8.5 Thyroid profile

(a) Quality Control

Quality control parameters for the TT_3, TT_4 and fT_4 assessment assays are presented in Table 4.10, Table 4.11, and Table 4.12, respectively.

(b) <u>Comparative analysis of thyroid profile Before and after replacement therapy</u>

The Mean ± SE values for the thyroid profile before and after replacement therapy are presented in Table 4.20 and (Appendix 7).

The concentrations of TT_3, TT_4 and fT_4 were 0.82 ± 0.05 (nmol/L), 10.00 ± 1.81 (nmol/L) and 6.63 ± 0.30 (pmol/L) before replacement therapy which were increased to 1.17 ± 0.21 (nmol/L), 25.28 ± 2.87 (nmol/L) and 13.53 ± 1.29 (pmol/L) after replacement therapy respectively. The mean concentration of TSH before replacement therapy was 1.63 ± 0.32 (ng/mL) which decreased to 1.21± 0.26 (ng/mL) after replacement therapy.

Table 4.20 Comparative analysis of thyroid profile before and after replacement therapy in dogs with hypothyroidism associated neuromuscular disorders (n=7)

Sr. No.	Parameter	Before Mean ± SE	After Mean ± SE	t Value
1	TT_3 (nmol/L)	0.82 ± 0.05	1.17 ± 0.21	1.84
2	TT_4 (nmol/L)	10.00 ± 1.81	25.28 ± 2.87	6.73**
3	fT_4 (pmol/L)	6.63 ± 0.30	13.53 ± 1.29	5.15**
4	TSH (ng/mL)	1.63 ± 0.32	1.21 ± 0.26	1.33
**=p≤0.01 (Table t= 3.70)				

Density distribution of the data points for TT_3, TT_4, fT_4 and TSH are presented in Figure 4.11, Figure 4.12, Figure 4.13, and Figure 4.14 respectively. The violin plot density distribution in hypothyroid dogs before replacement therapy and after replacement therapy groups.

Significant (p≤0.01) increase in the concentrations of TT_4 and fT_4 was recorded after the replacement therapy with levothyroxine sodium compared to before therapy. These findings are in accordance with Dixon *et al.* (2002), Le Traon *et al.* (2009) and Naveen (2024). This improvement towards normalcy after levothyroxine supplementation indicates positive response to the therapy (Le Traon *et al.,* 2009).

The mean concentration of TT_3 showed non-significant (p≤0.05) increase

after the replacement therapy and that of TSH showed non-significant ($p \leq 0.05$) decrease after the replacement therapy. Findings with respect to TSH in the current study align with Pawar (2009), who reported non-significant alterations in the concentrations of TSH after treatment. Non-significant ($p \leq 0.05$) difference in TT_3 between the groups gained support from Kantrowitz *et al.* (2001) stated that sensitivity and accuracy of TT_3 was doubtful for diagnosis of hypothyroidism.

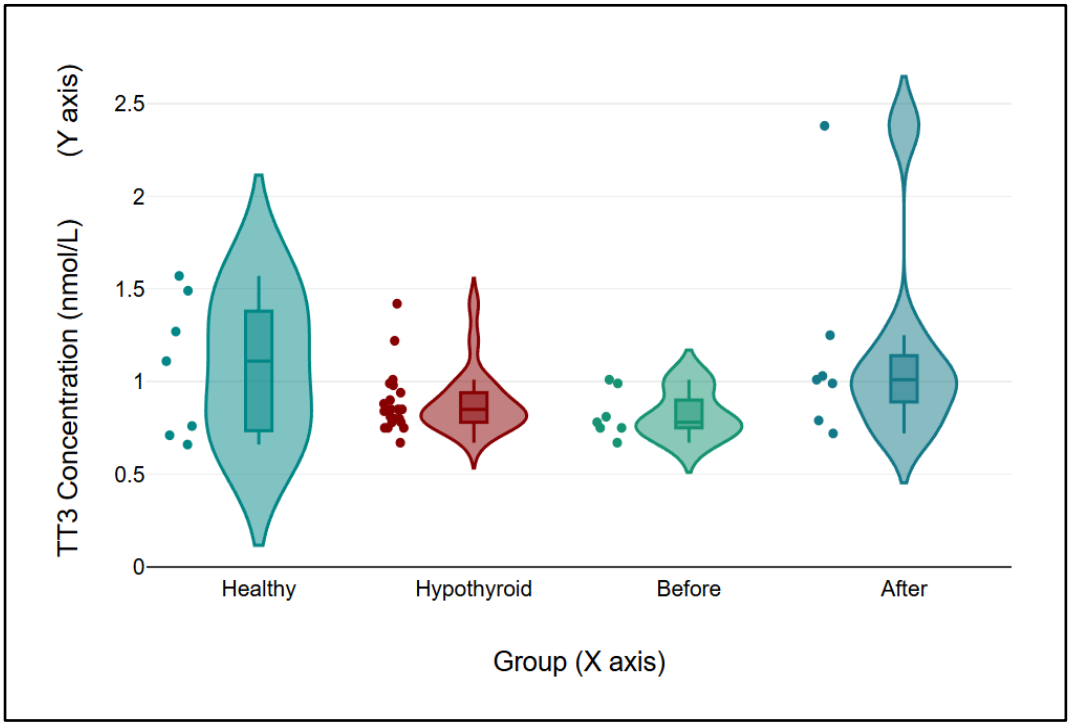

Figure 4.11 Comparative analysis of TT_3 between healthy (n=7), hypothyroid (n=21), before replacement therapy (n=7) and after replacement therapy (n=7) group of dogs

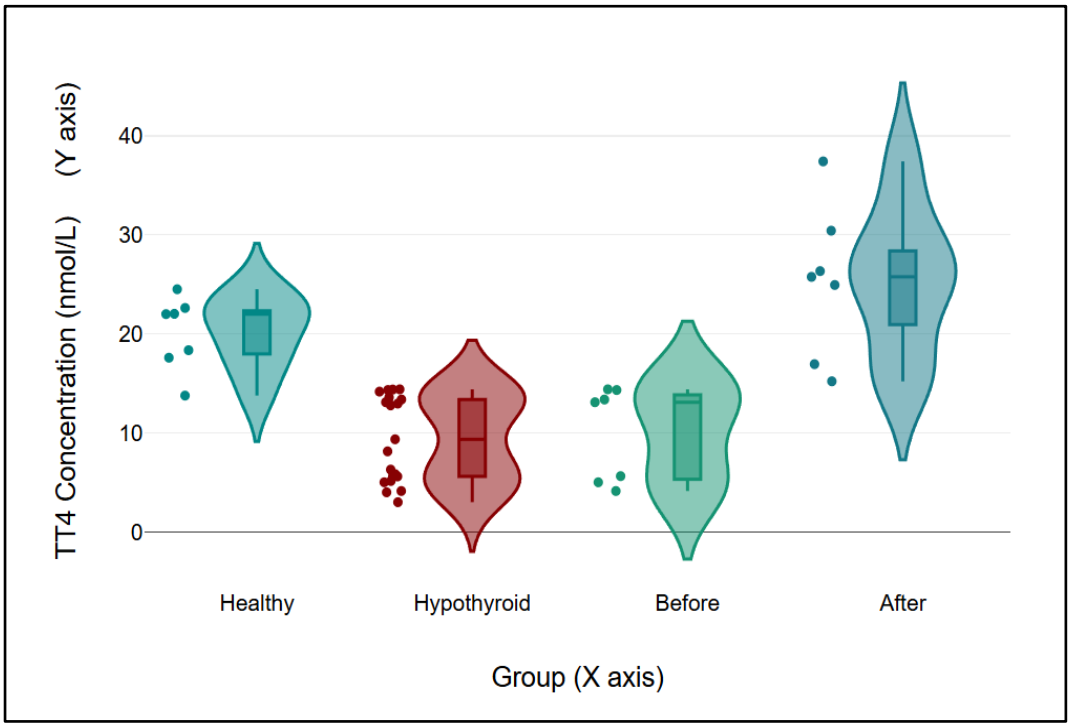

Figure 4.12 Comparative analysis of TT_4 between healthy (n=7), hypothyroid (n=21), before replacement therapy (n=7) and after replacement therapy (n=7) group of dogs

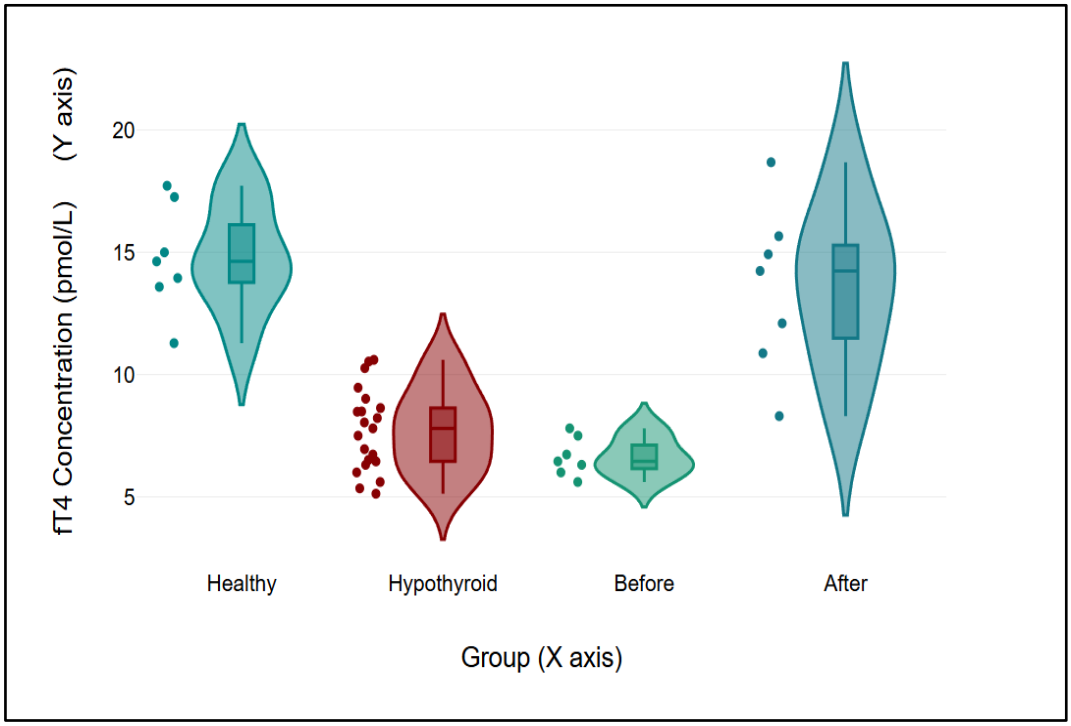

Figure 4.13 Comparative analysis of fT_4 between healthy (n=7), hypothyroid (n=21), before replacement therapy (n=7) and after replacement therapy (n=7) group of dogs

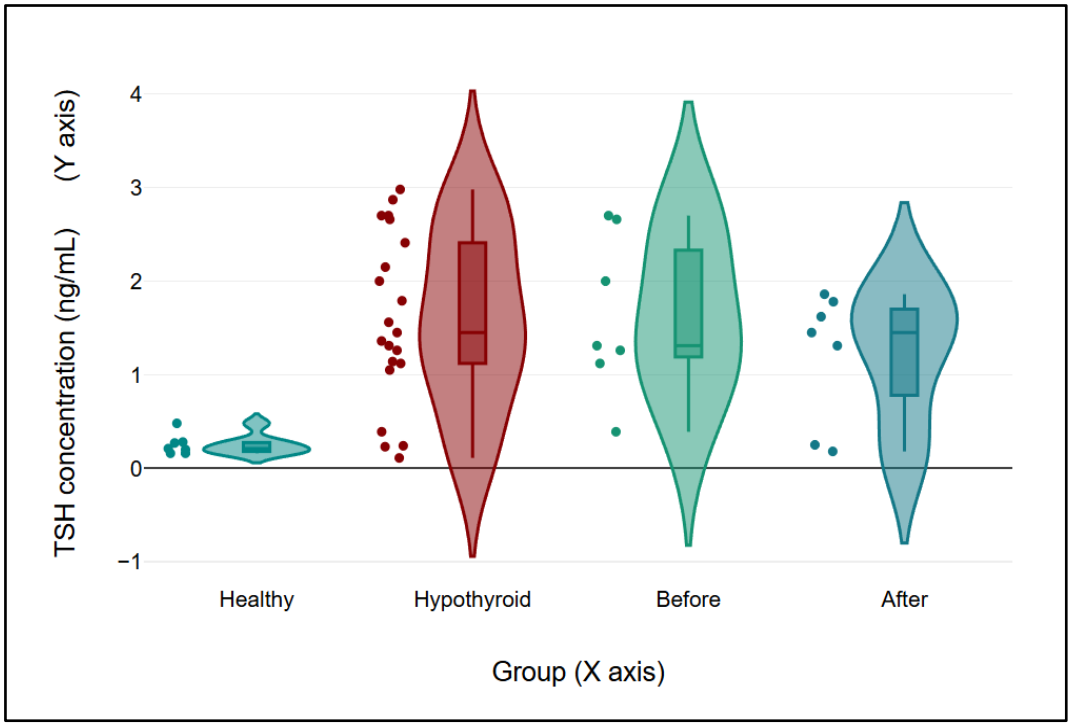

Figure 4.14 Comparative analysis of TSH between healthy (n=7), hypothyroid (n=21), before replacement therapy (n=7) and after replacement therapy (n=7) group of dogs

Summary & Conclusions

5. SUMMARY AND CONCLUSIONS

The present study entitled '**Studies on hypothyroidism in dogs with special reference to neuromuscular disorders**' has been carried out at Department Veterinary Clinical Medicine, Ethics and Jurisprudence and Department of Veterinary Nuclear Medicine including Radio Isotope Laboratory (Level-II; Research Purpose), Mumbai Veterinary College, Parel, Mumbai-400012, Maharashtra Animal and Fishery Science University Nagpur. The research aimed to investigate the neurological symptoms linked to hypothyroidism, analyse the changes in the clinico-hematobiochemical profile of neuromuscular disorders resulting from hypothyroidism, and evaluate the effectiveness of replacement therapy.

A total of 28 dogs were ethically included in the study after regulatory approval from the board of studies, Institutional Ethics Committee for Veterinary Clinical Research and the Institutional Biosafety Committee at Mumbai Veterinary College (Resolution no: VCM/5/2024. Dated: 14/06/2024, Ethics Committee No.- IEC-VCR/sub-committee/01/2024 IEC-VCR Dated: 28/12/2024 and IBSC no. MVC/IBSC/02/2024 Dated: 03/05/2024), as well as consent obtained from the pet owners. The dogs referred by the Bai Sakarbai Dinshaw Petit Hospital for Animals (BSDPHA) and field veterinarians were included in the study. The enrolled dogs were categorised into two groups based on clinical signs and symptoms viz, apparently healthy dogs (Group I; n=7) and hypothyroid dogs with neuromuscular disorders (Group II; n=21).

Hypothyroidism associated neurological signs documented in the current study were nystagmus (n=1/21; 4.76%), seizures (n=2/21; 9.52%), proprioceptive ataxia (n=3/21; 4.29%), head tilt (n=3/21; 14.29%), paraparesis (n=3/21; 14.29%), facial asymmetry (n=5/21; 23.81%), regurgitation due to megaesophagus (n=5/21; 23.81%) and tetraparesis (n=6/21, 28.57%).

The current study reported mean count of heart rate 108.57 $\pm$ 5.11 (beats/min) and 93.62 $\pm$ 3.78 (beats/min) in healthy and hypothyroid dogs respectively. The mean count of heart rate between the groups differed

significantly ($p \leq 0.05$), being comparatively lower in hypothyroid group.

The Mean ± SE values reported in the current study in healthy dogs for hemoglobin, total erythrocyte count and packed cell volume were 14.77 ± 0.90 (gm%), 6.55 ± 0.40 ($\times 10^6/\mu l$) and 43.23 ± 2.63(%) whereas, the mean concentrations of these parameters in hypothyroid dogs were 11.11 ± 0.58 (gm%), 4.80 ± 0.25 ($\times 10^6/\mu l$) and 32.29 ± 1.71 (%), respectively. Hemoglobin, total erythrocyte count and packed cell volume were significantly ($p \leq 0.01$) lower in hypothyroid group.

The lipid profile including serum cholesterol and serum triglycerides, had Mean ± SE values of 154.71 ± 17.95 (mg/dl) and 75.00 ± 9.91 (mg/dl) in healthy dogs and 219.14 ± 13.76 (mg/dl) and 85.43 ± 11.21 (mg/dl) in hypothyroid dogs, respectively. The mean values of serum cholesterol were significantly ($p \leq 0.05$) lower in hypothyroid group.

The mean concentrations of TT_3, TT_4, fT_4 and TSH were 1.08 ± 0.14 (nmol/L), 20.12 ± 1.40 (nmol/L), 14.77 ± 0.83 (pmol/L) and 0.25 ± 0.04 (ng/mL) in healthy dogs and 0.89 ± 0.04 (nmol/L), 9.45 ± 0.94 (nmol/L), 7.72 ± 0.37 (pmol/L) and 1.59 ± 0.20 (ng/mL) in hypothyroid dogs respectively. The study found that hypothyroid individuals had significantly ($p \leq 0.01$) lower concentrations of serum total and free thyroxine, while their thyroid stimulating hormone concentrations were significantly ($p \leq 0.01$) higher.

In the present study, seven (n=7) dogs with neuromuscular disorders associated with hypothyroidism were treated with thyroid hormone replacement therapy (Levothyroxine), and prominent improvements were documented. Dogs with various neuromuscular signs—such as head tilt, ataxia, facial asymmetry, seizures, limb weakness, and megaesophagus—showed notable clinical improvement following levothyroxine therapy. Symptoms like facial drooping, abnormal reflexes, difficulty in walking, regurgitation, and generalized weakness either significantly improved or resolved with appropriate thyroid hormone supplementation, highlighting the importance of considering hypothyroidism in dogs presenting with such neurological and neuromuscular signs.

In the current study the mean count of heart rate before replacement therapy was recorded as 95.43 ± 4.20 (beats/min). Whereas, after the

replacement therapy the mean value of heart rate was 102.71 ± 2.81 (beats/min). The study reported significant ($p \leq 0.05$) improvement in the heart rate after replacement therapy.

The Mean ± SE values for hemoglobin, total erythrocyte count, and packed cell volume in hypothyroid dogs were 10.53 ± 1.19 gm%, 4.52 ± 0.46 ($\times 10^6/\mu l$), and 30.86 ± 3.25% before replacement therapy, which increased to 14.07 ± 0.60 gm%, 5.94 ± 0.28 ($\times 10^6/\mu l$), and 40.71 ± 2.25% after replacement therapy. The improvement was highly significant ($p \leq 0.01$) in haemoglobin and significant ($p \leq 0.05$) in total erythrocyte count, and packed cell volume restoring towards the normalcy.

The concentrations of TT_3, TT_4 and fT_4 were 0.82 ± 0.05 (nmol/L), 10.00 ± 1.81 (nmol/L) and 6.63 ± 0.30 (pmol/L) before replacement therapy which were increased to 1.17 ± 0.21 (nmol/L), 25.28 ± 2.87 (nmol/L) and 13.53 ± 1.29 (pmol/L) after replacement therapy respectively. The mean concentration of TSH before replacement therapy was 1.63 ± 0.32 which decreased to 1.21 ± 0.26 after replacement therapy. Significant ($p \leq 0.01$) increase in the concentrations of TT_4 and fT_4 was recorded after the replacement therapy with levothyroxine sodium compared to before therapy. The mean concentration of TT_3 showed non-significant ($p \leq 0.05$) increase after the replacement therapy and that of TSH showed non-significant decrease after the replacement therapy.

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

1. Hypothyroidism in dogs is associated with neurological manifestation, viz. tetraparesis, megaoesophagus, facial asymmetry, paraparesis, head tilt, proprioceptive ataxia, seizures, and nystagmus. These symptoms should be considered as suggestive clinical signs of hypothyroidism.
2. Hypothyroid dogs exhibited significant ($p \leq 0.05$) hematological and biochemical alterations, including normocytic normochromic anemia and elevated cholesterol. They had significantly lower ($p \leq 0.01$) serum total and

free thyroxine concentrations, while their thyroid-stimulating hormone concentrations were significantly ($p \leq 0.01$) higher.

3. In hypothyroid dogs with neuromuscular disorders, levothyroxine replacement therapy was effective in improving clinical signs and restoration of hematobiochemical and thyroid profile.
4. The present study recommends 10 microgram/kg body weight, twice in a day as an initial starting dose of levothyroxine for successful therapeutic management in hypothyroid dogs with neuromuscular disorders.

Bibliography

6. BIBLIOGRAPHY

Alone, P. D. (2024). *Studies on Thyroid Profile in Dogs with Special Reference to Assessment of Canine TSH* (M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University).

Baker, K. (1986). Hormonal alopecia in dogs and cats. *In practice*, *8*(2), 71-78.

Baker, N (1997). Canine Practice: the journal of canine medicine and surgery for the practitioners. International symposium on canine hypothyroidism. Vol. 22.

Bell, J. S. (2003). Hereditary hypothyroidism: understanding the disease process. In *Tufts' Canine and Feline Breeding and Genetics Conference*.

Berson S. A and R. S. Yalow (1960). Immunoassay of endogenous plasma insulin in man. J. Clin. Invest.39:1157–1175.

Bertalan, A., M. Kent, and E. Glass (2013). Neurologic manifestations of hypothyroidism in dogs. *Compend Contin Educ Vet*, *35*(3), E2.

Bichsel, P., G. Jacobs, and J. E. Oliver Jr (1988). Neurologic manifestations associated with hypothyroidism in four dogs. *Journal of the American Veterinary Medical Association,* 192(12), 1745-1747.

Bharti, A., S. Shrestha, R. Rai, and M. K. Singh (2015). Assessment of serum minerals and electrolytes in thyroid patients. *Int J Adv Sci Res*, *1*(06), 259-263.

Blois, S. L., R. Poma, M. J. Stalker, and D. G. Allen (2008). A case of primary hypothyroidism causing central nervous system atherosclerosis in a dog. *The Canadian Veterinary Journal*, *49*(8), 789.

Bonagura, J. D., and D. C. Twedt (2009). Kirk's Current Veterinary Therapy XIV-E-Book. Elsevier Health Sciences.

Bonagura, J. D., and D. C. Twedt (2013). Kirks Current Veterinary Therapy XV. St Louis, Missouri.

Botman, J. W. M., and L. B. W. Jongkees (1955). Endotemporal branching of the facial nerve. *Acta Oto-laryngologica*, *45*(2), 111-114.

Catherine, J., R. Scott-Moncrieff, and L. Guptill-Yoran (2005). Endocrine disorders. Hypothyroidism. *Textbook of veterinary internal medicine. St. Louis (MO): Elsevier-Saunders*, 1535-43.

Chastain, C. B., and D. L. Panciera (1995). Hypothyroid diseases: In: Textbook of Veterinary Internal Medicine. p 1487.

Chaves, R. O., B. Copat, J. P. S. Feranti, D. A. Ferrarin, M. L.Schwab, R. T. França, and A. Mazzanti (2016). Facial paralysis secondary to hypothyroidism in a dog. Acta Scientiae Veterinariae, 44, 4-4.

Cuddon, P. A. (2002). Acquired canine peripheral neuropathies. *Veterinary Clinics: Small Animal Practice*, *32*(1), 207-249.

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).

Dambal, A. A., D. Sridevi, A. S. C. Sidrah, and S. K. Padaki (2016). Serum calcium and electrolytes in hypothyroidism-a case control study. *International Journal of Contemporary Medical Research Age (Years)*, *35*(8.85), 35-68.

Daminet, S., S. Croubels, L. Duchateau, A. Debunne, C. Van Geffen, Y. Hoybergs, ... and A. De Rick (2003). Influence of acetylsalicylic acid and ketoprofen on canine thyroid function tests. *The veterinary journal*, *166*(3), 224-232.

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

De Lahunta, A., E. N. Glass, and M. Kent (2020). *de Lahunta's veterinary neuroanatomy and clinical neurology*. Elsevier Health Sciences.

de Oliveira, J. T. S., A. A. Wenceslau, E. B. da Silva, M. S. L. de Lavor P. E. B. Guedes, and R. S. A. Carlos (2022). Canine hypothyroidism with neurological disorders. *Acta Scientiae Veterinariae*,*50*.

Dixon, R. M., S. W. J. Reid, and C. T. Mooney (2002). Treatment and therapeutic monitoring of canine hypothyroidism. *Journal of small animal practice*, *43*(8), 334-340.

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

Dubova, O., A. Dubovyi, I. Goralska, S. Zaika, and L. Kovalova (2023). Morphofunctional changes in the canine thyroid gland under low-intensity radiation exposure. *Japanese Journal of Veterinary Research*, *71*(1), 12-19.

Ettinger, S. J. and , E. C.Feldman (2000). Textbook of Veterinary Internal Medicine, 5th ed, Philadelphia: WB Saunders.,1419–1429.

Feldman, E. C., R. W. Nelson, C. Reusch and J. C. Scott-Moncrieff (2014). *Canine and feline endocrinology-e-book*. Elsevier health sciences.

Fenner, W. R. (1995). Textbook of Veterinary internal Medicine, Ettinger SJ, Feldman EC eds, 578-628.

Ferguson, D. C. (1994). Update on diagnosis of canine hypothyroidism. *Veterinary Clinics of North America: Small Animal Practice*, *24*(3), 515-539.

Fernandez, Y., and M. Seth (2016). Diagnosis and treatment of canine hypothyroidism. *The Veterinary Quarterly*, *18*(1), 1-11.

Fors, S. (2007). Neuromuscular manifestations of hypothyroidism in dogs. *EJCAP*, *17*, 173-178.

Fracassi, F., and A. Tamborini, (2011). associated with primary hypothyroidism in a dog. *Veterinary Record*, *168*, 329b.

Gaalova M, M. Fialkovicova, M. Kozak and S. Mateova (2008 Cardiovascular system abnormalities in a dog with primary hypothyroidism. Medycyna Weterynaryjna 64: 156-160.

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 at College of Veterinary and Animal Science, Pookode, Wayanad from 22.01.2015 to 24.01.2015

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., S. Chandra, A. Dadke, R. Gaikwad and A. Sarode, (2021). Metabolic and hormonal changes in water buffaloes during postparturient peak lactation. Buffalo Bulletin. 40: 565-570.

Galdhar, C. N., S. M. Vaidya, and, R. V. Gaikwad (2022). Serum triiodothyronine (T3) and thyroxine (T4) levels in healthy goats. *Indian Journal of Small Ruminants (The)*, *28*(2), 403-404.

Ghallab, E. H., H. Abdel-Saeed, H. Farghali, and T. Baraka, (2021). Epidemiological study on hypothyroidism in dogs in Egypt. *Veterinary Medical Journal (Giza)*, *67*(1), 113-121.

Giza, E. G., M. Płonek, J. M. Nicpoń, and M. A. Wrzosek (2015). Electrodiagnostic studies in presumptive primary hypothyroidism and polyneuropathy in dogs with reevaluation during hormone replacement therapy. *Acta Veterinaria Scandinavica*, *58*, 1-10.

Gnanasekar R., U. H. Nagvekar and N. Sivaprasad (2010) Development and validation of a two-step free thyroxine radioimmunoassay based on antibody coated tubes. Journal of Liquid Chromatography & Related Technologies.33:1576–1586.

Goldsmith S.J (1975) Radioimmune assay: Review of basic principles. Seminars in Nuclear Med.5:125-152.

Gori, E., P. Gianella, I. Lippi, and V. Marchetti (2023). Retrospective Evaluation of Gastrointestinal Signs in Hypothyroid Dogs. *Animals*, *13*(16), 2668.

Guglielmini, C., M. Berlanda, F. Fracassi, H. Poser S. Koren, and M. Baron Toaldo (2019). Electrocardiographic and echocardiographic evaluation in dogs with hypothyroidism before and after levothyroxine supplementation: A prospective controlled study. *Journal of Veterinary internal Medicine*, *33*(5), 1935-1942.

Gulzar, S., R. Khurana, D. Agnihotri, A. Aggarwal and G. Narang (2014). Prevalence of hypothyroidism in dogs in Haryana. *Indian J Vet Res.,*23(1):1-9.

Gupta, B., A. Moolchandani and M. Sareen (2010). Effect of induced Hypothyroidism on plasma cholesterol and bilirubin in Marwari Sheep. *Veterinary World*, *3*(7), 323.

Gupta, H., G. K. Sawale, C. N. Galdhar and S. D. Moregaonkar (2023). Pathology and diagnosis of hypothyroidism in dogs.

Hare, J. E., C. M. K. Morrow, J. Caldwell, J. and W, E. Lloyd (2018). Safety of orally administered, USP-compliant levothyroxine sodium tablets in dogs. *Journal of veterinary pharmacology and therapeutics*, *41*(2), 254-265.

Heseltine, J. (2019). Canine hypothyroidism: diagnosis and treatment. *Today's Veterinary Practice,* 2019, Vol. 9, No. 2, 46-50, 52 ref. 28

Higgins, M. A., J. H. Rossmeisl Jr, and D. L. Panciera (2006). Hypothyroid-associated central vestibular disease in 10 dogs: 1999–2005. *Journal of veterinary internal medicine,* 20(6), 1363-1369.

Huber, E., W. Armbrust, J. L. Forster, T. Ribiere and P. Grosclaude (2001). Resolution of megaesophagus after treatment of concurrent hypothyroidism in a dog. *Schweizer Archiv fur Tierheilkunde*, *143*(10), 512-514.

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

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 and S. Rajkhowa (2021). Radio immune assay (RIA) enabled thyroid profile (TT). *Indian J. Vet. Med. Vol*, *41*(2), 9-15.

Johnson, B. M., R. C. DeNovo and E. A. Mears (2009). Canine megaesophagus. *Kirk's Current Veterinary Therapy. 14th ed., Saunders Elsevier, St. Louis*, 486-92.

Kahn, S. E., R. L. Hull and K. M. Utzschneider (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 444(7121), 840-846.

Kantrowitz, L. B., M. E. Peterson, C. Melián and R. Nichols (2001). Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. *Journal of the American Veterinary Medical Association*, *219*(6), 765-769.

Kemppainen, R. J. and E. N. Behrend (2001). Diagnosis of canine hypothyroidism. *Veterinary Clinics: Small Animal Practice*, *31*(5), 951-962.

Kour, H., S. Chhabra and C. S. Randhawa (2020). Prevalence of hypothyroidism in dogs. *Pharma Innov J*, *9*, 70-72.

Kour, H., S. Chhabra and C. S. Randhawa (2021). Clinical and haemato-biochemical characteristics of hypothyroidism in canines. *Indian Journal of Veterinary Sciences and Biotechnology*, *17*(3), 1-5.

Kumar, K. S., D. S. Rao and G. Sharma (2007). A clinical study on hypothyroidism in dogs. *Intas Polivet*, *8*(2), 460-464.

Lathan, P. (2012). *Canine hypothyroidism. Clinician's brief Missisipi State University. pp. 25-28.*

Le Traon, G., S. F. Brennan, S. Burgaud, S. Daminet, K. Gommeren, L. J. I. Horspool, ... and C. T. Mooney (2009). Clinical evaluation of a novel liquid formulation of L-thyroxine for once daily treatment of dogs with hypothyroidism. *Journal of veterinary internal medicine*, *23*(1), 43-49.

McKeown, H. M. (2002). Hypothyroidism in a boxer dog. *The Canadian Veterinary Journal*, *43*(7), 553.

McCann, T. (2015). Canine hypothyroidism. *Companion animal*, *20*(10), 572-578.

Mooney, C. T. (2011). Canine hypothyroidism: a review of aetiology and diagnosis. *New Zealand Veterinary Journal*, *59*(3), 105-114.

Nachreiner, R. F., K.R. Refsal, P. A. Graham and M. W. Bowman (2002). Prevalence of serum thyroid hormone autoantibodies in dogs with clinical signs of hypothyroidism. *Journal of the American Veterinary Medical Association*, *220*(4), 466-471.

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).

NAVEEN, N. (2024). *STUDIES ON HYPOTHYROIDISM IN DOGS AND ITS THERAPEUTIC MANAGEMENT* (Doctoral dissertation, SRI VENKATESWARA VETERINARY UNIVERSITY).

Nelson, R. W., and C. G. Couto (2019). *Small Animal Internal Medicine-E-Book: Small Animal Internal Medicine-E-Book*. Elsevier Health Sciences.

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. (1997). Treating hypothyroidism. *Journal of the American Veterinary Medical Association, 92(1),* 58 - 68.

Panciera, D. L. (1999). Is it possible to diagnose canine hypothyroidism. *Journal of Small Animal Practice*, *40*(4), 152-157.

Papich, M. G. (2016). Saunders handbook of veterinary drugs. *North Carolina*, *12*, 162-171.

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

Peterson, M. E. (1988). Canine and feline endocrinology and reproduction. *Journal of Veterinary Internal Medicine, 2 (3),* 162.

Pettigrew, R., J. C. Fyee, B. L. Gregory, D. Lipsitz, A. Delahunta, B. A. Summer and G. D. Shelton (2007). CNS hypomyelination in Rat Terrier dogs with

congenital goiter and a mutation in the thyroid peroxidase gene. *Veterinary pathology*, *44*(1), 50-56.

Pöppl, A. G., N. D. S. Nunes, J. P. Matheus, L. Machado, S. D. F. Valle and S. P. Pavarini (2015). Dilated cardiomyopathy in a Fila Brasileiro with hypothyroidism secondary to lymphocytic thyroiditis.

Raja, R., D. Mondal, J. M. Velayudhan, R. S. K. Mandal, S. Bhatt V. Joshi and U. Dimri (2021). Studies on role of Thyroperoxidase (TPO) Enzyme in Primary Hypothyroidism Affected Dogs. *J. Anim. Res.,* 11(05): 909-914.

Reusch, C., R. W. Nelson, J. C. R. Scott-Moncrieff and E. C. Feldman (2015). *Canine and feline endocrinology*. Fourth edn. Elsevier Saunders, St. Louis, Missouri, 2015;137–187.

Romão, F. G., M. L. Poci Palumbo, J. C. Oshika and L. H. D. A. Machado (2012). Facial paralysis associated to hypothyroidism in a dog. *Semina: Ciências Agrárias*, 351-355.

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

Rossmeisl, J. H., R. B. Duncan, K. D. Inzana, D. L. Panciera and G. D. Shelton (2009). Longitudinal study of the effects of chronic hypothyroidism on skeletal muscle in dogs. *American journal of veterinary research*, *70*(7), 879-889.

Ryad, N., E. Ramadan, N. Salem and I. Saleh (2020). Canine adult-onset hypothyroidism: Prevalence, clinical and laboratory findings with special reference to therapeutic approach. *Veterinary Medical Journal (Giza)*, *66*(1), 10-18.

Salutgi, P., C. Galdhar, R. Sonigra, K. Natu, N. Mumbarkar, S. Mathkar, ... and 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.

Scarlett, J. M. (1994). Epidemiology of thyroid diseases of dogs and cats. *Veterinary Clinics of North America: Small Animal Practice*, *24*(3), 477-486.

Scott-Moncrieff, J. C., J. Azcona-Olivera, N. W. Glickman, L. T. Glickman and H. HogenEsch (2002). Evaluation of antithyroglobulin antibodies after routine

vaccination in pet and research dogs. *Journal of the American Veterinary Medical Association*, *221*(4), 515-521.

Snedecor, G. W. and W. G. Cochran (2009). *Statistical Methods,* 8th ed. Wiley Blackwell.

Srikala, D., K. S. Kumar and D. S. Rao (2011). Clinico-therapeutic management of thyroxine responsive polyneuropathies in dogs. Intas Polivet, 12(1), 96-99.

Stephan, I., I. Nolte and H. O. Hoppen (2003). Einfluss der Hypothyreose auf die kardiale Funktion beim Hund. *DTW. Deutsche tierärztliche Wochenschrift*, *110*(6), 231-239.

Suraniti, A. P., L. R. Gilardoni, M. G. R. Llal, M. Echevarría and M. Marcondes (2008). Hypothyroid associated polyneuropathy in dogs: Report of six cases. Braz J Vet Res Anim Sci, 45, 284-288.

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

Van Dijl, I. C., G. Le Traon, B. D. A. M. van De Meulengraaf, S. Burgaud, L. J. I. Horspool, and H. S. Kooistra (2014). Pharmacokinetics of total thyroxine after repeated oral administration of levothyroxine solution and its clinical efficacy in hypothyroid dogs. *Journal of veterinary internal medicine*, *28*(4), 1229-1234.

Vitale, C. L. and N. J. Olby (2007). Neurologic dysfunction in hypothyroid, hyperlipidemic Labrador Retrievers. Journal of Veterinary Internal Medicine, 21(6), 1316-1322.

Williams, D. A., J. C. Scott-Moncrieff, J. Bruner, D. Sustarsic, N. Panosian-Sahakian, E. Unver and A. S. El Shami, A. S. (1996). Validation of an immunoassay for canine thyroid-stimulating hormone and changes in serum concentration following induction of hypothyroidism in dogs. *Journal of the American Veterinary Medical Association*, *209*(10), 1730-1732.

Woelfel, C. W., C. L. Mariani, M. W. Nolan, E. K. Keenihan, S. P. Topulos, P. J. Early, ... and N. J. Olby (2023). Presumed pituitary apoplexy in 26 dogs: clinical findings, treatments, and outcomes. *Journal of Veterinary Internal Medicine*, *37*(3), 1119-1128.

Appendix

7. APPENDICES

Appendix 1. Owner Consent form

MUMBAI VETERINARY COLLEGE
PAREL, MUMBAI - 400 012.
Phone No:022-24131180, 24137030 Ext. 143/132
Fax: 022 – 24172301

Date: / /

Title of the project : STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS

CONSENT FORM

Owner's Name: _____ Contact No: _____
Email ID:_____

Address: _____

Pet : _____ Name : _____Breed: ____

Age : _____ Sex : M/F Colour: _____

Referred By: _____

I, _____, hereby authorize the Department _____ to evaluate and assess my dog.

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

I have also been informed by the doctors that there could be certain risks and complications associated with such procedure(s). The limitations of the procedures have been explained to me as well and for which I will not create any legal issue.

I further understand that during the procedure, any unforeseen complications may arise, which necessitate administration of medicines or performance of additional procedures. I authorize the use of appropriate medications or additional procedures as needed by the patient; before, during or after the procedure.

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

(Sign of Veterinarian) **(Signature of Owner)**

Appendix 2. Case Sheet

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, Nagpur
MUMBAI VETERINARY COLLEGE
Parel, Mumbai-12
DEPARTMENT OF VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

Case Sheet

Title - STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DISORDERS

Patient Demographic Data

Owner's Name:		**Mob. No.:**	
Dog's Name:	**Reg. No.:**	**Kennel:**	**Case no.:**
Sex:	**Age:**	**Weight:**	**Breed:**

History and clinical/physical examination

Vaccination status:		**Deworming status:**	
Neutered / Intact	**Heart Rate:**	**RR:**	**Temp:**

History, duration of illness, previous treatment (if done) and present complaint:

_____
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Clinical Examination:

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_____
_____

Laboratory Findings:

CBC

Sr. no	Elements	Result
1.	HB (gm %)	
2.	TEC (millions/cmm)	
3.	PCV (%)	
4.	MCV (fl)	
5.	MCH (pg)	
6.	MCHC (g/dl)	
7.	TLC ($\times 10^3$/cmm)	
8.	Neutrophils %	
9.	Eosinophils %	
10.	Lymphocytes %	
11.	Monocytes %	
12.	Basophils %	
13.	Platelets (/cmm)	

Serum Biochemistry

Sr. no	Elements	Result
1.	B. Total (mg/dl)	
2.	B. Direct (mg/dl)	
3.	B. Indirect (mg/dl)	
4.	SGOT (IU/L)	
5.	SGPT (IU/L)	
6.	ALP (IU/L)	
7.	Total protein (gm/dl)	
8.	Albumin (gm/dl)	
9.	Globulin (gm/dl)	
10.	BUN (mg/dl)	
11.	Creatinine (mg/dl)	
12.	Sr. Cholesterol (mg/dl)	
13.	Sr. Triglycerides (mg/dl)	

Thyroid Profile

fT_4 (ng/dl):	TT_3 (ng/mL):	TT_4 (µg/dL):	TSH (µIU/ml):
fT_4 (pmol/L):	TT_3 (nmol/L):	TT_4 (nmol/L):	TSH (µIU/ml):

Neurological Examination/Observations

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Observations

Gait:	Normal	Ataxic	Paresis	Paralysis	Comments:
Posture:	Normal	Head tilt	Kyphosis	Scoliosis	Comments:
Mentation:	Alert	Depressed	Stuporous	Comatose	Comments:

Cranial Nerve Examination

Cranial Nerve	**Test**	**Result**	**Comments**
Olfactory (CN I)	Response to odors	Yes / No	
Optic (CN II)	Menace response/ Cotton Ball Test (Right)	Present / Absent	
	Menace response/ Cotton Ball Test (Left)	Present / Absent	

Optic (CN II)	Pupillary light reflex (Right)	Normal / Abnormal	Contralateral- Present/ Absent
	Pupillary light reflex (Left)	Normal / Abnormal	
	Pupil Size (Right)	Normal/ Constrict/Dilated	Equal/Unequal
	Pupil Size (Left)	Normal/ Constrict/Dilated	
Oculomotor (CN III), Trochlear (CN IV), Abducent (CN VI)	Eye position and movement	Normal / Abnormal	
Trigeminal (CN V)	Jaw tone	Normal / Deficit / Increased	
Trigeminal (CN V)	Facial sensation	Normal / Deficit	
Facial (CN VII)	Facial symmetry	Yes / No	
Facial (CN VII)	Palpebral reflex	Present / Absent	
Vestibulocochlear (CN VIII)	Head tilt	Yes / No	
Vestibulocochlear (CN VIII)	Nystagmus	Yes / No	
Glossopharyngeal (CN IX), **Vagus (CN X)**	Gag reflex	Present / Absent	
Accessory (CN XI)	Muscle atrophy	Yes / No	

Hypoglossal (CN XII)	Tongue movement	Normal / Abnormal	

Spinal Reflexes

Reflex	Right	Left	Comments
Patellar Reflex	Normal / Exaggerated / Deficit / Absent	Normal / Exaggerated / Deficit / Absent	
Withdrawal Reflex (Thoracic Limb)	Normal / Exaggerated / Deficit / Absent	Normal / Exaggerated / Deficit / Absent	
Withdrawal Reflex (Pelvic Limb)	Normal / Exaggerated / Deficit / Absent	Normal / Exaggerated / Deficit / Absent	
Nociception (Thoracic Limb)	Superficial/Deep	Superficial/Deep	
Nociception (Pelvic Limb)	Superficial/Deep	Superficial/Deep	
Crossed Extensor Reflex	Present / Absent		
Perineal Reflex	Present / Absent		

Postural Reactions

Test	Right Front	Left Front	Right Rear	Left Rear	Comments
Proprioceptive Positioning	Normal / Abnormal	Normal / Abnormal	Normal / Abnormal	Normal / Abnormal	
Hopping	Normal / Abnormal	Normal / Abnormal	Normal / Abnormal	Normal / Abnormal	

Wheelbarrowing	Normal / Abnormal				
Hemistanding /Hemiwalking	Normal / Abnormal				

Muscle Tone and Strength

Limb	Right	Left	Comments
Thoracic Limbs	Normal / Deficit / Exaggerated	Normal / Deficit / Exaggerated	
Pelvic Limbs	Normal / Deficit / Exaggerated	Normal / Deficit / Exaggerated	
Muscle Atrophy	Present / Absent		
Esophageal Achalasia	Present / Absent		

Lesion Localization –
Additional Comments and Observations-

Neuro-Muscular Observations After Replacement Therapy

_____
_____
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Appendix 3. Hematological findings in healthy dogs (n=7)

Sr. No.	1	2	3	4	5	6	7
Hb (gm%)	18.30	16.60	11.40	14.70	12.40	14.20	15.80
TEC($\times 10^6/\mu l$)	7.77	7.18	4.66	6.63	5.60	6.77	7.22
PCV (%)	54.30	48.50	33.40	41.60	37.80	41.10	45.90
MCV (fl)	69.88	67.55	71.67	62.75	67.50	60.71	63.57
MCH (pg)	23.55	23.12	24.46	22.17	22.14	20.97	21.88
MCHC (g/dl)	33.70	34.23	34.13	35.34	32.80	34.55	34.42
TLC($\times 10^3/\ \mu l$)	9.80	16.10	17.80	17.00	15.40	9.30	6.70
Neutrophils %	77.00	75.00	57.00	80.00	65.00	66.00	60.00
Eosinophils %	1.00	5.00	3.00	4.00	6.00	2.00	2.00
Lymphocytes %	21.00	19.00	39.00	15.00	28.00	30.00	37.00
Monocytes %	1.00	1.00	1.00	1.00	1.00	2.00	1.00
Basophil %	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Platelets	195000.00	185000.00	315000.00	274000.00	502000.00	215000.00	328000.00

Appendix 4. Hematological findings in hypothyroid dogs (n=21)

Sr. No.	Hb (gm%)	TEC(×10^6/µl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	TLC(×10^3/ µl)	Neutrophils %	Eosinophils %	Lymphocytes %	Monocytes %	Basophil %	Platelets (×10^3/ µl)
1	11.00	5.41	32.90	60.81	20.33	33.43	7.90	65.00	0.00	34.00	1.00	0.00	184
2	15.90	6.73	45.20	67.16	23.63	35.18	13.70	73.00	4.00	22.00	1.00	0.00	1590
3	8.50	3.89	25.90	66.58	21.85	32.82	2.40	53.00	2.00	44.00	1.00	0.00	98
4	7.90	3.49	23.00	65.90	22.64	34.35	16.30	60.00	3.00	36.00	1.00	0.00	207
5	7.60	3.54	23.90	67.51	21.47	31.80	14.90	58.00	0.00	41.00	1.00	0.00	349
6	13.10	5.43	36.90	67.96	24.13	35.50	11.00	75.00	6.00	18.00	1.00	0.00	215
7	8.80	3.70	24.80	67.30	23.78	35.48	31.50	74.00	1.00	23.00	2.00	0.00	202
8	11.90	4.89	36.30	74.23	24.34	32.78	10.60	74.00	0.00	24.00	2.00	0.00	186
9	7.50	3.25	21.90	67.38	23.08	34.25	10.10	93.00	2.00	4.00	1.00	0.00	368
10	10.40	4.25	28.80	67.60	24.40	36.10	18.80	74.00	1.00	22.00	3.00	0.00	310
11	10.40	4.21	29.40	69.83	24.70	35.37	20.80	80.00	4.00	15.00	1.00	0.00	245
12	7.90	3.05	21.40	70.16	25.90	36.92	57.00	90.00	1.00	7.00	2.00	0.00	658
13	8.70	3.80	24.30	65.93	22.89	35.80	40.70	91.00	0.00	8.00	1.00	0.00	447
14	12.50	5.74	38.60	67.25	21.78	32.38	11.00	80.00	5.00	14.00	1.00	0.00	246
15	14.40	6.48	41.40	63.89	22.22	34.78	22.30	88.00	0.00	11.00	1.00	0.00	400
16	10.40	4.54	29.80	65.64	22.91	34.90	11.40	81.00	1.00	16.00	2.00	0.00	402
17	10.60	4.38	29.70	67.81	24.40	35.69	13.30	70.00	3.00	26.00	1.00	0.00	322
18	14.90	5.90	43.00	72.88	25.25	34.65	26.60	80.00	2.00	17.00	1.00	0.00	153
19	14.00	6.32	43.60	68.99	23.10	33.49	11.60	57.00	0.00	42.00	1.00	0.00	288
20	13.60	6.26	38.90	62.14	21.73	34.96	6.30	75.00	8.00	15.00	2.00	0.00	162
21	13.30	5.53	38.40	69.44	24.05	34.64	12.70	80.00	0.00	19.00	1.00	0.00	172

Appendix 5. Biochemical findings in healthy dogs (n=7)

Sr. No.	1	2	3	4	5	6	7
T. bilirubin (mg/dl)	0.20	0.30	0.30	0.20	0.30	0.30	0.30
D. bilirubin (mg/dl)	0.10	0.20	0.10	0.10	0.10	0.10	0.10
I. bilirubin (mg/dl)	0.10	0.10	0.20	0.10	0.20	0.20	0.20
SGOT (IU/L)	49.00	61.00	32.00	35.00	31.00	19.00	21.00
SGPT (IU/L)	129.00	28.00	32.00	26.00	57.00	28.00	26.00
ALP (IU/L)	166.00	171.00	351.00	278.00	66.00	157.00	245.00
Total protein (gm/dl)	5.60	5.70	5.30	5.60	5.40	5.70	7.60
Albumin(gm/dl)	2.60	2.30	2.10	2.80	2.10	2.70	2.20
Globulin (gm/dl)	3.00	3.40	3.20	2.80	3.30	3.00	5.40
BUN (mg/dl)	8.10	14.10	17.10	10.70	18.10	13.20	21.10
Creatinine (mg/dl)	0.70	0.80	1.50	0.45	0.60	1.10	1.20
Cholesterol (mg/dl)	134.00	107.00	191.00	107.00	131.00	232.00	181.00
Triglycerides(mg/dl)	42.00	118.00	76.00	74.00	48.00	96.00	71.00
Sodium (mEq/L)	145.70	147.00	148.70	148.00	147.90	145.50	146.10
Potassium (mEq/L)	4.60	4.50	4.30	4.50	4.50	5.20	4.90
Chlorides (mEq/L)	113.00	103.00	109.20	114.00	108.40	107.10	107.30
Calcium (mg/dl)	10.80	11.20	10.60	11.40	9.70	9.90	11.10
Phosphorus (mg/dl)	4.20	5.10	3.40	4.50	5.00	4.80	4.40

Appendix 6. Biochemical findings in hypothyroid dogs (n=21)

Sr. No.	1	2	3	4	5	6	7	8	9	10	11
T. bilirubin (mg/dl)	0.20	0.30	0.20	0.20	0.30	0.30	0.20	0.50	0.30	0.48	0.40
D. bilirubin (mg/dl)	0.10	0.10	0.10	0.10	0.20	0.20	0.10	0.20	0.10	0.13	0.20
I. bilirubin (mg/dl)	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.30	0.20	0.35	0.20
SGOT (IU/L)	41.00	37.00	23.00	21.00	26.00	21.00	104.00	94.00	28.00	54.87	43.00
SGPT (IU/L)	91.00	93.00	96.00	31.00	37.00	106.00	415.00	37.00	89.00	20.35	151.00
ALP (IU/L)	361.00	156.00	218.00	159.00	311.00	161.00	388.00	22.00	325.00	23.20	312.00
Total protein (gm/dl)	4.90	6.10	5.40	7.10	7.80	6.20	4.50	5.80	6.10	5.37	6.10
Albumin(gm/dl)	2.10	2.80	2.40	2.10	2.30	2.60	2.40	2.80	2.20	2.43	2.40
Globulin (gm/dl)	2.80	3.30	3.00	5.00	5.50	3.60	2.10	3.00	3.90	3.30	3.70
BUN (mg/dl)	8.90	23.10	19.10	8.30	16.10	26.10	28.10	22.10	8.10	17.24	21.20
Creatinine (mg/dl)	0.90	1.50	1.00	0.80	1.00	0.50	0.80	1.30	0.70	0.85	0.80
Cholesterol (mg/dl)	138.00	198.00	344.00	175.00	117.00	234.00	221.00	288.00	181.00	183.00	223.00
Triglycerides(mg/dl)	58.00	71.00	215.00	89.00	97.00	109.00	132.00	31.00	71.00	95.00	101.00
Sodium (mEq/L)	157.30	148.00	145.90	147.00	149.20	145.90	148.20	152.80	149.40	159.70	146.40
Potassium (mEq/L)	4.40	4.30	4.70	4.40	4.50	5.10	4.00	4.10	4.70	4.70	3.90
Chloride (mEq/L)	116.30	112.30	111.00	110.90	107.60	114.10	109.40	114.00	110.10	120.20	108.50
Calcium (mg/dl)	10.40	11.20	10.50	9.90	10.70	10.70	8.40	10.00	10.10	5.30	10.30
Phosphorus (mg/dl)	3.50	5.80	2.90	3.40	7.20	3.80	8.10	3.20	3.40	10.40	4.10

Appendix 6. Biochemical findings in hypothyroid dogs (n=21) (Continued.)

Sr. No.	12	13	14	15	16	17	18	19	20	21
T. bilirubin (mg/dl)	0.20	0.20	0.20	0.20	0.30	0.20	0.30	0.40	0.20	0.30
D. bilirubin (mg/dl)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.10	0.20
I. bilirubin (mg/dl)	0.10	0.10	0.10	0.10	0.20	0.10	0.20	0.20	0.10	0.10
SGOT (IU/L)	39.00	31.00	36.00	59.00	62.00	32.00	124.00	52.00	23.00	46.00
SGPT (IU/L)	45.00	26.00	21.00	81.00	27.00	37.00	186.00	43.00	31.00	52.00
ALP (IU/L)	372.00	521.00	71.00	186.00	268.00	245.00	534.00	65.00	49.00	178.00
Total protein (gm/dl)	5.20	6.50	7.10	7.10	8.40	5.50	5.10	5.80	7.10	5.80
Albumin(gm/dl)	1.90	2.40	2.60	2.80	2.30	2.40	2.30	2.60	2.40	2.70
Globulin (gm/dl)	3.30	4.10	4.50	4.30	6.10	3.10	2.80	3.20	4.70	3.10
BUN (mg/dl)	66.20	15.10	20.10	66.10	8.10	8.90	14.10	10.10	11.20	14.10
Creatinine (mg/dl)	2.50	0.80	1.60	2.90	0.80	0.70	0.70	0.60	0.80	1.20
Cholesterol (mg/dl)	279.00	257.00	241.00	233.00	131.00	256.00	112.00	307.00	271.00	213.00
Triglycerides (mg/dl)	48.00	89.00	66.00	214.00	21.00	67.00	41.00	91.00	59.00	29.00
Sodium (mEq/L)	145.20	146.90	147.20	132.20	147.90	146.00	143.40	146.10	145.10	150.00
Potassium (mEq/L)	5.50	4.60	4.30	3.50	4.20	4.30	4.10	5.00	4.40	4.00
Chlorides (mEq/L)	108.40	112.90	106.90	94.20	114.50	110.40	111.20	107.90	115.60	112.70
Calcium (mg/dl)	9.60	10.70	10.20	12.20	9.10	9.80	11.10	10.30	10.40	10.20
Phosphorus (mg/dl)	4.78	4.60	4.30	6.90	3.90	3.90	5.40	3.90	5.50	3.10

Appendix 7. Thyroid profile findings in healthy (n=7), hypothyroid (n=21) and after replacement therapy (n=7) dogs

	TT_3 (nmol/L)			TT_4 (nmol/L)			fT_4 (pmol/L)			TSH (ng/mL)		
Group	Healthy	Hypothyroid	After	Healthy	Hypothyroid	After	Healthy	Hypothyroid	After	Healthy	Hypothyroid	After
1	0.76	0.78	-	22.61	3.01	-	17.71	6.51	-	0.16	1.05	-
2	0.71	0.67	0.72	21.99	5.02	15.21	14.62	7.80	10.87	0.16	1.26	1.45
3	1.11	0.78	0.79	17.59	13.37	25.75	14.99	6.00	12.09	0.28	2	0.18
4	1.27	0.81	0.99	18.35	4.14	16.94	17.25	6.45	8.30	0.27	2.66	1.62
5	0.66	0.75	1.25	13.77	14.40	37.40	11.28	6.31	15.65	0.48	1.31	1.31
6	1.57	1.01	1.01	22.02	14.33	24.93	13.58	5.61	14.23	0.21	1.12	1.78
7	1.49	0.75	1.03	24.5	5.64	30.41	13.94	7.50	14.91	0.2	0.39	0.25
8		0.99	2.38		13.10	26.33		6.73	18.67		2.7	1.86
9		0.85			5.84			8.63			1.36	
10		0.90			6.30			9.46			2.87	
11		0.80			14.38			8.48			0.11	
12		0.88			5.61			10.60			2.15	
13		1.22			8.14			5.35			2.41	
14		0.80			13.18			8.22			2.7	
15		0.84			12.96			5.13			1.45	
16		0.98			9.36			10.53			0.24	
17		0.85			14.17			8.49			1.79	
18		0.94			4.01			8.04			1.14	
19		0.85			12.78			9.01			1.56	
20		1.42			13.59			10.26			2.98	
21		0.75			5.15			6.95			0.23	

Appendix 8. Hematological findings in dogs after replacement therapy (n=7)

Sr. No.	1	2	3	4	5	6	7
Hb (gm%)	14.30	14.80	14.30	11.10	16.40	13.60	14.00
TEC($\times 10^6$/µl)	5.68	7.12	5.81	5.25	6.81	5.67	5.24
PCV (%)	42.30	45.80	37.10	32.40	50.36	38.30	38.70
MCV (fl)	74.47	64.40	63.90	61.71	73.95	67.55	73.85
MCH (pg)	25.18	20.70	24.60	21.14	24.08	23.99	26.72
MCHC (g/dl)	33.81	32.30	38.60	34.26	32.57	35.51	36.18
TLC ($\times 10^3$/ µl)	11.60	6.50	23.37	16.40	22.50	12.40	7.50
Neutrophils %	70.00	78.50	91.00	52.00	85.00	80.00	77.00
Eosinophils %	9.00	1.70	8.00	8.00	1.00	5.00	2.00
Lymphocytes %	20.00	17.00	0.00	39.00	13.00	14.00	20.00
Monocytes %	1.00	2.80	1.00	1.00	1.00	1.00	1.00
Basophil %	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Platelets	185000.00	170000.00	220000.00	305000.00	344000.00	207000.00	208000.00

Appendix 9. Biochemical findings in dogs after replacement therapy (n=7)

Sr. No.	1	2	3	4	5	6	7
T. bilirubin (mg/dl)	0.30	0.28	0.52	0.20	0.30	0.30	0.30
D. bilirubin (mg/dl)	0.20	0.05	0.19	0.20	0.10	0.10	0.20
I. bilirubin (mg/dl)	0.20	0.23	0.33	0.10	0.20	0.20	0.10
SGOT (IU/L)	29.00	23.16	44.26	12.00	32.00	18.00	24.00
SGPT (IU/L)	311.00	64.65	404.00	34.00	61.00	43.00	41.00
ALP (IU/L)	627.00	431.00	377.90	98.00	789.00	232.00	112.00
Total protein(gm/dl)	5.80	5.15	7.73	7.10	6.10	5.10	5.20
Albumin(gm/dl)	3.20	2.80	2.67	2.40	2.50	2.70	2.70
Globulin (gm/dl)	2.60	2.35	5.06	4.70	3.60	2.40	2.50
BUN (mg/dl)	30.10	15.95	21.72	17.10	8.10	18.20	11.20
Creatinine (mg/dl)	0.80	1.38	0.46	0.70	1.00	0.50	0.80
Cholesterol (mg/dl)	267.00	347.00	209.00	162.00	171.00	223.00	286.00
Triglycerides(mg/dl)	147.00	172.00	75.00	48.00	69.00	68.00	101.00
Sodium (mEq/L)	144.80	147.10	147.50	146.90	148.10	146.80	148.10
Potassium (mEq/L)	5.90	4.40	5.00	5.10	4.90	4.90	4.50
Chlorides (mEq/L)	107.10	108.40	107.30	109.00	110.70	106.90	111.70
Calcium (mg/dl)	10.90	10.40	10.70	9.40	9.80	8.80	10.40
Phosphorus (mg/dl)	5.60	4.20	4.30	4.10	4.70	5.60	2.20

Abstract

Appendix - G

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	**:**	**STUDIES ON HYPOTHYROIDISM IN DOGS WITH SPECIAL REFERENCE TO NEUROMUSCULAR DIORDERS**
b)	Full name of student	**:**	Kawade Jaydeep Ramdas
c)	Name and address of Major Advisor	**:**	Dr. C. N. Galdhar Professor and RadiologicalSafety Officer (VNM), Veterinary Clinical Medicine, Ethics and Jurisprudence
d)	Degree to be awarded	**:**	M. V. Sc.
e)	Year of award of degree	**:**	2025
f)	Major subject	**:**	Veterinary Clinical Medicine, Ethics and Jurisprudence
g)	Total number of pages in the thesis	**:**	93
h)	Number of words in the abstract	**:**	295
i)	Signature of Student	**:**	
j)	Signature, Name, and address of forwarding authority (HOD / SH)	**:**	
k)	Signature of the Associate Dean	**:**	

ABSTRACT

The present study entitled '**Studies on hypothyroidism in dogs with special reference to neuromuscular disorders**' has been carried out at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence and Department of Veterinary Nuclear Medicine including Radio Isotope Laboratory, Mumbai Veterinary College, Parel, Mumbai-400012, MAFSU, Nagpur.

The current research primarily aimed to study neuromuscular manifestations associated with hypothyroidism and to evaluate the efficacy of hormonal replacement therapy in these cases. The study enrolled a total of twenty-eight (n=28) dogs, with seven (n=7) apparently healthy and twenty-one (n=21) hypothyroid associated neuromuscular disorders.

Among the neuromuscular signs exhibited by the dogs, tetraparesis contributed the most 28.57% (n=6/21), followed by megaoesophagus 23.81% (n=5/21), facial asymmetry 23.81% (n=5/21), paraparesis 14.29% (n=3/21), head tilt 14.29% (n=3/21), proprioceptive ataxia 14.29% (n=3/21), seizures 9.52% (n=2/21) and nystagmus 4.76% (n=1/21).

On comparison with apparently healthy group of dogs, the hypothyroid group showed normocytic normochromic anemia and statistically higher concentrations of cholesterol. Thyroid hormones, including TT_3, TT_4, and fT_4, were measured using radioimmunoassay, while TSH was assessed with a canine-specific ELISA kit. The mean concentrations of TT_4 and fT_4 in the hypothyroid group were 9.45 ± 0.94 (nmol/L) and 7.72 ± 0.37 (pmol/L), respectively, which were significantly ($p \leq 0.01$) lower compared to those in the healthy dogs, where TT_4 and fT_4 concentrations were 20.12 ± 1.40 (nmol/L) and 14.77 ± 0.83 (pmol/L), respectively. The mean concentration of TSH in the hypothyroid group was 1.59 ± 0.20 (ng/mL), which was significantly higher ($p \leq 0.01$) compared to 0.25 ± 0.04 (ng/mL) in healthy dogs. Mean concentration of TT_3 showed non-significant ($p \leq 0.05$) difference between the groups.

In hypothyroid dogs with neuromuscular disorders, levothyroxine replacement therapy with initial dose of 10 mcg/kg body weight was started and was found effective in improving clinical signs and restoration of hematobiochemical and thyroid profile.

प्रबंध सारांश

a)	प्रबंधाचे शीर्षक	**:**	**मज्जातंतु-स्नायूसंबंधी विकारांच्या विशेष संदर्भात श्वानांमध्ये हायपोथायरॉईडीझमवरील अभ्यास**
b)	विद्यार्थ्याचे पूर्णनाव	**:**	कवडे जयदीप रामदास
c)	प्रमुख मार्गदर्शकाचे नाव व पत्ता	**:**	डॉ. चंद्रकांत ना. गलधर प्राध्यापक आणि रेडिओलॉजिकल सुरक्षा अधिकारी, चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र विभाग मुंबई पशुवैद्यकीयमहाविद्यालय, मुंबई
d)	पदवी	**:**	पदव्युत्तर पदवी
e)	पदवीप्रदान करण्याचे वर्ष	**:**	२०२५
f)	मुख्यविषय	**:**	चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र
g)	प्रबंधतील एकूण पृष्टांची संख्या	**:**	९३
h)	सारांशाचे एकूण शब्द	**:**	२७२
i)	विद्यार्थ्याची सही	**:**	
j)	प्रबंध पाठविणाऱ्या अधिकाऱ्याचे संपूर्ण नाव, पत्ता व सही	**:**	
k)	सहयोगी अधिष्ठाता, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई ४०००१२		

सारांश

'मज्जातंतु-स्नायूसंबंधी विकारांच्या विशेष संदर्भात श्वानांमध्ये हायपोथायरॉईडीझमवरील अभ्यास', या शीर्षकाचा हा अभ्यास पशुवैद्यकीय चिकित्सालयीन औषधवैद्यक शास्त्र, नितीशास्त्र आणि न्यायवैद्यक शास्त्र, विभाग आणि पशुवैद्यकीय अणुऔषध विभाग, रेडिओ आइसोटोप प्रयोगशाळा, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई-४०००१२, मपमविवि, नागपूर येथे करण्यात आला आहे.

सदर संशोधनाचा मुख्य उद्देश हायपोथायरॉईडीझमशी संबंधित मज्जातंतु-स्नायूसंबंधी विकारांचा अभ्यास करणे आणि या प्रकरणांमध्ये संप्रेरक प्रतिस्थापण उपचार पद्धतीची प्रभावीता मूल्यांकन करणे हा होता. या अभ्यासात एकूण अठ्ठावीस (n=२८) श्वानांची नोंद करण्यात आली होती, ज्यात सात (n=७) निरोगी आणि एकवीस (n=२१) हायपोथायरॉईडीझमशी संबंधित मज्जातंतु- स्नायूसंबंधी विकार ग्रस्त श्वान होते.

श्वानांमध्ये आढळणाऱ्या मज्जातंतु-स्नायूसंबंधी लक्षणांमध्ये, टेट्रापेरेसिसने सर्वाधिक २८.५७% (n=६/२१) व त्यानंतर मेगाओसोफॅगस २३.८१% (n=५/२१), चेहऱ्यावरील असममितता २३.८१% (n=५/२१), पॅरापेरेसिस १४.२९% (n=३/२१), डोके झुकणे १४.२९% (n=३/२१), ऊतिअंतर्गतसंवेदनाग्राही गतिविभ्रम १४.२९% (n=३/२१), आकडी ९.५२% (n=२/२१) आणि नेत्रदोल ४.७६% (n=१/२१) यांचा क्रमांक लागतो.

श्वानांच्या निरोगी गटाशी तुलना केली असता, हायपोथायरॉइड गटात नॉर्मोसाइटिक नॉर्मोक्रोमिक रक्तक्षय आणि कोलेस्टेरॉलचे सांख्यिकीयदृष्ट्या जास्त प्रमाण दिसून आले. TT_3, TT_4 आणि fT_4 यासह थायरॉईड संप्रेरकांचे रेडिओ इम्युन प्रखनीद्वारे मोजमाप करण्यात आले, तर TSH चे मूल्यांकन कॅनाइन-स्पेसिफिक ELISA संच वापरून करण्यात आले. हायपोथायरॉइड गटात TT_4 आणि fT_4 चे सरासरी प्रमाण अनुक्रमे ९.४५ ± ०.९४ (nmol/L) आणि ७.७२ ± ०.३७ (pmol/L) होते, जे निरोगी श्वानांच्या तुलनेत लक्षणीयरीत्या ($p\leq०.०१$) कमी होते, जिथे TT_4 आणि fT_4 चे प्रमाण अनुक्रमे २०.१२ ± १.४० (nmol/L) आणि १४.७७ ± ०.८३ (pmol/L) होते. हायपोथायरॉइड गटात TSH चे सरासरी प्रमाण १.५९ ± ०.२० (ng/mL) होते, जे निरोगी श्वानांमध्ये ०.२५ ± ०.०४ (ng/mL) च्या तुलनेत लक्षणीयरीत्या जास्त ($p\leq०.०१$) होते. TT_3 चे सरासरी प्रमाण गटांमध्ये अलक्षणीय ($p\leq0.05$) फरक दर्शविते.

मज्जातंतु-स्नायूसंबंधी विकार असलेल्या हायपोथायरॉइड श्वानांमध्ये, १० mcg/kg शरीराच्या वजनाच्या सुरुवातीच्या डोससह लेव्होथायरॉक्सिन प्रतिस्थापण उपचार पद्धती सुरू करण्यात आली आणि चिकित्सालयीन लक्षणे सुधारण्यात आणि रक्त-रासायनिक आणि थायरॉईड प्रोफाइल पुनर्संचयित करण्यात प्रभावी आढळले.

Vita

VITA

Dr. Kawade Jaydeep Ramdas was born on February 28, 1999, in Ashti, Beed, Maharashtra. He excelled academically, securing 93.20% in S.S.C. (2015) and 86.00% in H.S.C. (2017) under the Aurangabad Board. He pursued his Bachelor of Veterinary Science and Animal Husbandry (B.V.Sc. & A.H.) at Mumbai Veterinary College, graduating with distinction and a C.G.P.A. of 7.89.

Driven by his interest in Veterinary Clinical Medicine, he joined Mumbai Veterinary College (MAFSU) in 2023 for an M.V.Sc. in Veterinary Clinical Medicine, Ethics, and Jurisprudence. His research focused on hypothyroidism in dogs with special reference to neuromuscular disorders. Under expert guidance, he successfully completed his work.

Beyond academics, he actively contributed to departmental research projects and participated in animal health camps and conferences, showcasing his dedication to veterinary medicine and community service.

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