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### Case Report

## Radio Immune Assay (RIA) Enabled Total Triiodothyronine (TT<sub>3</sub>) and Total Thyroxine (TT<sub>4</sub>) in Canine Trypanosomiasis: First Case Report from Maharashtra (India)

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#### **Abstract**

Radio Immune Assay (RIA) is an extremely sensitive in vitro assay technique to measure concentrations of antigen viz. hormones in biological fluids using antibodies. The present study reports the status of total triiodothyronine (TT<sub>3</sub>) and total thyroxine (TT<sub>4</sub>) in *Trypanosoma evansi* infection in a dog, year 2022. An adult, non-descript, male dog was referred to the Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, (MAFSU), Parel, Mumbai (India) with a history of inappetence, weakness, and ataxia of the hind limbs. Inspection revealed cachexia, anemia, bedsores, and mild mucopurulent ocular and nasal discharge. Clinical examination revealed pyrexia, polypnoea and tachycardia. There was an enlargement of popliteal, pre-scapular, and submandibular lymph nodes. The blood smear examination revealed severe infection of extracellular *T. evansi*. Laboratory investigations showed an altered haemato-biochemical profile. RIA-enabled thyroid hormone profile revealed a reduced concentration of TT<sub>3</sub> (0.57 nmol/l) and TT<sub>4</sub> (22.52 nmol/l). The present study reports a reduction in the concentration of TT<sub>3</sub> and TT<sub>4</sub> in a dog suffering from trypanosomiasis. The drop in TT<sub>4</sub> concentration was within the normal limit, this could be a cause for the non-appearance of usual clinical symptoms of hypothyroidism in the present case.



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## Introduction

Alterations in thyroid hormone concentrations in response to Non-Thyroid Illness (NTIs) are well recognized in veterinary clinical practice (1, 2). In severe NTIs, there is a decreased concentration of Triiodothyronine ( $TT_3$ ) and Total thyroxine ( $TT_4$ ). *Trypanosoma evansi* has a wide host range in most companion animals. The disease may occur in both acute and chronic forms. The reports on alterations in haemato-biochemical profile by acute and chronic infection are well documented (3, 4). However, reports on the status of thyroid function during canine trypanosomiasis have been little studied in veterinary clinical practice (5, 6).

Present clinical case record is first reported from Maharashtra (India) about the status of total triiodothyronine ( $TT_3$ ) and total thyroxine ( $TT_4$ ) in *T. evansi* infection in a dog.

## Case Report

A Non-descript, adult, male, neutered dog was presented to the Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Parel-Mumbai 400012 (India) in the year 2022.

Institutional Biosafety committee (IBSC) approved the study based on the Declaration of Helsinki. The owner presented this dog with the complaint of non-ambulatory paraparesis. Anamnesis of the patient revealed inappetence, progressive weakness, and ataxia of the hind limbs for a fortnight. Inspection of the patient revealed evidence of cachexia, anaemia (Fig. 1 and 2), decubital ulcers/pressure ulcers (bed sores) (Fig. 3), and mild mucopurulent ocular and nasal discharge. Clinical examination of the patient revealed pyrexia ( $103^{\circ}\text{F}$ ), polypnoea (29 breaths/minute), and tachycardia (102 bpm).



**Fig. 1:** Pale conjunctival mucous membranes



**Fig. 2:** Pale oral mucous membranes

There was an enlargement of popliteal, pre-scapular, and submandibular lymph nodes. On neurological examination, pain sensation (deep pain) reflexes were absent in rear limbs. Abdominal palpation revealed splenomegaly and urinary incontinence. After clinical examination of the patient, blood was collected for complete blood count (CBC), liver function test (LFT), kidney function test (KFT), and thyroid profile testing (by Radio Immune As-

say-RIA). Additionally, thin blood smears were also made from ear tips and stained with Giemsa stain to detect peripheral parasitemia. The results of the laboratory investigations are presented in Table 1. The microscopic examination of the blood smear revealed the presence of *T. evansi* (slender forms of trypanosomes with subterminal kinetoplasts, large undulating membrane, central nucleus and a long free flagellum) (Fig. 4).



**Fig. 3:** Cachexia and bed sores

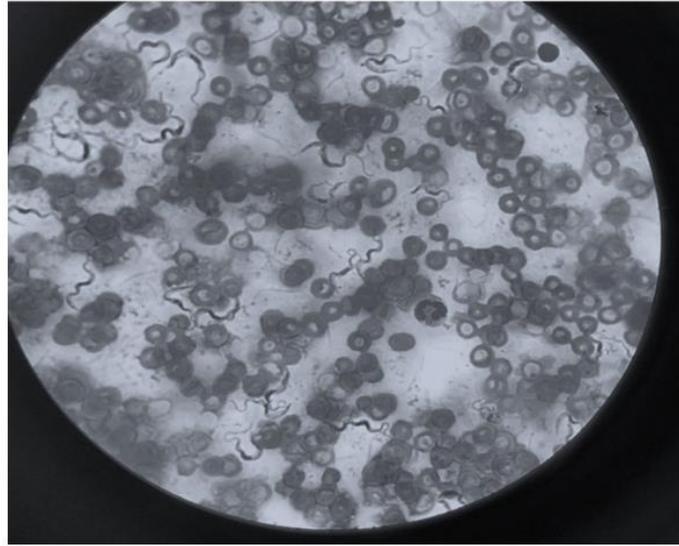


Fig. 4: Demonstrating *Trypanosoma evansi*

Table 1: Laboratory Investigation of a dog suffering from *T. evansi* infection and reference value for thyroid profile

<i>Sr. No.</i>	<i>Parameter</i>	<i>Result</i>
1	Hb (gm%)	4.30
2	TEC (million/cmm)	1.87
3	PCV (%)	12.60
4	MCV (fl)	67.38
5	MCH (pg)	22.99
6	MCHC (g/dl)	34.13
7	TLC (*10 <sup>3</sup> /cmm)	3.80
8	Platelets ( /cmm)	22000
9	Blood Parasites (peripheral Blood smear examination)	<i>Trypanosoma evansi</i> (++++)
10	BUN (mg/dl)	20.10
11	Serum Creatinine (mg/dl)	1.10
12	Bilirubin Total (mg/dl)	0.20
13	Bilirubin Direct (mg/dl)	0.10
14	Bilirubin Indirect (mg/dl)	0.10
15	SGPT (IU/L)	171.00
16	SGOT (IU/L)	107.00
17	Alkaline Phosphate (IU/L)	195.00
18	Total Proteins (gm/dl)	6.40
19	Albumin (gm/dl)	2.30
20	Globulin (gm/dl)	4.10
21	A/G	0.56
22	TT <sub>3</sub> (nmol/l)	0.57
23	TT <sub>4</sub> (nmol/l)	22.52
24	Reference Value TT <sub>3</sub> (nmol/l)	1.29 ± 0.04**
25	Reference Value TT <sub>4</sub> (nmol/l)	28.17±1.18**
26	TT <sub>3</sub> (nmol/l): Interquartile range (25 <sup>th</sup> to 75 <sup>th</sup> percentile)	0.88–1.51**
27	TT <sub>4</sub> (nmol/l): Interquartile range (25 <sup>th</sup> to 75 <sup>th</sup> percentile)	15.70– 35.29**

\*\* Galdhar C.N. (2021)

Thyroid hormones ( $TT_3$ , and  $TT_4$ ) were estimated using RIA and the procedure was carried out at the Radio Isotope Laboratory, Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai (India). Commercial RIA kits for human purposes manufactured by the Board of Radiation and Isotope Technology (BRIT), Vashi, Mumbai (India) were procured for thyroid profile estimation. Thyroid hormones were assayed of the collected sample giving paired observation.  $TT_3$  and  $TT_4$  were measured as per the standard procedure outlined by the manufacturers. Quality control parameters viz. magnitude of control samples and recovery percentage were studied to validate the assay.

## Discussion

Based on clinical and laboratory findings, the dog was suffering from *T. evansi* infection. In the present study, the authors are reporting the status of  $TT_3$  and  $TT_4$  in a dog suffering from *T. evansi* infection, for the first time from Maharashtra (India). The estimation of thyroid profile, was undertaken by Radio Immune Assay (RIA). RIA is extremely sensitive in vitro assay technique to measure concentrations of antigen viz. hormones in biological fluids using antibodies. The technique is impressively sensitive and specific with the ability to measure small molecules.  $I^{125}$  is the radioisotope used in RIA procedures. The method offers a convenient assay of large numbers of samples with good precision. It can assay materials that are difficult to detect and measure using other techniques (7). Since 1975, technological advances in RIA have progressively improved the specificity, reproducibility, and sensitivity of thyroid testing methods and thus RIA has been the method of choice for measuring circulating levels of thyroid hormones in vertebrates (8). Further, RIA is considered the best assay to estimate hormones as compared to

other techniques viz. ELISA and CLIA (Chemiluminescence Immunoassay). RIA technique is sensitive, cost-effective and the use of long half-life radioactive materials ( $I^{125}$ ; physical half-life 60 days) facilitates its use for a long time. Though RIA is the best test to estimate hormones. However, the use of radioactive materials and their disposal is critically important. It is compulsory that assay procedures must be undertaken under the supervision of a Radiological Safety Officer (9).

In the present investigation, RIA-enabled concentrations of  $TT_3$  and  $TT_4$  were 0.57 nmol/l and 22.52 nmol/l, respectively. These estimated hormonal concentrations were lower in magnitude as compared to the reference range reported (2), Table 1. When authors apprise thyroid profile critically, a reduction in the concentration of  $TT_3$  was noticed along with a reduction in the concentration of  $TT_4$ , but a drop in  $TT_4$  concentration was in the normal reference range. Therefore, no clinical signs of hypothyroidism were seen in the ailing dog. The reports on impairment in thyroidal function after experimental infection with trypanosomiasis have been documented in goats and cattle (5, 10), however, mechanisms by which trypanosomes affect thyroid functions are not completely understood.

## Conclusion

The present study reports a reduction in the concentration of  $TT_3$  and  $TT_4$  in a dog suffering from trypanosomiasis. The drop in  $TT_4$  concentration was in the normal limit, this could be a cause for the non-appearance of clinical symptoms of hypothyroidism in the present case.

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### Conflicts of interest

The authors declare that there is no conflict of interest.

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