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Estimation of Thyroid profile in cats through Radio Immunoassay

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Abstract

Radioimmunoassay (RIA) is a highly sensitive and specific *in vitro* technique for quantifying hormones and other small antigens in biological fluids. The present study was undertaken to establish reference values for total Triiodothyronine (TT3), total Thyroxine (TT4), and free Thyroxine (fT4) in healthy cats. A total of thirty-one (31) healthy cats were ethically enrolled for the establishment of the thyroid hormone reference range. Serum concentrations of TT3, TT4, and fT4 were measured using RIA. The estimation of TT3 was carried out using the procedure recommended by the manufacturers. However, TT4 and fT4 were estimated by doubling the serum and standard volumes and halving the final results. The results of the present study recorded mean serum TT3, TT4, and fT4 values in healthy cats as 0.69 ± 0.13 nmol/l, 28.29 ± 3.78 nmol/l and 31.41 ± 2.88 pmol/l respectively. The reference interval (25th to 75th percentile) for TT3, TT4, and fT4 was found to be 0.31 – 0.84 nmol/l, 13.84 – 34.75 nmol/l and 19.31 – 37.32 pmol/l respectively. The median for TT3, TT4, and fT4 was found to be 0.38 nmol/l, 19.95 nmol/l and 33.46 pmol/l respectively.

Keywords: Feline thyroid function, Radioimmunoassay (RIA); Triiodothyronine (TT3); Thyroxine (TT4) and Free Thyroxine (fT4).

Introduction

Feline hypothyroidism is rare, whereas hyperthyroidism is the most common endocrine disorder of older cats (Crowe, 2004; Peterson *et al*., 1983; Mooney, 2010). There is increase in the recognition of hyperthyroidism and other thyroid-responsive conditions in cats. The present study was designed to establish reference ranges for TT3, TT4, and fT4 in clinically healthy cats using Radio Immunoassay (RIA).

Materials and Methods

In the present study, thirty-one (n=31) healthy cats (average age in years: 3.35 ± 0.46; average body wt. in kg: 4.55 ± 0.31; Sex:14 males & 17 females; recorded: since year 2016) were enrolled ethically with written consent from the pet owner. The cats included in the study were referral cases (presented in year 2016) to the Department of Medicine, Outpatient Department, and Teaching Veterinary Clinical Complex (Goregaon and Parel, Mumbai Veterinary College, Mumbai), private practitioners, and pets of voluntarily participating owners. Inclusion criteria: comprised of Adult cats (male or female) aged 1 year or older, including purebred, mixed-breed, and non-descript cats, showing no clinical signs of illness, systemic disease, or endocrine dysfunction. Exclusion criteria were Cats receiving treatment for any endocrinopathy, including thyroid disorders; pregnant or lactating cats; cats treated with drugs known to affect thyroid function (e.g., steroids, methimazole, L-thyroxine); and cats with non-thyroidal illnesses such as viral infections, neoplasia of non-thyroidal origin, hepatic dysfunction, or severe renal disease that could secondarily affect thyroid function. The present study was initiated after permission from the Institutional Ethics Committee for Veterinary Clinical Research and the Institutional Biosafety Committee of Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University-Nagpur, Mumbai-India. Five ml of blood was collected from each cat. Two millilitres were placed in EDTA vials for haematological analysis, and the remaining 3 ml was used to harvest serum for biochemical and thyroid hormone estimation. Serum samples for thyroid profile analysis were stored at −20 °C until assayed. Thyroid hormone estimation (TT3, TT4 and fT4) was performed using RIA at the Radio Isotope Laboratory and Veterinary Nuclear Medicine facility of Mumbai Veterinary College, Mumbai. Commercial human RIA kits, manufactured by the Board of Radiation and Isotope Technology (BRIT), Department of Atomic

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Energy, Mumbai, were used for the measurement of feline thyroid hormones. The RIA kits used for thyroid hormone estimation included MAG-3B, a magnetisable particle-based kit for TT3; MAG-4B, a magnetisable particle-based kit for TT4; and BRIACT-1, a coated tube-based kit for fT4. In the present study, total thyroxine (TT4) and free thyroxine (fT4) were initially estimated using the standard procedures provided by the kit manufacturers. However, these assays yielded undetectable or subnormal concentrations. Therefore, the procedures were modified by doubling the volume of serum and standards and halving the final calculated values, as recommended by Anderson and Brown (1979), to obtain accurate TT4 and fT4 measurements. Thyroid hormones were assayed for each sample, providing paired/ duplicate observations. Quality control parameters, including the levels of control samples and recovery percentages, were assessed to validate each assay.

Results and Discussion

Assay passed all recommended quality control parameters, viz., magnitude of control samples provided with kits and percent 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., 25th to 75th percentile), and median of TT3, TT4, and fT4 in healthy cats (n=31) enrolled in the study, and levels of thyroid hormones reported by other authors are presented in Table -1. The mean value of TT3 in healthy cats was found to be 0.69 ± 0.13 nmol/l. Figure -1 depicts a box plot of TT3 concentration in healthy (n=31) cats. For each box plot, 'T bars' represent the data, which is equal to the range (0.15 – 1.31 nmol/l). The box represents the middle half of the data (Interquartile range; 25th to 75th percentile, as 0.31 – 0.84 nmol/l). The horizontal bar in the box is the median of the data (0.38 nmol/l). The mean concentration and interquartile range (25th to 75th percentile) of TT3 recorded in the present study falls within the previously reported normal ranges of 0.60–1.73 nmol/l (Reap *et al.,* 1978), 0.23–1.23 nmol/l (Reimers *et al.,* 1982), 0.13–1.24 nmol/l (Thoday *et al.,* 1984), and 0.4–1.8 nmol/l (Peterson *et al.,* 2001), respectively. The mean value of TT4 of healthy cats was recorded as 28.29 ± 3.78 nmol/l. Figure -2 depicts a box plot of TT4 concentration in healthy cats (n=31). For each box plot, 'T bars' represent the data, which is equal to the range (8.37 – 54.70 nmol/l). The box represents the middle half of the data (Interquartile range; 25th to 75th percentile, as 13.84 – 34.75 nmol/l). The horizontal bar in the box is the median of the data (19.95 nmol/l). The mean concentration and interquartile range (25th to 75th percentile) of TT4 recorded in the present study falls within the previously reported normal ranges 18-83.7 nmol/l (Anderson and Brown, 1979), 9.7-56.3 nmol/l (Reimers *et al.,*1982), 5-54.1 nmol/l (Thoday *et al,* 1984) and of 12.9-38.7 nmol/l (Peterson *et al.,* 1981).

The mean value of fT4 of healthy cats was found to be 31.41 ± 2.88 pmol/l. Figure 3 depicts a box plot of fT4 concentration in healthy cats (n = 31). For each box plot, 'T bars' represent the data, which is equal to the range (10.94 – 55.34 pmol/l). The box represents the middle half of the data (Interquartile range; 25th to 75th percentile, as 19.31 – 37.32 pmol/l), the horizontal bar in the box is the median of the data (33.46 pmol/l). The mean concentration and interquartile range (25th to 75th percentile) of fT4 recorded in the present study fall within the previously reported normal ranges 15-50 pmol/l (Peterson *et al.,* 2001) and 10-51 pmol/l (Peterson *et al.,*2015).

Measurements of circulating thyroid hormones using RIA are now widely employed in the investigation of thyroid disease in humans. These techniques can also be applied in feline practice, provided the methods are modified to accommodate the much lower circulating concentrations of total T4 and T3 found in cats compared with humans. Reap *et al.,* (1978) and Anderson and Brown (1979) have reported details of the RIA methods they used to determine plasma total T4 and T3 levels in normal cats. Reap *et al.,* (1978) measured total TT4 and TT3 in only 10 animals, whereas Anderson and Brown (1979) evaluated TT4 but not TT3 in 92 cats. Both groups used commercially produced RIA kits designed for human serum and recognized the challenges of assaying the low thyroid hormone levels in cats. To address this, they doubled the serum volume used in the assays and halved the measured values to obtain the correct hormone concentrations.

In the present study, commercial RIA kits intended for human serum was used to assess the thyroid profile of cats. Using the manufacturer's recommended protocol, only TT3 results showed clinical correlation in healthy cats, whereas TT4 and fT4 values were low and poorly correlated clinically. Following the approach described by Anderson and Brown (1979), we adapted our RIA procedures for TT4 and fT4 by doubling the

volumes of both the standards supplied with the kits and the serum samples, and then halving the resulting values to obtain the precise hormone concentrations. In our study, the adjusted total volumes of standards and serum samples were 50 µL for TT4 and 100 µL for fT4, respectively. This modification produced acceptable concentrations of both TT4 and fT4, with improved clinical correlation in healthy cats.

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Table 1: TT3, TT4 and fT4 concentration in healthy cats (n=31) using RIA

Parameters	Mean and range recorded in the present study	International published data about thyroid profile			
TT3 (nmol/l)	0.69±0.13	0.60-1.73 (Reap *et al*, 1978)	0.23-1.23 (Reimers *et al*, 1982)	0.13-1.24 (Thoday *et al*, 1984)	0.4-1.8 (Peterson *et al* 2001)
TT4 (nmol/l)	28.29±3.78	18-83.7 (Anderson & Brown, 1979)	9.7-56.3 (Reimers *et al*, 1982)	5-54.1 (Thoday *et al*, 1984)	12.9-38.7 (Peterson *et al*, 1981)
f T4 (pmol/l)	31.41±2.88	15-50 (Peterson *et al* 2001)	10-51 (Peterson *et al* 2015)	--	--

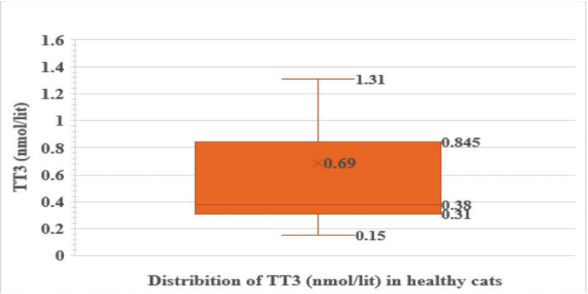

Figure 1: Box Plot of TT3 in healthy cats (n=31)

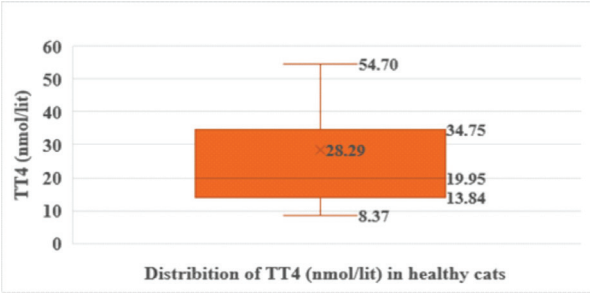

Figure 2: Box Plot of TT4 in healthy cats (n=31)

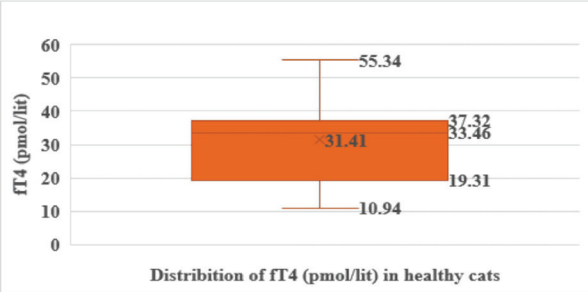

Figure 3: Box Plot of fT4 in healthy cats (n=31)

References

Anderson, J.H. and Brown, R.E. 1979. Serum thyroxine (T4) and triiodothyronine (T3) uptake values in normal adult cats as determined by radioimmunoassay. *Am. J. Vet. Res.*. **40(10)**: 1493–1494.

Crowe, A. 2004. Congenital hypothyroidism in a cat. *Canadian Vet. J,* **45**: 168–170.

Dadke, A.R., Galdhar, C.N., Gaikwad, R.V., and Kadam, D.P. 2018. Studies on biochemical alterations in hypothyroid dogs. *Indian J. Vet.Med.,* **38**: 35–38.

Galdhar, C., Chandra, S., Dadke, A., Gaikwad, R. and Sarode, A. 2021. Metabolic and hormonal changes in water buffaloes during postparturient peak lactation. *Buffalo Bulletin*. **40**: 565–570.

Galdhar, C.N., Gaikwad, R.V., Masare, P.S., Garud, K.V., Vaidya, S., Bhojne, G.R., et al. and Parkhe, A.G. (2024). Triiodothyronine (TT3), total thyroxine (TT4) and free thyroxine (FT4) reference range in healthy dogs by radioimmunoassay (RIA). *Indian Journal of Animal Research*. 58(1): 46–50.

Galdhar, C.N., Vaidya, S.M. and Gaikwad R.V. 2022. Serum Triiodothyronine (T) And Thyroxine (T) Levels in Healthy Goats. *Indian J. Small Ruminants*, 28(2): 403-404.

Galdhar C.N. Shubham Vyas, R.V. Gaikwad, J.R. Kawade, and P.M. Sapkal. 2024. Studies on the Assessment of Hair Cortisol Concentration in Dogs by Radioimmunoassay (RIA). *Indian J. Anim. Res*., doi: 10.18805/IJAR.B-5221. Online: 10-05-2024

Jayabhaye, R.M., Galdhar, C.N., Dadke, A.R., Gaikwad, R.V., Banik, S. and Rajkhowa, S. 2021. Radio immune assay (RIA) enabled thyroid profile (TT3, TT4 and fT4) in pigs. *Indian J. Vet. Med.,* **41**: 9–15.

Mooney, C.T. 2010. Hyperthyroidism. In: Ettinger, S. and Feldman, E. (eds.), *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. W.B. Saunders, Philadelphia. pp. 1761–1779.

Peterson, M.E., Guterl, J., Nichols, R. and Rishniw, M. 2015. Evaluation of serum thyroid stimulating hormone concentration as a diagnostic test for hyperthyroidism in cats. *Journal of Veterinary Internal Medicine*. 29: 1169–1170.

Peterson, M.E., Kintzer, P.P. and Cavanagh, P.G. 1983. Feline hyperthyroidism: pretreatment clinical and laboratory evaluation of 131 cases. *J. Vet. Internal Med.,***183**: 103–110.

Peterson, M.E., Kintzer, P.P., Fox, P.R., Ferguson, D.C. and Johnson, G.F. 1981. Feline hyperthyroidism: pretreatment clinical and laboratory evaluation of 131 cases. *J. Am. Vet. Med. Assoc.,* **183**: 103–110.

Peterson, M.E., Melián, C. and Nichols, R. 2001. Measurement of serum concentrations of free thyroxine, total thyroxine and total triiodothyronine in cats with hyperthyroidism and cats with non-thyroidal disease. *J. Am. Vet. Med. Assoc.,* **218(4)**: 529–536.

Reap, M., Cass, C. and Hightower, D. (1978). Thyroxine and triiodothyronine levels in ten species of animals. *Southwest Veterinary*. **31**: 31.

Reimers, T.J., Cowan, R.G., Davidson, H.P. and Colby, E.D. (1982). Validation of radioimmunoassays for triiodothyronine, thyroxine and hydrocortisone (cortisol) in canine, feline and equine sera. *Am. J. Vet.Res.,***42(11)**: 2016–2021.

Salutgi, P., Galdhar, C., Sonigra, R., Natu, K., Mumbarkar, N., Mathkar, S., Dalvie, A. and Gaikwad, R. (2023). Radio immune assay (RIA) enabled total triiodothyronine (TT3) and total thyroxine (TT4) in canine trypanosomiasis: first case report from Maharashtra (India). *Iranian J. Parasitol.,* **18**: 107–112.

Snedecor, G. W., and Cochran, W. G. 2004. Statistical methods, 8thEdn. *Ames: Iowa State Univ. Press Iowa*, *54*, 71-82.

Thoday, K.L., Setht, J. and Elton, R.A. 1984. Radioimmunoassay of serum total thyroxine and triiodothyronine in healthy cats: assay methodology and effects of age, sex, breed, heredity and environment. *J. Small Anim. Pract.,* 25: 451–412.

Yalow, R.S. and Berson, S.A. (1960). Immunoassay of endogenous plasma insulin in man. *J. Clinical Investigation*. 39: 1157–1175.

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