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Triidothyronine (TT3), Total Thyroxine (TT4) and Free Thyroxine (FT4) Reference Range in Healthy Dogs by Radioimmunoassay (RIA)

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ABSTRACT

Background: The thyroid function of the dog has been little studied in comparison with that of the human. This may have resulted, in part, from the apparent lack of reporting of thyroid-related disorders in the Indian canine population. There is, therefore, a need for a more detailed study of thyroid hormone levels (establish reference interval) in normal dogs using a specific and accurate technique like radioimmunoassay (RIA).

Methods: Total 192 healthy dogs from the clinical setup of constituent colleges of MAFSU (*viz*. Mumbai, Nagpur, Parbhani, Shirwal, Udgir and Akola) were ethically enrolled to examine thyroid hormones levels (TT3, TT4 and FT4). Thyroid hormones were estimated using RIA.

Result: The results of the present study recorded mean serum TT3, TT4 and FT4 values in healthy dogs as 1.29±0.04 nmol/l, 28.17±1.18 nmol/l and 13.03±0.68 pmol/l respectively. The reference interval (25th to 75th percentile) for TT3, TT4 and FT4 was found to be 0.88-1.51 nmol/l, 15.70-35.29 nmol/l and 7.80-14.75 pmol/l respectively. The median for TT3, TT4 and FT4 was found to be 1.13 nmol/l, 24.54 nmol/l and 10.00 pmol/l respectively. The baseline serum thyroid hormone concentration would be valuable for identifying thyroid dysfunction in dogs.

Key words: Free thyroxine (FT4), Indian canine population, Radioimmunoassay (RIA), Triiodothyronine (TT3), Total thyroxine (TT4).

INTRODUCTION

Thyroid hormones are critically important for the regulation of various metabolic processes. Calorigenic thyroid hormones are important in foetal life specifically for the development of the neural and skeletal system. Thyroid hormone deficiency or surplus indirectly affects the majority of systems in the body (McCann, 2015). Globally recorded prevalence of canine hypothyroidism was 0.20-0.80% (Catherine *et al*., 2005). However, in India, the prevalence of canine hypothyroidism in Hisar was 0.40% (Gulzar *et al*., 2014) and to be specific in Mumbai it was 0.206% (Pawar, 2009).

Radioimmunoassay (RIA) is a sensitive *in vitro* method for assessment of antigen *i.e*., hormones, minerals, vitamins *etc*. from biological fluids. ^{125}I is a commonly used radioisotope among others for RIA due to its long half-life (t1/2=60 days). This method offers a convenient assay of large numbers of samples with excellent precision. Researchers around the globe have studied thyroid profiles in dogs by using human-based RIA kits. Commercially developed RIA kits for human use are readily available across India. But reported literature brings to the light necessity of physiological and pathological validation of these kits before their use in veterinary clinical practice in India (Galdhar and Gaikwad, 2015). Addressing this prerequisite, Dadke (2008) studied thyroid profiles in 59 healthy dogs in and around Mumbai city (India) and he documented mean serum concentration of TT3, TT4 and FT4 in healthy dogs as 1.03±0.02 nmol/l (Range: 0.68-1.40 nmol/l); 29.67±1.43 nmol/l, (Range: 11.71- 49.26 nmol/l) and 9.07±0.52 pmol/l (Range: 4.06-18.72 pmol/l) respectively. Thus, reported data strongly suggests human-based commercial RIA kits have strong diagnostic potential in veterinary clinical practice for the assessment of thyroid dysfunction in companion animals. Furthermore, these

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techniques need to be emphasized in routine veterinary diagnostics. The purpose of the present study is to report thyroid profile (TT_3, TT_4 and FT_4) reference range in healthy dogs in clinical setup using a specific and accurate technique like RIA.

MATERIALS AND METHODS

The present study was initiated after permission from the project monitoring cell and Institutional Bio-safety Committee of Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University (MAFSU), Mumbai-India. Total 192 healthy dogs from the clinical setup of constituent colleges of MAFSU (*viz*. Mumbai, Nagpur, Parbhani, Shirwal, Udgir and Akola) were ethically enrolled to examine thyroid hormones levels (TT_3, TT_4 and FT_4) from December 2019 to March 2021. Inclusion and exclusion criteria for the establishment of thyroid profile in healthy dogs were (a) Inclusion criteria: Dogs of both sex and various age groups, any breed including nondescript dogs, clinically healthy dogs with no clinical endocrinopathy. (b) Exclusion criteria: Dogs recently being treated for any endocrinopathies including thyroid dysfunction, pregnant bitches, dogs on medicines that can potentially alter thyroid functioning and thyroid hormone estimation (*viz*. steroids and L-thyroxine, perchlorate or iodine therapy, *etc*). After enrolment of clinical cases, history was taken regarding appetite, diet, age, sex, breed and pregnancy. Additionally, all enrolled cases were examined clinically for any apparent abnormalities. After litigation, sick dogs were recognized and isolated which were not incorporated in the assessment of thyroid profile in healthy dogs.

Blood samples were collected from each dog (cephalic vein or saphenous vein). Serum samples for thyroid profile estimation were stored at -20°C until the time of analysis. Thyroid hormones were estimated using commercial RIA kits for human purposes manufactured by the Board of Radiation and Isotope Technology (BRIT), Vashi, Mumbai (India) and the analysis was carried out at the Radio Isotope Laboratory, Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai (India). Thyroid hormones were assayed of each sample giving paired observation. TT3 and TT4 were measured as per the standard procedure outlined by the manufacturers. FT4 was estimated as per modifications recommended (Dadke, 2018 and Dadke *et al.,* 2018). Quality control parameters *viz*. magnitude of control samples and recovery percentage were studied to validate every assay. Statistical analysis of all data was performed using methods outlined by researchers (Snedecor and Cochran, 2004). In the present study reference ranges for hormone concentration were established by the nonparametric method of percentile estimates (25^{th} to 75^{th} percentile).

RESULTS AND DISCUSSION

Assay passed all recommended quality control parameters *viz*. magnitude of control samples provided with kits and per cent recovery. The standard curve of the assay was plotted and the hormonal magnitude of unknown samples was interpolated from the standard curve. Mean, interquartile range (*i.e*. 25^{th} to 75^{th} percentile) and median of TT3, TT4 and FT4 in healthy dogs (n=192) enrolled in the study and levels of thyroid hormones reported by other authors is presented in Table 1.

The mean value of TT3 of healthy dogs was found to be 1.29±0.04 nmol/l. Fig 1 depicts a box plot of TT3 concentration in healthy (n=192) dogs. For each box plot 'T bars' represent the data which is equal to range (0.26-2.45 nmol/l). Box represent the middle half of data (Interquartile range; 25^{th} to 75^{th} percentile, as 0.88-1.51 nmol/l), Horizontal bar in the box is the median of the data (1.13 nmol/l).

The mean value of TT4 of healthy dogs was recorded as 28.17±1.18 nmol/l. Fig 2 depicts a box plot of TT4 concentration in healthy dogs (n=192). For each box plot, 'T bars' represent the data which is equal to the range (3.86 -64.35 nmol/l). Box represent the middle half of data (Interquartile range; 25^{th} to 75^{th} percentile, as 15.70-35.29 nmol/l), Horizontal bar in the box is the median of the data (24.54 nmol/l).

The mean value of FT4 of healthy dogs was found to be 13.03±0.68 pmol/l. Fig 3 depicts a box plot of FT4 concentration in healthy (n=192), dogs. For each box plot, 'T bars' represent the data which is equal to the range (3.7 -25 pmol/l). Box represent the middle half of data (Interquartile range; 25^{th} to 75^{th} percentile, as 7.80-14.75 pmol/l), Horizontal bar in the box is the median of the data (10.00 pmol/l).

In dogs, hypothyroidism is usually caused by dysfunction of the thyroid gland. Levels of circulating thyroid hormone (TT3, TT4 and FT4) are now widely used in the investigations of thyroid diseases in human and veterinary clinical practice. Allen (1991) reported that determination of basal serum TT4 concentration by RIA may provide important information to diagnose hypothyroidism. As TT4, is only produced from the thyroid gland, dogs with hypothyroidism can in most cases, be distinguished from normal dogs on the basis of a low serum TT4 concentration. Serum TT3 is the most potent thyroid hormone at the cellular level and its concentration in serum is the best discriminator between euthyroid and hypothyroid dogs when interpreted with TT4. The FT4 concentration reflects the hormone available to cells at equilibrium and its serum concentration is the best indicator for tissue thyroid status and its interpretation along serum concentration of TT4 will differentiate non-thyroid illness.

RIA, a convenient and inexpensive assay for large numbers of samples with excellent sensitivity, is widely used in veterinary hospital diagnostic laboratories. In the present investigation, measurement of circulating thyroid hormone (TT3, TT4 and FT4) by RIA in 192 normal dogs is not only more comprehensive but also the first largest study so far in India addressing the status of thyroid dysfunction in the Indian canine population. The results of levels of circulating hormones reported here are in agreement with previously

Table 1: Reported levels of TT3, TT4 and FT4 by RIA in healthy dogs (n=192).

Reference	Method used for detection	No. of dogs	Particular	TT3 (nmol/l)	TT4 (nmol/l)	FT4 (pmol/l)
Present study recorded levels of TT3, TT4 and FT4 by RIA						
Present study	RIA	192	Mean±SE	1.29±0.04	28.17±1.18	13.03±0.68
			Interquartile range (25th to 75th percentile)	0.88-1.51	15.70-35.29	7.80-14.75
			Median	1.13	24.54	10
Reported values from other authors						
Dadke, 2018 and Dadke *et al.* (2018)	RIA	59	Mean±SE	1.03±0.02	29.67±1.43	9.07±0.52
			Range	0.68-1.40	11.71-9.26	4.06-18.72
Aicher *et al.* (2019)	Equilibrium dialysis	NR	Reference Range	0.8 to 2.1	11-60	6-42
Hegstad-Davies *et al.* (2015)	NR	692	Mean	NR	24.10	16.60
Higgs *et al.* (2014)	Fluorescent enzyme immunoassay	60	Range	NR	10.3 to 43.3	NR
Shadwick *et al.* (2013)	NR	NR	Reference Interval	NR	12.9-51.6	08-40
Fialkovicova *et al.* (2013)	RIA	310	Average in large, medium and small dog breeds	1.80, 1.77 and 1.81	29.11, 36.31 and 39.11	13.09, 14.32 and15.26
Suarez and Ramirez (2012)	AIA-360-Automated Immunoassay Analyser	122	Interquartile range	0.69-1.64	11.58-50.19	6.18-41.18
Peterson *et al.* (1997)	RIA	150	Median	1.50	28 .00	22.00
Paradis *et al.* (1996)	TT4 by RIA and FT4 by CLIA	30	Median	NR	40.00	19.60

ND: Not Determined, NR: Not Reported.

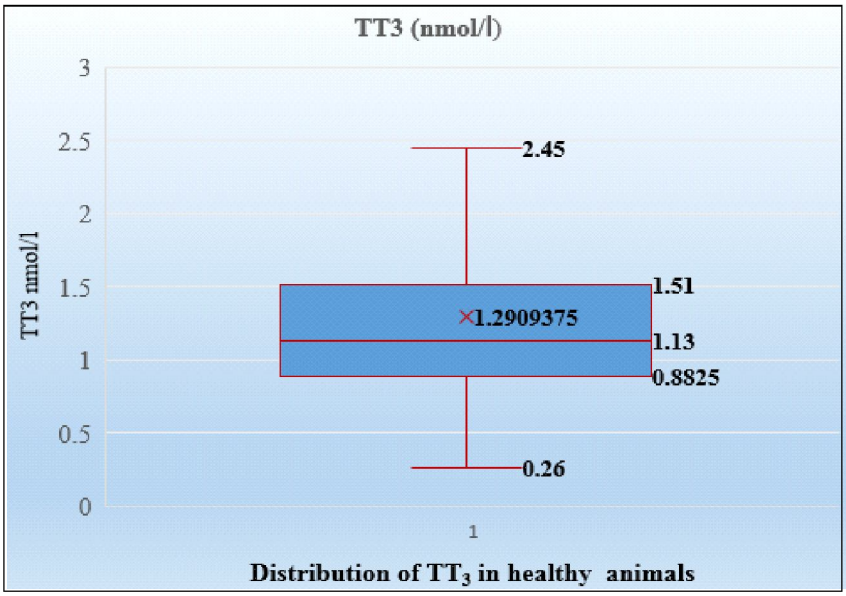


Fig 1: Box plot of TT3 in healthy dogs (n=192).

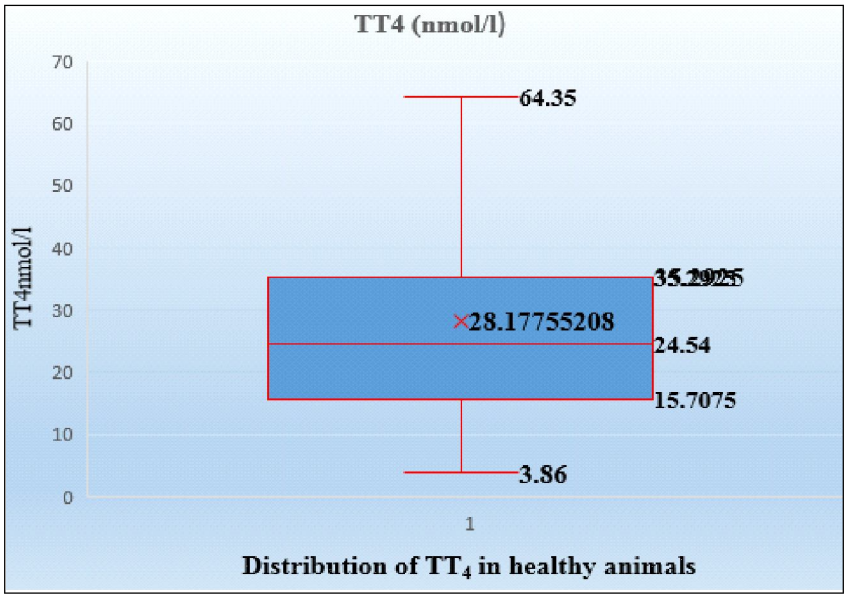


Fig 2: Box Plot of TT4 in healthy dogs (n=192).

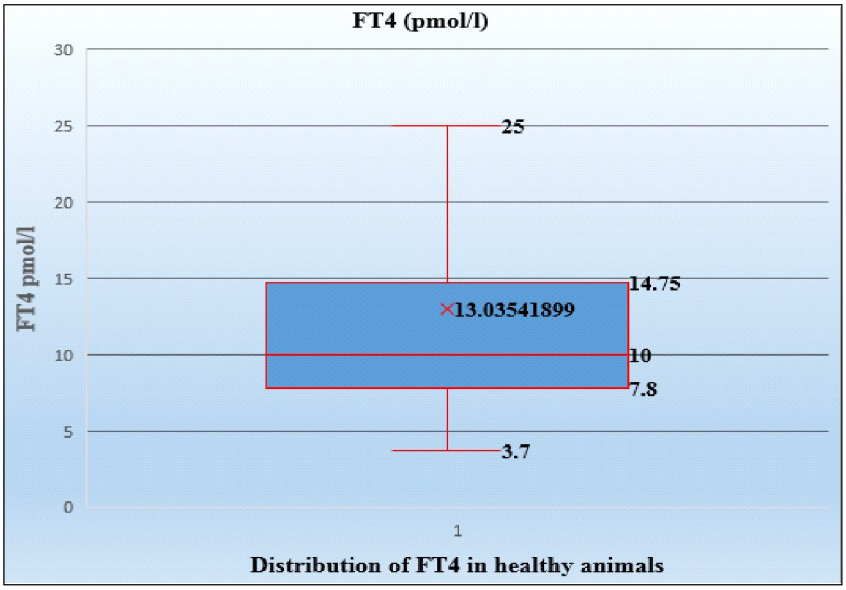


Fig 3: Box plot of FT4 in healthy dogs (n=192).

described (Table 1). The mean, interquartile range (*i.e.* 25th to 75th percentile) and median values of circulating thyroid hormone levels stated here must be considered trustworthy as they were obtained with a well-validated RIA method on a substantial canine population. We presume that the reporting of this baseline serum thyroid hormone concentration would be a valuable set of information/data in clinical setup to identify thyroid dysfunction (thyroid diseases) in the dogs which might be euthyroid, hypothyroid, or suffering from non-thyroidal illness.

CONCLUSION

The present study reports RIA enabled measurement of thyroid hormone levels in healthy dogs (n=192) in Maharashtra, India. Currently presented data has not been previously reported in the literature. Hopefully, this valuable and impactful data might help clinicians and the research community for their diagnostic, therapeutic and research work. This documented thyroid profile reference range in healthy dogs could be used as reference data to ascertain thyroid health in the canine population available in India and central East Asia.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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